EFFECTS OF OCULAR HYPOTENSIVE DRUGS AND THEIR MECHANISM OF ACTIONS TO CONTROL EXPERIMENTAL INDUCED MYOPIA IN CHICKEN

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Effects of Ocular Hypotensive Drugs and Their Mechanism of Actions to Control Experimental Induced Myopia in Chicken

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CERTIFICATE OF ORIGINALITY

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Abstract

Myopia is a common cause of correctable vision loss and uncorrected myopia has become one of the leading causes of blindness worldwide. The overall prevalence of myopia is increasing rapidly. It is estimated that 33.9% of the world' s population is myopic and by 2050 this is predicted to reach nearly 50%. In South-East Asia, myopia affects more than 80% to 90% of young adults, of whom approximately 10-20% are high myopes. High myopia increases the risk of ocular pathological changes, including retinal degeneration, retinal detachment, cataracts, and glaucoma.

Despite substantial research, the exact mechanism of myopia development remains unclear. There is also no universally accepted pharmaceutical therapy to control myopia progression. Research in this area found that atropine and only a few muscarinic acetylcholine receptors (mAChR) antagonists can effectively inhibit myopia. Currently, topical atropine 1% is the most effective pharmaceutical therapy for myopia control in humans, but adverse side effects include photophobia, glare, paralysis of accommodation, allergies and rebound effects. The underlying mechanism through which atropine inhibits myopia development also remains unclear. More recently, a few pharmacological agents, originally approved for glaucoma, have been shown to be effective in treating induced myopia in animal models. This PhD thesis describes work that aimed to unravel the signaling pathways involved in myopia development in the posterior eye by using a chicken model of myopia and a pharmacological approach. The study commenced with an experiment delineating the dose-response of atropine (Chapter 3). This extended to an investigation of non-canonical molecular targets of atropine, and examined the relations between glaucoma and myopia molecular pathways.

There is significant evidence that myopia-inhibiting non-MAChR antagonist drugs, such as atropine and mamba toxin-3 (MT3), not only react with M4 mAChR, but also bind

with α-adrenoceptors. The MT3 component of the mamba venom is especially selective for M4 mAChR subtypes in humans and is the most effective anti-myopia agent. Therefore, the next stage of the study was an investigation as to whether α1A (metaraminol), α2A (lofexidine), and a non-specific adrenergic receptor drug (cirazoline) may be involved in regulation of excessive eye growth (Chapter 4). The results provided novel evidence that α1 adrenergic agonists and nonspecific agonists had a similar inhibitory effect against myopia in chickens, suggesting their potential as pharmacological therapies. The data also revealed that α2-adrenoceptor agonist inhibited lens-induced myopia (LIM) as well as form-deprivation myopia (FDM) in a dose-dependent manner.

Other evidence suggested that few α-adrenoceptor drugs are most effective at reducing myopia through IOP-independent mechanisms. It affects the extracellular matrix of the sclera and is a relatively new IOP-lowering drug. Further study revealed that the link between the development of myopia and axial length elongation was associated with extracellular remodeling of the sclera. Therefore, the effects of one of the most potent IOP-lowering prostaglandin analogue drugs, topical latanoprost, were investigated and were found to be very effective in slowing the progression of myopia in chicks with either FDM or LIM (Chapter 5). Although the exact underlying mechanism of topical latanoprost in FDM and LIM involved in myopia control remains unknown, the initial results demonstrated that daily topical administration of latanoprost was effective in inhibiting both experimental myopia models of FDM and LIM in chicks. Further study revealed that its effect was associated with reduced scleral thinning and relatively upregulated EGR1 protein expression in the retina. Furthermore, it was demonstrated that the chosen concentration of latanoprost also reduced IOP in chicks.

Later study investigated the effects of another ocular hypertensive medication that effectively reduces IOP.

Rho-kinase is a serine/threonine kinase that plays a significant role in regulating and modifying cell size and shape. Rho-kinase inhibitors increase aqueous outflow facility by altering the ultrastructure of the trabecular meshwork leading to a reduction in IOP, which is similar to effects of prostaglandin analogues. The potential myopia-controlling effects of different doses of the Rho-kinase inhibitor (Y-33075 dihydrochloride) were examined in chicken eyes using LIM and FDM models (Chapter 6). This work provided the first evidence that intravitreal injection of Rho-kinase inhibitor (Y-33075 dihydrochloride) could significantly inhibit ocular growth and myopic refractive error in both LIM and FDM models in a dose-dependent manner. The result also showed that all tested doses of Rho-kinase inhibitor (Y-33075 dihydrochloride) significantly lowered IOP compared to control groups. This evidence may open up new opportunities to explore IOP-lowering drugs as future treatment options for myopia.

Publications Arising from this Thesis

1. Mezbah Uddin, Bing Zuo, KK Li, Chi-ho To, and Dennis Y. Tse, "Alpha-1 and alpha-2 adrenergic receptor agonists inhibit lens-induced myopia in chicks" *Association for Ocular Pharmacology and Therapeutics (AOPT);* March 4th-7th, 2021.

2. Mezbah Uddin, Samantha Sze Wan Shan, King-Kit Li, Bing Zuo, Jiajun Wang, Kirk Patrick Carreon Catral, Chi-ho To, and Dennis Yan-Yin Tse, "Topical latanoprost inhibits the developments of form-deprivation myopia and lens-induced myopia in the chick model." Presented at *The Association for Research in Vision and Ophthalmology (ARVO)* Annual Meeting, May 11-12, 2022.

3. Mezbah Uddin, Rachel Ka-Man Chun, King-Kit Li, Carly Siu-Yin Lam, Chi-Ho To, and Dennis Yan-Yin Tse. "Combined effects of atropine and myopic defocus against lens-induced myopia in the chicks model." Presented at *The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting;* April 23 – 27, *2023.*

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Chapter 1

Introduction

1.1 Myopia

Myopia, also known as short-sightedness, is one of the major causes of ocular refractive disorders worldwide. In myopes, parallel rays of light entering the eye are focused in front of the photoreceptors on the retina and, as a result, there is difficulty in seeing distant objects clearly. This condition arises from a combination of two factors: either an excessive curvature of the anterior part of the eye or an enlargement of the eyeball from front to back [1]. Previous studies have characterized myopia as a condition, defining it by a spherical equivalent of refractive error equal to or greater than -0.50D, -0.75D, and -1.0D in each respective eye [1, 2].

1.1.1 Classification of Myopia

Early in the decade myopia was classified according to its degree and the refractive power of the ideal correction. Myopia was clinically classified into three categories: low myopia defined as ≤ -3.00D, moderate myopia > -3.00D to ≤ -6.00D, and high myopia > -6.00D [3]. Other studies have proposed that myopia may be classified as non-pathological or pathological myopia [4]. These groups have different clinical features, disease processes and prognoses. Pathological myopia commonly occurs at an early stage of life and progresses very rapidly. It is characterized by degenerative changes in the back of the eyes. Other clinical and epidemiological studies of myopia suggested categorization based on the combined results of descriptive characteristics including primary myopia, secondary myopia, refractive myopia, and axial myopia [5]. The study used a results-based approach and its relation to the management of myopia. Classification may also be based on the power of the optical components of the eyes, including the cornea, crystalline lens, and axial length of the eyeball. Optical power of the cornea and crystalline lens may be too strong (more than 60D) while axial length of the eyeball is normal. In contrast, myopic refractive error may be associated with a longer axial length, while the refractive error of the eye is normal due to the excessive enlargement of the vitreous chamber depth [1, 6]. Classification may be made based on the age of onset of myopia between 7-15 years/ This divides patients into 4 groups based on the risk of developing high myopia [7]. It was found that myopia significantly increased more than 50% for 7-8 years of old, 30% for 9 years of old, and 20% for 10 years old. Finally, myopia may be characterized as congenital, which is present at birth or diagnosed at 6 years of age or less, or as developmental myopia, which may develop before age 18 and is termed, school onset or youth-onset myopia, adult-onset myopia, developing from 20-40 years of old and late adult-onset myopia over 40 years of old [8].

1.1.2 Prevalence of Myopia

Myopia prevalence is increasing very rapidly across the globe. There are several risk factors for development of myopia including geographic regions, ethnicity, environmental factors, lifestyle, insufficient time outdoors, socioeconomic status, educational achievement, and urban versus rural populations, that may be possible factors responsible for this increasing prevalence [9]. However, several epidemiological studies

have shown it may also be associated with the patients level of education, lifestyle, use of electronic devices, university attendance and profession may play a role in the development of myopia [10, 11]. In addition, the World Health Organization (WHO) noted that global prevalence of myopia was around 30% and has dramatically increased over the past few decades. However, in South Asia, myopia affects more than 80% to 90% of young adults in China [12]. The current prevalence of high myopia in young adults is around 10-20%, which is approximately 1-3% in South Asia and 1% in Europeans. High myopia is often accompanied by signs of retinal pathological or atrophic changes [13]. Table 1.1 provides a brief overview of the prevalence of myopia based on age.

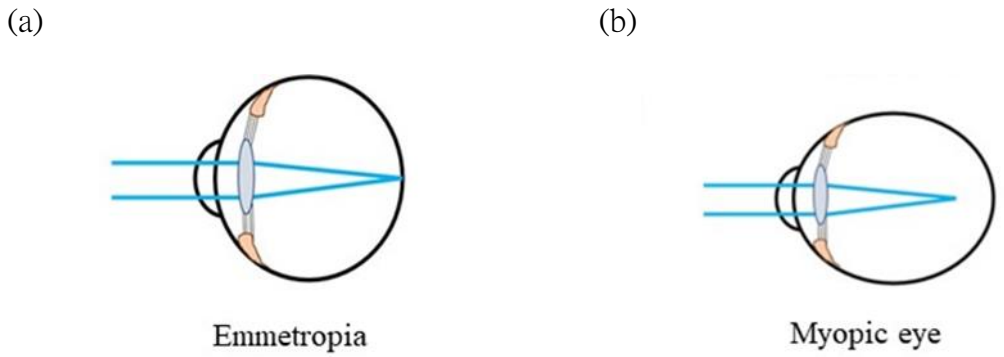


Figure 1. Schematic diagram of the emmetropic eye and the myopic eye. (a) Emmetropia: Normal vision occurs when light is focused upon the retina. (b) Myopia: where parallel rays are focused in front of the retina when accommodation is at rest.

Table 1.1: Overview of myopia prevalence by age

Authors	Country	Year	Age group	Prevalence
Zhang et al. [14]	China	2022	6 years	14.5%
			6-12 years	36%
			13-18 years	71%
			19-24 years	81%
Karuppiah et al. [15]	Singapore	2021	Preschool	29%
			7-8 years	34.7%
			9-10 years	53.1%
			18 years	80% - 90%
Yoon et al. [16]	South Korea	2011	6-10 years	50%
			12-18 years	72% - 84%
			19-24 years	96%
Yotsukura et al. [17]	Japan	2019	6-12 years	40%
			13-18 years	76.5%
			19-24 years	70% - 94.9%
Lam et al. [18]	Hong Kong, China	2012	6 years	18.3%
			12 years	61.5%
Fortin et al. [19]	United States	2022	6-17 years	41%
			13-18 years	15.7%
Lin et al. and Tan et al. [20, 21]	Taiwan	2004-2005	6 years	5.8% - 20%
			15 years	62.2% - 81%
			16-18 years	84%
Cumberland et al. [22]	United Kingdom	2022	6-7 years	1.9%
			12-13 years	16.4%
			15-16 years	25%
Hartwig et al. [23]	Germany	2018	6 years	18%
			7-12 years	30%
			13-18 years	72%
Fu et al. [24]	Australia	2020	4-12 years	8.8%

Hussain et al. [25]	Malaysia	2021	7 years	9.8%
			15 years	34.4%
Dandona et al. [26]	India	2002	5-15 years	21.1%
Sapkota et al. [27]	Nepal	2008	5-15 years	10.9% - 27.4%
Barliana et al. [28]	Indonesia	2023	Preschool	4.9%
Shah et al. [29]	Pakistan	2008	30 years	36.5%
Bourne et al. [30]	Bangladesh	2004	30 years	22.1%
Kleinstein et al. [31]	Asians Americans	2003	5-7 years	18.55
	Hispanics	2003	5-7 years	13.2%
	African Americans	2003	5-7 years	6.6%
	Europeans	2003	5-7 years	4.4%
O' Donoghue et al. [32]	Europeans	2010	10-12 years	3% - 5%
			12-13 years	20%
Barria et al. [33]	Chile	2018	5 years	3% - 4%
			15 years	14.7% -19.4%
Villarreal et al. [34]	Sweden	2000	12-14 years	49.7%
Plainis et al. [35]	Bulgaria and Greece	2009	10-15 years	13.5% -37.2%

1.1.3 Etiology of Myopia

The exact mechanism of myopia progression is not completely understood, but several risk factors has been reported to contribute to myopia development, including genetics, the environmental, activities which increase the exposure to optical blur, near work or excessive reading, accommodation or accommodative lag, higher intelligence, ambient lighting, lack of outdoor activities, and nutrition.

1.1.4 Genetic Factors of Myopia

Genetic mutations thought to increase the risk of myopia are generally involve biological pathways that mediate extracellular matrix remodelling and regular connective tissue composition. These genes are involved in different pathways including glycosylation, mannosylation, gliogenesis, lens development and Schwann cell differentiation. Previous genetic studies have discovered approximately 600 gene loci associated with refractive error and myopia development. Of these, 22 loci are on autosomal chromosomes and 3 are on the X chromosome [36]. Previously, twin and family studies had reported that children with parents who has a high myopic refractive error had an increased risk of developing some degree of myopia due to strong genetic factors [37, 38]. Genetic studies identified a new molecule, the Cacna1f, which has a diversity of functions. Its site of expression seems to include whole layers of the retina that are involved in myopia genesis [39].

Analysis of the myopia development signaling pathway indicated that it had a role in synaptic transmission, calcium ion binding, plasma membrane function, cation channel activity, and cell to cell adhesions. Although some forms of high myopia or secondary myopia, including Stickler syndrome, congenital stationary night blindness, Marfan syndrome, Weill-Marchesani syndrome, Ehlers – Danlos syndrome, and Donnai-Barrow syndrome, are very rare. The Northern Ireland Childhood Errors of Refraction (NICER) study, a population-based study which explored the risk of childhood myopia in 12- to 13 year-old school children, concluded that children who had one myopic parent had nearly 2.91 times increased chance of developing myopia, whilst children whose both parents were myopic had considerably higher chance for myopia (approximately 7.79 times) compared with those having emmetropic or hypermetropic parents [40]. Additional evidence has supported these findings showing that there was a rapid increase in myopia progression due to high genetic risk compared with a non-myopic person at low genetic risk [41]. The second study estimated the relationship between parental myopia and increased likelihood of myopia in children was controlled by decreased levels of physical activities, more time spent indoors, and homework or near work activities (37). Previous gene studies

influence both intraocular pressure (IOP) and axial length, which are closely connected to myopia and refractive error [42]. However, similar gene studies have also reported associations between KCNJ2 and SIX6 gene, whereas KCNJ2 regulates ion channel activity, and the SIX6 gene is crucial for ocular development [43]. It was suggested that genes influence ocular growth and refractive growth outcomes through pathways that regulate neurotransmitter functions, retinoic acid metabolism, and growth-regulating mechanisms. Although a recent multiethnic genome-wide association study (GWAS) identified that PRSS56 variants are associated with refractive errors, high hyperopia, and angle-closure glaucoma, suggesting the gene's role in both IOP regulation and eye growth [44]. PRSS56 is expressed in retinal Muller cells, where it contributes to the regulation of axial growth. In addition, genes involved in corneal structure, such as LTBP2 and COL4A3, play a significant role in maintaining ocular shape and integrity, which also changes in IOP [45]. These types of structural genes play a crucial role in preventing abnormal ocular growth, which is necessary for focusing light accurately on the retina. Dysregulation of genes can lead to an elongated axial length and increase the risk of myopia.

1.1.5 Environmental Factors of Myopia

Environmental factors have been demonstrated to play a vital role in myopia development. These include reading, writing, various other near work including using mobile phones or computers for playing games and watching television, which led to a higher progression of myopia. However, various studies have indicated that longer time spent outdoors and involvement in various physical activities may be associated with reduced incidence of myopia development in children [46, 47]. Progression of myopia has been shown to increase in the winter and to slow down in summer months because of the shorter period of day light, more near work, decreased outdoor time, and limited sunlight exposure [48]. Similar results were reported in other studies including the Avon Longitudinal Study of Parents and Children, the Singapore Cohort Study of the Risk Factors for Myopia and the Sydney Myopia Study [49-51]. All found that students who

spent more time outdoors had slower progression of myopia compared to those who spent longer time indoors. They also reported that less time outdoors and maximum time indoors was associated with considerable changes in ocular elongation and rapid myopia progression in teenagers and school children. In addition, other influences may lead to higher levels of myopia, including higher educational achievement, due to more time spent in education and performing homework leading to a decrease in less outdoor activities [52]. It has been suggested that more years in education could lead to an increase in myopic progression of nearly -0.27D every year due to the minimum amount of natural light exposure [53]. Physical activity may also effect myopia development as it has been found that participation in more physical activities may result in reduced levels of myopia due to sunlight exposure and increased blood flow in the choroidal layers of inner parts of the eye. It was suggested the increased blood flow might inhibit axial elongation and thereby prevent the development of myopia [54].

It has also been suggested that heavier body weight may increase the risk of myopia. The body stature study suggested that heavier subjects had an increased risk of myopia compared to lighter subjects [55]. In contrast, low socioeconomic status and lifestyle have been linked with myopia development in later life, due to low birth weight and prematurity [56]. In addition, large populations based longitudinal studies have led to better understanding of the effects of prenatal, social and early life biological influences on development of myopia and their association with genes which effect visual outcomes in later life [57]. The suggested effects of parental smoking on myopia development remains unknown, despite studies having concluded that smoking during pregnancy smoking might influence refractive error development process in children [58]. Animal studies have provided clear evidence that cigarette smoking can affect myopia development via nicotinic acetylcholine receptors which inhibit induced form-deprivation myopia in chicks. A relationship between birth and reduced unaided vision has been observed. The risk of myopia in first-born individuals was low due to more time in outdoor activity and less time for reading [59]. A recent environmental study investigated the effect of residential housing on myopia progression among school children in China [60]. The study suggested that housing type was independently associated with myopia in school children who lived in small flats, lower living areas

and time spent outdoors. Interesting evidence also suggested that parents with higher education had significantly more myopia than parents with lower education [61]. These results can be interpreted in different ways because parents with higher educational levels had more near work in the office and spent more time indoors, which can lead to more progression of myopia. In addition, controversial data on the effects of breastfeeding on later development of myopia suggested that children who were breastfed had optimum vision in infancy or early childhood while formula fed children were more likely to develop myopic refractive errors [62]. Human milk provides polyunsaturated fatty acids, bioactive components and essential vitamins, which may aid brain development and retinal growth, by maintaining high concentrations of these factors during development of all tissues which could lead to better vision and neurodevelopmental outcomes for infants. However, population-based studies revealed that close reading distance, continuous reading, and excessive homework could lead to increased mechanical stress to the eye, stimulating myopia development in children, resulting in more enlargement of the eyes [63].

1.1.6 Accommodation and Accommodative Lag

Human and animal studies have suggested that during myopic progression retinal defocus or blur images may act as a stimulus to axial enlargement of the eyeball because of excessive accommodation. However, early longitudinal studies did not find any relationship; more recent longitudinal evidence indicates a modest association between near work and myopia [64]. Various studies have investigated the strong relationship between lag of accommodation and myopia progression because of peripheral retinal defocus [65, 66]. Gwiazda et al. discovered that lag of accommodation increased in myopes and thus could stimulate axial enlargement leading to myopia progression [67]. Although it has been concluded that an increase in accommodation lag may be a consequence rather than a cause of myopia [68]. Several clinical trials studies that suggested that use of multifocal spectacle lenses to control myopia development found decreased lag of accommodation during near work to be a result of reduced hypermetropic retinal blur [69, 70]. Similar clinical trials have investigated limited treatment effects of an excessive lag of accommodation and near esophoria [71]. In

contrast, a correlation between dynamic accommodation and myopic refractive error has been reported that accommodation was significantly less to the original target compared with emmetropic children [72, 73]. Increased accommodative variability in myopia studies suggested that retinal defocus during near work could be integrated over time, resulting in axial elongation and, hence, onset of myopia.

1.2 Animal Models of Myopia

Animal models has been extensively used to study myopia. Although experimental animals are widely used in the study of human refractive error, these can be criticized due to anatomical, structural and biological characteristics of few animals are similar to human spontaneous refractive errors. However, insights into eye growth mechanisms, use of a range of techniques can help to answer the specific research questions and provide results which might be applicable for further human studies. In the 1970s, animal models were developed for ocular studies to help determine the underlying mechanisms of refractive error and ocular diseases. It was concluded experimental interventions led to refractive error developed in chicks which became more myopic and had increased eyeball length [74]. Wallman, Turkel and Trachtman (1977) developed a chicken model for experimental myopia that demonstrated that visual cues can make eyes myopic or other tissues and organs used for systematic investigation. Subsequently, refractive error development studies suggested that different techniques could be used for study of potential mechanisms of eye growth and emmetropization in animal models. These include forced near environments, lens-induced myopia, form-deprivation myopia, and myopic defocus. Studies using animal models have reported the development of experimental induced myopia in marmoset tree shrews, cats, monkeys, kestrel, marmosets, mice, fish, rabbit, squid, guinea pigs and chickens [75, 76]. The chicken model is frequently used as it allows development of myopia up to -20D and axial elongation increase of more than 2 mm within two weeks [74]. Other experiments produced surprising clinical outcomes which allowed development of techniques which may markedly reduce prevalence of myopia and prevented progression of myopia in humans worldwide. Chicken is an ideal model for induction of experimental myopia because they are inexpensive and co-operative in nature, easy to maintain. They have

large eyeballs (8 to 14 mm), similar structure of the cornea and choroid, excellent retinal image quality, ocular focusing system, and control of refractive state, faster eye growth compared to other animals (approximately 100 μm per day), active accommodation (Nealy 17D), limited pupil size (about 2 mm), and high visual acuity (7cycles/degree). The large eye allows easy drug delivery by intravitreal injection.

1.2.1 Form-deprivation Myopia (FDM)

The form deprivation myopia (FDM) model has been used in experimental animal research to induce myopia, which is the result of the breakdown of the visually controlled growth mechanism. FDM is produced by attaching a diffuser or filter in front of the eye of a young animal with an occlusion foil. However, low levels of light pass through the filter. During the process that blocks sharp vision and produces low contrast retinal image triggers unusually exaggerated eye growth, which results in induced negative refractive errors. This phenomenon is known as FDM [77, 78]. The exact mechanism of FDM and the related refractive development process are not well understood in animals. Several alternative techniques have been used to produce FDM by using translucent goggles, diffuser spectacle lens, lid suture surgery, and manipulating contrast of the visual occurrence, which alters the retinal input in peripheral and central sectors that can lead to myopia development [79, 80]. The results also revealed that FDM-treated animals had ocular growth and changes in the refractive state due to the blurred image of the retina, which affected contrast and resolution. Nevertheless, various animal studies have reported differing amounts of myopic response to FDM. The response is most rapid in chickens, which develop myopia up to -9D within 5 days of FDM. Other rapid responders include young tree shrews (-8D within 12 days of FDM) and guinea pigs (-6.6D after 11 days of FDM) [81]. In contrast, slower and relatively lower refractive error was noted in marmosets (up to -8D after 4.5 weeks of FDM) and rhesus monkeys (-5 to -6D after 17 weeks of FDM) [82, 83]. In humans, FDM associated with conditions including corneal opacity, neonatal ptosis, congenital cataract, and vitreous haemorrhage leads to development of more myopia. These outcomes may result from a similar mechanism of FDM in animals [84, 85].

1.2.2 Lens-induced Myopia (LIM)

Research has determined that lens-induced myopia (LIM) is an ideal, effective model of experimentally induced myopia in many kinds of animals. These models provide tools for development of new strategies for prevention and therapy for refractive error insight, as well as research into the eye mechanisms and maintaining ocular growth. LIM involves use of minus lenses, which induce myopic refractive error equal to the inducing power of a lens [86, 87]. It was reported that myopia developed slowly, but hyperopia developed more rapidly. The refractive errors produced by LIM are caused mainly by increases and decreases in ocular growth, although a high amount of hyperopic refractive error is associated with corneal flattening. This phenomenon has been extensively investigated in tree shrews, rhesus monkeys, marmosets, guinea pigs, mice, and chicks [88-90]. It has been determined that the regulating mechanism of eye growth of LIM is associated with defocus signs of anterior to posterior movement of the retina towards the image plane because of changes in choroidal thickness and scleral growth. Myopic defocus (Plus lens) pushes the image to form in front of the retina, which results in shortening of the ocular length and the development of hyperopia, whilst hyperopic defocus (Minus lens) causes the image to form behind the retina, leading to the development of myopia through axial elongation [91]. Interestingly, a novel study of defocus revealed that myopic ocular growth might be reduced by applying myopic defocus lenses due to defocus produced by out-of-focus or blurry pictures on the retina to slow down myopia progression by reducing excessive eye growth [92]. Although previous evidence suggests that there are differing amounts of refractive error response to negative or positive lens wear [93]. Response to LIM is higher in chickens which develop refractive error between +15D to -10D after 5 days of lens wear, tree shrews (-10D to +4D within 8 weeks of LIM), guinea pigs (-7D to +4 D after 10 days lens wear), marmosets (-8D to +4D after 21 weeks of LIM) and monkey (-2D to +8D within 30 days of LIM) [88, 94, 95].

1.2.3 Form-deprivation Myopia Versus Lens-induced Myopia

It has been suggested that FDM and LIM share the same physiological mechanisms of action [96], but another study reported opposing underlying mechanisms of these techniques [97]. The basic difference between the techniques is that FDM occurs in localized areas of the retina where the visual feedback system is compromised, while induced myopia can occur in an eye with blocked vision, which is independent of the central nervous system [96]. In contrast, LIM requires emmetropization procedures for this visual feedback system to have an effect on the retina and central nervous system [98]. Although many differences between the FDM and LIM have been reported including effects on the dopamine system, circadian rhythm, kinetics of eye growth, and ERG responses [99, 100]. Recent knockout mouse studies reported that FDM reduced lower levels of dopamine secretion due to fitting a diffuser or blocking the light, which results in an increased rate of eye growth [101]. However, findings of a chick study supported the suggestion that apomorphine reduced the development of FDM and LIM, but that LIM needs a higher dose of the drug compared to FDM. The dopamine D2 receptor antagonist spiperone has inhibitory effects on the growth of the anterior chamber and disinhibitory effects on the vitreous chamber in the FDM model, whereas there are different inhibitory effects on LIM due to the individual dopamine mechanisms of action in anterior chamber growth and vitreous chamber growth [102]. Effects of other molecules have been investigated including kainic acid, nitric oxide synthase inhibitor, and retinal hormone melatonin revealed that spiperone has an inhibitory effect in the front of the eye, but opposite effects on posterior part of the eye [103]. In contrast, quisqualic acid and insulin produced different mechanisms, which led to increased depth of the anterior chamber, but no changes in the posterior of the eye. This was attributable to D1 and D2 receptors being localized in the anterior segment of the eyeball [104]. Although epidemiological studies have suggested that children who spend a longer time outdoors and perform less near work are less likely to develop myopia. This may be because longer outdoor time leads to exposure to more bright light, which may increase retinal dopamine levels and release more dopamine into the retina and thereby inhibit myopia progression [105]. A similar study reported bright light inhibition of myopia progression in FDM treated tree shrews and chickens, but not monkeys [106, 107]. Other evidence suggested that constant light was more responsible for development of FDM, but not LIM because the disruption of emmetropization caused by the absence of

environmental light led to excessive ocular elongation [108]. An electroretinography (ERG) study comparing effects of FDM and LIM models on retinal function in chicken eyes demonstrated that the a and b wave reflected amplitudes were uniform. However, oscillatory potentials markedly decreased in the FDM chicken model, because the retinal image is regularly defocused whereas LIM chicken model images are finally focused upon the retina.

1.3 Myopia Control Interventions

1.3.1 Optical Interventions

Traditional optical interventions for control of myopia include spectacle lenses, special contact lenses, and refractive surgery, which can adjust the focus of the eye to provide clear distance vision but cannot stop or slow down the progression of myopia. In contrast, newer optical and pharmacological interventions, including under refractive correction, part-time spectacle wear, bifocal and multifocal spectacle correction, progressive-addition spectacle lenses, peripheral retinal defocus, contact lenses, orthokeratology (OK) and pharmaceutical therapy can slow myopia progression with promising results. Under-correction of refractive error can reduce accommodative stimulus and make more demands at near distance, which produce defocused retinal images that may cause a greater rate of myopia progression due to form deprivation myopia effect [109]. A previous study concluded that the increase in development of myopia and progression of myopia could be caused by annual correction of refractive error due to geography, environment, ethnicity, and socioeconomic development [110]. Under correction spectacle therapy requires that spectacles should be prescribed slightly lower than full spectacle correction. This causes the light to focus in front of rather than at the back of the retina. Another therapeutic study demonstrated that under correction may decrease accommodative stimulus, which involves more demand at near focus, decrease the blur drive for the near accommodative response, and mechanical stress at the posterior pole, which may have a significant role in myopic progression [111]. However, the study concluded that there were no significant outcomes in myopia progression in children with under correction compared with full correction spectacles. In 1947, Dr. Robert Wick

suggested bifocal glasses could be used to control myopia progression. Bifocal glasses can slow down progression of myopia in children compared to single vision spectacles because the bifocal segment line provides appropriate prompt to the children to ensure the upper portion (upper part of the lens) of the lenses should be used for distance work and the reading portion (Lower part of the lens) of lenses for near work [112]. It was observed myopic children using prismatic bifocals with low lags had a significant treatment outcome in reduced myopia due to lower portion of the lenses having plus power and thus produced a greater visual field. However, progressive addition lenses (PALs), which are multifocal glass lenses (line-free) initially used for presbyopia treatment in adults to provide more natural vision. The Correction of Myopia Evaluation Trial (COMET) study suggested the effect of PALs compared to single vision lenses was significant in all myopic children with first 3 years of PALs treatment, but after five years treatment effect, there was no longer a significant difference even in children who continued to wear the same lenses [113]. These findings suggest that PALs treatment reduced myopia progression by foveal hyperopic blur in the periphery retina resulting in a decreased eye growth and limited axial elongation. Experimental animal studies have provided strong evidence that lens-induced refractive error alters retinal image quality of hyperopic defocus or myopic defocus due to different mechanisms, emmetropization process, defocus varies with viewing distance and changes the accommodative status that leads to a decrease in refractive error during development of eye growth [92]. A similar finding was reported about the effect of hyperopic and myopic defocus lenses on the refractive development of chicks [114]. It was noted that intra-ocular differences in refraction at day 6 were +13.5, +4.75, -0.6 and -3.9 respectively and that changes in the vitreous chamber depth correlated with refractive error difference between the two eyes. Importantly, previous studies include one involving ten marmosets and defocus lenses with different powers (+5/-5D, 50:50 ratio for a moderate pupil diameter of 2.80 mm over the right eye and plano over the control eye for 10 to 12 weeks) [115]. The results showed there was more hyperopic refractive error and a slower growth rate in the treated eye compared to the untreated eye. Another study using two groups of 14 monkeys treated with dual focus spectacle lenses, with a central zone of 2 mm diameter with altering power of +3 D and plano (pl or 0D) (+3 D/pl group) or -3D and plano (-3D/pL group), with 9 further monkeys serving as compression controls with +3.0 or --3.0D lenses. The study showed that the +3D/plano groups developed hyperopic refractive errors and had a

slow ocular growth rate which was similar to the +3D single vision lenses groups [116]. In contrast in the -3D/pl group), refractive error was not significant different from the comparison groups, hence more hyperopic than monkeys treated with −3D single-vision lenses. A recent study showed that bright light and myopic defocus could significantly inhibit excessive ocular growth induced by hyperopic defocus [117]. However, consistent with the previous studies, clinical trials have been performed using myopic defocus lenses to reduce myopia progression in children partial success rate with effectiveness ranging from 30% to 60%. The outcomes were dependent on the lens design and wearing schedules [118]. Interestingly, a two year study of defocus incorporated multiple segments (DIMS) spectacle lenses significantly inhibited myopia progression in children by up to 52% and axial elongation by 62% when compared to single vision lenses [119]. The success of the clinical trials of DIMS and DISC (defocus incorporated segment contact lenses (DISC lenses) has led to their commercial launch and growing use in the community. A randomized clinical trial demonstrates that Essilor Stellest™ lenses, which incorporate highly aspherical lenslets (HAL), significantly reduce myopia progression in children over two years. It was concluded that children who used HAL lenses showed a 55% reduction in myopia progression (0.80D) and a 51% reduction in axial elongation (0.35 mm) compared to single-vision lenses (SVL) [120]. It has also been suggested that children who wore HAL lenses consistently at least 12 hours per day, a significant 67% reduction in myopia progression (0.99D) and a 60% reduction in axial elongation (0.41 mm).

Orthokeratology (ortho-k) is a non-surgical method of myopia control and myopia control treatment using overnight wear of special gas permeable contact lenses. Several studies have investigated ortho-k use for full or partial refractive correction and shown to significantly slow progression of myopia in children by between 36% to 56% compared to single vision glasses or contact lens wear [121, 122]. It was also noted that ortho-k reduced refractive error changes to 0.2 - 0.3D per/ year, while single vision spectacle wear leads to greater increases in refractive error 0.4- 0.5D per/ year. Ortho-k decreased vitreous chamber depth and axial length elongation up to 50% in comparison to soft contact lenses. In contrast, ortho-k treatment over 14-months on 66 myopic children of three groups of children (6 to 9 years; 9 to13 years; and adolescents 13 to 16

years) with myopia between -0.75D to -4.50D and astigmatism less than 2.0D, Average axial elongation over the three groups was 0.14 ± 0.12, 0.03 ± 0.09 and -0.01 ± 0.08 mm for the test groups when compared to 0.31 ± 0.08, 0.14 ± 0.09 and 0.07 ± 0.08 mm for single vision spectacle wearers [123]. In addition, they confirmed ortho-k does require parental supervision for younger children and appropriate training in lens care and handling. Another study observed axial elongation of myopic eyes was 0.07 + 0.21 mm in ortho-k eyes compared to 0.36 + 0.23 mm in none treated partner eyes over one year of study of 108 myopic anisometropic children aged between 8-16 years [124]. Previous findings from a two-year randomized clinical trial study demonstrated that MiSight lenses significantly reduce myopia progression and axial elongation in children [125]. MiSight lenses were also shown to inhibit 39.32% of myopia progression and 36.04% reduction in axial elongation compared to those using single-vision spectacles from between 8-16 years.

1.3.2 Drug Intervention

Several pharmacological methods to slow the onset of myopia have been investigated, including atropine eye drops and pirenzepine eye gel. Anticholinergics (anticholinergic agents) are a class of medications that prevent smooth muscle contraction by blocking the actions of acetylcholine at muscarinic receptors in the central and peripheral nervous systems. Muscarinic acetylcholine receptors (mAChRs) are a subfamily of G-protein-coupled receptors (GPCRs) that control a wide range of intracellular functions. In mammals, mAChRs comprise five distinct genes expressed in the brain and peripheral organs, including M1, M2, M3, M4, and M5 [126]. The Galpha(q/11)-phosphatidylinositol pathway is preferentially activated by M1, M3, and M5 mAChR subtypes, whereas M2 and M4 receptors block adenylyl cyclase via Galpha I [127]. In contrast, mAChRs, present in different structures within the eye, including the lacrimal gland, conjunctiva, cornea, lens, uvea, retina, and sclera. In chickens, four mAChR subtypes (M2, M3, M4, and M5) have been investigated. There is no M1 receptor, but the M2 ortholog exhibits M1-like characteristics, such as a high affinity for pirenzepine [128]. However, affinity-purified type-specific antibodies to chicken muscarinic receptors M2, M3, and M4 or CM2, CM3, and CM4 are used to locate mAChRs in

chicken ocular tissues [129]. However, only the amacrine, outer plexiform layer, ganglion cells, and inner plexiform layer of the chicken retina contain CM2. The retinal pigment epithelium, bipolar cells, outer plexiform layer choroidal blood vessels, and a small number of cells in the inner nuclear layer and ganglion cell layer have been confirmed to contain CM3, while they are present in the posterior segment of the eye, including the retinal pigment epithelium, bipolar cells, outer plexiform layer choroidal blood vessels, and a small number of cells in the inner nuclear layer and ganglion cell layer. In the inner plexiform layer, CM4 is present in the ciliary epithelium, ciliary ganglion, choroidal blood vessels, amacrine, and ganglion cells. How mAChRs exert their influence on ocular growth is unknown, but the use of atropine and other mAChR antagonists can limit myopia advancement by blocking muscarinic receptors. Furthermore, atropine dilates the pupil and paralyzes accommodation. Except in chicken, the muscarinic receptor suppresses accommodation and sphincter pupillary muscle activities. Due to the lack of M1 receptors, chicken ciliary muscle and pupil are controlled by nicotinic receptors rather than muscarinic receptors. Excessive expansion of the eyeball may be due to decreased thickness or increased synthesis of the extracellular matrix of the sclera [130]. In contrast atropine can reduce extracellular matrix manufacturing in scleral and choroidal fibroblasts cells, increasing scleral blood circulation through the choroid due to high permeability and slow myopia progression. Previous findings from animal studies on chickens showed that atropine solution release increased neurotransmitter dopamine levels in the extracellular space and vitreous chamber depth, suppressed form-deprivation myopia, and increased neurotransmitter dopamine levels in the extracellular space and vitreous chamber depth [131].

Atropine treatment has been extensively tested in myopic eyes in animal studies since 1977 to understand how myopic eyes change. These tests have employed diverse strategies including blocking one eye and blacking one eye using black contact lenses to generate experimental myopia [132]. Despite this, the studies all revealed that atropine, in combination with pirenzepine, inhibits cellular and extracellular matrix in the whole scleral cartilage layer. 4-DAMP (1,1-dimethyl-4-diphenylacetoxypiperidinium iodide) is a selective muscarinic acetylcholine receptor M_3 antagonist initially used for pharmacological studies to investigate the function of muscarinic acetylcholine receptor

M_3 in various physiological processes. Its primary function is to inhibit the effects of acetylcholine by blocking muscarinic receptors in the body. When administered sub-conjunctively, 4-DAMP was found to be a substantial inhibitor of scleral chondrocytes but had no effect on FDM in chicks. Only atropine has been studied in humans as a medication to effectively control myopia progression in a dose-dependent manner [133, 134]. Over a 2-year study, high dosages of atropine (0.5 % and 1%) considerably inhibited myopia progression by up to 75%, but there were no differences in axial elongation between atropine and placebo-treated eyes [134]. However, higher concentrations of atropine have several ocular side effects, including eye pain, photophobia, allergy, blurred reading vision, decreased contrast sensitivity, decreased tear production, decreased visual acuity, and a significant rebound effect [135, 136]. A trial of atropine 1% eye drops Atropine in the Treatment of Myopia (ATOM) reported (0.28 ± 0.92D) in the myopia progression group compared to the placebo (1.20 ± 0.69) group [137]. A lower concentration of atropine was also shown to slow myopia development by up to 67% [138, 139]. The ATOM2 study found that atropine eye drops using different concentrations including 0.5% (-0.30 ± 0.60D), 0.1% (-0.38 ± 0.60D), and 0.01% (-0.49 ± 0.63D) were associated with less myopia progression over a two-year period with axial elongation of 0.5% (0.27 ± 0.25 mm), 0.1% (0.28 ± 0.28 mm), and 0.01% (0.41 ± 0.32 mm), respectively [140]. Low concentrations of atropine cause pupil size to expand by 0.5% (3.11 mm), 0.1 % (2.42 mm), and 0.01% (0.91 mm), respectively. However, the study also found that a 0.01% concentration of atropine had a 60% rebound effect in children aged 8 to 10, 30% in children aged 10 to 12, and 8% in children aged 12 and over. When atropine eye drop use was discontinued, following a washout period, myopia returned. When compared to high doses of atropine eye drop groups, the atropine 0.01 % group revealed myopia progression was more successful with fewer ocular adverse effects during a 5-year trial period. As a result, low-dose atropine eye drops are currently the most popular pharmaceutical treatment for slowing myopia progression; this is partly based on the findings of the ATOM 2 investigations. However, over the 2-year study showed that lower doses of atropine considerably limited myopia progression and axial length in children by 0.5 % (0.55 ± 0.86D versus 0.39 ± 0.35 mm), 0.25 % (0.85 ± 0.73D versus 0.50 ± 0.33 mm), and 0.1% (1.12 ± 0.85D versus 0.59 ± 0.38mm) respectively [141]. Evaluation of the results of atropine at 0.05 % and 0.025 % had the same effects as 2-year outcomes and concluded that 0.05 % atropine concentration had the best effect for

myopia control after a year of follow-up [142]. It was also noted that low doses of atropine cause less pupil dilatation and minimal impact on near vision and accommodation, making it more tolerable for children. Atropine alters the structure of Bruch's membrane, choroidal thickness, choroidal blood flow, and the scleral matrix, perhaps reducing excessive axial elongation [143, 144]. Atropine also prevents choroidal thinning induced by hyperopic retinal defocus by increasing choroidal thickness in myopic children via a direct or indirect channel [145]. After considering the risks, a higher dose of atropine is the best option for myopia control. However, previous animal research investigated that another muscarinic antagonist intravitreal injection of pirenzepine is also effective in preventing myopia progression and inhibiting axial elongation in a dose-depend manner. Pirenzepine is currently unavailable on the market for myopia therapy in humans. The underlying mechanism of the action of atropine and pirenzepine remains unclear, but it is believed that their primary function independently affects through accommodation and posteriorly involves receptors in the retina or sclera. The biomechanical properties of the sclera are determined by the composition or structural organization of proteoglycans and collagen [146, 147].

Previous studies have investigated whether the nonselective adenosine receptor antagonist, 7-methylxanthine (7-MX), may inhibit myopia progression in children and adolescents. In Danish school children, studies found that 7-methylxanthine (7-MX) significantly inhibited myopia progression and reduced axial elongation over two years [148]. It was also suggested that 7-MX may impact the retinal dopamine system, which plays a critical role in controlling ocular growth and refractive development. To determine the effects of adenosine receptor antagonists on myopia development in animals has shown inconsistent results. In chickens, 7-methylxanthine (7-MX) did not affect form-deprivation myopia but lens-induced myopia reduced the myopia approximately 23% [148]. It was noted that there have been no studies on the use of topical atropine to reduce myopia in chickens. In addition, due to ocular side effects and rebound effects, previous human studies only employed dosages of atropine up to 1%. To determine the effects of higher concentrations, a chick model was used to test different dosages of topical atropine up to 100% (Chapter 3).

1.3.3 Ocular Alpha Adrenoceptors

Muscarinic acetylcholine receptor subtype M4 can inhibit myopia progression in the chick model treated with MT3, a component of the mamba snake venom, MT3 is selective for M4 receptor subtypes over all other mAChRs in mammals and inhibited myopia in tree shrews and chicks using a lower concentration than required by atropine injection of 17.5-70 nmol versus 20-2000 nmol per injection respectively [149]. Atropine can block α-adrenoceptors at concentrations $\geq$ 0.1 mM which strongly effects myopia inhibiting ligand MT3 and properly binds to human mAChR M4, Alpha α1A-, and α2A-adrenoceptors. Alpha-1 adrenergic receptor is G-protein coupled receptor, that makes up the sympathetic response in the autonomic nervous system. When a ligand binds to the alpha-1 receptor it activates corresponding Gq protein leading to downstream cascade effect by secondary messengers, including phospholipase C, Inositol trisphosphate (IP3), and diacylglycerol (DAG). Since Alpha-1 adrenergic receptor is found in nearly all peripheral tissues, the smooth muscle function of these receptors leads to muscle contraction, vasoconstriction, pupil dilation, and sphincter muscle contraction. The α1-adrenoceptors are responsible for various physiological activities in and around the eye, for example its involvement in regulation of protein secretion in the lacrimal gland, blood flow regulation, aqueous humour dynamics, pupil diameter increases, and ocular blood vessels [150-152]. Although pharmacological studies have concluded that use of selective adrenoceptor agonist in individuals with α1 subtypes plays a major role in controlling the pupil size by contraction of the iris dilator muscle through α1-adrenoceptors being blocked by phentolamine [153, 154]. While α1-adrenoceptors located in the ciliary body are responsible for aqueous humour formation, outflow and inflow via the pupil into the anterior chamber and then to drain out of the eye through both the conventional outflow route and non-conventional or uveoscleral outflow, they also have a role in reducing the intraocular pressure [155]. A recent human study reported the concentration of α2-adrenoceptors in the iris, ciliary body, ciliary muscle, retina, and retinal pigment epithelium (RPE). In these areas, the mechanism of α2-adrenoceptors subtypes is consistently negatively coupled to adenylate cyclase via Gi. When Gi is activated by α2-adrenoceptors, the level of cyclic

adenosine monophosphate (cAMP) decreases and protein kinase a is deactivated. Signaling mechanisms of α 2-adrenoceptors are responsible for opening of K+ channels, leading to hyperpolarization of cells and closing or opening of Ca2+ channels causing vasoconstriction. However, previous studies have reported the use of α 2 adrenoceptors for management of glaucoma and to control experimentally induced FDM. The finding showed that brimonidine and clonidine (20 and 200 nmol) significantly reduced optically induced myopic refractive error and axial elongation, providing direct evidence that α 2-adrenoceptors agonists can be an effective anti-myopia therapy [156]. Although studies have suggested that brimonidine and guanfacine had similar effects to atropine in inhibiting FDM in chickens. However, higher concentrations are required which increase the risk of binding to an off-target receptor. Previous in-vitro studies suggested that alpha-2-adrenergic receptors may underlie the inhibitory effect of atropine against myopia development, due to non-specificity of atropine, and that α 2a agonists inhibition induced FDM myopia in chicks [157]. This study also suggested that inhibitory drugs may have different effects against LIM, which involves an image-guided process. The present study aimed to examine whether α 2 agonists (lofexidine) could suppress LIM in the chick model. In addition, the study aimed to investigate whether an alpha-1 adrenoceptor agonist, metaraminol, and a non-specific adrenergic agonist, cirazoline, would produce similar effects to alpha-2-adrenergic agonists (Chapter 4).

1.4 Intraocular Pressure (IOP)

Intraocular pressure (IOP) plays a very important role in the physiology or pathophysiology of the eye to maintain its consistent form and shape to preserve vision and prevent sight-threatening diseases. The normal range of IOP is between 10 to 22 mm/hg which is maintained by balancing the concentration of aqueous humour. If the balance is disrupted due to impairment of IOP, cellular pressure can increase, damaging the optic nerve and resulting in glaucoma. Aqueous human drainage comprises both a conventional pathway (trabecular outflow) and an unconventional pathway (uveoscleral outflow), (Figure 2). The conventional pathway involves passage of the aqueous humour through the trabecular meshwork into Schlemm's canal, collector channels, and the episcleral venous system [158]. In contrast, the unconventional pathway involves

passage of the aqueous humour through the anterior chamber, uveal meshwork, ciliary muscle, and, finally, entry into the suprachoroidal space [158]. However, the exact mechanism and dysfunction of the conventional pathway are not fully understood. An increase trabecular meshwork then changes in extracellular matrix (ECM), lower porosity of the inner wall endothelium of Schlemm's have been suggested as reasons for impaired drainage, as observed in glaucoma [159].

1.4.1 Association Between Intraocular Pressure, Myopia, and Ocular Elongation

Several studies have investigated the association between IOP and myopia progression in humans and animals. The role of IOP and development of myopia is very controversial, and the mechanism is unknown, but some studies have reported that longer axial length is associated with higher IOP [160]. Elevated IOP can affect the sclera, as elasticity of the scleral wall, which becomes thinner, may change leading to stress on the optic nerve head or lamina cribrosa. In a myopia study of 636 Singaporean children reported no relationship between elevated IOP and refractive error (16.7 mm Hg) emmetropia, (16.7 mm Hg) hypermetropia, (16.4 mm Hg) low myopia, and (16.7 mm Hg) high myopia [161]. Over the 2 years of the study, 49 children aged 9 to 12 years were observed to have high IOP. These children had higher levels of myopia and a relatively rapid myopia progression rate of 1.14D per 2 years and IOP 16 mm Hg [162]. An adult study reported that elevated IOP was significantly correlated with longer axial length [163]. In China, the Lingtou Eye Cohort Study reported a positive correlation between IOP and axial length in adults. This result suggested that myopia and IOP in middle aged subjects may be related to tissue biomechanics. A further study found that IOP was associated with sex, body mass index, younger age, maternal myopia and more time spent indoor with writing or reading, axial length and corneal horizontal diameter [164], but that IOP was not associated with refractive error. More recently, a study of young guinea pigs revealed no association between IOP and axial length [165]. There is some evidence elevated that IOP can lead to damage of the optic nerve head, altered ocular shape and volume due to axial elongation leads to an increased corneal diameter, decreased anterior chamber depth. It has previously been reported that there was a significantly reduction of IOP and

decreased axial length after a trabeculectomy surgery, suggesting a link between IOP and axial length [166, 167]. Trabeculectomy surgery leads to changes in axial length due to anatomical and physiological changes in the sclera and choroid. During the development of myopia, the choroid becomes thinner, but after trabeculectomy the choroidal thickness increases, because of increased blood flow and scleral stiffness.

Diurnal variation in IOP has been investigated in humans and animals, including rabbits, chicks, marmosets, mice, rats, rabbits, and monkeys [168, 169]. An experimental study found that variation of IOP over 24 hours plays an important role in axial length association with myopia progression [170]. IOP and myopia have a strong relationship with moderate to high myopia due to mechanical strain and lamina cribrosa deformation, thus limiting retinal ganglion cells function. However, investigation found that during the daytime IOP was higher in myopic children, adults, and older people and that IOP was lower at night, but the causes of these observations are unknown [171, 172]. Possible reasons are postural changes, which cause an increase of episcleral venous pressure, globe size, and an enlarged lens. In the chicken model of FDM study noted diurnal variation of IOP changes following myopic elongation compared with control eyes. Furthermore, diurnal variation of IOP changes scleral structures and biomechanics, including altered collagen fibres, tissue loss, decreased collagen synthesis, increased matrix metalloproteinase functions, changed proteoglycans, reduced potential and decreased scleral strength [173, 174].

1.4.2 Anti-glaucoma Drugs and Their Mechanisms of Action to Control Myopia.

Beta-blockers (topical ß-adrenergic antagonists), Alpha agonists (Brimonidine), topical carbonic anhydrase inhibitors (dorzolamide), Cholinergic agents (Pilocarpine), prostaglandins (latanoprost), and Rho kinase inhibitors are IOP-lowering drugs used to treat glaucoma and ocular hypertension patients. Ocular hypertension medicines lower aqueous humour production from the ciliary body and increase aqueous humour production through the trabecular meshwork and the uveoscleral route, or a combination

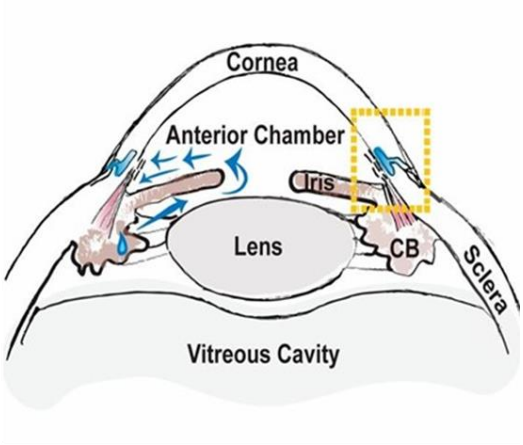
of the two methods. According to the World Health Organization (WHO) has listed drugs suitable for use as first-line therapy based on those with the fewest adverse effects, require single doses of the drug, have a maximum IOP reducing impact of 8 to 12 hours, and reduce IOP within 24 hours. Studies based on the history of IOP reducing efficacy, tolerance, and safety, and pharmacotherapeutic alternatives for myopia control therapy are limited. Recent myopia therapy possibilities are listed in Table 1, together with a summary of each drug's mechanism of action.

Table 1.2 Summary of Intraocular Pressure (IOP) lowering drugs used in myopia model.

Drug Name	Drug Class	Mechanism of actions	ECM remodelling or Receptor mediated	Effect	Model	Myopia Progression
Timolol [175]	Beta-blockers	Block effect of epinephrine	Receptor	Decreases aqueous humour production	FDM/ LIM	Not effective
Brimonidine [176]	Alpha 2 agonists	Selective alpha 2 adrenoceptor agonists	Receptor	Increases uveoscleral outflow, decreases aqueous humour	FDM/ LIM	Inhibition
Latanoprost [177]	Prostaglandin analogues	Extracellular matrix (ECM) remodelling within the uveoscleral (UV) outflow pathway	ECM remodelling	Increase uveoscleral outflow	FDM	Inhibition
Carteolol [178]	Beta-blockers	blocks both ß1 and ß2 adrenergic receptors	Receptor	Decreases aqueous humour production	LIM	Not effective

ECM: extracellular matrix; FDM: form-deprivation myopia; LIM: lens-induced myopia

(a) Aqueous humour drainage pathway



(b) Conventional and Unconventional pathways

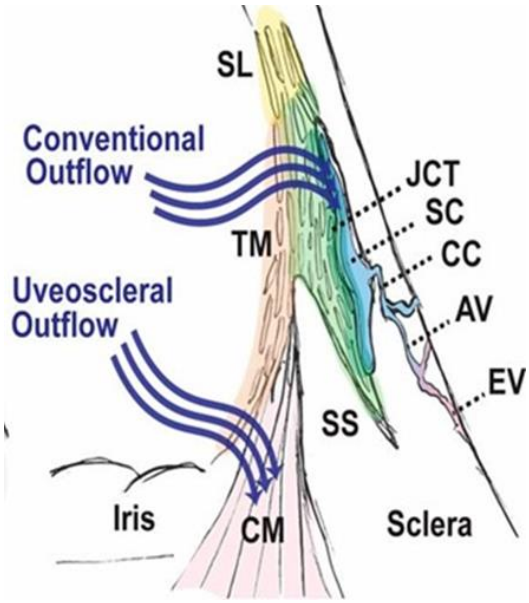


Figure 2: Aqueous humour drainage pathway [179]. Abbreviations: TM, Trabecular Meshwork; SS, Scleral Spur; CM, Ciliary Muscle; JCT, Juxtacanalicular Tissue; SC, Schlemm's Cana; CC, Collector Channels; AV, Aqueous Veins; EV, Episcleral Veins and SL, Schwalbe's line. (a) Schematic diagram of the anterior segment of the eye including the cornea, iris, ciliary body, and lens. (b) Unconventional pathway passes aqueous humour through the anterior chamber, uveal meshwork, ciliary body, lens and finally enters the suprachoroidal space. The conventional pathway passes the aqueous humour through the trabecular meshwork into the Schlemm's canal, collector channels, and episcleral venous.

1.4.3 Beta-blockers (Timolol)

Timolol is a medication used to treat open-angle glaucoma and ocular hypertension. Beta-blockers lower IOP by inhibiting the activity and binding of adrenaline or nor-epinephrine to beta 1 and beta 2 adrenergic receptors on the non-pigmented ciliary epithelium, which reduces the production of aqueous humour by the ciliary body. Interestingly, early studies reported that topical beta-blockers reduce IOP by 20% to 30% during the day, but have no effect during the night [180]. In a chicken investigation, timolol was found to lower IOP, but it had no effect on ocular hypertension compared to the control group [175]. In humans in a 12-month study of beta blocker treatment using timolol 0.25 percent eye drops twice daily, for 10 children aged 7 to 12 years old, there was no significant effect on myopia progression or IOP reduction [181].

1.4.4 Alpha Agonists (Brimonidine)

Alpha-adrenergic agonists are a group of selective α_2-adrenoceptor drugs, offering an innovative pharmacological intervention for glaucoma therapy. The mechanism of action and application of the α_2-adrenoceptor were briefly mentioned above. In contrast, Brimonidine can lower IOP by 2.5 to 29 percent by decreasing aqueous humour production and increasing uveoscleral outflow of the fluid inside the eye. Four glaucomatous medicines have been shown to reduce IOP in guinea pigs: brimonidine, carteolol, latanoprost, and brinzolamide, with brimonidine having the greatest effect [182]. Initially, different concentrations of α_2-adrenergic agonist (brimonidine 1% and 2%) were tested on lens-induced myopia in guinea pigs, with results indicating that they decreased experimental myopia and inhibited axial length elongation via IOP-dependent or -independent pathways [176]. It was observed that IOP-lowering medication lowered IOP, while lens-induced myopia eyes had higher IOP during the experiment. Brimonidine prevents scleral thinning by inhibiting extracellular matrix remodeling, which may be useful in reducing pathological myopia complications. Due to elevated retinal dopamine levels, high-concentration intravitreal injection of brimonidine consistently reduced myopic refractive error and resulted in shorter axial length in chickens with FDM [156]. Brimonidine was recently studied in guinea pigs using topical

drops, subconjunctival injection, and intravitreal injection for 21 days, with results indicating that intravitreal injection was effective in reducing IOP, greatly inhibiting myopia progression and resulted in shorter axial length [183]. The expression of Col1a1 and MMP2 genes was also elevated.

1.4.5 Prostaglandins (Latanoprost)

Prostaglandin analogues are a new first-line medication with minimal side effects for medical management of ocular hypertension and primary open-angle glaucoma. The mechanism of action of prostaglandin F2-alpha analogues, that acts on FP receptors expressed on the ciliary muscle epithelium, results in increased aqueous humour flow and activation of matrix metalloproteinases causes a decrease in IOP. Latanoprost lowered IOP 3 to 4 hours after administration, with a 24-hour effect. Topical treatment of latanoprost one drop at a time in the evening results in a 30 to 35 % reduction in IOP with no risk of systemic side effects. In comparison to timolol eye drops, latanoprost was effective in reducing IOP by up to 35 % when applied in the evening and by 27 % when applied in the morning. Due to the scleral thickness of chicks, latanoprost intravitreal injections were initially evaluated on FDM chick model, and were shown to have a modest effect on reducing myopia [184]. The inner cartilaginous layer creates more extracellular matrix during myopia development in chicks, but the outer fibrous layer produces less matrix and lowers its synthetic activity. However, when topical latanoprost was tested in Rhesus monkeys with lens-induced myopia, there was no difference in IOP between the treated and control eyes [185].

In contrast, daily administration of topical latanoprost has been shown to successfully prevent myopia progression and is significantly connected to axial elongation. In guinea pigs with FDM, additional topical latanoprost was also beneficial in slowing the progression of myopia and decreasing IOP [186]. Reduced stress on the scleral wall, increased MMP activity, and remodeling of the ECM could all be factors that impede ocular elongation. A previous study found that latanoprost inhibits myopia progression in guinea pig models [187]. “The current study included an investigation that

latanoprost inhibits ocular biometric elongation and the underlying mechanism in both lens-induced and form-deprivation myopia" (Chapter 5).

1.4.6 Rho kinase inhibitor

Rho kinase inhibitors were recently developed as therapeutic agents for glaucoma, diabetic retinopathy, Fuchs endothelial dystrophy, age-related macular degeneration, and retinal detachment. Through its effect on the cytoskeleton, the serine/threonine kinase protein kinase known as ROCK inhibitor plays a significant role in the regulation and modification of cell size and cell shape [188]. Rho kinase has two primary domains that bind to the amino-terminal kinase domain through their pleckstrin homology and Rho-binding domains, respectively [189]. There are two types of Rho kinase inhibitors: Rock 1 and Rock 2. Rock1 is found in the liver, lung, blood, testicles, and immune system while Rock 2 is predominantly found in the muscles and brain. The amino acid sequences of Rock 1 and Rock 2 isoforms are approximately 65% similar overall and they share a 92% similarity specifically within their kinase domains [190]. This high degree of similarity in the kinase domains suggests that both isoforms perform similar functions in regulating cellular processes, but their distinct tissue distributions imply that they may also have specialized in different physiological contexts. However, myosin light chain, CP1-17, LIM kinase, calponin, and the ezrin-radixin-myosin proteins tau, MAP2, and adducin are all phosphorylated because Rho kinase activation is controlled by myosin phosphatase substrate 1. However, guanine nucleotide exchange factors and GTPase activating proteins regulate the connection between the two forms of RhoA, GDP-bound and GTP-bound RhoA, respectively.

Rho kinase inhibitors directly affect changes in cell shape, loss of focal adhesions, changes in the extracellular matrix, loss of actin stress fibers in the trabecular meshwork, and Schlemm's canal cells, which reduce IOP and increase aqueous humour outflow [188]. It is still unclear how Rho kinase inhibitors work to boost the aqueous outflow capacity through the traditional method. The cellular cytoskeleton of the smooth tissue of the traditional pathway is the site of Rho kinase, which lowers its contractile tone.

These two methods may improve paracellular fluid outflow through the inner wall of the Schlemm's canal, altering cell shape, cell junction, and relaxing the trabecular meshwork and juxtacanalicular connective tissue. This may increase aqueous outflow capacity and lower IOP. In trabecular meshwork cells, rho kinase inhibitors increase the antioxidant action, which modestly reduces oxygen species and cell mortality [191]. A common physiological occurrence, known as oxidative stress, involves the generation of reactive oxygen species, including peroxides, radicals, superoxide, hydroxyl, and singlet oxygen. Reactive oxygen species are important for pathogens and cell homeostasis during biological processes. In addition, various visual illnesses, including glaucoma, are associated with an increase in reactive oxygen species [192]. Additionally, Rho kinase inhibitors decreased aqueous humour by inhibiting the norepinephrine transporter and causing dilated uveoscleral and episcleral veins [188]. Research on the effects of Rho kinase inhibitors on the trabecular meshwork in animals showed promise for the future development of glaucoma treatments that lower IOP [193]. Rho kinase inhibitors have been shown in a rabbit model to be effective in reducing the need for anti-scarring medications after filtration surgery, increasing blood flow to the optic nerve head by relaxing vascular endothelial smooth muscle cells, and increasing the number of retinal ganglion cells (RGCs) that regenerate in rats and humans [194, 195]. Previous studies on diabetes found that a Rho kinase inhibitor can impede endothelial migration and proliferation, which prevents the development of retinal detachment [196]. This study also revealed that by eliminating transforming growth factor beta (TGF-), Rho kinase inhibitors significantly reduce the development of proliferative vitreoretinopathy. Interestingly, Rho kinase inhibitor reduced fibrosis in a mouse retina, which stopped neovascularization in age-related macular degeneration and reversed the pathology of the eye [197]. A Rho kinase inhibitor drug (Netarsudil-AR-13,324), which decreased IOP by boosting outflow through trabecular meshwork, was first approved for clinical use in the USA in 2017. [198]. Previous research from the Rocket-1 to Rocket-4 phase III studies evaluated the effectiveness and safety of once-daily administration of topical drug Netarsudil (0.02%) compared to timolol 0.5% twice daily in patients with open-angle glaucoma or ocular hypertension [199]. The results demonstrated that netarsudil effectively reduced IOP to levels comparable to twice daily timolol with generally mild and self-resolving ocular serious adverse events. Although the study found that latanoprost 0.005% and Netarsudil 0.02% used alone or in combination also significantly

lowered IOP. Rho kinase inhibitors noted limited systemic and ocular adverse effects including conjunctival hyperemia, subconjunctival haemorrhage, blurred vision, lacrimation and ocular irritation. The current study investigated whether the Rho-kinase inhibitors could be involved in inhibiting myopia progression in FDM and LIM in a chicken model (Chapter 6).

Chapter 2

Methods and Materials

2.1 Animals

All animal experiment procedures were approved by the Animal Ethics subcommittee of the Hong Kong Polytechnic University (PolyU). These procedures complied with the government of the Hong Kong special administrative region and adhered to the Association for Research in Vision and Ophthalmology (ARVO) guideline for the use of experimental animals in vision and ophthalmology research. The White Leghorn chicks (*Gallus gallus*) used in the study were hatched from specific pathogen-free eggs (SPF, Jinan, China). They were housed in standard stainless-steel cages in the Central Animal Facility of PolyU, with the room temperature maintained at 25°C. The central ambient luminance over the cages was maintained at around 300 lux. The experimental period commenced on postnatal day 7 (PN 7) and extended to post-natal day 21 (PN 21). Food and water were provided *ab libidum* undera 12h:/12h light/dark cycle (lights on between 7 am and 7 pm. The chicks were monitored twice daily, once by the researcher to check the lens attachment or drug delivery and once by the technician responsible for cleaning the room, cages and supplying food or water.

2.2 Experimental Induced Myopia

Lens-induced Myopia (LIM) and Form-deprivation Myopia (FDM) were induced for each experimental animal which started PN day 5 or 7. Myopia was induced in the right eyes of chicks using diffuser lenses (FDM) and -10D single vision lenses (LIM) for 7 to 12 days of treatment, while the left eyes were untreated as a control. The lens used for induction was attached to the right eye using Velcro rings (generic) and held in place by cyanoacrylate glue to the feathers around the eye. This arrangement allowed the lenses to be removed for cleaning, installation of eye drops, or injection of intravitreal drugs.

2.3 Refractive Error

Experimental measurements were performed under semi-darkness to provide better visualization of light reflection. The chick was gently held and comfortably positioned to immobilize its head during the retinoscopy examination. For retinoscopy two reflex movements were observed, when the instrument was moved and the eye movement was in the same direction it indicated hyperopic refractive error, whilst movement in the opposite direction indicated myopic refractive error. During the observation, several characteristics of the light reflex were noted, including bright reflex (normal eye), and hazy or dull light reflex, suggesting the presence of a high refractive error and irregular or distorted light reflexes, which were typical of astigmatism. Refractive correction for each animal was measured without cycloplegia using streak retinoscopy (Model HEINE BETA 200; Heine Optotechnik, Gilching, Germany). Loose lenses were used to neutralize refractive error in the two principal meridians separately (horizontal meridian, then vertical meridian). The working distance was approximately 50 cm (lens power of +2.0D or -2.0D depending on the experiment), and results were recorded as spherical equivalent refractive error.

2.4 High-frequency A-scan Ultrasonography

A custom-made A-scan ultrasonography system, originally designed for animal experiments (Figure 2.1), was used to facilitate imaging of anatomical structures of the eye, specifically for ocular biometry measurements, with the following modifications (Voltage level 200 mV, Scope width 20 microseconds, Delay 30 microseconds). Ocular

biometry was measured using high-frequency A-scan ultrasonography with a 30 MHz transducer (Panametrics Inc., Waltham, MA) to detect anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL) and choroidal thickness (ChT). Previous studies have provided detailed descriptions of A-scan ultrasonography techniques [170].

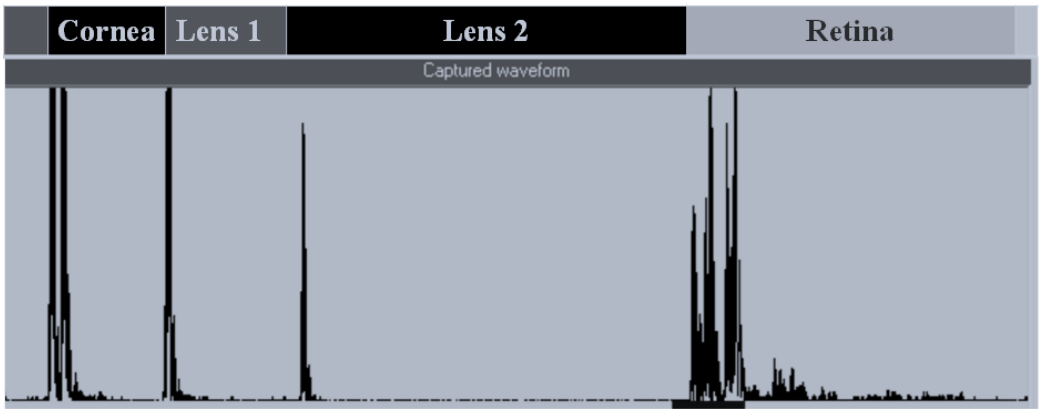


Figure 2.1 Schematic diagram of ocular A-Scan ultrasonography. Recorded images from chick experiment by high-frequency A-Scan ultrasonography. A-line shows anatomical structures of the eye from the anterior part of the cornea to the back of the sclera, which from left to right includes the cornea, lens 1, lens 2, and retina.

An arrangement of small light-emitting diodes (LEDs) that generated a ring pattern when focused on the cornea facilitated repeatable telescope measurements. By centering this ring within the pupil, probe alignment along the pupillary axis. The telescope was mounted on a rotary platform attached to a manipulator, which made it easy to switch between it and the probe. Alignment and reliable tracing were completed within 1 minute. For each successful alignment, nearly eight measurements were recorded. Acceptable data were defined as readings obtained from three separate successful alignments. Ultrasound traces were converted to corresponding lengths using the following velocity values: approximately 1534 meters per second for the aqueous chamber, vitreous chamber, retina, and choroid, and 1608 meters per second for the lens [170]. Previous studies identified the peak selection strategy to accurately pinpoint the location of these interfaces, which is essential for obtaining precise measurements

of the various ocular structures [200].

2.5 Choroidal Thickness

Custom-made PolyUChoroid software was designed to measure the posterior eyeball thickness. The choroid measurements were obtained from posterior A-Scan ultrasound traces were divided into four sectors to measure individual thickness of back of the eyes including the choroid, sclera, and retina to total sclera (Figure 2.2).

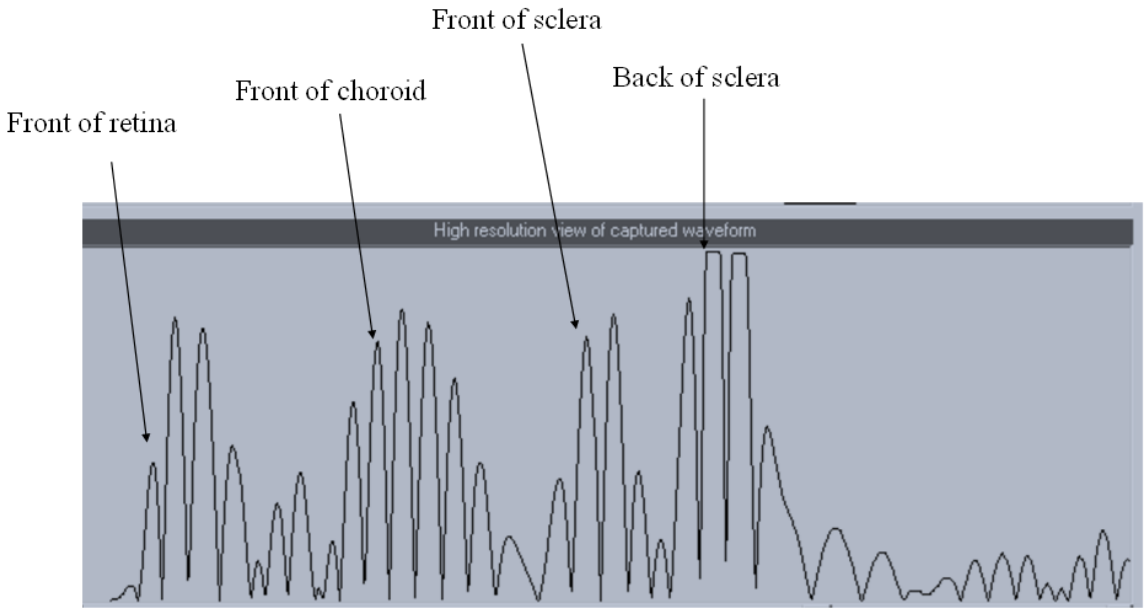


Figure 2.2: Schematic diagram of posterior ocular A-Scan ultrasonography. Recorded images from chick experiments by high-frequency A-Scan ultrasonography. The arrow indicates different parts of the posterior of the eye including the front of retina, front of choroid, front of sclera and back of sclera.

2.6 Body Weight

Body weight of all chicks was measured consistently throughout the experimental period. Initial measurements were taken at baseline to establish a starting point, with

subsequent measurements conducted on days 4, 8, and 12 to monitor growth and ensure overall health.

2.7 Drug Administration

Topical administration (atropine, latanoprost, and saline) was applied directly to the surface of the chicken eyes. Each drop was carefully measured to ensure a consistent volume of approximately 20 to 25 μl per drop, which was dispensed using a micropipette. The drugs were applied once a day in the evening, and the chicks were allowed to blink, facilitating absorption.

Intravitreal injections were performed under general anesthesia using 0.5% isoflurane in 50% oxygen (O2) and 50% nitrous oxide (N2O). Ethanol was used to disinfect and clean the surface of the injected eye and the lid retractor. A 10-20 μl aliquot of the solution was injected into the dorsal quadrant of the eye using a 30-gauge needle attached to a 25 μl Hamilton Gastight Syringe. The needle was inserted in the chick's eye approximately 3 to 4 mm deep, held for three seconds, and slowly removed to prevent backflow. Each intravitreal injection used a different site to avoid conjunctival scarring and backflow through the injection holes.

2.8 Intraocular Pressure (IOP) Measurement

Intraocular pressure (IOP) was measured using an iCare tonometer (Model: TV02, Icare Finland Oy, Vantaa, Finland). The baseline IOP was measured before myopia induction. Subsequent measurements were taken on experimental days between 9 am and 11 am. IOP was measured three times for each eye to ensure consistency and reliability. The average measurements were calculated and utilized for statistical analysis.

Chapter 3

Effect of Different Concentrations of Topical Atropine on Lens-Induced Myopia in a Chick Model

Abstract. This study aimed to determine the dose-effect relationship of topical atropine against lens-induced myopia (LIM) in chickens. Myopia was induced in the right eyes of chicks by using -10D (single vision lenses) while the left eyes were left untreated to serve as a control. A total of 60 chicks were randomly allocated into six groups which received atropine eye drops at a range of concentrations: 5%, 3%, 1%, 0.1%, 0.01% or 0% (PBS). One drop of topical atropine, or PBS was administered daily (morning or evening) to the treated and control groups, respectively. Refractive errors and ocular biometry were measured by retinoscopy and high-frequency A-scan ultrasonography on days 0, 4, 8 and 12 respectively. Data are presented as mean interocular differences ± standard deviation (SD). At the end of the treatment, recipients of all concentrations of atropines showed significant inhibition of myopia progression. The retardation was in a dose-dependent manner with the atropine concentrations administered. Interocular differences (IOD, treated versus -control eye) in refractive error on day 12 were: PBS -11.05 ± 1.41D versus 5% atropine: -6.25 ± 2.77D, $p < 0.0001$; 3% atropine: -6.30 ± 1.79D, $p < 0.0001$; 1% atropine: -

6.10 ± 1.79D, $p < 0.0001$; 0.1% atropine: -7.70 ± 1.50D, $p < 0.0001$; and 0.01% atropine: -8.02 ± 1.26D, $p < 0.0001$; respectively. All tested concentrations of atropine showed a markedly reduced anterior chamber depth, shorter vitreous chamber depth and axial elongation compared to the control group. Our data suggests that all five concentrations of atropine (5%, 3%, 1%, 0.1%, and 0.01%) were partially effective at controlling induced myopia.

3.1 Introduction

Anticholinergics (anticholinergic agents) refer to a group of medications that inhibit the action of acetylcholine at muscarinic receptors in both the central and peripheral nervous systems and, thus, control the contraction of smooth muscles. Muscarinic acetylcholine receptors (mAChRs) or acetylcholine receptors are part of the G-Protein coupled receptors (GPCRs). Previous studies have suggested mAChRs receptors serve as promising pharmacological targets for the control of myopia and other ocular diseases [172]. In recent years, pharmacological intervention using atropine has been shown to be effective for myopia control in humans and animals, but the exact mechanism is still not well understood [173, 174]. Initially, atropine was used for ophthalmic eye examination as it dilates the pupil (cycloplegia) and for treatment of malignant glaucoma, amblyopia, and iridocyclitis [175]. However, atropine is the only drug to have been shown to effectively control myopia progression in a dose-dependent manner [112, 113]. Although high concentrations of atropine has several ocular side effects, including ocular pain, photophobia, allergy, blurred reading vision, decreased contrast sensitivity, decreased tear production, and decreased visual acuity. In addition, discontinuation of its use led to a significant rebound in myopia [115, 116].

An early study, ATOM-1 investigated atropine treatment of children ages 6 to 12 years, over a 2-year- period and found that 1% atropine could slow progression of myopia by up to 77% when compared with the placebo group [114]. A further study, ATOM2. showed that lower concentrations of atropines 0.5%, 0.25%, and 0.1% could also significantly control myopia progression in children [176]. Currently, low-dose atropine eye drops are the favored pharmacological treatment to slow progression of myopia. This is largely justified based on the outcome of the ATOM2 studies [114, 177]. Low-dose atropine has been described as a straightforward, effective treatment that rapidly increased in acceptance, because low dose atropine has minimum significant ocular side effects and slow rebound effects. Daily low doses of 0.01% and 0.05% atropine groups were shown to slow progression by 30-50% in normal school children. Therefore, low dose atropine is currently the most popular treatment prescribed by clinicians in North America and Southeast Asia [116, 178]. However, a recent clinical study reported that

0.01% atropine had no effects on accommodation before and after treatment [201]. The findings suggested that atropine may not affect blocking accommodation, but its action on muscarinic receptors could involve biochemical and molecular effects on the retina or sclera. Thus, further investigation of the outcome of different concentrations of topical atropine on myopia control and safety efficacy is necessary. A previous study reported that effects of atropine on accommodation are still controversial because atropine inhibits myopia progression in chicks, but chicks do not have muscarinic receptors in their ciliary muscles [179]. It has been shown that atropine inhibits myopia in chicks, although chick M4 muscarinic receptor, but does not affect the accommodation. Although animal models have shown that atropine has different sites of action in the eyes including the retina, choroid and sclera. In addition, there are no universally acceptable concentrations of this pharmaceutical therapy to control myopia progression. Currently, 1% atropine is considered to be the most effective therapy to control myopia in humans, being able to halt progression of myopia by up to 50%. It is not possible to use more than 1% atropine in humans due to adverse side effects that include photophobia, glare, paralysis of accommodation, allergies, and rebound effects. Therefore, in the current study higher concentrations of atropine 3% and 5% were examined to determine whether higher concentrations of atropine could effectively reduce or prevent experimentally induced myopia in chicks.

3.2 Methods

3.2.1 Animals

The ethical guidelines and procedures for lens-induced myopia (LIM), as well as the methods of refractive error and Choroidal thickness determination are described in Chapter 2.

3.2.2 Preparation of Topical Atropine

Atropine sulfate powder (Sigma-Aldrich; St Louis, MO, USA United States Pharmacopeia, USP) was dissolved in 0.9% Sodium chloride (NaCl) solution. Atropine eye drops were prepared fresh for every group of experiments. For 5% atropine, eye drop was prepared by atropine sulfate powder (0.075 mg/ml), 3% atropine eye drop (0.045 mg/ml), 1% atropine eye drop (0.015mg/ml), 0.1% atropine eye drop (0.0015mg/ml) and 0.01% atropine eye drop (0.00015mg/ml) dissolved with 1.5 ml of 0.9% NaCl solution. In addition, Benzalkonium chloride (BAK) 0.01% = 10 gm in 100 ml was used to provide longer action. During the experiment atropine eye drops were kept at normal room temperature. 1 drop of atropine eye drop solution was administered once a day (evening) into their LIM eyes, starting from day 0 (Baseline) to the end of the treatment (Day 12). For the control group, LIM eyes received PBS once a day from once a day from baseline to the end of the experiment.

3.2.3 Ocular Biometry Measurement

Ocular biometry was assessed by using high-frequency A-scan ultrasonography employing 20 MHz probe (custom made) on days 0 (Baseline), 4, 8 and 12 respectively. Each measurement involved the capture of three traces of each eye, which were subsequently analyzed offline. The reported values calculated as the sum of anterior

chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL).

3.2.4 Refractive Error Measurement

Refractive errors were measured using streak retinoscopy in both eyes of each chick before the insertion of lens-induced myopia (baseline). Measurements were taken again on days 4, 8, and 12, respectively. Spherical equivalent values were calculated as the averages of results from the two principal meridians for data analysis.

3.3 Statistics

GraphPad Prism (8) software programs were used for analysis. Data were expressed as the means of interocular difference (IOD) between values for the treated eyes minus untreated eyes ± SD. Normally distributed data were analyzed using a two-way repeated-measures ANOVA to compare the different concentration groups, and Tukey's post hoc test was used to determine the least significant difference between groups when p was less than 0.05. The strength of the association between the refractive error and axial length was assessed by using Pearson's correlation coefficient (R2).

3.4 Results

3.4.1 Effect of Atropine on Lens-Induced Myopia in Chicks

Table 3.1 presents baseline (Day 0) biometric data before the treatment. The results indicated that there were no statistically significant differences in refractive error and ocular parameter outcomes between treated eyes minus untreated eyes for any of the groups. The results of different concentrations of topical atropine indicate that lens-induced myopia is significantly inhibited in a dose-dependent manner compared to control groups of chicks. Interocular difference (IOD), treated eye minus untreated eye) in refractive errors (Diopter, mean ± SD) at day 12 when compared to the controls PBS were -11.05 ± 1.41D versus 5% atropine: -6.25 ± 2.77D, $p<0.0001$; 3% atropine: -6.30±1.79D, $p<0.0001$; 1% atropine: -6.10±1.79D, $p<0.0001$; 0.1% atropine: -7.70 ±1.50D, $p<0.0001$; and 0.01% atropine: -8.02 ±1.26D, $p<0.0001$; respectively, (Table 3.2 and Figure 3.1). Only higher concentrations of 5%, 3% and 1% atropine groups resulted in significant changes, IOD of refractive error compared to low concentrations of 0.1% atropine group: (-8.02 ± 1.26D versus -6.25 ± 2.77D, $p< 0.05$; -6.30 ± 1.79D, $p< 0.05$; and 0.01% atropine group: -6.10 ± 1.79D, $p< 0.001$) respectively (Figure 3.1). However, 1% atropine group showed a significantly different IOD refractive error change compared to the 0.1% atropine group (-6.10 ± 1.79D versus -7.70 ± 1.50D, $p< 0.05$). All tested concentrations of atropine led to significantly shorter ACD compared to PBS control (IOD of ACD: 0.27 ± 0.10 mm versus 5% atropine: 0.12 ± 0.08 mm, $p=0.0001$; 3% atropine: 0.07 ± 0.07 mm, $p= 0.0001$; 1% atropine: 0.13 ± 0.04 mm, $p=0.0001$; 0.1% atropine: 0.15 ± 0.07 mm, $p=0.0001$ and 0.01% atropine: 0.16 ± 0.10 mm, $p=0.0001$ respectively (Table 3.2 and Figure 3.2). In contrast, IOD of ACD significantly inhibited differences between higher concentrations of 3% atropine compared to lower concentrations of 0.1% atropine and 0.01% atropine (0.07 ± 0.07 mm versus 0.15 ± 0.07 mm and 0.16 ± 0.10 mm, $p=0.001$, respectively. For lens thickness, no statistically significant differences were observed between all concentrations of

atropine groups compared to the PBS group.

IOD of VCD elongation significantly inhibited all concentrations of atropine eye drop groups compared to control PBS. (IOD of VCD: 0.75 ± 0.04 mm versus 5% atropine: 0.44 ± 0.21 mm, p= 0.0001; 3% atropine: 0.46 ± 0.11 mm, p=0.0001; 1% atropine: 0.40 ± 0.12 mm, p=0.0001; 0.1% atropine: 0.55 ± 0.10 mm, p=0.001 and 0.01% atropine: 0.57 ± 0.16 mm, p=0.01 respectively; Table 3.2 and Figure 3.4. However, on day 12, IOD of VCD a significant difference was found between 1% atropine group and 0.1% atropine and 0.01% atropine groups (0.40 ± 0.12 mm versus 0.55 ± 0.10 mm, p= 0.05) or (0.40 ± 0.12 mm versus 0.57 ± 0.16 mm, p= 0.01) respectively. All concentrations of atropine effectively inhibited axial elongation compared to control PBS. (IOD of AL: 0.87 ± 0.12 mm versus 5% atropine: 0.56 ± 0.23 mm, p= 0.0001; 3% atropine: 0.59 ± 0.15 mm, p=0.0001; 1% atropine: 0.51 ± 0.11 mm, p= 0.0001; 0.1% atropine: 0.65 ± 0.10 mm, p=0.001 and 0.01% atropine: 0.67 ± 0.16 mm, p= 0.01 respectively (Table 3.2 and Figure 3.4). In contrast, at the end of experiment, the IOD of AL significant difference was found between 1% atropine group and 0.1% atropine and 0.01% atropine groups (0.51 ± 0.11 mm versus 0.65 ± 0.12 mm, p=0.05) or (0.51 ± 0.11 mm versus 0.67 ± 0.14 mm, p=0.01), respectively. IOD of choroidal thickness (ChT) elongation significantly inhibited all concentrations of atropine groups compared with control PBS. (IOD of ChT: -58.90 ± 7.51 μm versus atropine 5% atropine: -23.83 ± 5.72 μm, p= 0.0001; 3% atropine: -18.23 ± 5.43 μm, p= 0.0001; 1% atropine: -18.20 ± 5.70 μm, p= 0.0001; 0.1% atropine: -24.13 ± 8.98 μm, p= 0.001 and 0.01% atropine: -33.73 ± 5.66 μm, p= 0.01 respectively (Table 3.2 and Figure 3.4). In contrast, end of the treatment significantly difference of IOD of ChT was found between 3% atropine group and 0.01% atropine groups (-18.23 ± 5.43 μm versus -33.73 ± 5.66 μm, p= 0.05). In addition, at the end of the experiment, the IOD of ChT significantly differed between 1% atropine group and 0.01% atropine group (-18.20 ± 5.70 μm versus -33.73 ± 7.51 μm, p= 0.05).

3.4.2 Coefficient of determination between refractive error versus vitreous chamber depth and axial length

In this study, linear regression analysis was conducted to examine the coefficient of determination between refractive error versus vitreous chamber depth and axial length elongation data over a 12-day treatment period. The interocular differences in refractive errors between treated eye minus untreated eye correlated with the interocular differences in vitreous chamber depth or axial length elongation between treated eye - untreated eye as shown in Figure 3.7; a and b (R^2 = 0.78 and 0.80, respectively).

3.4.3 Body Weight

There were no significant differences in body weight between the treatment and the control groups at any time (Table 3.5). All groups of chicken showed a normal increase in weight over time, which is consistent with normal chick development. There is no difference between body weight and refractive error or axial length elongation.

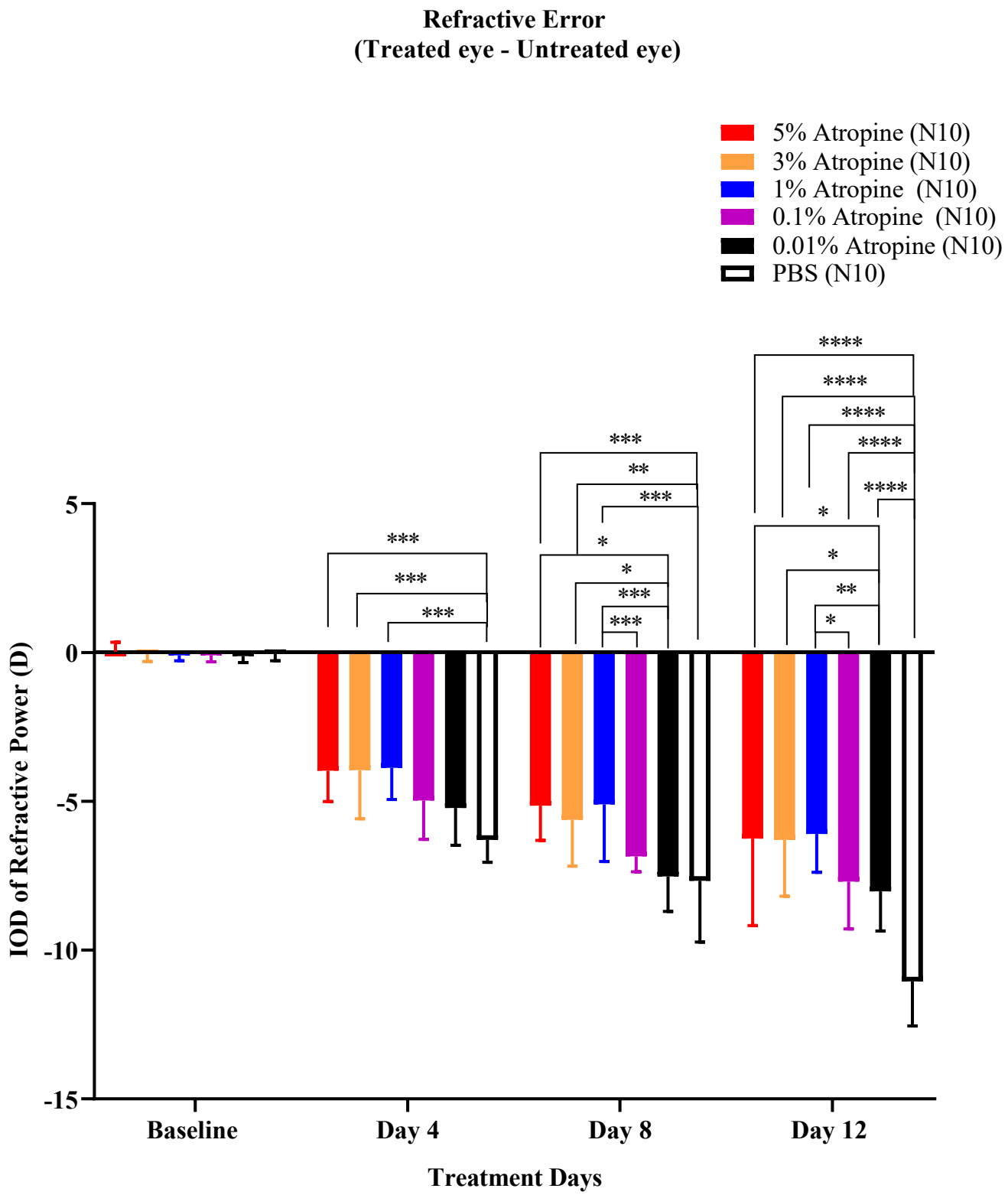

Refractive Error
(Treated eye - Untreated eye)
5% Atropine (N10)
3% Atropine (N10)
1% Atropine (N10)
0.1% Atropine (N10)
0.01% Atropine (N10)
PBS (N10)
IOD of Refractive Power (D)
5
0
-5
-10
-15
Baseline
Day 4
Day 8
Day 12
Treatment Days

Figure 3.1 Interocular differences (IOD, treated eye minus untreated eye) in refractive errors (Diopter, mean ±SD) on day 0 (baseline), 4, 8, and 12, respectively. IOD of refractive error on day 4, only higher concentrations of atropine 5%, 3%, and 1% significant difference compared to the control group. 1% atropine treated group showed a statistically significant difference between 5% atropine and 3% atropine groups. On day 8, only higher concentrations of 5%,3%,1% and 0.1% atropine groups significantly inhibited myopic refractive error compared to control PBS. However, 5%, 3%, and 1% atropine groups showed statistically significant differences between 0.01% atropine group. In addition, the 1% atropine group was significantly different from the 0.1% atropine group. At the end of the treatment, all concentrations of atropine were found to have significantly inhibited myopia refractive error compared to control PBS. In addition, higher concentrations of 5%, 3% and 1% atropine differed significantly from the lower concentration of 0.01% atropine group. Finally, 1% atropine group showed a significant difference from the 0.1% atropine group.

Bar graph shows (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p< 0.0001$.

IOD: Interocular difference; PBS: Phosphate-buffered saline.

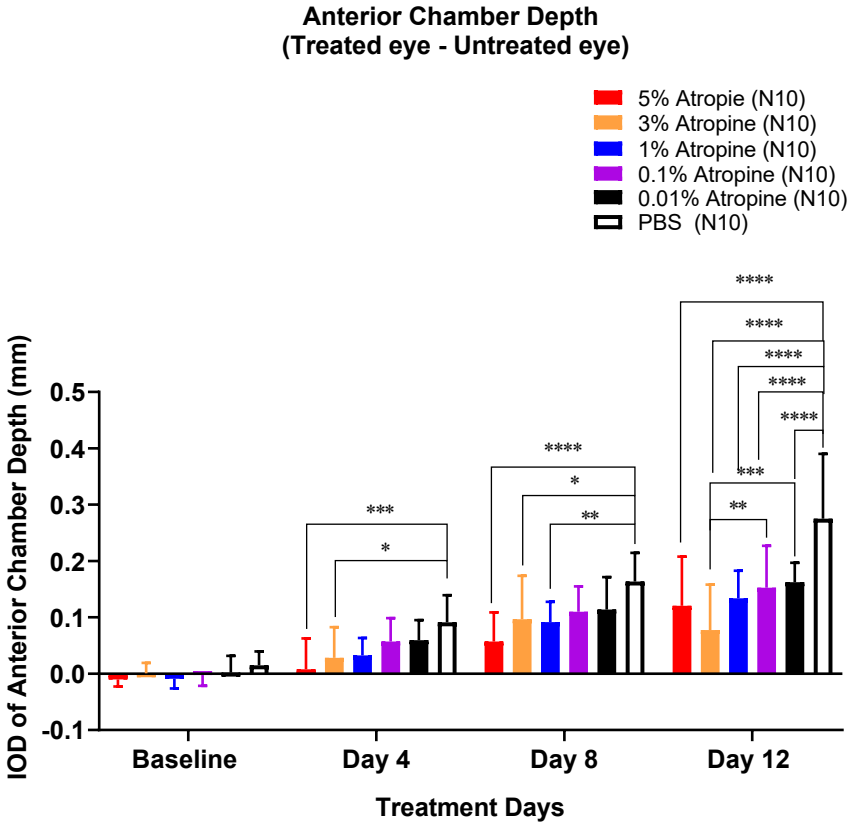


Figure 3.2 Interocular differences (IOD) (treated eye minus untreated eye) in anterior chamber depth (mm, mean ±SD) on day 0 (baseline), 4, 8 and 12, respectively. IOD of anterior chamber depth on day 4, only higher concentration of 5% atropine group and 3% atropine group had shorter anterior chamber depth compared to control PBS group. On day 8, only higher concentrations of 5%,3%, and 1% atropine groups significantly inhibited anterior chamber depth compared to the control PBS group. At the end of the treatment all tested concentrations of atropine had significantly shorter ACD compared to PBS control. However, the IOD of anterior chamber depth (ACD) significantly inhibited difference between higher concentrations of the 3% atropine group compared to the lower concentrations of 0.1% atropine and 0.01% atropine groups respectively. Bar graph showing (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine, and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p< 0.0001$.

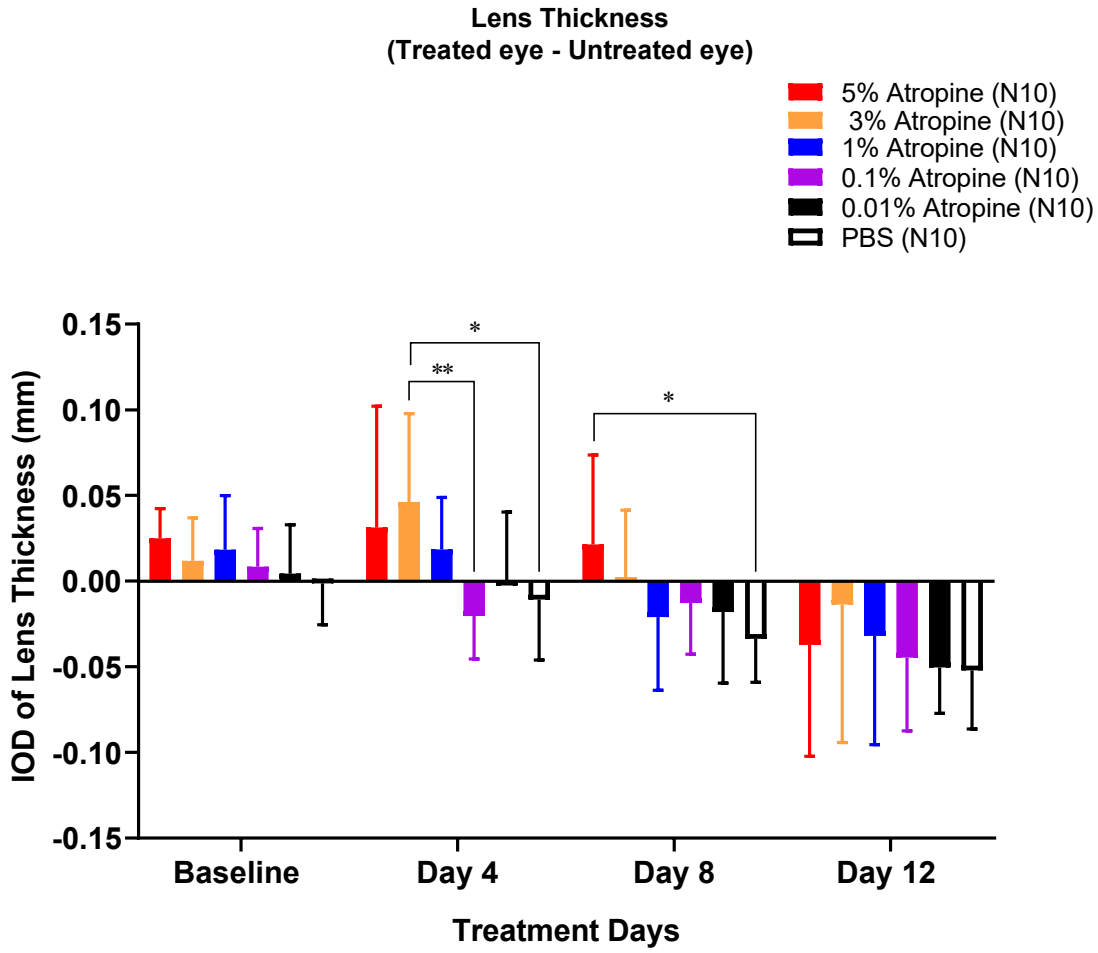


Figure 3.3 Interocular differences (treated eye minus untreated eye) in the lens thickness (mm, mean ±SD) on days 0 (baseline), 4, 8, and 12 respectively. On day 4, only a higher concentration of 3% atropine was found to have a significantly thicker lens thickness between 0.1% atropine group and control PBS group has a thinner lens thickness. However, on day 8, only the higher concentration of 5% atropine group had a significantly thicker lens thickness difference from the control PBS group. On day 12, no significant differences were observed in the effects of all tested concentrations of atropine when compared to the PBS group. Bar graph showing (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

PBS: Phosphate-buffered saline

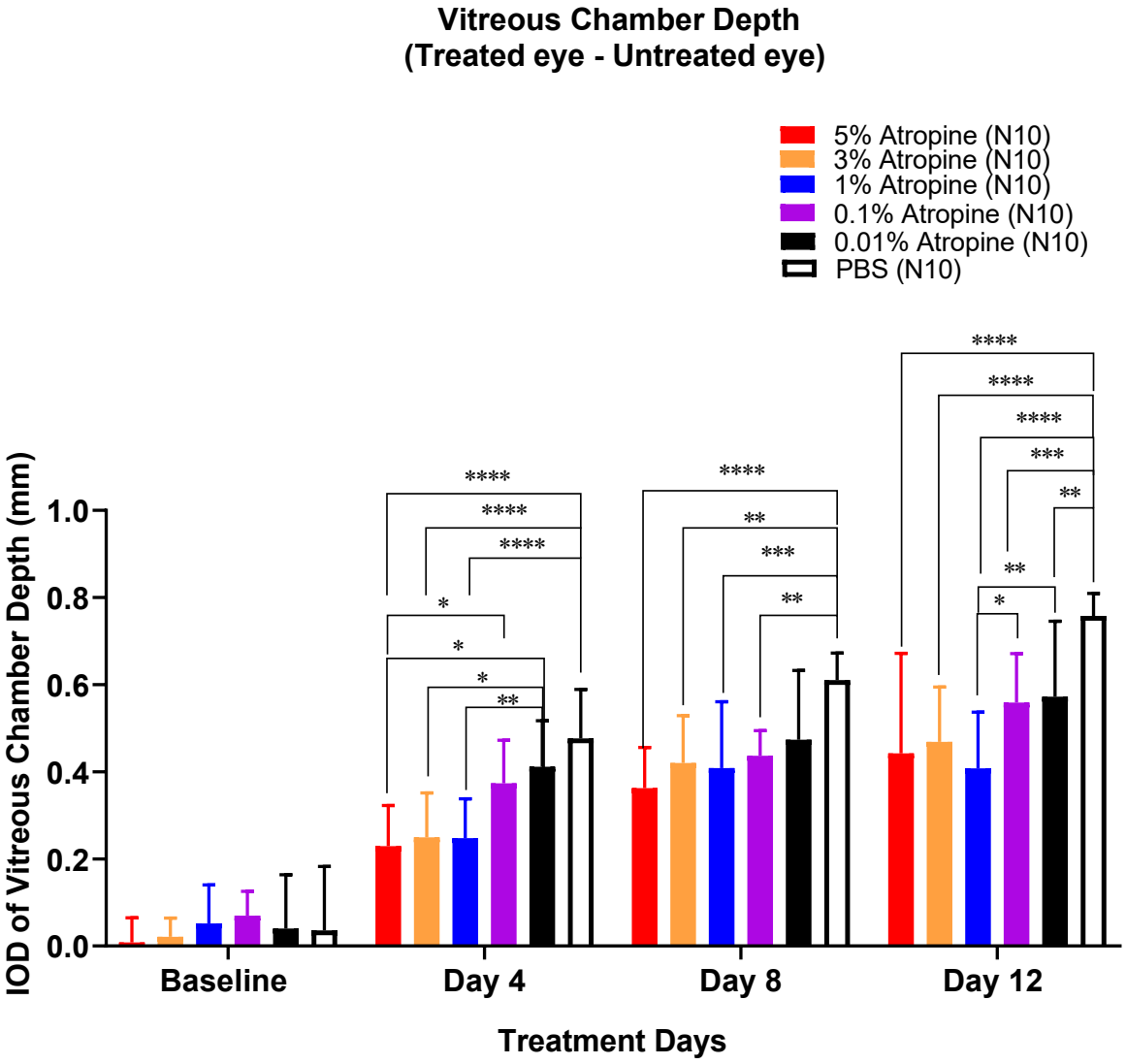

Vitreous Chamber Depth
(Treated eye - Untreated eye)
5% Atropine (N10)
3% Atropine (N10)
1% Atropine (N10)
0.1% Atropine (N10)
0.01% Atropine (N10)
PBS (N10)
IOD of Vitreous Chamber Depth (mm)
1.0
0.8
0.6
0.4
0.2
0.0
Baseline
Day 4
Day 8
Day 12
Treatment Days

Figure 3.4 (Interocular differences (IOD) (treated eye minus untreated eye) in the vitreous chamber depth (VCD) (mm, mean ±SD) on day 0 (baseline), 4, 8, and 12, respectively. On day 4, higher concentrations of 5%, 3%, and 1% atropine groups were found to have significantly shorter VCD compared with the control PBS group. However, the IOD of VCD is significantly shorter in higher concentrations of 5%, 3%, and 1% atropine groups, compared to the lower concentrations of atropine 0.01% atropine group. Although higher concentrations of atropine 5% atropine group have shorter VCD compared to lower concentrations of 0.1% atropine group. In contrast, on day 8, only higher concentrations of 5%, 3%, 1% and 0.1% atropine groups were found to significantly inhibit VCD compared with control PBS. At the end of the experiment, all concentrations of atropine significantly inhibited VCD elongation compared to control PBS. In addition, VCD significantly differed between 1% atropine group compared to 0.1% and 0.01% atropine groups respectively.

Bar graph showing (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine, and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p< 0.0001$.

PBS: Phosphate-buffered saline)

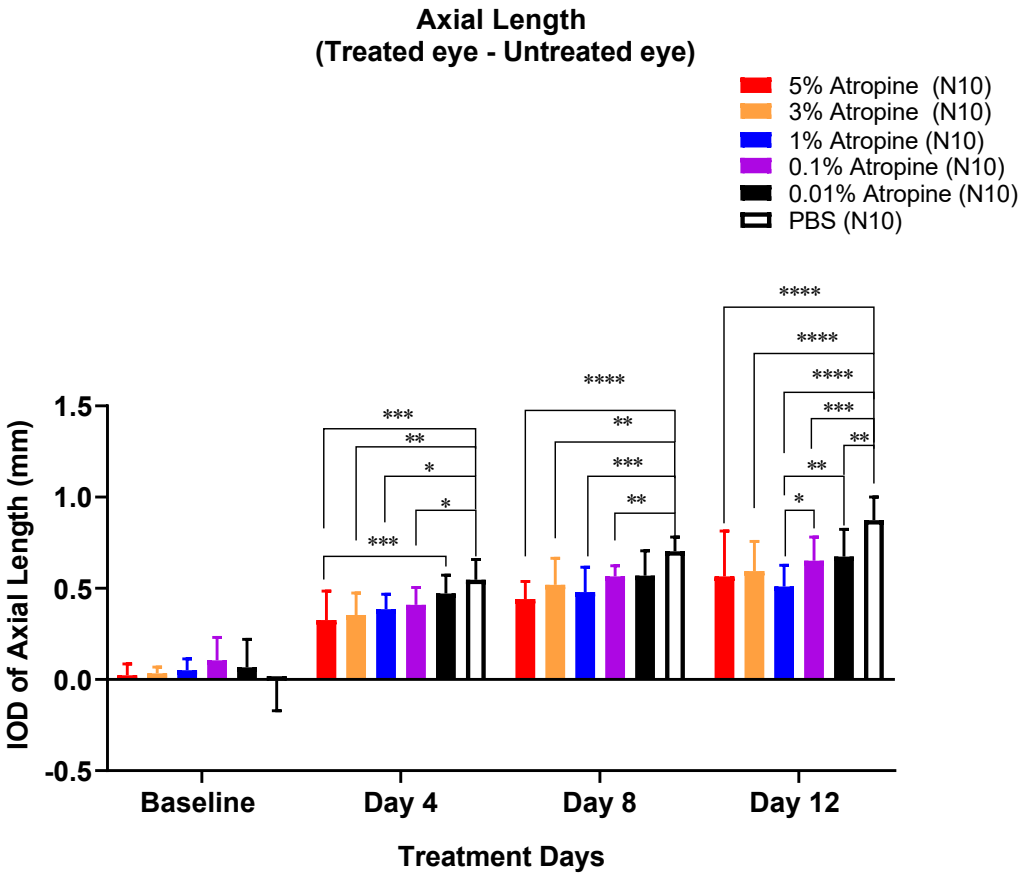


Figure 3.5 Interocular differences (IOD) (treated eye minus untreated eye) in the axial elongation (mm, mean ±SD) on day 0 (baseline), 4, 8, and 12 respectively. On day 4, higher concentrations of atropine, 5%, 3%, 1%, and 0.1%, were found to have a significantly less axial elongation compared with the control PBS group. However, the higher concentration of 5% atropine group had less axial elongation compared to lower concentration of 0.01% atropine group. In contrast, on day 8, only higher concentrations of 5%, 3%, 1% and 0.1% atropine groups were found to significantly inhibit axial elongation compared with control PBS. At the end of the experiment, all concentrations of atropine groups effectively inhibited axial length elongation compared to control PBS. Although a significant difference was noted between the AL of 1% atropine treatment and the 0.1% and 0.01% atropine groups.

Bar graph showing (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine, and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p< 0.0001$.

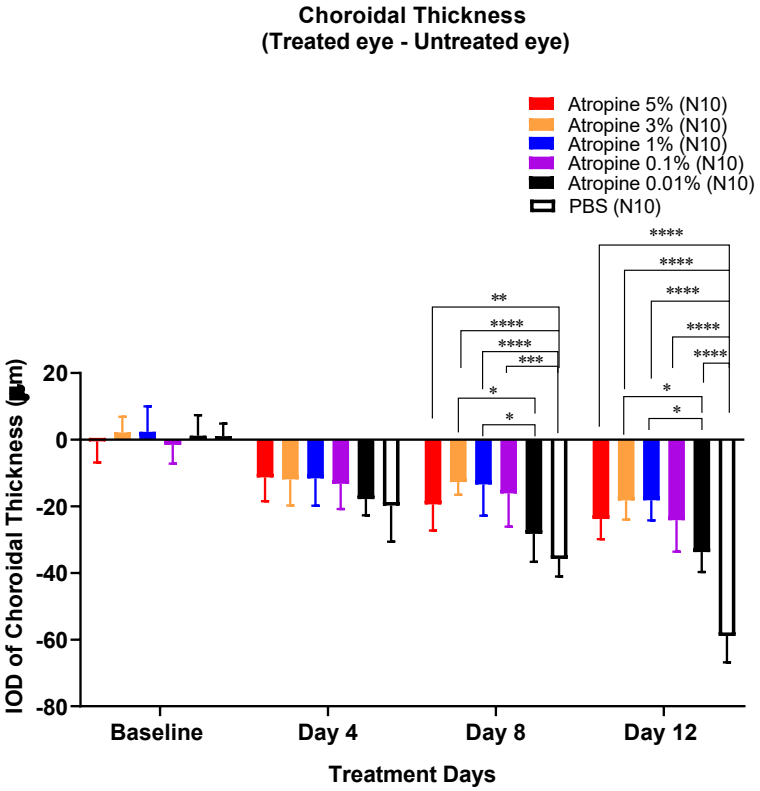


Figure 3.6 Interocular differences (IOD) (treated eye minus untreated eye) in choroidal thickness (μm, mean ±SD) on day 0 (baseline), 4, 8, and 12, respectively. On day 8, only higher concentrations of 5%, 3%, 1%, and 0.1% atropine groups significantly inhibited axial elongation compared with control PBS. However, the higher concentrations of 3% and 1% atropine significantly reduced the increase in choroidal thickness than the lower concentration 0.01% atropine. The end of the experiment shows all concentrations of atropine effectively inhibited choroidal thickness increase compared to control PBS. In addition, for IOD of ChT, a significant difference was found between 3% atropine group and the 0.01% atropine group. In addition, IOD of ChT 1% atropine group significantly inhibited choroidal thickness more than the 0.01% atropine group. Bar graph showing (Red bar = 5% atropine, yellow bar = 3% atropine, blue bar = 1% atropine, violet bar = 0.1% atropine, black bar = 0.01% atropine, and white bar = PBS). Statistics - Two-Way repeated measures ANOVA + Tukey's honestly-significant-difference. Error bar expresses standard deviation (SD), and the sample size is denoted by (n). ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, and **** $p< 0.0001$.

IOD: Intra-ocular differences; PBS: Phosphate-buffered saline; ChT: Choroidal thickness; SD: Standard deviation.

Table 3.1 Summarized baseline data for all tested concentrations of atropine. The data represents the difference (IOD) in refractive error (D) and ocular parameters (mm).

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of CT (μm)
Atropine 5%	0.02 ± 0.30	-0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.05	0.02 ± 0.05	-0.5 ± 6.03
Atropine 3%	-0.05 ± 0.24	0.00 ± 0.01	0.01 ± 0.02	0.02 ± 0.04	0.03 ± 0.03	2.2 ± 4.46
Atropine 1%	-0.10 ± 0.17	-0.00 ± 0.01	0.01 ± 0.02	0.05 ± 0.08	0.05 ± 0.05	2.38±7.24
Atropine 0.1%	-0.01 ± 0.21	-0.00 ± 0.01	0.00 ± 0.02	0.06 ± 0.05	0.10 ± 0.11	-1.56±5.35
Atropine 0.01%	-0.11 ± 0.21	0.00 ± 0.02	0.00 ± 0.02	0.03 ± 0.11	0.06 ± 0.14	1.16±5.86
PBS	-0.05 ± 0.21	0.01 ± 0.02	-0.00 ± 0.02	0.03 ± 0.13	-0.00 ± 0.15	1.00±3.70

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 3.2 Summarized data for the outcomes of different concentrations of atropine on day 4, interocular difference (IOD) in refractive error (D), and ocular parameters (mm).

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of CT (μm)
Atropine 5%	-3.97 ± 0.98	0.00 ± 0.05	0.03 ± 0.06	0.22 ± 0.08	0.32 ± 0.15	-11.33 ± 6.79
Atropine 3%	-3.95 ± 1.55	0.02 ± 0.05	0.04 ± 0.04	0.24 ± 0.09	0.35 ± 0.11	-11.86 ± 7.46
Atropine 1%	-3.87 ± 1.00	0.03 ± 0.02	0.01 ± 0.02	0.24 ± 0.08	0.38 ± 0.07	-11.60 ± 7.78
Atropine 0.1%	-4.97 ± 1.23	0.05 ± 0.03	-0.02 ± 0.02	0.37 ± 0.09	0.40 ± 0.08	-13.23 ± 7.18
Atropine 0.01%	-5.22 ± 1.18	0.05 ± 0.03	0.00 ± 0.03	0.41 ± 0.09	0.47 ± 0.09	-17.83 ±4.59
PBS	-6.30 ± 0.71	0.09 ± 0.04	-0.01 ± 0.03	0.47 ± 0.10	0.54 ± 0.10	-19.75 ± 10.32

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 3.3 Summarized data for the outcome of different concentrations of atropine on day 8, interocular difference (IOD) in refractive error (D), and ocular parameters (mm).

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of CT (μm)
Atropine 5%	-5.15 ± 1.10	0.05 ± 0.04	0.02 ± 0.04	0.36 ± 0.08	0.44 ± 0.09	-19.43 ± 7.42
Atropine 3%	-5.62 ± 1.47	0.09 ± 0.07	0.00 ± 0.03	0.42 ± 0.10	0.51 ± 0.13	-12.73±3.60
Atropine 1%	-5.1 ± 1.82	0.09 ± 0.03	-0.02 ± 0.04	0.40 ± 0.14	0.47 ± 0.12	-13.40 ± 8.94
Atropine 0.1%	-6.85 ± 0.48	0.10 ± 0.04	-0.01 ± 0.02	0.43 ± 0.05	0.56 ± 0.05	-16.10 ± 9.49
Atropine 0.01%	-7.52 ± 1.11	0.11 ± 0.05	-0.01 ± 0.03	0.47 ± 0.15	0.56 ± 0.12	-28.26 ±7.98
PBS	-7.67 ± 1.95	0.16 ± 0.04	-0.03 ± 0.02	0.61± 0.05	0.70 ± 0.07	-35.80 ± 5.25

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 3.4 Summarized data on day 12 for the outcomes of different concentrations of atropine, interocular difference (IOD) in refractive error (D), and ocular parameters (mm).

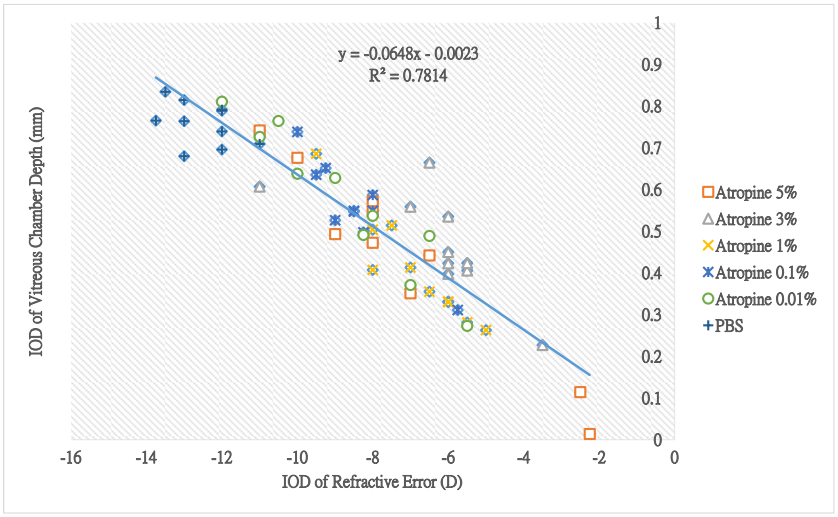
Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of CT (μm)
Atropine 5%	-6.25 ± 2.77	0.12 ± 0.08	-0.03 ± 0.06	0.44 ± 0.21	0.56 ± 0.23	-23.83 ± 5.72
Atropine 3%	-6.30 ± 1.79	0.07 ± 0.07	-0.01 ± 0.07	0.46 ± 0.11	0.59 ± 0.15	-18.23± 5.43
Atropine 1%	-6.10 ± 1.22	0.13 ± 0.04	-0.03 ± 0.06	0.40 ± 0.12	0.51 ± 0.11	-18.20 ± 5.70
Atropine 0.1%	-7.70 ± 1.50	0.15 ± 0.07	-0.04 ± 0.04	0.55 ± 0.10	0.65 ± 0.12	-24.13 ± 8.98
Atropine 0.01%	-8.02 ± 1.26	0.16 ± 0.03	-0.05 ± 0.02	0.57 ± 0.16	0.67 ± 0.14	-33.73 ± 5.66
PBS	-11.05 ± 1.41	0.27 ± 0.10	-0.05 ± 0.03	0.75 ± 0.04	0.87 ± 0.12	-58.90 ± 7.51

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 3.5 Average body weight (mean±SD) of chickens at baseline, days 4, 8, and 12

Weight of chicken				
Experiment groups	Baseline/g	Day 4/g	Day 8/g	Day 12/g
Atropine 5%	41.76 ± 4.02	70.29 ± 3.55	97.09± 6.61	152.29 ± 10.9
Atropine 3%	41.34 ± 2.81	72.33 ± 3.65	99.80 ± 3.30	148.72 ± 7.93
Atropine 1%	40.33 ± 4.25	76.08 ± 7.20	98.17± 3.28	141.48± 8.76
Atropine 0.1%	44.35± 3.98	78.94 ± 5.74	105.59 ± 6.16	151.09 ± 13.55
Atropine 0.01%	38.69± 2.58	74.89± 4.92	99.08± 5.07	142.4± 7.25
PBS	40.49±5.09	76.37±3.98	101.28±5.72	144.07±11.92

(a) Correlation of interocular differences (IOD) in refractive errors and vitreous chamber depth in chicks treated with different doses of atropine



(b) Correlation of interocular differences (IOD) in refractive errors and axial length in chicks treated with different doses of atropine

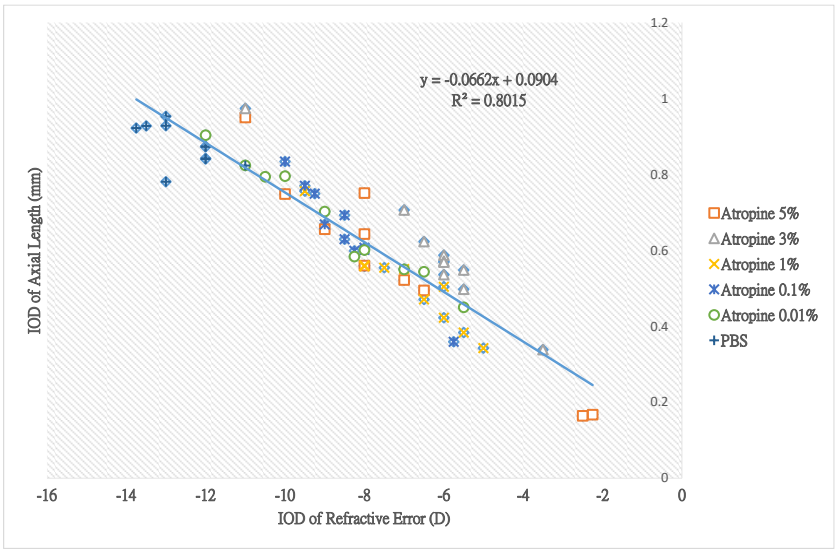


Figure 3.7 Scatter plots showing the coefficient of determination between interocular differences in refractive errors and vitreous chamber depth at day 12 (a), as well as interocular differences in refractive errors and axial elongation (b), treated chicken (R^2 = 0.78 and 0.80, respectively).

3.5 Discussion

3.5.1 Atropine Changes Refractive Error

This non-clinical study investigated induced myopia using different concentrations of atropine for treatment effect of experimental induced myopia in chicken. The refractive error results showed that all concentrations of atropine effectively inhibited experimental induced myopic refractive error in chicks. This is the first study to investigate the treatment effect of topical atropine at different concentrations from high to low (5%, 3%, 1% 0.1% and 0.01%). The results revealed that higher concentrations of atropine had a maximum effect on controlling induced myopic refractive error but among these, 1% had a greater effect in myopia progression in chicken. Previous experimental study showed atropine intravitreal injection could inhibit myopia progression by approximately 60% with 2.5 μg and 100% with 250 μg which was an opposite result to the current study findings, possibly due to different drug delivery routes [202]. Based on the results, the current study found that higher concentrations of 3% atropine and 5% atropine were less effective. Possible explanations could be blockage of cell membranes at higher concentrations of drug, which may not permit endosmosis, whereas at lower concentrations the process is facilitated. Alternatively, at higher concentrations of the drug, the pharmacokinetics and pharmacodynamics are shifted to toxicokinetics or toxicodynamics producing overdose manifestations or toxicity [203]. Several studies have suggested that topical atropine could control myopia progression in humans or animals in a dose dependent manner [143, 204, 205]. A randomized controlled trail study revealed that 1% atropine significantly inhibited myopia progression in children compared to cyclopentolate 1% and placebo [96]. These results showed that 1% atropine changed mean progression of myopia was -0.22D per year compared to 1% cyclopentolate -0.58D per year and placebo group -0.91D per year. Another clinical study found children aged 5-10 years receiving 1% atropine eye ointment had reduced progression of myopia (+0.06D per year) while the control group experienced 1.19D per year [206]. Similar findings were observed in a study conducted in Asia, in which 1% atropine use led to a difference in progression of myopia between study and control group of approximately 0.92D [134]. The current study results also showed that higher

concentration of atropine (1%) was effective to arrest the progression of myopia than the lower concentrations of atropine (0.1% and 0.01%). A similar finding was observed by Song et al., who suggested that higher concentrations of atropine, 0.5 % and 1 %, were most effective in stopping the progression of myopia compared to lower concentrations of atropines of 0.05 %, 0.1 %, and 0.25 % [204]. Another study reported that 61% of children in the 0.5% atropine group had no changes in myopia, 49% children in the 0.25% atropine group, and 42% children in the 0.1% atropine group indicating that higher concentrations of atropine were more powerful [207]. However, higher concentrations of atropine have several ocular side effects including photophobia, allergy, blurred reading vision, decreased contrast sensitivity, decreased tear production, and decreased visual acuity, which may limit the use of higher concentrations of medications [208]. In addition, the ATOM 1 study also reported common ocular side effects of atropine included allergic reactions 4.5%, glare 1.5%, and blurred near visual acuity 1% [209]. ATOM 2 study found that atropine side effects were dose dependent as those receiving higher concentrations had more side effects compared to lower concentration of the drug. A study conducted by Cooper et al. reported that the maximum concentration of atropine which did not create any clinical signs and symptoms related with high doses of atropine was 0.02%. They observed that 0.02% atropine caused dilation of the pupil of around 3 mm and maintained the amplitude of accommodation of approximately 8D, which minimize the ocular side effects or clinical signs and symptoms [210]. In addition, the current study indicated that lower concentrations of atropine (0.1% and 0.01%) also effectively retarded the progression of myopia. Numerous studies have reported that lower doses of atropine can effectively reduce myopia and had fewer side effects [211-213]. Therefore, lower concentrations of atropine should be used to control myopia progression as these can minimize ocular side effects and improve tolerance.

3.5.2 Changes in Ocular Biometry

Anterior chamber depth (ACD) of the eye gradually decreases with age, with an average reduction of approximately 0.011 mm per year [214]. This finding decrease indicates age-related anatomical changes in the eye, contributing to shifts in ocular dimensions over

time. Topical administration of atropine led to positive changes in the ocular biometry in induced myopic eyes which differed from those of control eyes. The ATOM 1 study reported atropine treatment caused changes in ocular biometry, including corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth, and axial length elongation. The results of the current study suggest that all administered doses of topical atropine significantly inhibited the elongation of ACD when compared to the control group. In the ATOM 1 study, which used 1% atropine, a mean decrease in ACD of -0.17 mm was observed after 36 months of follow-up [215]. The Correction of Myopia Evaluation Trial (COMET) reported that myopic children from 6 to 11 years of age changed their ACD by 0.035 mm every year [216]. A previous myopia study demonstrated that younger myopic children had increased ACD, but after 20 years of age it became less [217]. Overall lens thickness decreases in the first 7 years of life, remains stable from ages 8 to 16, and subsequently increases at an approximate rate of 27 μm per year [218]. The current study revealed little decrease in LT in the atropine treated groups and only a slightly greater decrease in LT in the control group, but these differences were not statistically significant. However, the lenses of myopic children decreased between 7-11 years of age, but little increased from 11 to 15 years of age and become thicker thereafter [217]. The Singapore Cohort of Risk Myopia study, a 5-years study, found that LT changes the lens into a U shape due to LT between 9 to 10 years of age [49]. A study conducted in Taiwan reported similar findings with LT reduction from 7 to 11 years of age [219]. A study of use of atropine demonstrated that during the treatment, LT decreased, but resumed when treatment was withdrawn. It was hypothesized that LT was increased because atropine could paralyse the ciliary body, resulting in absence of accommodation [215]. Consequently, the ciliary body or process to moves outward and tension of the lens increases thereby resulting in LT.

Previous studies have described an association between high myopia and increasing VCD and increased AL [215, 219], whilst atropine-treated eyes demonstrated that less myopic shift was associated with a shorter VCD and AL. The reduction in VCD is due to a posterior shift of the iris-lens apparatus through the posterior chamber. Other changes could be due to mechanical forces associated with paralysis of ciliary muscle leading to a change from prolate to oblate shape. Although a higher concentration of

atropine (1%) led to mean changes in AL of 0.02± 0.35 mm when compared to the placebo group in which the AL change was significantly greater at 0.38 ± 0.38 mm after 24 months of follow-up [134]. At the end of third year of study, the higher concentration of atropine group had a mean change in AL of 0.29± 0.37 mm, compared to the placebo groups markedly increased AL of 0.52 ± 0.45 mm. These findings demonstrated that atropine treatment slowed progression of myopic symptoms and reduced the growth rate of VCD and axial elongation. In another study, ATOM 2, different concentrations of atropine, 0.5%, 0.1%, and 0.01% treated groups, revealed changes in AL after 2 years of follow-up were 0.27 ± 0.25, 0.28 ± 0.28 and 0.41 ± 0.32 mm, respectively [137]. At the end of the third year of the study the 0.01% (atropine treated group had slight increase in AL 0.19 ± 0.13 mm), compared with 0.5% (0.35 ± 0.20 mm) and 0.1% (0.33 ± 0.18 mm) groups, respectively. However, it was considered that after 5 years of study there were no significant differences in AL change between the three atropine treatment groups, 0.5%, 0.1%, and 0.01%, 0.75 ± 0.48, 0.85 ± 0.83 and 0.87 ± 0.49 mm, respectively.

The current study investigated that all concentrations of atropine significantly increased choroidal thinning. Atropine, a nonselective muscarinic antagonist, has been used as an anti-myopia treatment for some time. It was initially confirmed by Yen et al. in 1989, who demonstrated that 1% atropine effectively slowed myopia progression in children [133]. At present, 0.01% atropine is generally used for myopia treatment, especially in school children due to its reduced side effects compared to higher concentrations [140]. The current study also found that a higher concentration of atropine 1% significantly preserved choroidal thinning compared to lower concentrations of atropine. Similarly, a study by Luyao et al. reported that 1% atropine significantly increased choroidal thickness after one week of treatment, while the lower concentration of atropine 0.01% decreased in choroidal thickness over the 6 months of the treatment period [220]. However, another clinical study suggested that a higher concentration of atropine (1%) gel used for 1 week resulted in an average increase in sub-foveal ChT of 20.33 ± 28.41 μm in Chinese children [145]. Supporting this, relevant to changes in choroidal thickness are associated with faster eye growth in chicks, while a thicker choroid may contribute to inhibited eye growth in children who are treated with atropine [221]. Woodman et al. observed choroidal thickness changes during accommodation when using Spectralis OCT. The authors

suggested that atropine could change regional variations in parafoveal thickness that correspond to the distribution of nonvascular smooth muscle cells within the choroid, which indicates that these cells may regulate choroidal thickness during accommodation [222]. Therefore, atropine may alter choroidal thickness by blocking accommodation or modifying the tone of nonvascular smooth muscle. A result of an animal study found that antimuscarinic drugs could change choroidal thickening due to changes in the tone of nonvascular smooth muscle [223]. This occurs when the choroid receives extensive autonomic innervation by both adrenergic and muscarinic pathways. In chicken, parasympathetic stimulation from the ciliary ganglion produces choroidal contraction, which was blocked by atropine, resulting in the role of acetylcholine affecting choroidal smooth muscle contraction [224]. Nickla et al. observed that atropine significantly inhibits myopia progression and increases choroidal thickening in lens-induced myopia chicks due to reduced cholinergic input that normally prevents osmotic swelling in the choroid, thus maintaining a balance that prevents excessive thickening of the choroid [225]. Another possible cause could be the structural similarity between muscarinic and adrenergic receptors [226]. Muscarinic toxin 3 (MT3), a selective M4 antagonist, also binds with high affinity to α-adrenergic receptors in mammals [227]. Previous studies have shown that atropine, at higher concentrations, exhibits direct α-adrenoceptor blocking activity, which may partly occur for the atropine flush [228]. This cross-reactivity could also influence choroidal vasculature, as both sympathetic and parasympathetic nerves innervate it. As a result, α-adrenoceptor blockades within the vasculature of the choroid would play a significant role in preventing choroidal thickening. Atropine may induce choroidal thickening through a non-adrenergic, non-cholinergic mechanism by triggering vasodilation in ocular blood vessels through presynaptic muscarinic receptor blockade M2, which enhances nitric oxide (NO) release [229]. Additionally, atropine may also indirectly affect the retina by stimulating the release of dopamine or other neurotransmitters, and dopamine itself has been shown to increase in choroidal thickness [230]. Atropine, a nonselective muscarinic antagonist, influences choroidal thickness by altering muscarinic and adrenergic receptor activity, blocking accommodation, stimulating nitric oxide release, and affecting neurotransmitters like dopamine, which together contribute to its role in myopia control.

3.6 Conclusions

In conclusion, the results demonstrated that lens-induced myopia was significantly inhibited in a dose-dependent manner by different concentrations of atropine. Higher concentrations of atropine (5%, 3% and 1%) were more effective in reducing myopic refractive error compared to lower concentrations (0.1% and 0.01%). Only the 1% atropine group showed a significant difference compared to the 0.1% atropine group. All tested concentrations of atropine significantly inhibited anterior chamber depth compared to the control group. Although all tested concentrations of atropine effectively reduced the vitreous chamber elongation a significant difference in VCD was found between the 1% atropine group and 0.1% atropine group. In addition, all tested concentrations of atropine effectively inhibited axial elongation although significant differences were observed between the 1% atropine and the 0.1% and 0.01% atropine groups. Finally, all tested concentrations of atropine groups effectively reduced choroidal thickness changes, whereas 3% atropine markedly inhibited choroidal thickness changes.

Chapter 4

Alpha-1, alpha-2, and nonspecific adrenergic receptor agonists inhibit lens-induced myopia in chicks.

Abstract. Alpha-adrenergic receptor agonists have been shown to inhibit the effect of atropine against myopia development. The study aimed to test the efficacy of α1-adrenoceptor, α2-adrenoceptor, and non-specific adrenergic receptor agonists in inhibiting myopia progression in a chick model. Lens-induced myopia was induced optically in the right eyes of chickens using -10D single vision lenses for 7 days, while the left eyes had no lens fitted, acting as controls. The α1-adrenoceptor agonist (metaraminol), α2-adrenoceptor agonist (lofexidine), and non-specific adrenergic agonist (cirazoline) were intravitreally injected at concentrations of 3, 30, and 300 nmol in 10 μl solution into the right eyes on days 0, 2, 4 and 6. Phosphate-buffered saline (PBS) was injected into the untreated control eyes. On day 7, all lenses were removed and the eyes allowed to recover from induced myopia until day 14. Refractive errors were assessed, and ultrasound biometry was performed on days 0, 7, and 14. Metaraminol significantly inhibited lens-induced myopia and reduced axial elongation in a dose-dependent manner. Significant interocular differences in

refraction on day 7 were observed: PBS (-8.57± 1.13D) versus (-3.43 ± 2.76D, $p = 0.001$) and 300 nmol: (-2.61 ± 1.84D, $p < 0.001$). Lofexidine inhibited lens-induced myopia and reduced axial elongation at all tested doses. Interocular differences of refraction on day 7 were: -PBS (9.17 ± 1.44D) versus 3 nmol: (-5.67 ± 2.64D, $p = 0.0001$), 30 nmol: (-4.25 ± 1.92D, $p < 0.001$), and for 300 nmol: (-3.25 ± 1.92D, $p < 0.001$). Interocular differences of choroidal thickness compared to the control PBS on day 7 were (PBS: -65.12 ± 26.31 μm, $n = 13$; versus 30 nmol: -12.98 ± 21.59 μm, $p = 0.001$, $n = 19$; 300 nmol: -14.35 ± 10.45 μm. In contrast, cirazoline significantly inhibited the lens-induced myopic refractive error and reduced ocular elongation at doses of 30 nmol and 300 nmol. Interocular differences of refraction on day 7 were: PBS (-7.08 ± 0.58D) versus 30 nmol: -5.31 ± 1.39D, $p < 0.001$), and for 300 nmol: -4.09 ± 1.18D, $p < 0.001$). All tested groups induced myopia in the treated eyes showed significant recovery on day 14 following lens removal on day 7. α1-adrenoceptor, α2-adrenoceptor, and non-specific adrenergic receptor agonists were partially effective in preventing optically induced experimental myopia in the chick.

4.1 Introduction

Myopia is a common refractive disorder worldwide and has become a major public health concern due to a dramatic increase in its prevalence over the past few decades [13, 231]. Uncorrected refractive errors can cause irreversible visual impairment in both adults and children. The refractive error could be corrected with spectacles, contact lenses, or ocular refractive surgery. However, although these corrections can provide better visual acuity, they cannot reverse or control myopia progression [232]. A few pharmacological treatments are available for partially slowing the progression of human myopia using a non-selective muscarinic acetylcholine receptor (mAChR) antagonist drug. The most commonly used agent is atropine which has been demonstrated to be an effective intervention to prevent the structural causes of myopia by paralysis of ocular accommodation during near work [233]. Muscarinic receptors are widely distributed within the eye, including the ciliary muscle, choroid, retina, and sclera [234]. Studies have suggested that muscarinic antagonists inhibit myopia progression and prevent ocular structural growth through their action at the choroid, retinal pigmented epithelium, and sclera but not through an accommodative mechanism [235]. There is some evidence that the accommodative apparatus is altered in myopia and ciliary muscular hypertrophy resulting in abnormal ocular enlargement of the eye and progression to high myopia [236]. While higher concentrations of atropine, 0.5% to 1%, are very effective in delaying the development of myopia progression, there is a higher rate of muscarinic acetylcholine receptor M3 mediated side effects, including glare, photophobia, paralysis of accommodation, and allergies [133, 237]. These studies reported a high dropout rate of approximately 16 to 58%.

While atropine has proven effective, its application is limited by side effects such as photophobia, blurred near vision, allergic conjunctivitis, and systemic reactions. The exact mechanism of atropines action against myopia remains unclear. However, atropine is poisonous in higher doses, causing the FDA to be hesitant to approve its clinical use for myopia management. Currently, only the lowest concentration is

permitted to be used under strict regulations. Understanding the mechanism behind recovery from induced myopia is crucial for developing effective therapeutic strategies to prevent myopia progression. While many studies have effectively focused on the induction of myopia and methods for prevention, there is an opportunity to explore the recovery process after the removal of induced lenses. To address this significant gap in myopia treatment, optometry researchers are looking for an alternative pharmaceutical option with comparable effectiveness, lower toxicity, myopia-related changes in the eye, and fewer side effects. Previous studies have primarily focused on the effect of alpha 2-adrenoceptor agonists in the form-deprivation myopia (FDM) model in chicks [156]. However, there is a lack of evidence regarding their impact on the lens-induced myopia (LIM) model in chickens. Since FDM and LIM share similar pathophysiology but have different mechanisms, further investigation is necessary to determine if α2-adrenoceptor agonists produce similar effects in the LIM model. Additionally, this study aims to examine whether α1-adrenoceptor agonists, α2-adrenoceptor agonists, and non-specific adrenergic agonists can effectively control myopia progression and the recovery effect from lens-induced myopia. Further research in these areas is essential to understand the potential effects and develop comprehensive strategies for myopia management. The preliminary data of this study were presented in the Association for Ocular Pharmacology and Therapeutics (Mezbah et al., 2021; E-abstract AOPT XV Biennial Scientific Meeting), a study investigating the adrenergic receptor agonist inhibition of lens-induced myopia in chicks.

4.2 Methods

4.2.1 Animals

Chapter 2 explains the ethical guidelines and methods for lens-induced myopia (LIM), refractive error measurements, choroidal thickness, and body weight.

4.2.2 Intraviral Injections

An α1-adrenoceptor agonist (metaraminol), α2-adrenoceptor agonist (lofexidine), and nonspecific adrenergic agonist (cirazoline) were intravitreally injected at doses of 3, 30, or 300 nmol in 10 µl solution into the right eyes on days 0, 2, 4 and 6. Phosphate-buffered saline (PBS) was injected into the control eye. Intravitreal injections were performed at the same time point each day (11:00 am - 1:00 pm) to maintain the circadian rhythm and avoid growth-retarding effects or any types of discomfort and ocular infection. Chicks were anesthetized with 0.5% isofluorane in 50% oxygen (O2) and 50% nitrous oxide (N2O). Ethanol was used as disinfection and cleaning the surface of the injected eye and the lid retractor. A 10 µl aliquot of the agonist solution was injected into the dorsal quadrant of the eye using a 30-gauge needle attached to a 25 µl Hamilton Gastight Syringe. The needle was inserted in the chick's eye approximately 3 to 4 mm deep, held for three seconds, and slowly removed to prevent backflow. A different site was used for each intravitreal injection to avoid conjunctival scarring and backflow through the injection holes. On day 7, all lenses were removed, and injections ceased to allow the eyes to recover from lens-induced myopia for another 7 days. The percentage of refractive error (Rx) inhibition for each drug at each dose (low: 3 nmol, medium: 30 nmol, and high: 300 nmol) was determined by (IOD refractive error in PBS control – IOD refractive error treated group) / IOD refractive error in PBS control *100.

4.2.3 Preparation of the Intravitreal Injections

The drugs were dissolved at room temperature in sterile phosphate-buffered saline (PBS; Thermo Fisher Scientific, Waltham, MA, USA). Lower doses of the drugs used in the study, metaraminol, lofexidine, and cirazoline dissolve easily in PBS. However, gentle heating is required for these drugs to dissolve fully at the highest doses. Fresh solutions were prepared daily for each injection. The doses of the selected doses were based on a concentration of atropine treatment, which has been shown to significantly inhibit form-deprivation myopia in a chick model [157].

4.2.4 Rationale for the use of chosen Drugs

Metaraminol (M4773, Sigma-Aldrich, St Louis, Mo, USA) was selected primarily for its ability to stimulate the contraction of the iris dilator muscle, leading to mydriasis because α 1-adrenoceptors mediate longer-term actions of catecholamines along with cell growth and proliferation [238, 239]. This pupil dilation can directly influence abnormal ocular growth. Secondly, metaraminol induces vasoconstriction in the blood vessels supplying the ciliary muscle, reducing blood flow, and decreasing aqueous humour production, which may reduce mechanical stress on the sclera and retina, potentially influencing axial elongation. Previous studies have investigated the use of muscarinic receptor antagonists for controlled experimental induced form-deprivation myopia [156, 157]. These include brimonidine, clonidine, and guanfacine, all of which can bind to α 2A-adrenoceptors, resulting in their effects on myopia control. However, studies have demonstrated that metaraminol can modulate chick Muller cell activity and retinal neuroprotective pathways. Lofexidine hydrochloride (Sigma-Aldrich, SML1019-50MG) was chosen because of its retina neuroprotective effects and decreased intracellular cAMP levels. These effects increase uveoscleral outflow, potentially influencing intraocular pressure and controlling abnormal ocular growth or diseases [240]. Cirazoline hydrochloride (Sigma-Aldrich, C223) was chosen because of its combined action of alpha-1 and alpha-2 adrenergic receptor agonists. It primarily induces vasoconstriction by

binding to alpha-1 receptors, reducing blood flow in targeted tissues and pupil dilation. It has also been shown to activate α2-adrenoceptor receptors, which results in decreased intracellular cAMP levels and aqueous humour dynamics [241].

4.2.5 Ocular Biometry Measurement

Ocular biometry was assessed using high-frequency A-scan ultrasonography employing a 20 MHz probe (custom-made) on days 0 (Baseline), 7, and 14, respectively. Each measurement involved capturing three traces of each eye, which were subsequently analyzed offline. The reported values were calculated as the sum of anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL).

4.2.6 Refractive Error Measurement

Refractive errors were measured using streak retinoscopy in both eyes of each chick before the insertion of lens-induced myopia (baseline). Measurements were taken again on days 7 and 14, respectively. Spherical equivalent values were calculated as the averages of results from the two principal meridians for data analysis.

4.3 Statistics

GraphPad Prism (8) software was used for the data analysis. All measurements were expressed as the means of interocular difference (IOD), calculated as the values for the (treated eye minus the untreated eye in each chick, and presented as the mean ± SD). For the recovery of data, the IOD was calculated as the values for the recovery eye minus the treated eye in each chick and presented as the mean ± SD. One-way ANOVA was used to compare the different drug dose groups, and Tukey's post hoc test was applied to determine the least significant difference between the groups when the p was less than 0.05. The strength of the association between the refractive error versus VCD and refractive error versus AL was assessed using Pearson's correlation coefficient (R2).

4.4 Results

4.4.1 Effect of Alpha-1 Adrenoceptor Agonists on Lens-induced Myopia

On day 0 (Baseline), no statistically significant differences in refractive error and ocular parameter outcomes between treated and untreated eyes were found in any group (Table 4.1). The results presented here indicate that lens-induced myopia is significantly inhibited by α1-adrenoceptor agonist (metaraminol) in chicks in a dose-dependent manner. Interocular difference (IOD, treated eye minus untreated eye) in refractive errors (Diopter, mean ± SD) at day 7 when compared to the control PBS were -8.57 ± 1.13D (PBS), -7.46 ± 1.25D (3 nmol) -3.43 ± 2.84D (30 nmol), and -2.61 ± 1.91D (300 nmol), (Table 4.2 and Figure 4.1.a). All tested doses of metaraminol significantly inhibited elongation of the ACD compared to the PBS control (IOD of ACD: 0.19 ± 0.05 mm; versus 3 nmol 0.11 ± 0.04 mm, p = 0.001; 30 nmol: -0.00 ± 0.06 mm, p = 0.001 and 300 nmol: -0.01 ± 0.04 mm, p=0.001 (Table 4.3 and Figure 4.1.b). There were no changes in lens thickness with different tested doses of metaraminol treatment (Table 4.3 and Figure 4.1.c). In addition, only 30 and 300 nmol concentrations significantly reduced IOD of VCD and AL elongation (IOD of VCD: 0.55 ± 0.08 mm, 30 nmol: 0.35 ± 0.18 mm, p = 0.001, and 300 nmol: 0.28 ± 0.14 mm, p = 0.001 (Table 4.3 and Figure 4.1d). For IOD of AL, the results were PBS: 0.62 ± 0.08 mm versus 30 nmol 0.34 ± 0.22 mm, p = 0.001, 300 nmol 0.28 ± 0.18 mm, p = 0.001 (Table 4.3 and Figure 4.1.e). On day 7 only 30 nmol and 300 nmol tested doses significantly inhibited the reduction in choroidal thickness compared to the PBS control (PBS: -53.58 ± 13.87 μm versus 30 nmol: -20 ± 8.27 μm, p = 0.0001, 300 nmol: -16.95 ± 10.05 μm, p < 0.0001) (Table 4.3 and Figure 4.1.f).

4.4.2 Effects of Metaraminol on Recovery from Lens-induced Myopia

The recovery effects of Metaraminol from lens-induced myopia revealed that all treated groups of drugs showed signs of recovery in refractive error and ocular biometry after lens removal and cessation of Metaraminol injections, and the eyes became emmetropic, which was similar to the control eyes. As shown in Figure 2, myopia recovery varied significantly among the groups at the end of the 7-day recovery period. The IOD, (recovery eye minus treated eye) in refractive errors (Diopter, mean ± SD) at day 14 compared to the control PBS was 7.95 ± 0.73D versus 2.82 ± 2.99D (30 nmol), $p < 0.0001$; and 2.15 ± 1.99D (300 nmol), $p < 0.0001$ respectively (Table 4.2 and Figure 4.2.a). All tested doses of metaraminol slightly, but significantly reduced elongation of ACD compared to the PBS control (IOD of ACD: -0.15 ± 0.05 mm; versus 3 nmol -0.06 ± 0.05 mm, $p = 0.001$; 30 nmol: 0.01 ± 0.06 mm, $p = 0.0001$ and 300 nmol: 0.02 ± 0.11 mm, $p=0.0001$ (Table 4.4 and Figure 4.2.b). There were no significant differences in lens thickness during the recovery period between the treated and control groups (Table 4.4 and Figure 4.2.c). However, only 30 and 300 nmol tested groups showed slow recovery in IOD of VCD elongation (PBS: -0.41 ± 0.09 mm versus 30 nmol: -0.27 ± 0.21 mm, $p = 0.001$, and 300 nmol: -0.19 ± 0.16 mm, $p = 0.0001$ (Table 4.4 and Figure 4.2.d). In addition, only 30 and 300 nmol tested doses led to partial recovered IOD of AL elongation compared with the control: -0.46 ± 0.10 mm versus 30 nmol -0.39 ± 0.10 mm, $p = 0.001$, 300 nmol -0.21 ± 0.21 mm, $p = 0.001$ (Table 4.4 and Figure 4.2.e). In addition, 30 and 300 nmol tested doses of metaraminol resulted in incomplete recovery of choroidal thickness compared to the control: 56.40 ± 19.81 μm versus 30 nmol: 9.25 ± 20.59 μm, $p = 0.0001$, 300 nmol: 8.85 ± 10.78 μm, $p < 0.0001$), (Table 4.4 and Figure 4.2.f).

Table 4.1. Summarized data for interocular difference (IOD) between refractive error (D) and ocular parameters (mm) on day 0 (Baseline)

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)
Day 0 (Baseline) Intravitreal injection of Metaraminol					
PBS	0.03 (0.34)	0.01 (0.02)	0.00 (0.03)	0.03 (0.04)	0.04 (0.05)
3 nmol	- 0.07 (0.38)	-0.00 (0.01)	0.01 (0.01)	0.03 (0.08)	0.04 (0.08)
30 nmol	0.02 (0.34)	0.00 (0.04)	- 0.01 (0.05)	0.06 (0.13)	0.06 (0.21)
300 nmol	0.07 (0.34)	-0.00 (0.02)	0.01 (0.03)	0.01 (0.05)	0.02 (0.05)
Day 0 (Baseline) Intravitreal injection of Lofexidine					
PBS	- 0.22 (0.17)	-0.00 (0.01)	0.02 (0.01)	0.03 (0.03)	0.04 (0.02)
3 nmol	- 0.12 (0.27)	0.01 (0.01)	0.00 (0.02)	0.06 (0.02)	0.07 (0.02)
30 nmol	-0.18 (0.23)	0.00 (0.01)	-0.00 (0.01)	0.06 (0.04)	0.07 (0.04)
300 nmol	- 0.12 (0.27)	0.00 (0.01)	0.00 (0.02)	0.04 (0.05)	0.05 (0.05)
Day 0 (Baseline) Intravitreal injection of Cirazolin					
PBS	0.02 (0.36)	0.00 (0.04)	-0.02 (0.08)	0.05 (0.14)	0.04 (0.22)
3 nmol	- 0.02 (0.34)	0.01 (0.02)	0.00 (0.03)	0.02 (0.04)	0.04 (0.05)
30 nmol	- 0.05 (0.41)	0.01 (0.02)	- 0.00 (0.06)	0.06 (0.10)	0.06 (0.16)
300 nmol	- 0.08 (0.38)	-0.01 (0.02)	0.01 (0.03)	0.01 (0.04)	0.01 (0.04)

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length

Table 4.2. (Interocular difference (IOD, treated eye minus untreated eye) in refractive errors (D) Diopter, mean ± SD) on days 7 and 14.

Category	Metaraminol		Lofexidine		Cirazolin	
	Treatment Day 7/D	Recovery Day 14/D	Treatment Day 7/D	Recovery Day14/D	Treatment Day 7/D	Recovery Day14/D
PBS	-8.57±1.08	7.95 ± 0.73	-9.17±1.44	8.45±1.15	-7.08±0.58	5.93±0.74
3 nmol	-7.46±1.20	6.61±1.13	-5.67±2.74	5.19±2.72	-6.42±1.31	5.28±1.46
30 nmol	-3.43±2.76	2.82±2.99	-4.07±1.98	3.44±2.15	-5.31±1.39	4.23±1.24
300 nmol	-2.61±1.84	2.15±1.99	-3.23±2.67	2.39±2.63	-4.09±1.18	2.99±1.06

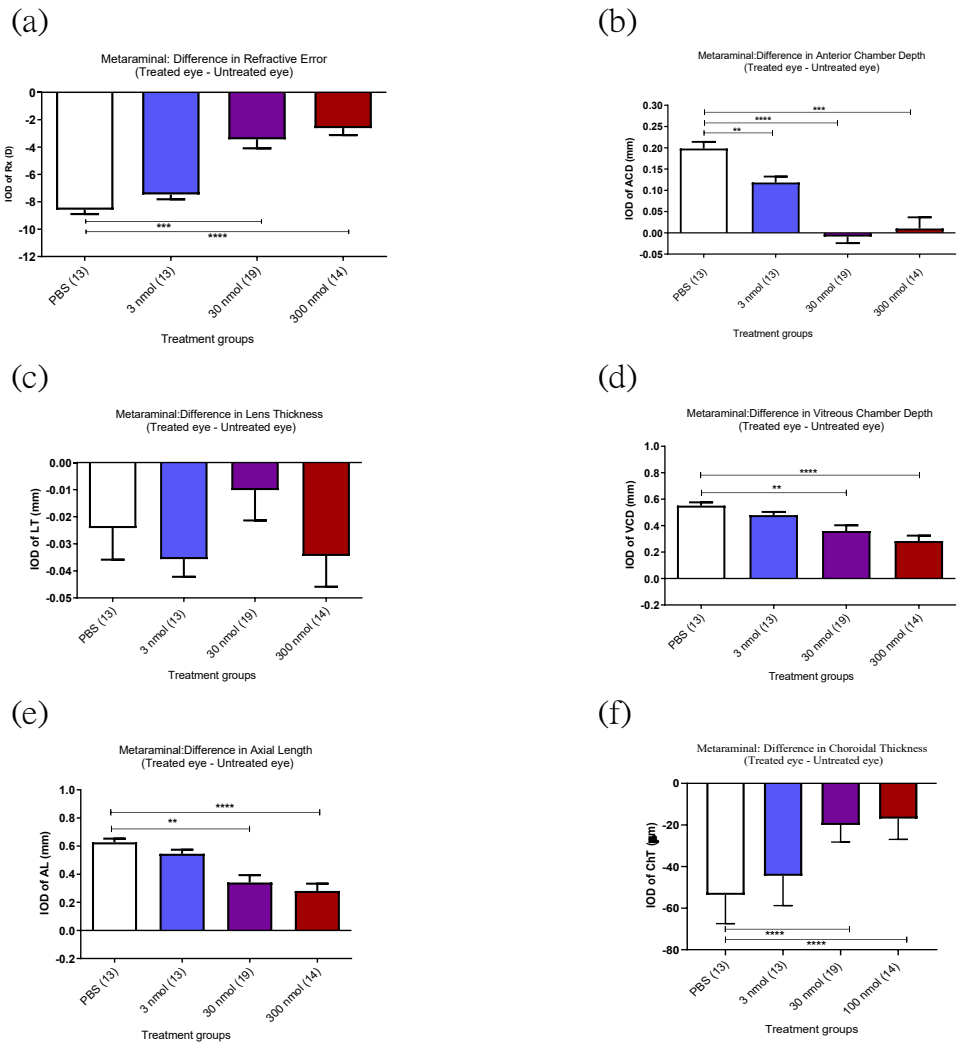
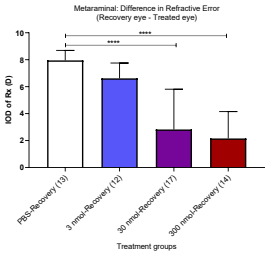


Figure 4.1 The interocular differences (treated eye minus untreated eye) in (a) myopic refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT after 7 days of treatment with metaraminol at doses of 3, 30 and 300 nmol on LIM in chicks. Only the 30 nmol and 300 nmol doses showed significant inhibition of induced myopic refractive error (a), reduced the reduction of VCD, (d) AL, and ChT (f). All tested doses of drugs effectively reduced the reduction of ACD (b). Conversely, no changes were observed in lens thickness (c). Statistics - One-Way ANOVA + Tukey's post-hoc. Error bars indicates standard deviation (SD), and sample size is denoted by (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness.

(a)



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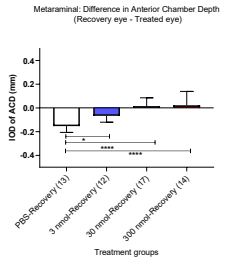
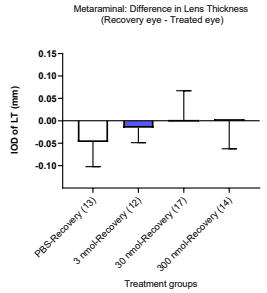
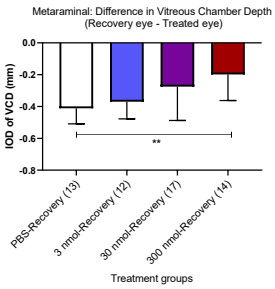


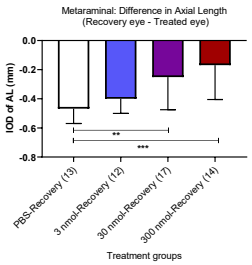
(c)



(d)



(e)



(f)

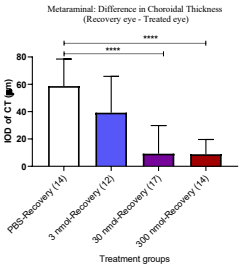


Figure 4.2 Recovery from lens-induced myopia. The interocular differences in (recovery eye minus treated eye) in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT after 7 days of following lens removal and cessation of injections of metaraminal. All tested doses of metaraminal significantly reduced the elongation of ACD (b). Only the 30 nmol and 300 nmol significantly reduced the induced myopia (a). However, only 300 nmol significantly reduced the elongation of VCD (d). Although 30 nmol and 300 nmol significantly reduced the AL elongation (e) and ChT (f). Statistics - One-Way ANOVA + Tukey's post-hoc. Error bars indicates standard deviation (SD) and Number in brackets denotes sample size (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness.

4.4.3 Effect of α2-Adrenergic Receptor Agonist Lofexidine on Lens-induced Myopia

All tested doses (3, 30, and 300 nmol) of intravitreal injection of lofexidine significantly reduced lens-induced myopia and inhibited axial elongation in a dose-dependent manner. Interocular difference (IOD, treated eye minus untreated eye) in refractive errors (Diopter, mean ±SD) at day 7 were -9.17 ± 1.44D (PBS) versus -5.67 ± 2.74D (3 nmol), $p = 0.0001$; -4.07 ± 1.98D (30 nmol), $p < 0.001$; and -3.23 ± 2.67D (300 nmol), $p < 0.001$ respectively (Table 4.2 and Figure 4.3.a). All tested doses of lofexidine results showed a shorter ACD compared to control PBS: 0.19 ± 0.07 mm, n = 15 versus 3 nmol: 0.06 ± 0.08 mm, $p = 0.0001$; 30 nmol: -0.02 ± 0.05 mm, $p < 0.001$; and 300 nmol: -0.07 ± 0.05 mm, $p < 0.001$. (Table 4.3 and Figure 4.3.b). However, only the 300 nmol tested dose group showed a significantly thinner lens (LT) than the control group (PBS: -0.03 ± 0.02 mm, versus 300 nmol: -0.00 ± 0.01mm, $p < 0.05$ (Table 4.3 and Figure 4.3.c). Additionally, all tested doses of lofexadine resulted in significantly less VCD elongation compared to the control group (PBS: 0.59 ± 0.12 mm, versus 3 nmol: 0.36 ± 0.16 mm, $p = 0.0001$, 30 nmol: 0.31 ± 009 mm, $p < 0.001$ and 300 nmol: 0.31 ± 0.09 mm, $p < 0.001$ (Table 4.3 and Figure 4.3.d). Furthermore, the IOD of AL was significantly reduced in the treated groups compared to the control group (PBS: 0.72 ± 0.15 mm, versus 3 nmol: 0.40 ± 0.18 mm, $p = 0.0001$; 30 nmol: 0.36 ± 0.13 mm, $p < 0.001$; and 300 nmol: 0.25 ± 0.20 mm, $p < 0.001$), (Table 4.3 and Figure 4.3.e). In contrast, the results showed that only higher tested doses of lofexidine 30 nmol and 300 nmol significantly reduced choroidal thickness compared to the control PBS on day 7 (PBS: -47.27 ± 14.23 μm versus 30 nmol: -21.74 ± 12.51 μm, $p = 0.001$; 300 nmol: 11.77 ± 6.32 μm, $p < 0.001$; Table 3 and Figure 3d). Table 4.3 and Figure 4.3.f).

4.4.4 Effects of Lofexidine on Recovery from Lens-induced Myopia

The recovery effects of lofexidine from lens-induced myopia showed that all treated groups demonstrated signs of recovery in refractive error and ocular biometry after lens removal and cessation of lofexidine injections, and the eyes became emmetropic, which is similar to the control eyes. As shown in figure 4, at the end of 7-day recovery period, myopia recovery differed significantly across groups, IOD, refractive error (Diopter, mean ± SD) at day 14 compared with the control PBS was 8.45 ± 1.15D versus 5.19 ± 2.72D (3 nmol), $p < 0.001$; 3.44 ± 2.15D (30 nmol), $p < 0.0001$; and 2.39 ± 2.63D (300 nmol), $p < 0.0001$ respectively (Table 4.2 and Figure 4.4.a). In addition, all tested doses of lofexidine slightly, but significantly, decreased the elongation of the ACD compared to the PBS control (IOD of ACD: -0.11 ± 0.05 mm; versus 3 nmol 0.01 ± 0.10 mm, $p = 0.01$; 30 nmol: 0.03 ± 0.06 mm, $p = 0.001$ and 300 nmol: 0.12 ± 0.10 mm, $p=0.0001$ (Table 4.4 and Figure 4.4.b). However, there were no significant differences in lens thickness between the treated and control groups during the recovery period (Table 4.4 and Figure 4.4.c). All tested doses of lofexidine also showed incomplete recovery in IOD of VCD elongation (PBS: -0.47 ± 0.13 mm versus 3 nmol: -0.26 ± 0.24 mm, $p = 0.01$; 30 nmol: -0.21 ± 0.13 mm, $p = 0.001$; and 300 nmol: -0.24 ± 0.18 mm, $p = 0.01$ (Table 4.4 and Figure 4.4.d). Furthermore, all tested doses of lofexidine showed slow recovery in IOD of AL elongation (PBS: -0.55 ± 0.10 mm versus 3 nmol: -0.28 ± 0.24 mm, $p = 0.001$; 30 nmol: -0.26 ± 0.15 mm, $p = 0.001$; and 300 nmol: -0.13 ± 0.17 mm, $p = 0.0001$ (Table 4.4 and Figure 4.4.e). In contrast, only partial recovery of choroidal thickness compared to the control PBS was observed for 30 and 300 nmol doses of lofexidine:41.76 ± 14.19 μm versus 30 nmol: 19.12 ± 13.05 μm, $p = 0.0001$, 300 nmol: 6.84 ± 7.65 μm, $p < 0.0001$) table 4.4 and figure 4.4.f.

4.3 Summarized data for the ocular outcome of metaraminal, lofexidine, and cirazolin, interocular difference (IOD) in ocular parameters (mm) at day 7

Category	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
Day 7 (Treatment outcomes of metaraminol)					
PBS	0.19 (0.05)	-0.02 (0.04)	0.55 (0.09)	0.62 (0.09)	-53.58 (9.70)
3 nmol	0.11 (0.05)	-0.03 (0.02)	0.47 (0.08)	0.54 (0.10)	-44.48 (14.36)
30 nmol	-0.00 (0.06)	-0.01 (0.04)	0.35 (0.18)	0.34 (0.22)	-20.00 (08.27)
300 nmol	0.01 (0.09)	-0.03 (0.04)	0.28 (0.15)	0.28 (0.19)	-16.95 (10.00)
Day 7 (Treatment outcomes of lofexidine)					
PBS	0.19 (0.07)	-0.03 (0.02)	0.59 (0.12)	0.72 (0.14)	-47.27 (14.23)
3 nmol	0.06 (0.08)	-0.02 (0.02)	0.36 (0.16)	0.40 (0.17)	-38.64 (11.80)
30 nmol	0.00 (0.04)	-0.01 (0.03)	0.31 (0.09)	0.36 (0.10)	-21.74 (12.51)
300 nmol	-0.07 (0.06)	0.01 (0.01)	0.31 (0.19)	0.25 (0.19)	-11.77 (6.32)
Day 7 (Treatment outcomes of cirazolin)					
PBS	0.22 (0.06)	-0.09 (0.05)	0.65 (0.09)	0.72 (0.08)	-48.79 (20.24)
3 nmol	0.16 (0.08)	-0.04 (0.05)	0.44 (0.12)	0.53 (0.44)	-37.78 (14.48)
30 nmol	0.10 (0.04)	-0.03 (0.07)	0.36 (0.18)	0.44 (0.17)	-23.56 (18.46)
300 nmol	0.07 (0.04)	-0.03 (0.03)	0.34 (0.11)	0.39 (0.11)	-22.90 (9.40)

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: choroidal thickness

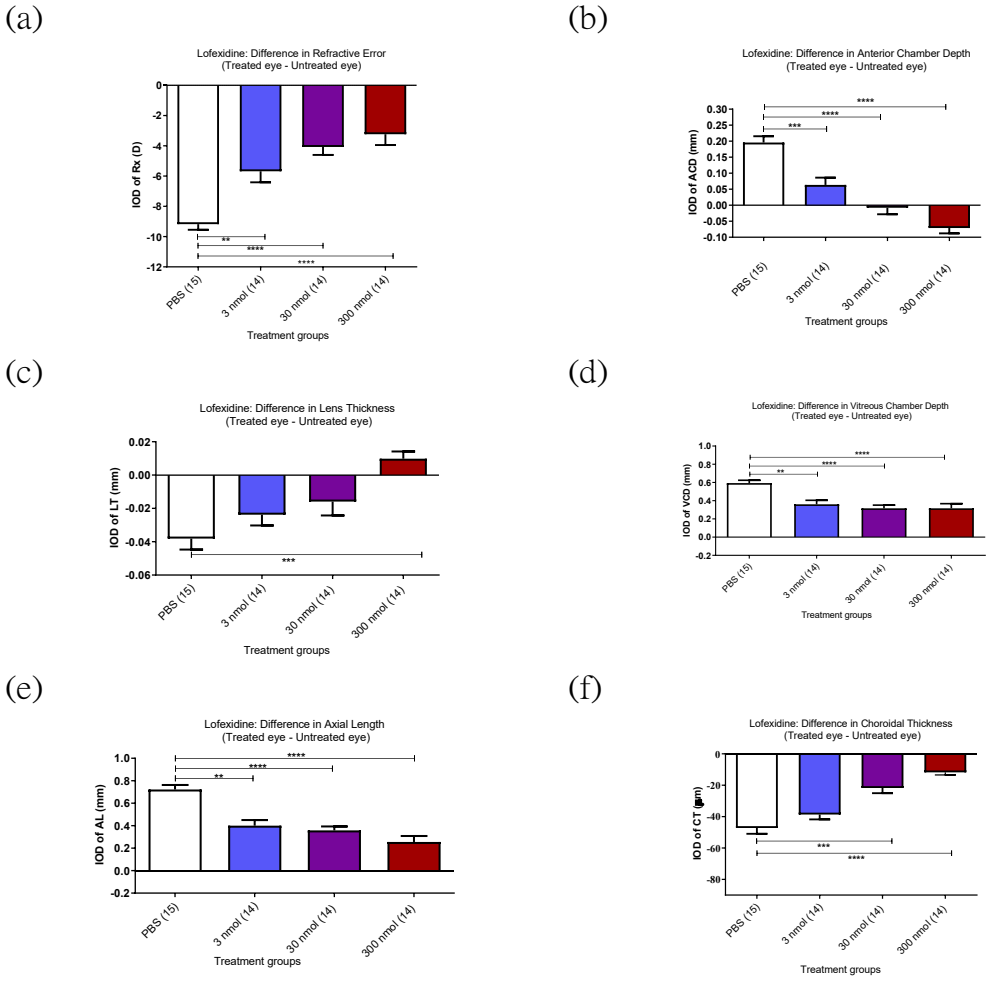


Figure 4.3 The interocular differences in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT after 7 days of lofexidine treatment (3, 30, and 300 nmol) on LIM in chicks. All tested dosages of lofexidine significantly inhibited the induced myopic refractive error (a) and impeded the elongation of ACD (b), VCD (d), and AL (e). Only 300 nmol doses of the drug significantly inhibited LT (c). In addition, only high doses (30 nmol and 300 nmol) significantly reduced the incidence of ChT (f). Statistics: *** $p < 0.001$, **$p < 0.01$ and * $p < 0.05$. Statistics - One-Way ANOVA + Tukey's post-hoc. Error bar expressed standard deviation (SD) and Number in brackets denoted sample size (n). **** $p < 0.0001$, *** $p < 0.001$, **$p < 0.01$ and * $p < 0.05$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness.

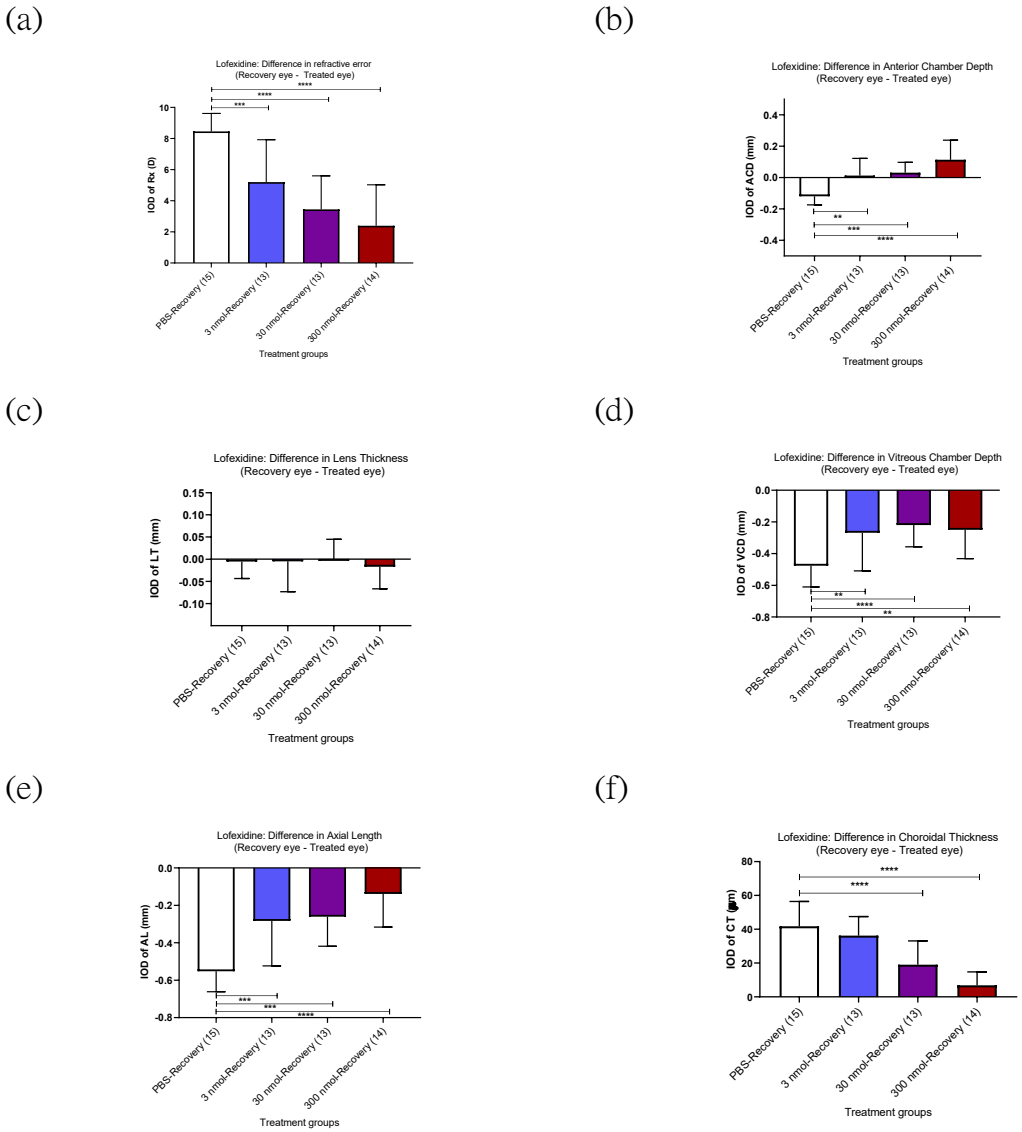


Figure 4.4 Recovery from lens-induced myopia. The interocular differences in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT 7 days after lens removal and cessation of injections of Lofexidine. All tested dosages of Lofexidine significantly reduced the effects of induced myopia (a), ACD (b), VCD (b), and AL (c). However, only 30 nmol and 300 nmol significantly reduced ChT thickness (f) reduction. Statistics - One-Way ANOVA + Tukey's post-hoc. Error bar shows standard deviation (SD). Numbers in brackets denote sample size (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness.

4.4.5 Effect of Nonspecific Adrenergic Receptor Agonist Cirazoline on Lens-induced Myopia

The effects of intravitreal injection of cirazoline also indicated that lens-induced myopia in chicks is significantly inhibited in a dose-dependent manner by this receptor agonist. Interocular difference in refractive errors (Diopter, mean ±SD) at day 7 were -7.08 ± 0.58D (PBS) versus -6.42 ± 1.31D (3 nmol), $p = 0.57$; -5.31 ± 1.39D (30 nmol), $p < 0.001$; and -4.09 ± 1.18D (300 nmol), $p < 0.001$, respectively (Table 4.2 and Figure 4.5.a). Higher doses of 30 and 300 nmol significantly shortened the IOD of ACD compared to PBS control: (- 0.22 ± 0.06 mm); versus 30 nmol (0.10 ± 0.04 mm), $p = 0.01$, and 300 nmol: (0.07 ± 0.04 mm), $p = 0.001$ (Table 4.3 and Figure 4.5.b). However, only the 30 nmol dose significantly reduced the difference in lens thickness (PBS: -0.09 ± 0.05 mm versus 30 nmol: -0.01 ± 0.02 mm, $p = 0.001$ (Table 4.3 and Figure 4.5.c). All tested doses of cirazoline significantly inhibited VCD and axial elongation (AL) compared to the PBS control. IOD of VCD (PBS: - 0.65 ± 0.09 mm versus 3 nmol: 0.44 ± 0.12 mm, $p = 0.0001$; 30 nmol 0.36 ± 0.18 mm, $p = 0.0001$; and 300 nmol: 0.34 ± 0.11 mm, $p = 0.001$; (Table 4.3 and Figure 4.5.d). Similarly, the IOD of AL was (PBS: - 0.72 ± 0.08 mm versus 3 nmol 0.55 ± 0.44 mm, $p = 0.000$; 30 nmol 0.44 ± 0.17 mm, $p = 0.0001$; and 300 nmol: 0.39 ± 0.11 mm, $p = 0.001$ (Table 4.3 and Figure 4.5.e). In contrast, only 30 and 300 nmol cirazoline significantly reduced choroidal thickness compared to the PBS control at day 7 (PBS: - 48.79 ± 20.24 μm versus 30 nmol: -23.56 ± 18.46 μm, $p = 0.0001$; and 300 nmol: -22.90 ± 9.401 μm, $p = 0.0001$, Table 4.3 and Figure 4.5.f).

4.4.6 Recovery Effects of Cirazoline on Lens-induced Myopia

Administration of cirazoline at all tested doses resulted in signs of recovery in refractive error and ocular biometry following lens removal and cessation of injections. As shown in Figure 6, after a 7-day recovery period, myopia symptoms

varied significantly among the groups, with interocular differences in refractive errors (Diopter, mean ± SD) at day 14 compared with the control PBS were 5.93 ± 0.74D versus 5.28 ± 1.46D (3 nmol), $p > 0.41$; 4.23 ± 1.24D (30 nmol), $p < 0.01$; and 2.99 ± 1.06D (300 nmol), $p < 0.001$ respectively (Table 4.2 and Figure 4.6.a). However, there were no significant differences in the IOD of ACD between the treated and control groups during the recovery period (Table 4.4 and Figure 4.6.b). Only 3 and 30 nmol tested doses significantly decreased the lens thickness compared to control (PBS: 0.04 ± 0.06 mm versus 3 nmol: -0.05 ± 0.06 mm, $p = 0.001$ and 30 nmol: -0.02 ± 0.03 mm, $p = 0.01$; (Table 4.4 and Figure 4.6.c). However, all concentrations of cirazoline resulted in slow recovery in IOD of VCD elongation (PBS: -0.52 ± 0.16 mm versus 3 nmol: -0.33 ± 0.11 mm, $p = 0.01$; 30 nmol: -0.28 ± 0.18 mm, $p = 0.001$; and 300 nmol: -0.21 ± 0.12 mm, $p = 0.0001$ (Table 4.4 and Figure 4.6.d). However, all concentrations showed incomplete recovery in IOD of AL elongation (PBS: -0.58 ± 0.14 mm versus 3 nmol: -0.40 ± 0.11 mm, $p = 0.01$; 30 nmol: -0.35 ± 0.20 mm, $p = 0.001$; and 300 nmol: -0.25 ± 0.11 mm, $p = 0.0001$ (Table 4.4 and Figure 4.6.e) and, only 30 and 300 nmol showed partial recovery of choroidal thickness compared to the control PBS: 44.13 ± 20.80 μm versus 30 nmol: 17.31 ± 19.99 μm, $p = 0.001$, 300 nmol: 15.29 ± 10.84 μm, $p < 0.001$) Table 4.4 and Figure 4.6.f.

4.4.7 Coefficient of determination Between Refractive Error versus Vitreous Chamber Depth and Axial Length

Strong correlation was observed between IOD in refractive errors and vitreous chamber depth for (a) metaraminol, (b) lofexidine, and (c) cirazoline ($R^2 = 0.75$, 0.79, and 0.67, respectively), as shown in Figure 4.7. Figure 4.8 shows the strong correlation between IOD in refractive errors and axial elongation at day 7 (a) metaraminol, (b) lofexidine, and (c) cirazoline ($R^2 = 0.80$, 0.84 and 0.70, respectively).

4.4.8 Dose Effects of Metaraminol, Lofexidine and Cirazolin

The study examined the inhibitory effects of three drugs, metaraminol, lofexidine, and cirazolin, on refractive error (Rx) outcomes across three dosage categories: Low (3 nmol), Medium (30 nmol), and High (300 nmol). Metaraminol inhibited refractive error by 12.96±1.68% at 3 nmol, 59.97±3.05% at 30 nmol, and 69.54±2.22% at 300 nmol. Lofexidine consistently showed the highest inhibition of myopia at all tested doses: 38.15±0.51% at 3 nmol, 55.61±0.51% at 30 nmol, and 64.77±0.84% at 300 nmol, respectively, indicating its strong and sustained efficacy. In contrast, cirazolin showed the lowest inhibition percentages among all tested doses, with inhibition rates of 9.32±0.22%, 25.00±0.27%, and 42.27±0.30%, respectively (Table 4.5). Overall, both metaraminol and lofexidine were significantly more effective in inhibiting refractive error, particularly at higher doses, whereas cirazoline showed weaker effects but remained consistent across doses. However, statistical analysis suggested no significant differences between the treatment groups ($p > 0.05$).

4.4.9 Body Weight

As shown in Figure 4.9, there were no significant differences in body weight between the treatment and the control groups at any time. All groups of chickens showed a normal increase in weight over time, consistent with normal chick development. There is no difference between body weight, refractive error, or axial length elongation.

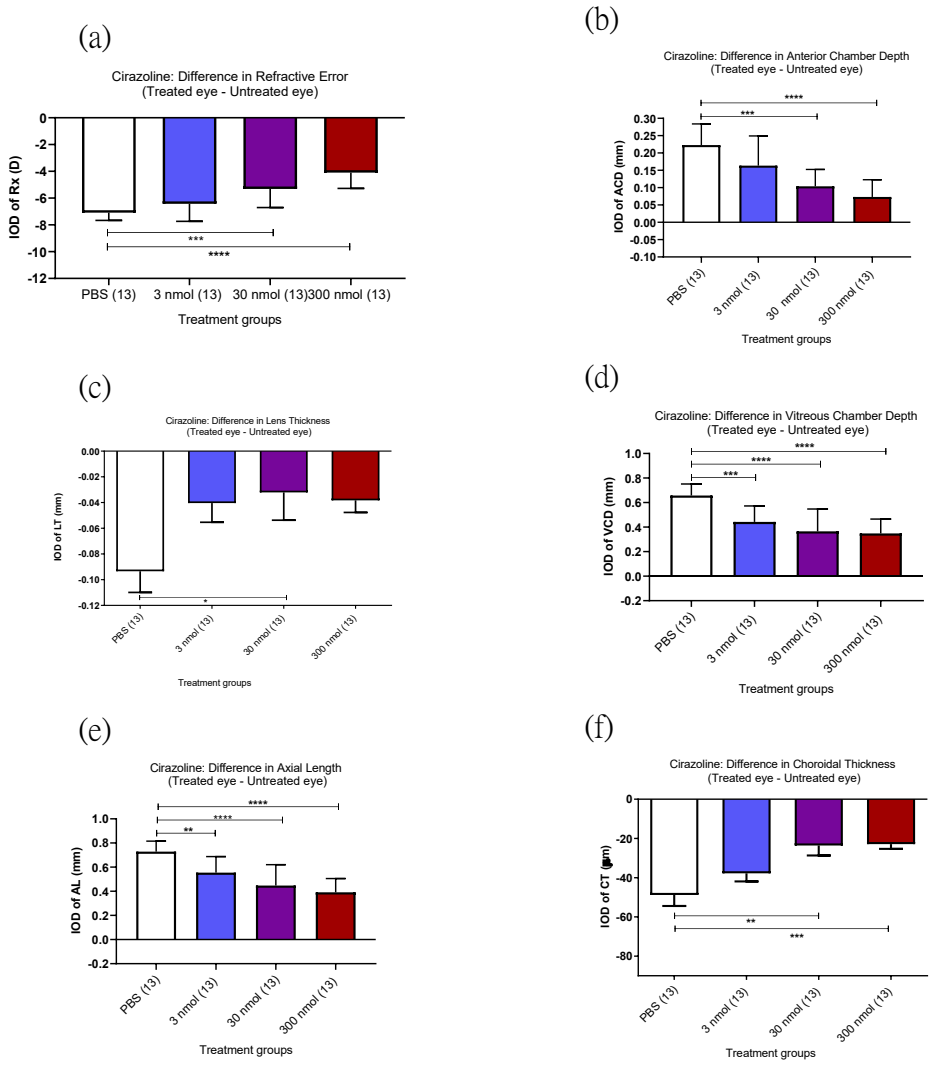


Figure 4.5 The interocular differences in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT after 7 days of cirazoline treatment (3, 30 and 300 nmol) on LIM in chicks. Only 30 and 300 nmol doses significantly inhibited the induced myopic refractive error (a) and led to reduced ACD elongation (b). Only 30 nmol doses of cirazoline significantly reduced lens thickness (c). All concentrations of cirazoline (3, 30, and 300 nmol) significantly reduced VCD elongation (d) and AL elongation (e). Only 300 nmol concentration significantly inhibited the reduction of ChT (f). Statistics: One-Way ANOVA + Tukey's post-hoc. Error bar denotes standard deviation (SD) and number in brackets is the sample size (n). *** $p < 0.001$, **$p < 0.01$ and * $p < 0.05$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness.

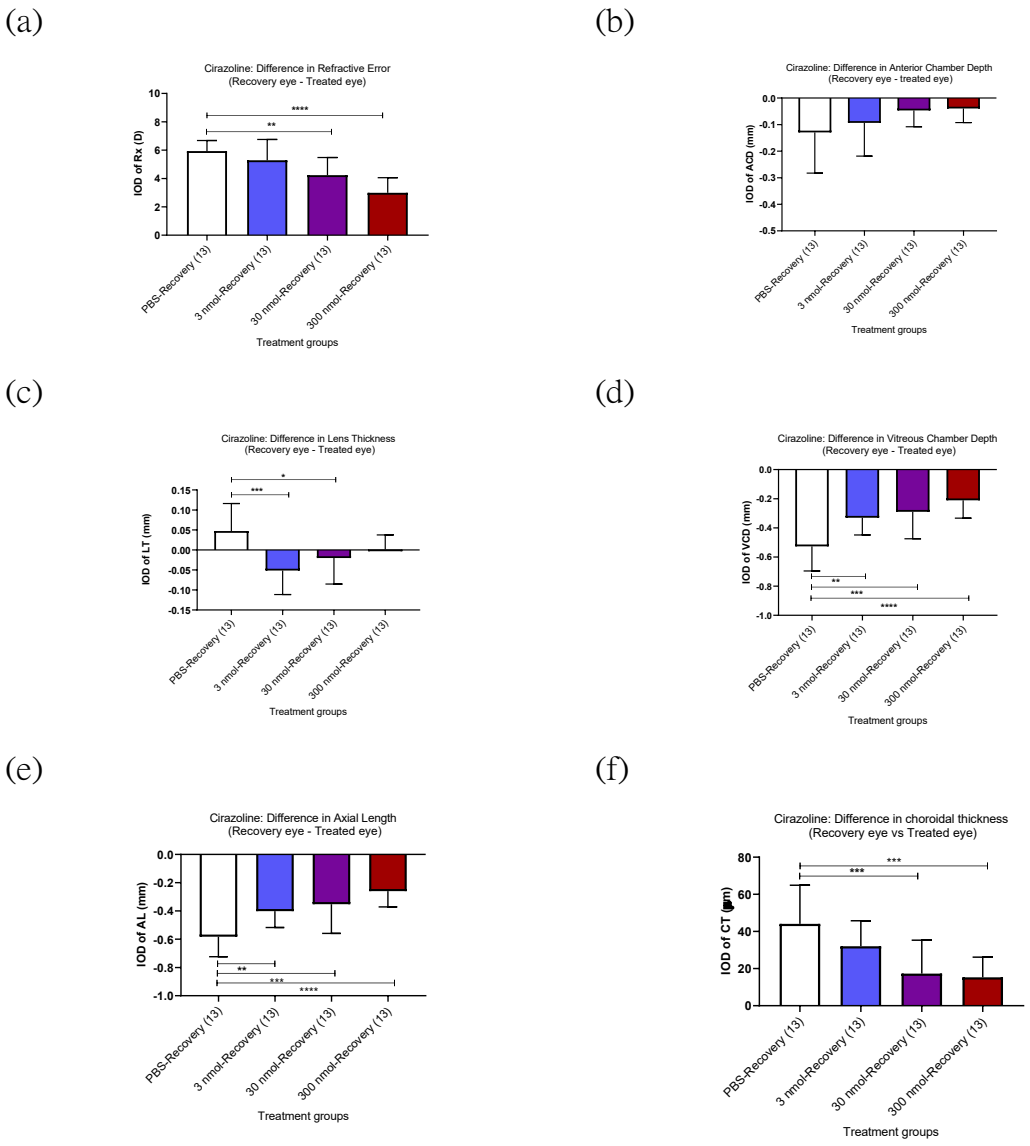


Figure 4.6 Recovery from lens-induced myopia. The interocular differences (IOD) in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT 7 days after lens removal and cessation of treatment with cirazoline. Only higher doses of 30 nmol and 300 nmol significantly reduced the induced myopia (a) and LT (c), but all concentrations. significantly reduced the length of the VCD (d) and AL (e). However, only 30 nmol and 300 nmol significantly halted the reduction of ChT thickness (f). Statistics - One-Way ANOVA + Tukey's post-hoc. Error bar denotes standard deviation (SD) and number in brackets is the sample size (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: Choroidal Thickness

Table 4.4 Summarized data for recovery on day 14 (7 days after discontinuation of drugs and lenses), interocular difference (IOD) in ocular parameters (mm).

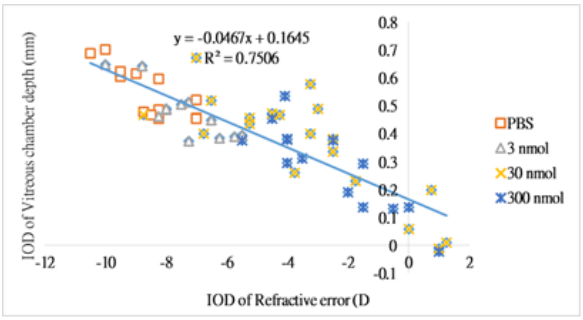
Category	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
Day 14 (Recovery of metaraminal)					
PBS	-0.15 (0.05)	-0.04 (0.05)	-0.40 (0.09)	-0.46 (0.10)	58.71 (19.81)
3 nmol	-0.06 (0.05)	-0.01 (0.03)	-0.36 (0.10)	-0.39 (0.10)	39.22 (26.63)
30 nmol	0.01 (0.06)	0.00 (0.06)	-0.27 (0.21)	-0.25 (0.22)	9.25 (20.59)
300 nmol	0.02 (0.11)	-0.00 (0.06)	-0.19 (0.16)	-0.16 (0.23)	8.85 (10.78)
Day 14 (Recovery of lofexidine)					
PBS	-0.11 (0.05)	-0.00 (0.03)	-0.47 (0.13)	-0.55 (0.10)	41.76 (14.19)
3 nmol	0.01 (0.10)	-0.00 (0.06)	-0.26 (0.24)	-0.28 (0.24)	33.11 (11.01)
30 nmol	0.03 (0.06)	0.00 (0.04)	-0.21 (0.13)	-0.26 (0.15)	19.12 (13.05)
300 nmol	0.12 (0.10)	-0.01 (0.04)	-0.24 (0.18)	-0.13 (0.17)	6.84 (7.65)
Day 14 (Recovery of cirazolin)					
PBS	0.12 (0.15)	0.04 (0.06)	-0.52 (0.16)	-0.58 (0.14)	44.13 (20.80)
3 nmol	0.09 (0.12)	-0.05 (0.06)	-0.33 (0.11)	-0.40 (0.11)	32.06 (13.71)
30 nmol	0.04 (0.06)	-0.02 (0.06)	-0.28 (0.18)	-0.35 (0.20)	17.31 (17.99)
300 nmol	0.03 (0.05)	-0.00 (0.03)	-0.21 (0.12)	-0.25 (0.11)	15.29 (10.85)

ACD: Anterior chamber depth; LT: lens thickness; VCD: vitreous chamber depth; AL: Axial length; ChT: choroidal thickness

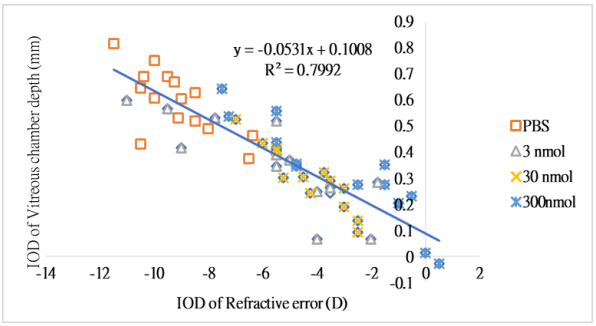
Table 4.5 Inhibition effects on interocular differences in refractive error between metaraminol, lofexidine and cirazoline

	Percentage of Rx inhibition (Mean ± SD%)		
Dose	Metaraminol (%)	Lofexidine (%)	Cirazoline (%)
3 nmol Low (L)	12.96 ± 1.68%	38.15 ± 0.51%	9.33 ± 0.22%
30 nmol Medium (M)	59.97 ± 3.05%	55.61 ± 0.51%	25.03 ± 0.27%
300 nmol High (H)	69.54 ± 2.22%	64.77 ± 0.84	42.26 ± 0.30%

(a) Correlation of interocular differences in refractive errors with vitreous chamber depth in chicks treated with different tested doses of metaraminol



(b) Correlation of interocular differences in refractive errors with vitreous chamber depth in chicks treated with different tested doses of lofexidine



(c) Correlation of interocular differences in refractive errors with vitreous chamber depth in chicks treated with different tested doses of cirazoline

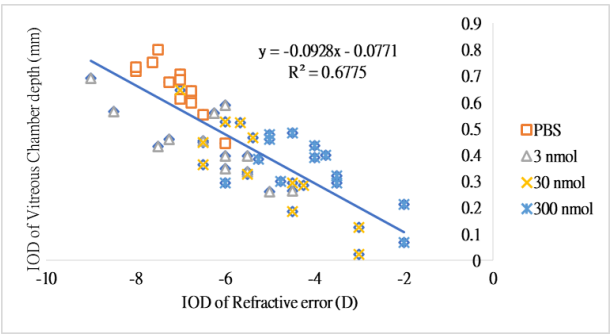
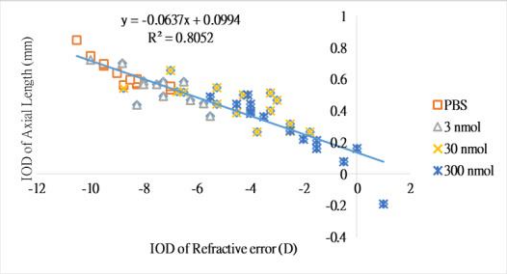
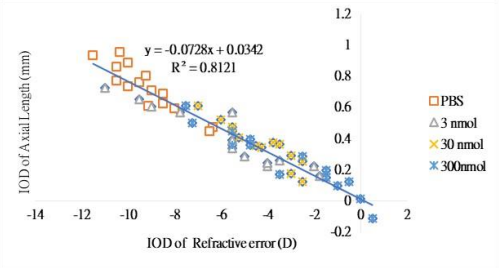


Figure 4.7 Coefficient of determination between interocular differences in refractive errors and vitreous chamber depth at day 7. (a) metaraminol-treated animals, (b) lofexidine-treated animals, and (c) cirazoline-treated animals (R^2 = 0.75, 0.79, and 0.67, respectively).

(a) Correlation of interocular differences in refractive errors with axial length in chicks treated with different tested doses of injections of metaraminol



(b) Correlation of interocular differences in refractive errors with axial length in chicks treated with different tested doses of injections of lofexidine



(c) Correlation of (interocular differences in refractive errors with axial length in chicks treated with different tested doses of injections of cirazoline

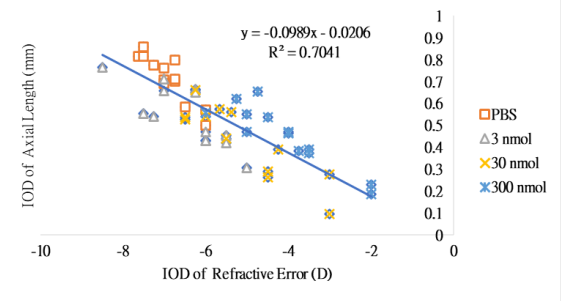
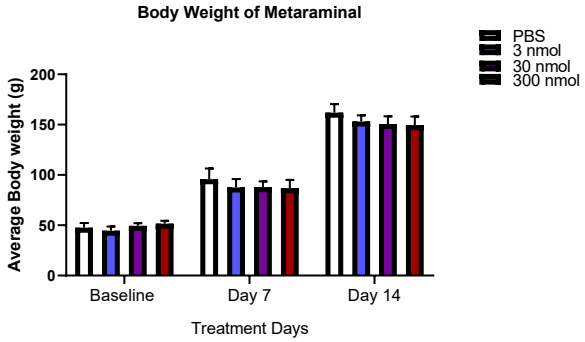
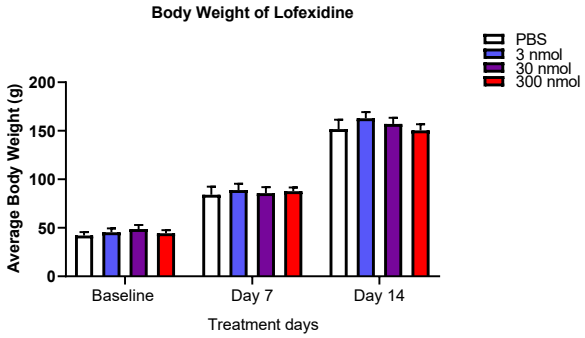


Figure 4.8 Coefficient of determination between interocular differences in refractive errors and axial length elongation at day 7. (a) metaraminol-treated animals, (b) lofexidine-treated animals, and (c) cirazoline-treated animals (R^2 = 0.80, 0.82, and 0.70, respectively).

(a) Average body weight of chickens in different tested doses of metaraminal



(b) Average body weight of chickens in different tested doses of lofexidine



(c) Average body weight of chickens in different tested doses of cirazoline

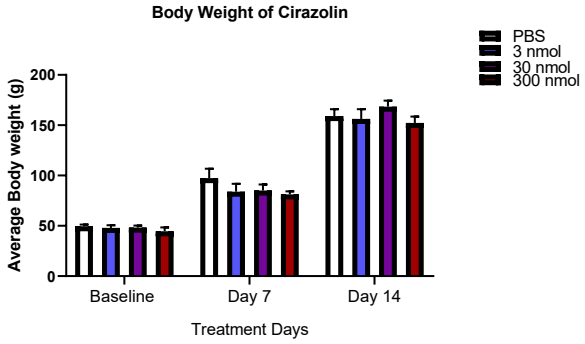


Figure 4.9 Average body weight (mean±SD) of chickens at baseline, 7, and 14 days old.

4.5 Discussion

This study presents the first evidence that lens-induced myopia (LIM) can be significantly inhibited by high concentrations of adrenoceptor agonists, including α 1-adrenoceptor agonists (metaraminol) and α 2-adrenoceptor agonists (lofexidine), and a non-specific adrenergic receptor agonist (cirazoline). These drugs effectively reduced myopia-associated changes in refractive error and inhibited excessive ocular elongation in a dose-dependent manner. The most prominent inhibitory effects were observed at doses of 30 and 300 nmol, significantly reducing vitreous chamber depth (VCD), axial length (AL), and choroidal thickness (ChT). These findings suggest that adrenergic receptor activation plays a crucial role in controlling ocular growth. Metaraminol significantly inhibited LIM in a dose-dependent manner. All tested doses of metaraminol affected ACD, but 30 and 300 nmol markedly decreased ACD, due to increased vascular tone and transient elevations in intraocular pressure (IOP). A previous study reported that intravitreal injections decreased ACD by shifting the ciliary body forward [242]. Another study has shown that higher IOP correlates with reduced ACD and narrowing of the anterior chamber angle, which may contribute to the progression of myopia [243]. A recent study found that long-term intravitreal injections do not change ACD [244]. In addition, α 1-adrenoceptor activation initiates smooth muscle contraction through downstream signalling pathways [245]. This leads to vasoconstriction, which increases systemic blood pressure and organ perfusion. Another downstream effect is mydriasis produced by contraction of the iris dilator muscle [245]. A previous study confirmed α 1-adrenoceptor expression in rabbit eyes at both RNA and protein levels in the iris, ciliary body, and choroid [246]. However, this expression in the retina was minimal due to low mRNA. It was also demonstrated that the in-situ hybridization signal for α 1-adrenoceptor mRNA in the epithelium of the ciliary body, which regulates IOP via an α 1-adrenoceptor, could change the rate of aqueous humour production and outflow. Based on the findings, metaraminol may be a promising candidate for stabilizing myopia progression due to its mydriatic effect, resulting from contraction of the iris dilator muscle. Additionally, dopamine has been to activate adrenergic receptors in the anterior chamber via D1 and D2 receptors, which are involved in ciliary flow, aqueous humour production, and

maintenance of IOP [247]. These effects also contribute to the inhibition of abnormal ocular growth. Dopaminergic modulation of accommodation may involve nicotinic acetylcholine receptors in the striated ciliary muscles, particularly in avian models. The current study provides further promising findings that an α2-adrenoceptor agonist inhibits lens-induced myopia in a dose-dependent manner. A recent study has shown that form-deprivation myopia can also be suppressed by administration of α2-adrenoceptor agonists such as brimonidine, clonidine, and guanfacine, which reduce both myopic refractive error and axial elongation [248]. These effects suggest a pharmacological pathway that may control ocular growth. α2-adrenoceptors regulate retinal dopamine activity, which plays a vital role in the regulation of ocular size [249]. Dopamine and nitric oxide (NO) have been reported to work with different dopaminergic mechanisms to control experimental myopia [250]. Interestingly, norepinephrine and dopamine share similar affinity for α2-adrenoceptor receptors, and dopamine may block myopia growth signals depending on receptor subtype activation and drug concentration [157]. α2-adrenoceptor receptors also influence the function of retinal tyrosine hydroxylase (TH), especially under photic stimulation [251]. Intravitreal injection of yohimbine increased retinal TH activity in light-exposed retinas, while clonidine decreased its activity, and both showed minimal effect in darkness [157]. These findings suggest yohimbine and clonidine may work together to antagonize the effects of one another with respect to dopamine-related mechanisms [252]. The present study proposed that lofexidine may similarly activate retinal dopamine in LIM, particularly under light conditions that maintain circadian rhythm. This contrasts with the dopamine activities seen in FDM by clonidine, brimonidine, and guanfacine [156]. The role of dopamine in myopia inhibition appears to vary across species, potentially due to differences in receptor subtypes such as D1 in the guinea pig and D2 in the chicken [253, 254].

Brimonidine has been investigated for its neuroprotective effects in guinea pigs, promoting basic fibroblast growth factor (bFGF) expression and influencing scleral remodeling [176]. It was also documented that decreased intraocular pressure inhibits

the formation of aqueous humour and aqueous outflow. Another guinea pig study reported that latanoprost, a prostaglandin analogue used to treat glaucoma, significantly reduced the development of induced myopia [186]. However, it is unclear how latanoprost affects scleral growth and controls myopia progression. Scleral ingenuity is crucial in myopia progression, as extracellular matrix (ECM) remodelling contributes to axial elongation [255, 256]. α2-adrenoceptors agonists activate neuroprotective mechanisms, including transforming growth factor-beta (TGFB) and basic fibroblast growth factor (BFGF), in various retinal cells [257]. These pathways may present a stop-and-go signal mechanism for ocular growth. However, LIM guinea pigs found that an increase in TGF-ß2 activity, whereas in FDM tree shrew, appeared to form inhibition of the bFGF rescue effect [258, 259].

The current study provided another promising finding that non-specific adrenergic receptor agonists (cirazoline) can inhibit LIM in a dose-dependent manner. However, higher doses of cirazoline (30 and 300 nmol) significantly inhibited LIM as well as ACD, VCD, and AL. Our findings indicated that cirazoline is less effective than metaraminol and lofexidine in controlling myopia. This has less effect due to cirazoline actions on multiple adrenergic receptor subtypes, specifically both α1 and α2 receptors, which may lead to mixed signaling outcomes [260]. Furthermore, its partial agonist activity at α1B and α1D receptors, combined with antagonistic effects on α2 receptors, may result in conflicting or opposite responses that limit its therapeutic effect [241]. Interestingly, similar pharmacological comparisons were observed with apomorphine and atropine. Atropine was slightly more effective than apomorphine, while the combined treatment did not offer greater inhibition than atropine alone [261]. These findings suggest that combining agents is not consistently effective and may depend on receptor specificity and downstream signaling. Previous study clearly demonstrates that combined therapy using α1-adrenoceptor and α2-

adrenoceptor agonists is effective in treating neuroinflammatory diseases, including Alzheimer's, Parkinson's, and multiple sclerosis [262]. These effects were dose-dependent and associated with reduced drug toxicity, lower resistance, and fewer side effects. Based on the results, lofexidine demonstrated the highest inhibitory effect on myopia, followed by metraminal and cirazoline due to selective α2-adrenoceptor activation, which changes choroidal perfusion and retinal neuroprotection.

Despite various animal models, research investigated the underlying mechanisms of myopia in LIM and FDM, including chick, mouse, tree shrew, primate, guinea pig, cat, and monkey [263]. These results underline many conflicts between LIM and FDM and whether they work through the same biological mechanism. In recent years, many studies have concluded that FDM is due to local alterations in the retinal mechanism, as the central nervous system does not affect the development of negative ocular growth [96]. Although circadian rhythm fluctuates between LIM and FDM in chickens, these variations confirm myopia recovery mechanisms [264]. In contrast, results of refractive error, axial length, abnormal growth of the sclera, and pathological changes indicate that different mechanisms are responsible for LIM and FDM. Pharmacological management of myopia remains controversial. While atropine is widely accepted for controlling myopia, it is effective in FDM but not in LIM in chicks [202]. A similar picture is seen in tree shrews M4 muscarinic antagonist and MT3 are effective at inhibiting FDM but have little or partial effect on LIM [265]. The reasons for these differences are unknown. Interestingly, muscarinic Toxin 7 (MT7), a highly selective M1 antagonist, was shown to be effective at inhibiting both FDM and LIM in tree shrews due to M4 and M1 receptor signaling pathways [266]. Different effects were also noted for the effectiveness of 6-Hydroxy dopamine, which does not affect LIM, but could inhibit FDM [267]. The current study concluded that atropine and α1-adrenoceptor agonist (metaraminol), α2-adrenoceptor agonist

(lofexidine), and non-specific adrenergic (cirazoline) work through different mechanisms but have the same effect on controlling the progression of myopia and ocular elongation. Further work is needed in a range of animal species.

In addition, the current study indicated that higher doses of metaraminol, lofexidine, and cirazoline (30 and 300 nmol) significantly inhibited the reduction in choroidal thickness. A similar finding was observed in a study of guinea pigs, which mentioned that α2-adrenoceptor agonists (brimonidine) effectively suppressed choroidal thinning and decline and the decrease in ocular blood perfusion [268]. Previous animal studies found that the choroid becomes thinner during myopia development due to choroidal blood perfusion and oxygenation, including chicks, guinea pigs, and marmosets [269-271]. Whereas a clinical study indicated that a single dose of brimonidine 0.15% did not significantly affect choroidal thickness or blood flow in humans [272].

The present study provides strong evidence that activation of α-adrenergic receptors helps recovery from LIM in chicks. Following lens removal and cessation of intravitreal injections, all tested groups of chicken eyes returned to emmetropia, similar to that of control eyes. Metaraminol and lofexidine led to complete recovery, while treatment with cirazoline resulted in slow recovery. This evidence suggests that opposite actions of α_1- and α_2-adrenergic pathways may weaken vascular restoration. Similar to previous findings, adrenergic signaling modulates choroidal blood flow and scleral matrix metabolism, both of which affect emmetropization [273]. However, lofexidine is more effective due to its direct action on α_2-receptors, which support choroidal perfusion and nitric oxide-mediated relaxation. This mechanism provides faster recovery compared to the vasoconstrictive effects

associated with α_1-receptor activation. Overall, these findings highlight that selective modulation of adrenergic pathways, particularly through α_2 pathways, plays a significant role in both the inhibition and recovery phases of myopia development. Previous animal studies have demonstrated that LIM can be partially or fully returned to a normal state of the eyes when minus lenses are removed [274]. It was also documented that young tree shrews showed a rapid and complete reversal of LIM following lens removal. However, a similar finding was observed in chicks, in which one week of induced myopia was corrected within 2 days of lens removal [275]. Although the guinea pigs studied recovered from 4 weeks of LIM within a week [272]. These findings not only deepen our understanding of LIM but also have significant implications for the development of myopia treatment. One limitation of the study is that we did not investigate intraocular pressure (IOP) measurement. The IOP plays a crucial role in the biomechanics of the eyes, which may influence the development of myopia.

4.6 Conclusions

In summary, this study results indicate that α2-adrenoceptor agonists inhibit LIM in a dose-dependent manner. Only high concentrations of metaraminol (30 and 300 nmol) significantly inhibited myopic refractive error and markedly reduced VCD elongation, axial elongation, and choroidal thickness. In contrast, all tested doses of lofexidine (3, 30 and 300 nmol) significantly reduced lens-induced myopia, with shorter ACD, thinner lens thickness, lower VCD, and axial elongation. In contrast, only high concentrations of cirazoline in intravitreal injections (30 and 300 nmol) significantly inhibited induced myopia, resulting in shorter VCD, and axial elongation, and only 300 nmol significantly reduced choroidal thickness. During the recovery phase, higher doses of metaraminol, lofexidine, and cirazoline showed partial recovery effects from induced myopia, while medium and lower doses showed complete recovery effects. This study also confirmed that α1 adrenergic and nonspecific agonists have similar inhibitory effects against myopia in chickens and indicated their potential as pharmacological treatment to prevent or treat myopia.

Chapter 5

Topical latanoprost inhibits the development of form-deprivation myopia and lens-induced myopia in the chick.

Prostaglandin analogues have recently been introduced as a first-line therapy with minimal side effects for the medical management of ocular hypertension. This study aimed to investigate the effects and the underlying mechanism of latanoprost on induced myopia in chickens. Myopia was induced in the right eyes of chicks using diffuser lenses (FDM) or -10D single vision lenses (LIM) for 12 days, while the left eyes acted as untreated controls. One drop of topical latanoprost 0.005% or saline was administered daily in the treated and control groups, respectively. Refractive errors, tonometry, and high-frequency A-scan ultrasonography were performed on days 0, 4, 8, and 12. Eyeballs of the chicks were then enucleated and processed for hematoxylin & eosin (H&E) staining and western blot. Data are presented as mean interocular differences (IOD) ± standard deviation (SD). After 12 days of treatment, latanoprost inhibited both FDM and LIM. FDM control: -16.02±2.80D versus FDM latanoprost: -10.86±2.54D, $p<0.0001$. LIM control: -10.90±1.37D versus LIM

latanoprost: -6.62±1.39D, $p<0.0001$) respectively. In the FDM and LIM latanoprost groups there was significantly reduced vitreous chamber depth (VCD) and axial elongation (AL) compared to control FDM and LIM saline groups. Intraocular pressure (IOP) was increased in FDM and LIM groups but decreased in those treated with latanoprost. Staining showed that latanoprost inhibited the thinning of sclera in the FDM and LIM groups. However, latanoprost treatment groups showed a relatively upregulated retinal early growth response-1 (Egr1) in mRNA and protein expression levels at the 12-day time point. In conclusion, topical latanoprost inhibits FDM and LIM in the chicken model, and its effect is associated with a reduced IOP and upregulated Egr1 protein expression.

5.1 Introduction

Myopia is a common ocular condition that leads to visual impairment. The mechanism of myopia development is unclear, but several possibilities have been hypothesized, including extracellular matrix remodeling of the scleral shell, retinal dopamine secretion, and RPE cell dysfunction [276-278]. Studies have indicated that prolonged near work plays a crucial role in myopia progression. Myopic vision is due to abnormal axial length caused by the stretching of the ocular tissues and morphological changes in the outer wall of the eye due to compromised scleral integrity [278]. Research focusing on the sclera has revealed that typical emmetropic eyes have varying degrees of stiffness, whilst myopic eyes exhibit less scleral stiffness [279]. Intraocular pressure (IOP) also plays a critical role in the physiology and pathophysiology of the eye, ensuring it maintains its stable form and shape to preserve vision and prevent sight-threatening disorders. Due to the stretching effect of increased IOP during myopia development, the scleral wall becomes thinner and weaker [186]. Several studies have examined the association between myopia and IOP, finding a strong relationship in myopic subjects compared to emmetropes [280]. Even though relatively normal daily IOP rhythms were recorded, IOP increased at night in young adults with moderate to severe myopia compared to those with emmetropes and low myopia. These findings were similar to prior investigations of from-deprived (FDM) myopic chicks [176]. The chicken experimental study investigated the causes of elevated IOP and axial elongation following intravitreal injection, which currently remain unknown [281]. Another study revealed a link between IOP and axial length before and after trabeculectomy surgery. [282]. It indicated that axial elongation and IOP both decreased significantly due to scleral relaxation. A recent meta-analysis of a genome-wide investigation found a substantial link between IOP and refractive error [45].

Several studies have reported that some glaucoma medications not only reduce IOP but also slow the progression of myopia [163, 283]. Timolol (beta-blockers) did not affect myopia status in the chick or guinea pig models, although it did lower IOP

[175]. Another clinical study in young school children using beta-blocker eye drops found no significant difference in myopia progression compared to single-vision lenses [284]. In contrast, brimonidine (2-adrenergic agonist), a novel ocular hypertensive medication, which lowers aqueous humour production while increasing its outflow via the uveoscleral pathway [285], significantly inhibited myopia progression and reduced axial elongation in lens-induced myopia (LIM) and form-deprivation myopia (FDM) in guinea pigs receiving eye drops and intravitreal injections [183]. In contrast, studies on latanoprost injections for FDM using a chick model indicate that the therapy may not have any effect on myopia progression due to the thickness of the sclera, which may limit drug efficacy [184]. In contrast, latanoprost was reported to inhibit myopia progression in other animal models, including guinea pigs and monkeys [185, 286]. The current study investigated whether topical administration of latanoprost would alter the development of FDM and LIM in chick models by inhibiting ocular elongation and modulating the expression of Early Growth Response Protein 1 (EGR1). The preliminary findings were presented at the Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting 2022, as Topical latanoprost inhibits the development of form-deprivation myopia and lens-induced myopia in the chick model" [287].

5.2 Methods

5.2.1 Animals

Chapter 2 described the ethical guidelines and methods for LIM, FDM, refractive error determination, choroidal thickness measurements, and body weight measurements. A total of 40 chicks were randomly divided into four groups (n=10) for LIM and DFM induction, other 48 chicks were used for western blot (Days 4, 8, and 12), and another 24 chicks for qPCR and hematoxylin-eosin staining on day 0 and day 12.

5.2.2 Latanoprost eye drops

Latanoprost (0.005% ophthalmic solution, Bausch + Lomb Incorporated, Tampa, FL, USA) was administered to both treatment groups. In the study, myopia was induced in the right eye using LIM and FDM lenses. For the treatment groups, topical latanoprost (0.005%) was applied to the right eye of the chicks, while the left eye served as an untreated control. A single drop of topical latanoprost was applied daily in the evening, starting at baseline and continuing for 12 days. In the control groups, FDM and LIM chicks were administered a topical saline solution every evening in place of latanoprost.

5.2.3 Ocular biometry measurement

Ocular biometry measurements were conducted using a high-frequency A-scan ultrasound system with a 30 MHz transducer (Panametrics Inc., Waltham, MA, USA). Measurements were taken at baseline, day 4, day 8, and day 12 to assess anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL).

5.2.4 Intraocular pressure (IOP) measurement

Intraocular pressure (IOP) was measured using an iCare tonometer (Model: TV02, iCare Finland Oy, Vantaa, Finland). The baseline IOP was measured before myopia induction. Subsequent measurements were taken on experimental days 4, 8, and 12, between 9:00 am and 10:00 am. To ensure consistency and reliability, IOP was measured three times for each eye. The average of these three measurements was calculated and utilized for statistical analysis.

5.2.5 Hematoxylin and eosin staining

Following 12 days of treatment, the chickens were sacrificed by carbon dioxide (CO2) overdose at the intended time point. Chick eyeballs were immediately enucleated and cut with a razor blade in the equatorial plane. A 5mm diameter biopsy punch was used to cut the center part of the sclera. The sclera tissue was fixed in a 4% paraformaldehyde solution (PFA). The tissue was then incubated overnight in 30% sucrose before embedding in an optimal cutting temperature compound (OCT, USA). 10 μm thick frozen sections were cut from the posterior pole regions and then mounted and stained with H & E. The scleral thickness measurement was performed using an upright microscope (BA210, LED Binocular W/O 100X China) with a digital Canon (Tokyo, Japan) camera, and the latest version of 2022 Image J software (National Institute of Health, USA) was used for image analysis.

5.2.6 Real-time reverse transcription PCR (RT-qPCR)

At the end of the experiment, the eyes were isolated, and the retinas were carefully separated from the choroid. The total retinal RNA was extracted using TRIzol™ reagent (Catalogue number 15596018; Thermo Fisher, Waltham, MA., USA). The retinal tissue was homogenized in 1 mL of TRIzol using a thermomixer. Following homogenization, 0.2 mL of chloroform was added for phase separation, and the

mixture was centrifuged at 12,000 × g for 15 minutes at 4 ˚C. The clear aqueous phase was carefully transferred to a new tube. RNA was precipitated by adding 0.5 mL of isopropanol, incubating the mixture at room temperature for 10 minutes, and centrifuging again at 12,000 × g for 10 minutes. The RNA pellet was then washed with 75% ethanol (prepared in RNase-free water), centrifuged at 7,500 × g for 5 minutes, air-dried for 10 – 15 minutes, and resuspended in 15 – 20 μL of RNase-free water. The sample was incubated at 55 – 60 ˚C for 10 – 15 minutes to ensure complete dissolution. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific), measuring absorbance at 260 and 280 nm using 2 μL of each sample. Only RNA samples with A260/A280 ratios between 1.8 and 2.0 were used for reverse transcription. cDNA was synthesized using the TaqMan RT Master Mix (Sigma-Aldrich) and gene-specific primers. Real-time PCR was performed using the synthesized cDNA and gene-specific primers with the TaqMan Master Mix on a real-time PCR system. The specific primers designed for the qPCR were: EGR-1 forward primer, TTGAGACCAGAGCACAGCAG, and EGR-1 reverse primer, CATCGGACCGTGAAAATCGC; β-actin forward primer, GCCCTCTTCCAGCCATCTTT, and reverse primer, CTGTCAGCAATGCCAGGGTA. Gene expression levels were normalized by β-actin, and relative quantification was calculated using the 2^ – Δ ΔCq method.

5.2.7 Western blot

Retinas from all experimental chicks were homogenized in 250 μL of ice-cold lysis buffer for protein extraction. Samples were centrifuged at 5800 RPM for 4 x 15 seconds at 4˚C, and the precipitate was collected and kept on ice. Protein concentration was determined using a commercial kit (Bio-Rad, Hercules, CA, USA). 50 micrograms of retinal proteins were separated by 10% SDS polyacrylamide gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes. Membranes were blocked with 1X TBS containing 0.1% Tween-20 and 5% nonfat milk powder for 1 hour at room temperature, then washed three times with Tris-buffered saline with Tween-20 (TBST) for 5 minutes each.

Membranes were incubated overnight at 4 ˚C with primary antibodies against EGR-1 (Polyclonal, 1:1,000; LS-C352150; LS-Bio, USA). After washing, the membranes were incubated for 1 hour at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (polyclonal, 1:5,000; PI-1000; Vector Laboratories). For internal controls, a membrane was incubated with Anti-GAPDH Mouse mAb (monoclonal; 1:1,000; Catalog number CB1001; Sigma-Aldrich) overnight at 4 ˚C, followed by incubation with a secondary antibody (Polyclonal; 1:5,000; Catalog number M300041; Invitrogen, Waltham, MA, USA) for 1 h at room temperature. Protein bands were visualized using Signal™ West Pico Plus chemiluminescent substrate (Catalog number 34579; Thermo Fisher Scientific).

5.3 Statistics

Data was analyzed using GraphPad Prism software (Prism 8; GraphPad Prism, La Jolla, CA, USA). All data were expressed as the mean interocular difference (IOD) between treated minus untreated eyes ± SD. A two-way repeated measures ANOVA was conducted to compare different time points and Tukey's post hoc test was applied to determine the least significant difference between groups when $p<0.05$. The correlation strength between refractive error, vitreous chamber depth, axial length, and IOP was assessed using Pearson's correlation coefficient (R^2). H&E staining outcomes were analyzed using ImageJ software. Scleral thickness was measured at ten consistent locations along a vertical axis within each image, focusing on both the cartilaginous and fibrous layers from bottom to top. These points were selected to ensure that the measurements capture the full depth of the sclera across different regions. The average thickness for each layer was calculated for image analysis.

5.4 Results

5.4.1 Effects of Latanoprost on Form-deprivation Myopia

At baseline, no significant differences were observed in refractive error and ocular biometry outcomes for the interocular difference between treated eyes minus control eyes, as summarized in Table 5.1. Topical latanoprost significantly reduced induced form-deprivation myopia in chicks compared to vehicle, as indicated by the interocular difference (IOD), treated eye minus untreated eye (Diopters, mean ± SD) on day 12. (-10.37 ± 2.51D versus -16.02 ± 3.07D, $p<0.0001$; Table 5.1 and Figures 5.1.a). However, the interocular difference of ACD was significantly shorter in the FDM latanoprost group compared with the FDM Vehicle group on day 12 (IOD of ACD: 0.28 ± 0.18 mm versus 0.51 ± 0.20 mm, $p<0.0001$; Table 5.1 and Figures 5.1.b). At the end of the treatment, there were no differences in lens thickness (LT) between the FDM latanoprost group compared with the FDM vehicle group (IOD of LT: -0.02 ± 0.04 mm versus -0.02 ± 0.05 mm, $p>0.99$; Table 5.1 and Figures 5.1.c). In contrast, the IOD of VCD elongation was significantly reduced between the FDM latanoprost group compared with the FDM vehicle group (IOD of VCD: 0.82 ± 0.22 mm versus 1.18 ± 0.22 mm, $p<0.0001$; Table 5.1 and Figures 5.1.d). The IOD of AL showed that latanoprost significantly inhibited AL with a highly significant difference between the FDM latanoprost and vehicle groups (0.97 ± 0.22 mm versus 1.52 ± 0.30 mm, $p< 0.0001$; Table 5.1 and Figures 5.1.e). In addition, the FDM latanoprost group showed markedly inhibited choroidal thickness, whereas the FDM vehicle group demonstrated an excessively decreasing trend of ChT (IOD of ChT: -30.30 ± 11.30 μm versus -64.26 ± 10.31 μm, $p< 0.0001$; Table 5.1 and Figures 5.1.f).

5.4.2 Effects of Latanoprost on Lens-induced Myopia

Baseline data revealed that there were no significant differences in refractive error and ocular biometry outcomes in the IOD between treated eyes minus control eyes, as summarized in Table 5.2. The topical latanoprost group was found to have a

significant reduction of slowing lens-induced myopia in chicks compared with LIM vehicle (IOD of refractive error on day 12: (-11.00±1.33D versus -7.17±1.06D, $p<0.0001$; Table 5.2 and Figures 5.2.a). There was also a significant difference in IOD of ACD between the LIM latanoprost and the LIM vehicle groups at day 12 (IOD of ACD: 0.16 ± 0.06 mm versus 0.24 ± 0.07 mm, $p<0.0001$; Table 5.2 and Figures 5.2.b). At the end of the treatment, there were no significant changes in LT between the LIM latanoprost and LIM vehicle groups (IOD of LT: -0.01 ± 0.02 mm versus -0.05 ± 0.07 mm, $p>0.17$; Table 5.2 and Figures 5.2.c), whilst, in contrast, the IOD revealed that VCD elongation was significantly inhibited in the LIM latanoprost treated chicks compared to the LIM vehicle group (IOD of VCD: 0.51 ± 0.11 mm versus 0.67 ± 0.07 mm, $p<0.01$; Table 5.2 and Figures 5.2.d). Latanoprost treatment also led to a significantly reduced AL compared to the control LIM vehicle group (IOD of AL: 0.66 ± 0.10 mm versus 0.87 ± 0.12 mm, $p <0.01$, Table 5.2 and Figure 5.2.e). In addition, the latanoprost group showed markedly inhibited CHT compared to the vehicle group (IOD of ChT: -27.16 ± 24.13 μm versus -53.90 ± 20.0 μm, $p>0.001$; Table 5.2 and Figures 5.2.f).

5.4.3 Coefficient of Determination Between Refractive Error Versus Vitreous Chamber Depth and Axial Length

At the end of treatment, a linear regression analysis showed a strong correlation between interocular differences in (FDM latanoprost versus FDM vehicle) refractive error and vitreous chamber depth ($R^2 = 0.76$, Figure 5.4.a) and IOD between (FDM latanoprost versus FDM vehicle) refractive errors and axial length ($R^2 = 0.87$, Figure 5.4.b). Linear regression analysis also showed a strong correlation between interocular differences in (LIM latanoprost group versus LIM vehicle) refractive error and VCD ($R^2 = 0.67$, Figure 5.5.a) and IOD between (LIM latanoprost versus LIM vehicle) refractive errors and AL ($R^2 = 0.71$, Figure 5.5.b).

5.4.4 Latanoprost effects on Intraocular pressure (IOP)

Topical latanoprost effectively reduced IOP in both FDM and LIM latanoprost groups. Higher IOP was observed in the FDM and LIM control groups. Baseline IOP did not differ statistically between the two groups (FDM Latanoprost 0.20 ± 2.59 mmHg versus FDM vehicle 0.26 ± 1.98 mmHg, $p>0.99$; Table 5.3). At the end of the treatment, the IOD in IOP in the treatment group decreased while increasing in the control group (IOD of IOP: FDM Latanoprost -2.46 ± 3.68 mm Hg versus FDM vehicle group 2.86 ± 3.09 mmHg; $p<0.0001$. There was no significant difference in baseline IOP between the two groups (LIM Latanoprost 0.26 ± 2.18 mmHg versus LIM vehicle group 0.46 ± 1.45 mmHg, $p>0.87$; Table 5.3), but IOD of IOP significantly decreased after the latanoprost treatment compared with the control group at day 12 (IOD of IOP: LIM Latanoprost -2.66 ± 2.76 mmHg versus LIM vehicle group 3.20 ± 2.98 mmHg, $p<0.0001$). At the end of the treatment, a linear regression analysis revealed a strong correlation between IOD of refractive error and IOD in IOP in the FDM vehicle group ($R^2 = 0.54$, Figure 5.6.a), as well as IOD of IOP and AL ($R^2 = 0.50$, Figure 5.6.b), while no significant correlations were observed in the FDM latanoprost group. However, LIM latanoprost versus LIM vehicle groups showed no significant correlation between the IOD in refractive error and IOD in IOP in either the LIM vehicle group ($R^2 = 0.04$, Figure 5.7.a) or LIM latanoprost group ($R^2 = 0.04$, Figure 5.7.a). Similarly, there is no strong correlation between IOD of IOP and IOD of AL in LIM vehicle or LIM latanoprost groups ($R^2 = 0.24$ and 0.14, Figure 5.7.b). H&E staining of the sclera is shown in Figures 5.8 and b. The sclera of both FDM latanoprost and LIM latanoprost was significantly thicker than the sclera of the control groups. H & E staining confirmed that the fibrous layer of the sclera changes during myopia development, whereas the cartilage layer of the sclera remains unchanged or more stable when compared between control groups.

5.4.5 Latanoprost Effects on Retinal EGR-1 Expression

Baseline EGR-1 expression levels are shown in Figure 5.9 (a and b). The results revealed no significant differences between the treated and control groups. However, after 12 days of treatment, retinal EGR-1 mRNA levels were significantly higher in both the FDM and LIM latanoprost-treated groups than in the control groups (P

<0.001; Fig. 5.9.a, b). Western blot analysis showed that retinal EGR1 protein expression was higher in the FDM + Latanoprost group compared to the control group on days 4 and 12. However, these differences were not statistically significant ($p > 0.05$; Fig. 5.10. a and b). Although retinal EGR-1 protein expression was higher in the LIM + Latanoprost group than in the vehicle group on day 4, the difference did not reach statistical significance ($p > 0.05$; Fig. 5.10. c and d).

5.4.6 Chicken Body Weight

There were no significant differences in body weight between the latanoprost-treated and control groups at baseline, as well as on days 4, 8, and 12 (Table 5.4). All groups of chickens demonstrated normal growth patterns over time, indicating that latanoprost administration did not affect overall physical development.

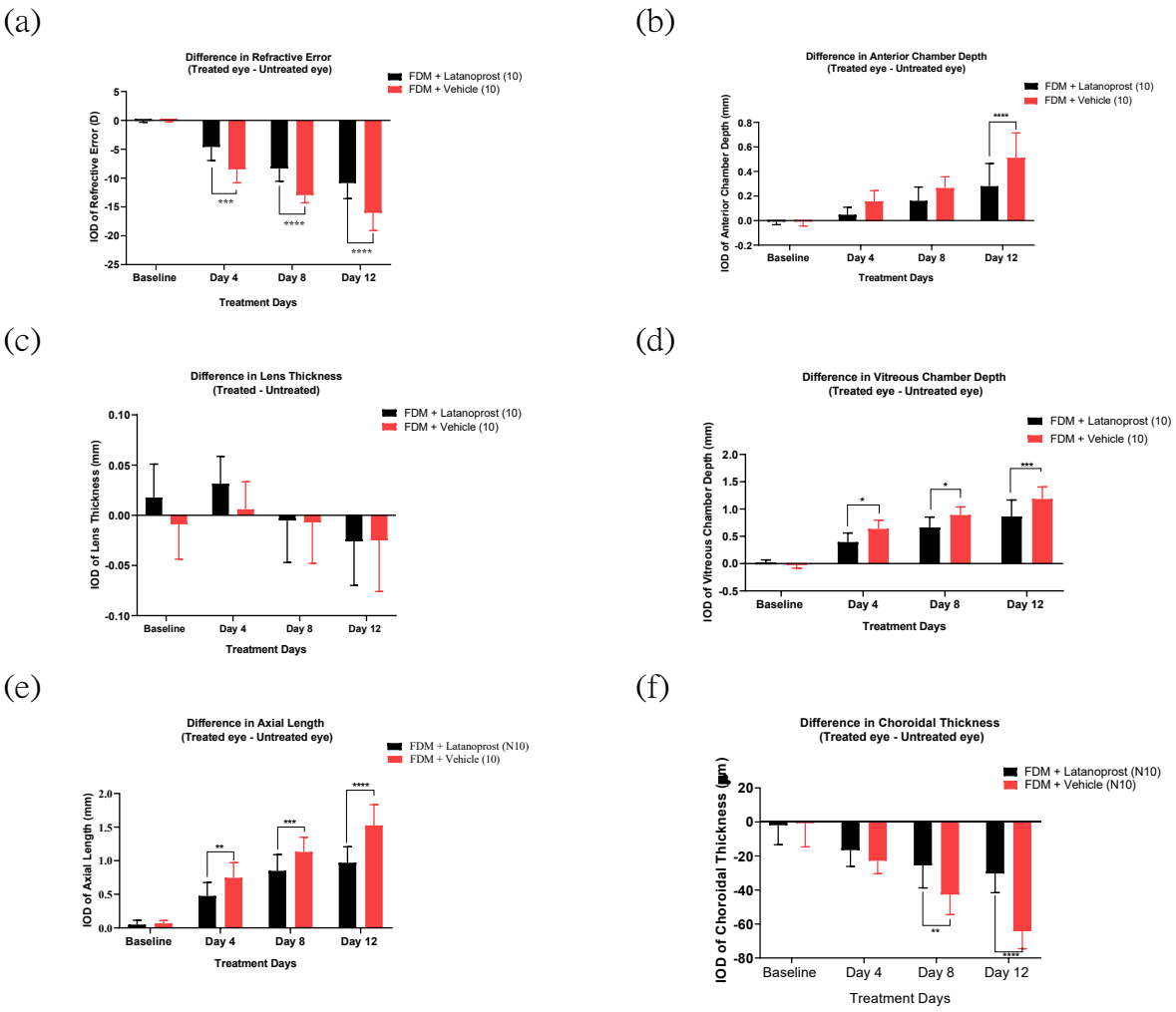


Figure 5.1 Bar charts showing the treatment effects of topical latanoprost on FDM Chicks. The interocular difference (IOD, treated eye minus an untreated eye) in refractive errors (Diopter, mean ± SD) demonstrated significant inhibition of experimentally induced myopia on days 4, 8, and 12, respectively (a). The IOD of ACD was significantly shorter chamber in treated chicks on day 12 compared to the vehicle group (b). The IOD of LT showed no significant changes (c). The IOD of VCD (d) and IOD of AL (e) were significantly shorter in the treatment group compared to the vehicle group on days 4, 8, and 12. In addition, the IOD of ChT thickness increased in the treatment group and decreased in the vehicle group on days 8 and 12. Statistical analysis was performed using a two-way repeated measures ANOVA followed by the Bonferroni post hoc test. Error bars represent SD, and the numbers in brackets indicate sample sizes. Significance levels are denoted as * $p < 0.05$, **$p < 0.01$ and *** $p < 0.001$.

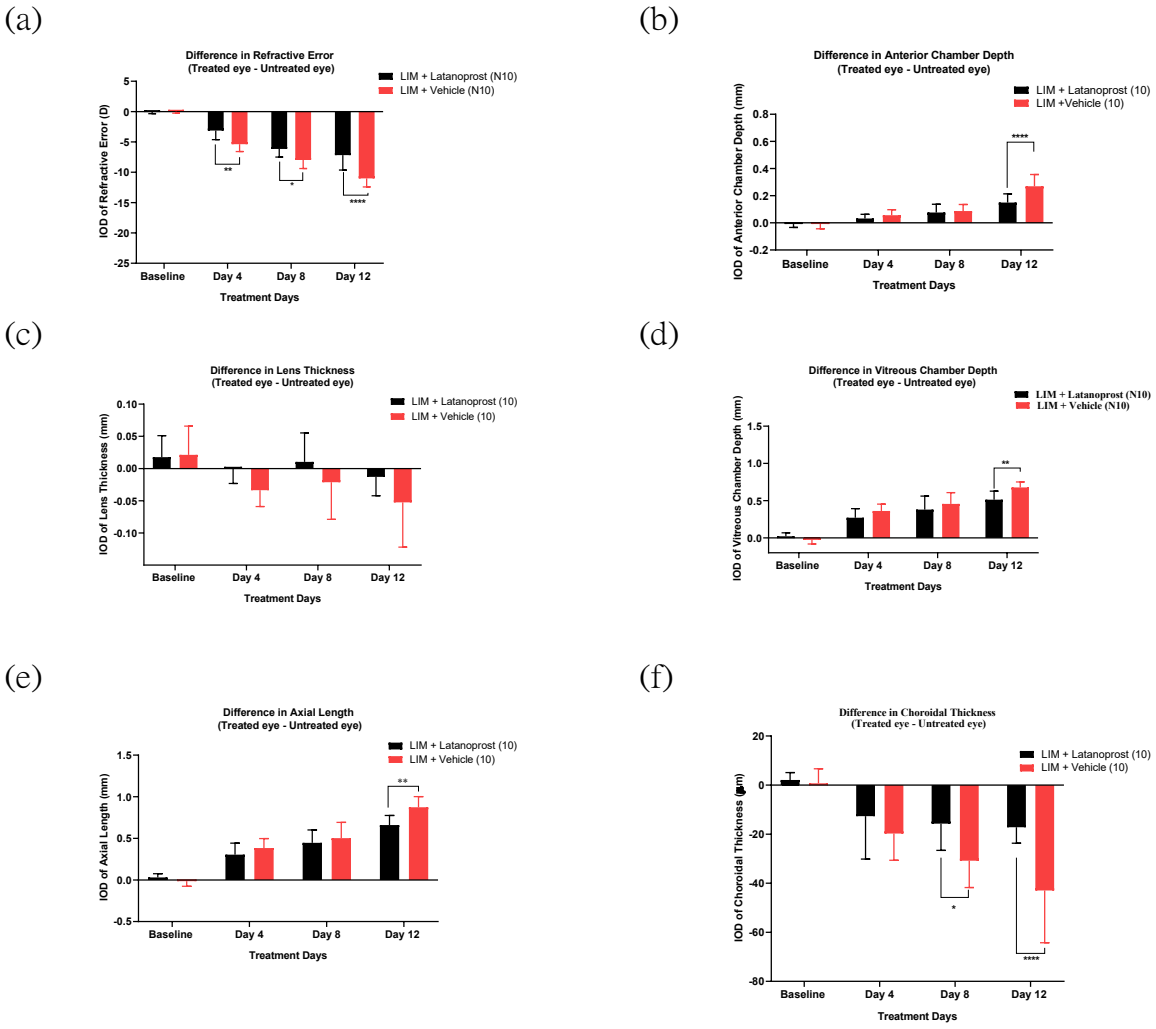
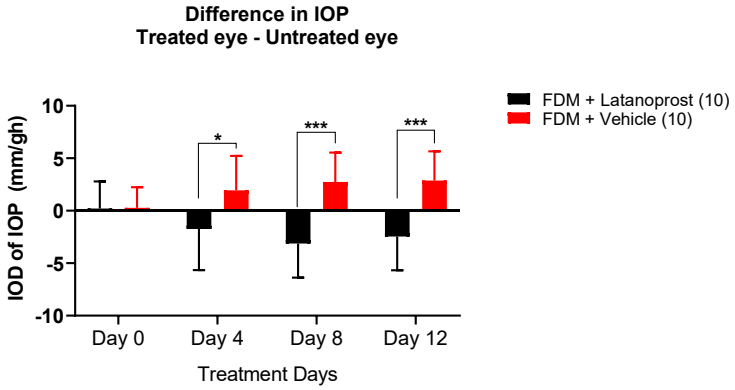


Figure 5.2 Bar charts showing the treatment effects of topical latanoprost on LIM in chicks. Interocular difference (IOD, treated eye minus untreated eye) in refractive errors (Diopter, mean ± SD) showed that latanoprost significantly inhibited experimentally induced myopia on days 4, 8, and 12 (a). The IOD of the ACD group was notably shorter on day 12 than in the vehicle group (b), with no significant changes in lens thickness (c). VCD (d) and AL (e) were significantly shorter in the treatment group compared to the vehicle group on day 12. Additionally, the IOD of choroidal thickness was greater in the treatment group than in the control group on day 12 (f). Statistical analysis was performed using a two-way repeated measures ANOVA followed by the Bonferroni post hoc test. Error bars represent SD, and the numbers in brackets indicate sample sizes. Significance levels are denoted as * $p < 0.05$, **$p < 0.01$, *** $p < 0.001$ and *** $p < 0.001$.

(a) Comparison of interocular difference (IOD) of IOP between FDM + Latanoprost and FDM + Vehicle



(b) Comparison of interocular difference (IOD) of IOP between LIM + Latanoprost versus LIM + Vehicle

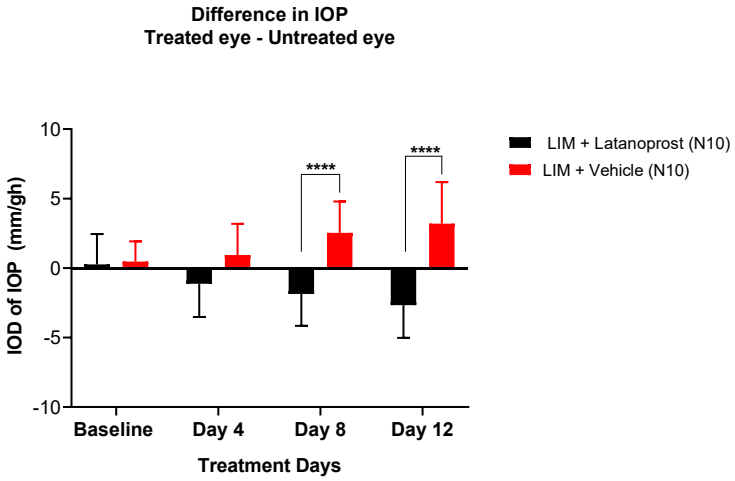
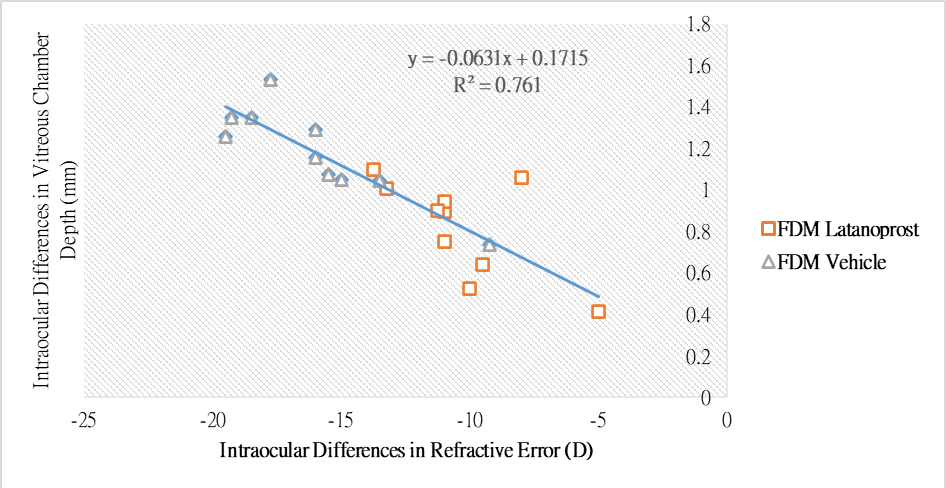


Figure 5.3 Line graph of the interocular pressure (IOP, mmHg) of FDM and LIM. The IOP (IOD, treated eye minus untreated eye, mean ± SEM, mmHg) measured over a 12-day treatment period (a) FDM + Latanoprost, (b) LIM + Latanoprost. The FDM + Latanoprost group showed a statistically significantly lower IOP than the FDM + Vehicle group on days 4, 8, and 12 (a). Similarly, the LIM + Latanoprost group exhibited lower IOP than the LIM + Vehicle group, with statistical significance observed on days 8 and 12 (b). Error bars represent SD, and sample sizes (n) are indicated in brackets. Statistical analysis was conducted using a two-way repeated measures ANOVA followed by Tukey's post hoc test. Significance levels are denoted as * $p < 0.05$, **$p < 0.01$, *** $p < 0.001$ and *** $p < 0.001$.

(a) Correlation of interocular differences in refractive errors and vitreous chamber depth in chicks treated with topical latanoprost



(b) Correlation of interocular differences in refractive errors and axial length in chicks treated with topical latanoprost

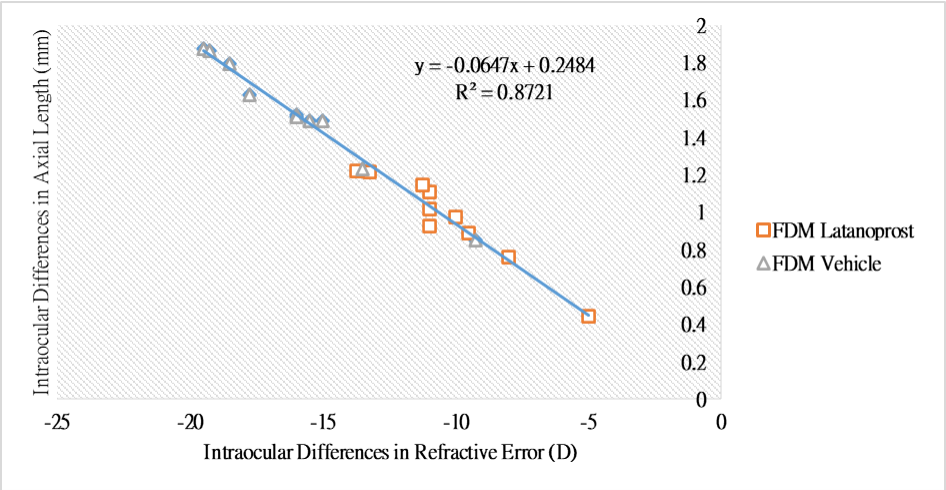
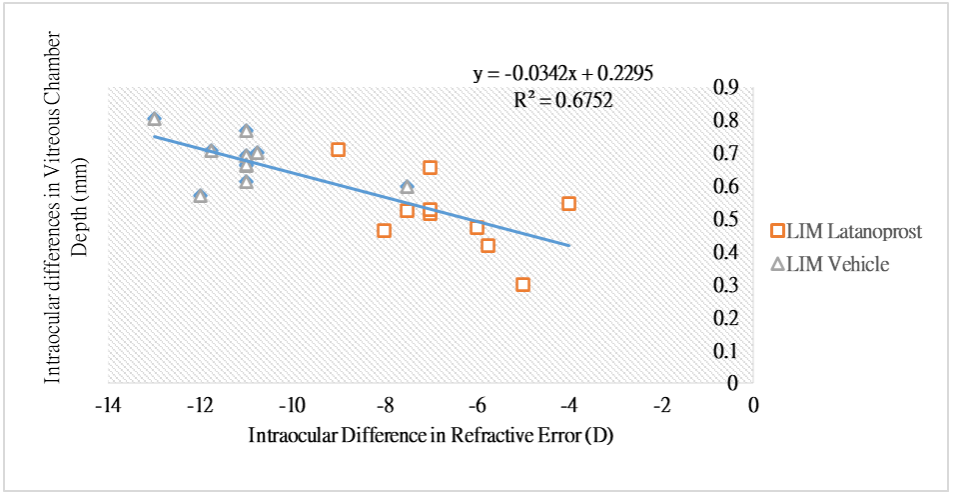


Figure 5.4 Coefficient of determination between interocular differences in refractive errors versus vitreous chamber depth (a) and axial elongation (b) in the FDM latanoprost control groups (R^2 = 0.76 and 0.87, respectively).

(a) Correlation of interocular differences in refractive errors and vitreous chamber depth in chicks treated with topical latanoprost



(b) Correlation of interocular differences in refractive errors and axial length in chicks treated with topical latanoprost

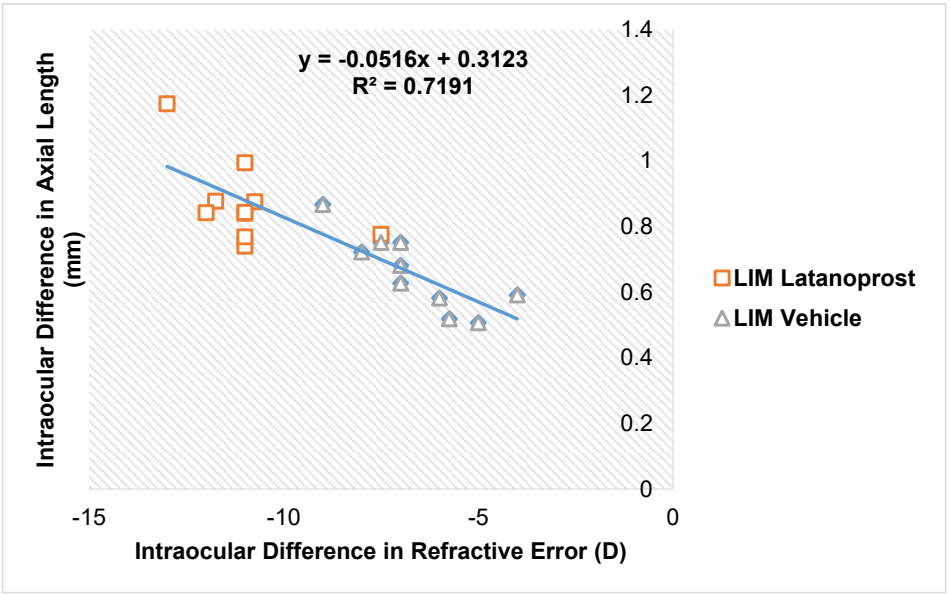
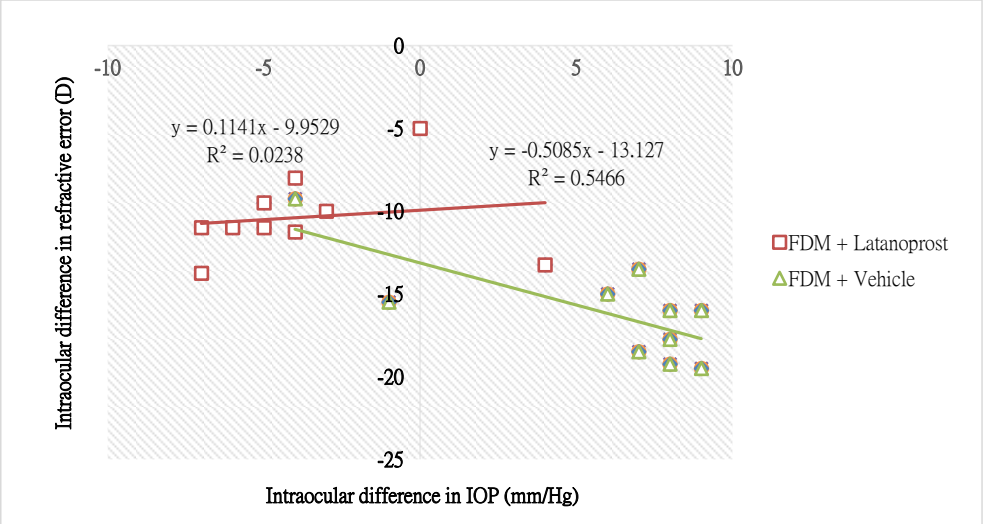


Figure 5.5 Coefficient of determination between interocular differences in refractive errors versus vitreous chamber depth (a) and axial elongation (b) in LIM + Latanoprost group and LIM + Vehicle group (R^2 = 0.67 and 0.71, respectively).

(a) Correlation between interocular differences in refractive errors and intraocular pressure (IOP) in chicks treated with topical latanoprost



(b) Correlation between interocular differences in axial length and intraocular pressure (IOP) in chicks treated with topical latanoprost

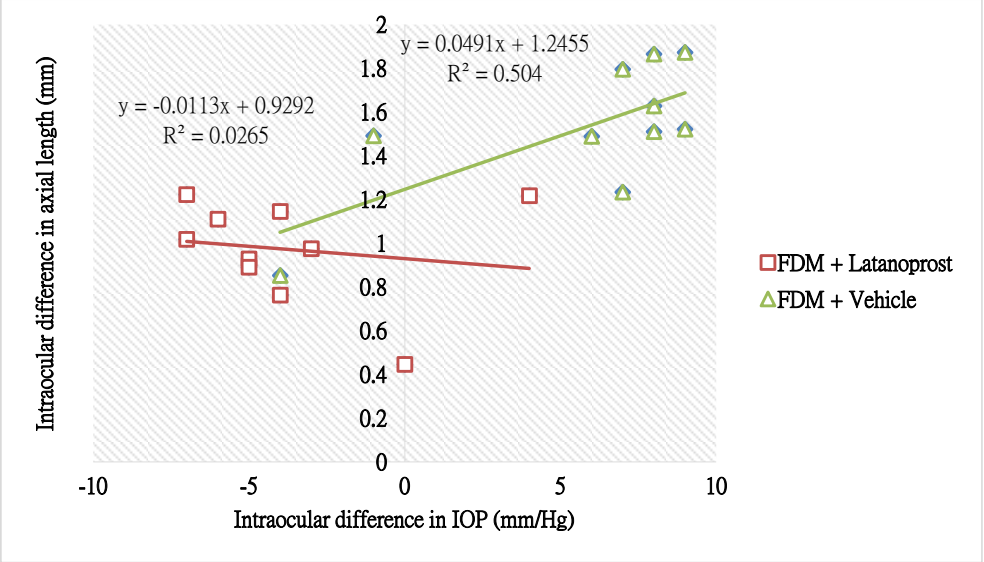
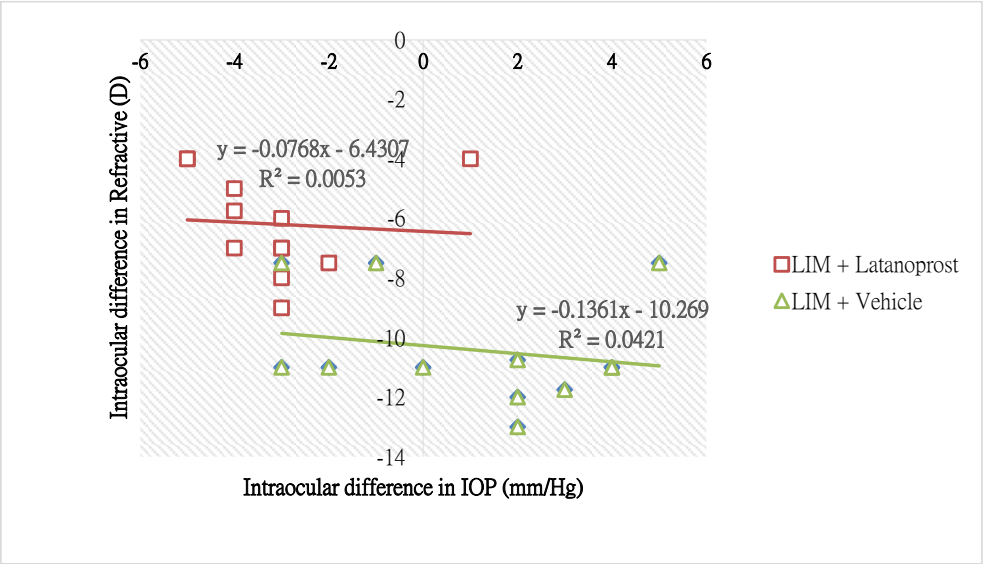


Figure 5.6 Coefficient of determination between interocular differences in refractive errors and interocular differences in IOP (a), as well as axial elongation (b) in both the FDM + Latanoprost and FDM + Vehicle groups ($R^2 = 0.54$; $p<0.001$ and 0.53; $p<0.001$, respectively).

(a) Correlation of interocular differences in refractive errors with intraocular pressure (IOP) in chicks treated with topical latanoprost for lens-induced myopia (LIM).



(b) Correlation of interocular differences in axial length with intraocular pressure (IOP) in chicks treated with topical latanoprost for lens-induced myopia (LIM).

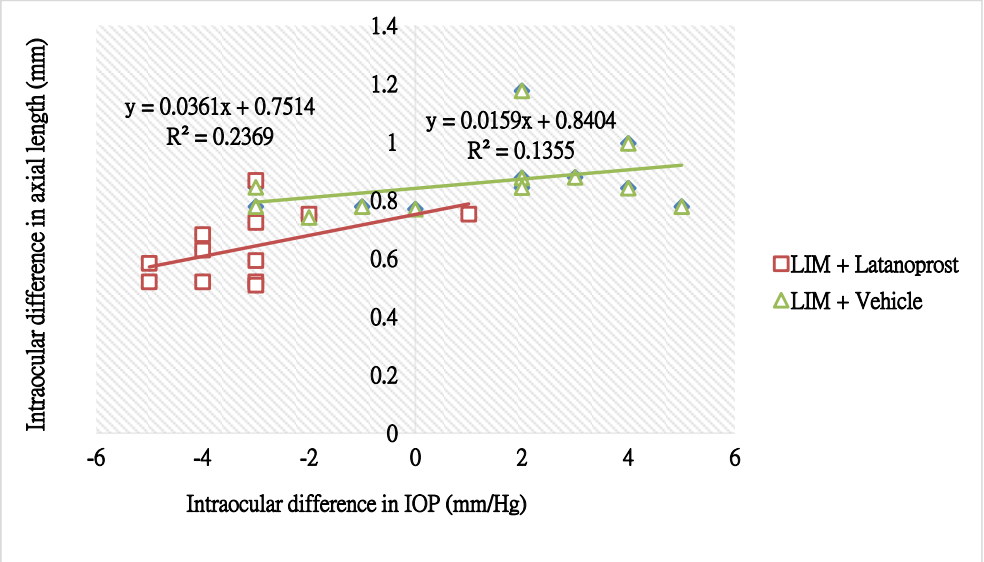
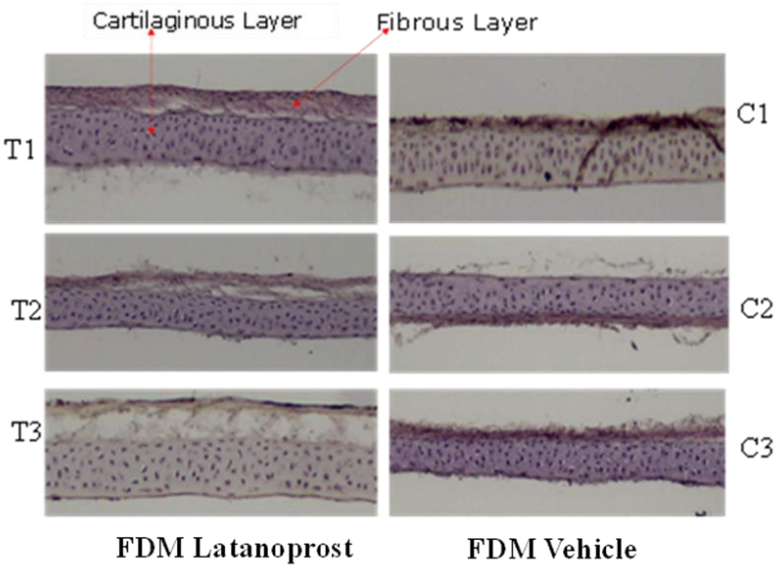


Figure 5.7 Coefficient of determination between interocular differences in refractive errors and interocular differences in IOP (a), axial elongation (b) in both the LIM + Latanoprost and LIM + Vehicle groups (R^2 = 0.37; $p<0.002$ and 0.47; $p<0.001$ respectively).

(a) FDM latanoprost versus FDM vehicle



(b) LIM latanoprost versus LIM vehicle

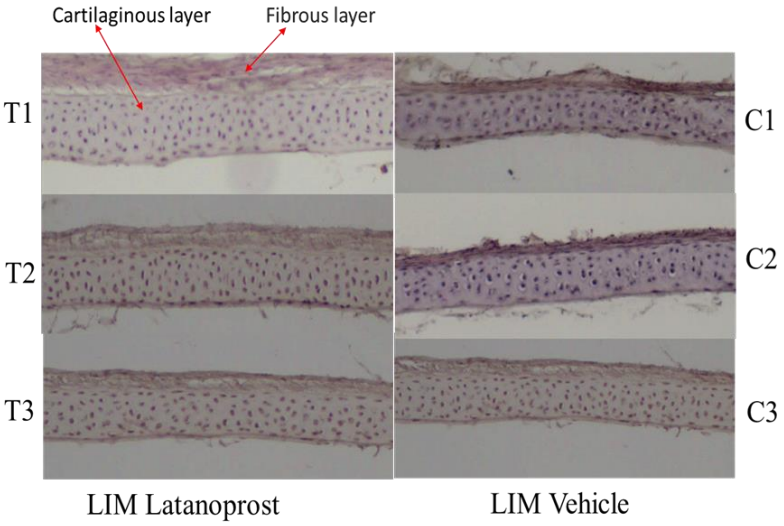
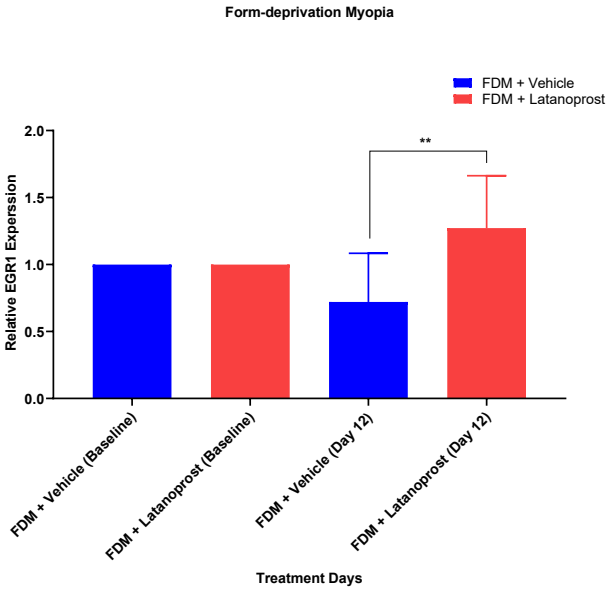


Figure 5.8 Hematoxylin and eosin (H&E) staining showing the differences in scleral thickness between treated samples (T1, T2 & T3) and control samples (C1, C2 & C3). Scleral thickness was compared between (a) FDM + Latanoprost versus FDM + Vehicle groups and (b) LIM + Latanoprost versus LIM + Vehicle groups. Both latanoprost-treated groups showed a significant reduction in the thinning of the scleral fibrous layers.

(a) mRNA expression levels of FDM + Latanoprost versus FDM + Vehicle groups



(b) mRNA expression levels of LIM + Latanoprost versus LIM + Vehicle groups

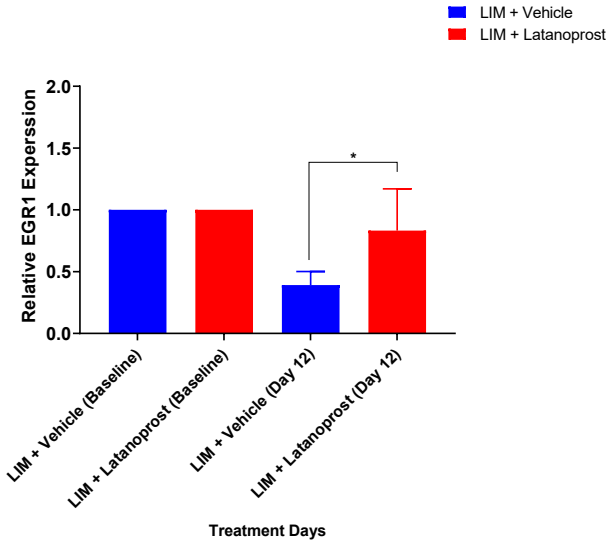
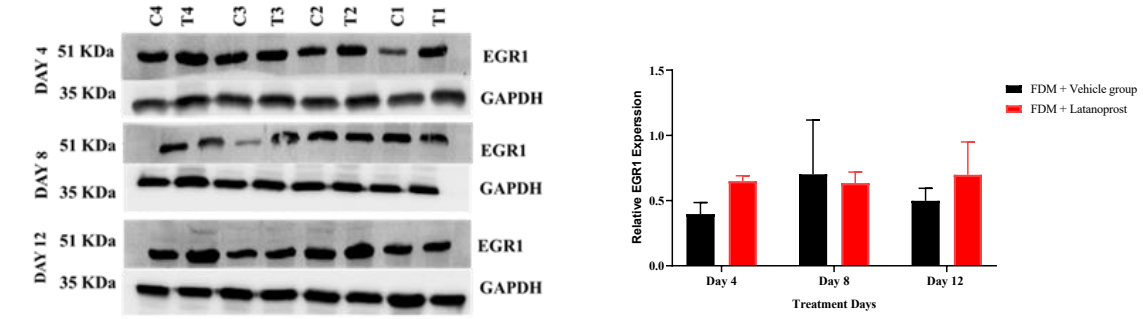


Figure 5.9. Graph of the qPCR results showing the effects of topical latanoprost after 12 days of treatment on (a) FDM + Latanoprost versus FDM + Vehicle groups and (b) LIM + Latanoprost versus LIM + Vehicle groups. In both treated groups, retinal EGR-1 mRNA levels were upregulated.

EGR1 expression levels of FDM + Latanoprost versus FDM + Vehicle group

(a) (b)



EGR1 expression levels of LIM + Latanoprost versus LIM + Vehicle group

(c) (d)

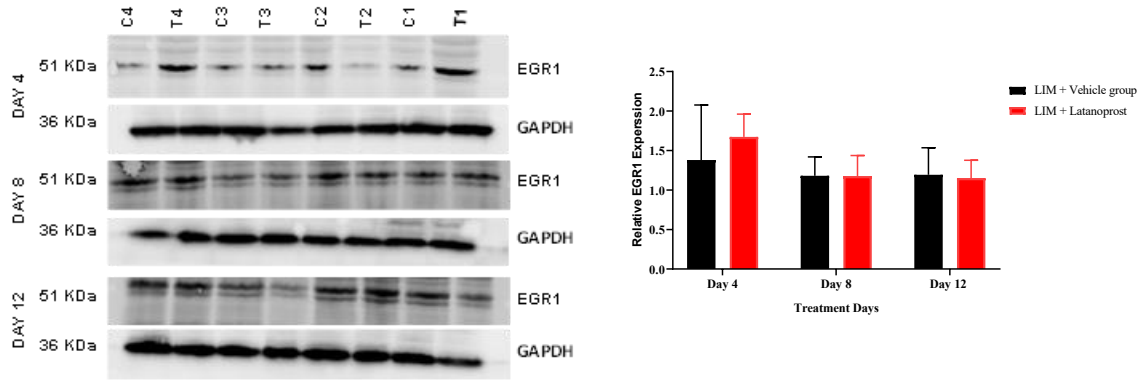


Figure 5.10: A representative western blot between treated samples (T1, T2, T3 & T4) and control samples (C1, C2, C3 & C4). Western blots and bar graphs representing a summary of EGR-1 protein expression levels (a and b) FDM + Latanoprost versus FDM + Vehicle and (c and d) LIM + Latanoprost versus LIM + Vehicle on days 4, 8, and 12, respectively.

Table 5.1 Summary of the data on the effect of topical latanoprost on FDM chicks. The interocular difference (IOD) in refractive error (D) and ocular parameters (mm) was presented for days 0 (Baseline), 4, 8, and 12.

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
FDM + Latanoprost						
Baseline	-0.06 (0.33)	0.00 (0.01)	0.00 (0.02)	0.03 (0.05)	0.03 (0.05)	1.86 (6.17)
Day 4	-4.60 (2.35)	0.04 (0.06)	0.03 (0.02)	0.39 (0.16)	0.47 (0.20)	-16.50 5.15)
Day 8	-8.35 (2.19)	0.19 (0.10)	-0.00 (0.04)	0.66 (0.19)	0.84 (0.22)	-25.53 (13.22)
Day 12	-10.37 (2.51)	0.28 (0.18)	-0.02 (0.04)	0.82 (0.22)	0.97 (0.23)	-30.30 (11.30)
FDM + Vehicle						
Baseline	-0.04 (0.35)	0.00 (0.02)	0.00 (0.03)	0.02 (0.04)	0.03 (0.05)	0.36 (4.58)
Day 4	-8.47 (2.34)	0.12 (0.07)	0.00 (0.02)	0.63 (0.15)	0.74 (0.22)	-22.80 (7.65)
Day 8	-12.95 (1.33)	0.26 (0.09)	-0.00 (0.04)	0.89 (0.15)	1.13 (0.21)	-42.73 (11.77)
Day 12	-16.02 (3.07)	0.51 (0.20)	-0.02 (0.05)	1.18 (0.22)	1.52 (0.30)	-64.26 (10.31)

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 5.2 Summary of the data on the effect of topical latanoprost on LIM chicks. The interocular difference (IOD) in refractive error (D) and ocular parameters (mm) was presented for days 0 (Baseline), 4, 8, and 12.

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
LIM + Latanoprost						
Baseline	-0.04 (0.25)	-0.00 (0.02)	0.01 (0.03)	0.03 (0.04)	0.03 (0.04)	2.10 (5.7)
Day 4	-3.12 (1.50)	0.03 (0.03)	0.00 (0.02)	0.27 (0.12)	0.30 (0.13)	-12.63 (19.21)
Day 8	-6.15 (1.34)	0.07 (0.06)	0.01 (0.04)	0.38 (0.18)	0.44 (0.15)	-22.46 (7.60)
Day 12	-7.17 (1.06)	0.16 (0.06)	-0.05 (0.02)	0.51 (0.11)	0.66 (0.11)	-27.16 (24.13)
LIM + Vehicle						
Baseline	-0.06 (0.16)	-0.01 (0.03)	0.02 (0.04)	-0.02 (0.05)	-0.01 (0.06)	1.23 (3.47)
Day 4	-5.37 (1.21)	0.05 (0.04)	-0.03 (0.02)	0.35 (0.09)	0.38 (0.11)	-19.76 (10.86)
Day 8	-7.67 (2.05)	0.06 (0.04)	0.00 (0.05)	0.45 (0.15)	0.49 (0.18)	-30.80 (10.97)
Day 12	-11 (1.33)	0.24 (0.07)	-0.01 (0.02)	0.67 (0.07)	0.87 (0.12)	-53.90 (20.0)

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 5.3 Summary of the data on the interocular difference (IOD) in interocular pressure (IOP) outcomes following latanoprost treatment (mmHg, mean ± SEM).

Experiment groups	Baseline (mean ±SD)	Day 4 (mean ±SD)	Day 8 (mean ±SD)	Day 12 (mean ±SD)
FDM+ Latanoprost	0.20 ± 2.59	-1.73 ± 3.91	-3.13 ± 3.24	-2.46 ± 3.68
FDM + Vehicle	0.26 ± 1.98	1.93 ± 3.28	2.73 ± 2.81	2.86 ± 4.09
LIM + Latanoprost	0.26 ± 2.18	-1.13 ± 2.38	-1.80 ± 2.29	-2.66 ± 2.76
LIM + Vehicle	0.46 ± 1.40	0.93 ± 2.17	2.53 ± 2.18	3.20 ± 2.87

Table 5.4 Average body weight (mean±SD) of chickens at baseline, day 4, 8, and 12

Weight of chicken

Experiment groups	Baseline/g	Day 4/g	Day 8/g	Day 12/g
FDM+ Latanoprost	40.47 ± 3.27	66.04 ± 4.07	90.47 ± 7.46	136. 09 ± 8.45
FDM + Vehicle	39.24 ± 4.00	65.02 ± 5.19	98.55 ± 8.87	146.79 ± 8.25
LIM + Latanoprost	41.86 ± 38.41	69.19 ± 8.27	94.97 ± 3.89	144.10 ± 8.16
LIM + Vehicle	38.41 ± 3.28	71.42 ± 8.27	99.45 ± 8.32	141.67 ± 8.79

5.5 Discussion

This preclinical study investigated the effects of topical latanoprost, a prostaglandin analogue, which significantly inhibits the progression of both FDM and LIM in chicken eyes. Our findings show that topical latanoprost treatment reduced excessive elongation of AL and decreased IOP, preserved scleral thickness, and upregulated retinal EGR-1 expression. Previous investigations into ocular hypotensive drugs for myopia control in animal models have included timolol, brimonidine, and latanoprost, but results were inconsistent. Timolol, a beta blocker, concluded that there was no effect on the development of experimental myopia, although it was able to lower IOP in both myopic eyes and normal eyes in animals [175]. Timolol was clinically evaluated in a study of Danish children (7 to 12 years), which confirmed that there were no significant effects on the development of myopia progression when compared to the single vision spectacle [176]. This study also reported that timolol treatment resulted in a reduction of IOP by approximately 3 mmHg. Brimonidine, an α2-adrenoceptor agonist, has been investigated for significant inhibition of myopia progression and axial elongation in guinea pigs [176, 183]. It also demonstrated that it effectively reduced IOP through inhibition of the cAMP-dependent aqueous inflow system and uveoscleral outflow system [288]. The effects of prostaglandin analogues in myopia control appear to depend on the specific drugs and route of administration. While prostaglandin E2 (PGE2) and latanoprost acid, administered through subconjunctival injections or topical routes, did not affect myopia development in chicks, prostaglandin F2α (PGF2α), administered via intravitreal injection, significantly inhibited myopia progression in chicks. [184]. Our results extend previous work by showing that topical latanoprost significantly inhibited myopia progression in FDM and LIM models. This could be possible because anti-glaucoma drugs are successfully delivered directly to the posterior segment through the blood-aqueous barrier and the vascular blood-retinal barrier. The underlying mechanisms of FDM and LIM differ. Previous studies demonstrated

that FDM leads to localized ocular elongation without direct feedback from the central nervous system [106]. At the same time, LIM involves visual feedback and disruption of the circadian rhythm, which enables the retina and central nervous system to work together to regulate ocular growth [289]. In this study, latanoprost was effective in both models, with a more pronounced and earlier effect observed in FDM. This could be more directly related to the uncontrolled ocular growth mechanisms in FDM, which were more rapidly modulated by pharmacological intervention, whereas LIM depends on gradual visual feedback adjustments. Previous pharmacology studies found that a dopaminergic agonist (apomorphine) inhibits FDM but exhibits inconsistent effects in LIM in both chicks and guinea pigs [273, 290].

The current study observed that latanoprost treatment resulted in significantly shorter anterior chamber depths (ACD) in both the FDM and LIM groups. This is possibly due to ciliary muscle contraction and zonular relaxation. A previous clinical observation study found that latanoprost decreases ACD in patients with ocular hypertension or glaucoma without changes in visual acuity and lens thickness [291]. Interestingly, a recent animal study also reported that another glaucomatous drug, topical pilocarpine 0.5% also significantly decreased ACD in both rabbits and guinea pigs [292]. The current study found no significant changes in lens thickness in either the FDM or LIM latanoprost groups. However, previous studies suggest that latanoprost reduces the thickness of the ciliary body, which in turn increases zonular tension and results in the lens moving posteriorly [293]. In contrast, latanoprost significantly inhibited vitreous chamber depth(VCD) and axial length elongation (AL), findings that were similar to those in FDM guinea pigs [186]. In the current study, choroidal thickness was significantly preserved in both latanoprost-treated groups, while the control groups showed a thinner choroid. This may be related to

increased ocular blood flow and decreased ocular perfusion pressure, as well as increased glycosaminoglycan and myofibroblast activity. However, previous clinical studies have suggested that topical latanoprost increased sub-foveal choroidal thickness after 3 months of treatment [294]. This funding suggests that topical latanoprost might elevate choroidal vascular permeability and disrupt choroidal blood flow.

Our study confirmed that latanoprost significantly reduced IOP in both FDM and LIM models of chicks. Previous studies have reported that topical latanoprost significantly decreased IOP in guinea pigs and rabbits [187, 295, 296]. However, studies in rhesus monkeys reported no significant difference in IOP between latanoprost-treated eyes and control eyes [185]. Previous studies provide contradictory results on IOP, which could influence early ocular eye growth in chicks and changes in IOP during normal development [297]. The present study showed that IOP increased in experimentally induced myopic eyes treated with saline from baseline to end of the treatment. This finding was similar to a report that noted an elevation in IOP in FDM and LIM in young guinea pigs that received artificial tears or saline [186]. The relationship between myopia and IOP remains controversial, with some studies have found no significant differences in IOP between myopic and emmetropic eyes or different types of myopia [161, 298]. However, other studies found that IOP was significantly higher in the high myopes compared to those with emmetropes [299-301]. Our data suggest that elevated IOP contributes to myopia progression due to anatomical changes in the posterior eye, including alterations in choroidal circulation and scleral remodeling. Previous study mentioned that a change in IOP may modulate axial length elongation by altering the scleral extracellular matrix through the scleral fibroblast activities [255]. Similarly, another study supports that IOP plays an important role in ocular growth and changes scleral structures and matrix metalloproteinase activities [302].

Topical latanoprost not only reduced IOP but also inhibited AL elongation in both FDM and LIM models of chicks. The effects of ocular hypotensive drugs varied in lowering IOP and reducing AL. Some studies suggested that high IOP in myopic chicks was induced by injected fluid, which also leads to increased AL [281]. However, AL elongation was dramatically decreased after IOP-lowering surgery [282]. This finding provided direct evidence of an association between IOP and AL. Topical timolol studies reported no correlation between myopia progression and AL in young guinea pigs [178]. In contrast, other study has shown that topical latanoprost significantly reduces myopia progression and AL in guinea pigs due to remodeling of scleral collagen and microstructures [303]. Furthermore, topical latanoprost treatment in guinea pigs at 10 weeks was more effective in reducing AL than intravitreal latanoprost injections in chicks at 4 weeks (0.29 ± 0.04 mm versus 0.06 ± 0.02 mm). This result was possibly due to the anatomical structures of chicks, which have two layers of sclera. The inner sclera layer was comprised of hyaline cartilage layers that were very thickened, providing extra support to the fibrous layer. Therefore, the mechanism of scleral growth, the role of IOP, and ocular enlargement in chicks differ from those in humans and mammals. In a glaucoma study, the prostaglandin analogue latanoprost acid reduced IOP by increasing uveoscleral outflow, while nitric oxide increased aqueous humour outflow via the trabecular meshwork and Schlemm's canal [304]. Brimonidine, used in topical or intravitreal injections, has been shown to effectively inhibit myopia and AL via a change in refractive error [176, 183]. However, a clinical study found that high myopia was significantly correlated with a higher AL [305]. An early onset of myopia study reported that school-age Chinese children with high myopia had a greater increase in AL [306]. Other studies have investigated the association between AL and changes in the scleral extracellular matrix and remodelling of the scleral shell, which is linked to decreased collagen and glycosaminoglycan [255]. In both FDM and LIM models, the sclera was relatively thicker after topical latanoprost administration compared to the control groups, where the sclera was thinner. These findings indicate that latanoprost can prevent excessive

thinning by inhibiting the extracellular matrix and scleral remodelling process. Similar findings were observed with brimonidine, which can inhibit scleral thinning and help to prevent myopia development in guinea pigs [176]. However, some studies have suggested that scleral thickness was thinner in the high myopic eye with or without excess AL due to a reduction in quantity of scleral tissue and decreased collagen fibril diameter [173, 307, 308]. A recent study reported that scleral thickness was significantly thinner in children with mild (534 μm) and moderate (515 μm) myopia [309].

EGR-1 (Early growth response protein 1) is a transcription factor that regulates ocular growth and responds to environmental stimuli [310]. Animal studies have shown that Egr-1 suppresses eye elongation with upregulation acting as a STOP signal and downregulation associated with increased AL [311]. The current study found that topical latanoprost treatment significantly increased Egr-1 levels in both FDM and LIM chicks. Topical latanoprost treatment may upregulate the molecular levels of the myopia-suppressive gene Egr-1 expression. However, topical latanoprost treatment helps regulate ocular growth by activating a molecular pathway that inhibits excessive axial elongation, creating a new possibility for controlling myopia progression. Previous human and chick model studies found that violet light upregulated the EGR1 gene and suppressed AL [99]. This upregulation reduces excessive eye growth, suggesting that environmental factors, such as light exposure, can positively influence EGR-1 activity. They also indicate that EGR-1 plays a crucial role in regulating controlled eye growth and may hold potential as a target for myopia prevention and treatment strategies. Previous chick study provides evidence that levels of Egr-1 mRNA are rapidly downregulated in FDM or LIM and remain consistently low during ongoing ocular growth [312].

5.6 Conclusion

This study observed that topical latanoprost effectively reduced the development of experimental FDM and LIM models in chicks. Importantly, it was shown that topical latanoprost effectively reduced ACD, VCD, and AL and increased choroidal thickness in both FDM and LIM. Notably, it was shown that the chosen concentration of topical latanoprost effectively reduced IOP in both FDM and LIM models. Importantly, at the molecular level, topical latanoprost upregulated the expression of EGR-1, a myopia-suppressive gene in both FDM and LIM models. This finding suggests new opportunities for clinical drug applications to control myopia progression. These findings indicate that topical latanoprost may play a significant role in effecting the development of both FDM and LIM. These findings provide valuable insights into potential treatments for myopia control.

Chapter 6

Rho kinase inhibitor (Y-33075 dihydrochloride) affects the development of Lens-induced myopia (LIM) or Form-deprivation myopia (FDM) in the chick model.

Rho kinase inhibitors are members of the serine/threonine kinase protein family, which play a significant role in regulating and modulating cell size and shape through their function on the cytoskeleton. This study was designed to investigate the impact of the Rho kinase inhibitor (Y-33075 dihydrochloride) on the development of lens-induced myopia (LIM) and form-deprivation myopia (FDM) in the chick model. Myopia was induced in chicks treated eyes (right eyes) using diffuser lenses or -10D (single vision lenses) for one week, while the left eyes remained untreated as controls. The Rho kinase inhibitor (Y-33075 dihydrochloride) was intravitreally injected into the right eye at doses of 1, 10, and 100 nmol in a 20 µl solution on days 0 (Baseline), 2, 4, and 6. The control-treated groups received intravitreal injections of a mixture of 10% DMSO + 90% Corn oil into the control eye as a control measure. After 7 days of treatment, all lenses were removed, and the eyes were allowed to

recover from the induced myopia for a further 7 days. On days 0, 7, and 14, Retinoscopy, iCare tonometer, and high-frequency A-scan ultrasonography were used to measure refractive errors, intraocular pressure, and ocular biometry, respectively. Data were presented as the mean interocular differences ± standard deviation (SD). After 7 days of treatment, it was observed that Rho kinase inhibitor (Y-33075 dihydrochloride) significantly inhibited myopic refractive error in both FDM and LIM models in a dose-dependent manner. In experiment I: Interocular differences (IOD, treated eye minus untreated eye) in refractive errors at day 7 when compared to the LIM controls (DMSO + Corn oil), were as follows -8.56 ± 2.70D versus (Y-33075 dihydrochloride at 1 nmol) -7.29±2.82D, $p>0.72$; (Y-33075 dihydrochloride at 10 nmol) -2.72±2.53D $p<0.001$; and (Y-33075 dihydrochloride at 100 nmol) -3.87±.69D, $p<0.01$ respectively. In experiment II, IODs in refraction on day 7 for FDM control (DMSO + Corn oil) were -7.51±3.95D versus (Y-33075 dihydrochloride at 1 nmol) -6.66 ±1.90D, $p>0.72$; (Y-33075 dihydrochloride at 10 nmol) -3.09 ±2.39D, $p<0.01$; and (Y-33075 dihydrochloride at 100 nmol) -2.94±2.42D, $p<0.01$, respectively. In addition, intraocular pressure (IOP) was elevated in both LIM and FDM groups treated with (DMSO + Corn oil) but decreased in those injected with different tested doses of Rho kinase inhibitor. In both LIM and FDM groups, the treated eyes recovered significantly by day 14 after removing the lenses. Overall, intravitreal injections of Rho kinase inhibitor (Y-33075 dihydrochloride) markedly inhibited LIM and FDM in chicken models, and its effect was associated with a decreased IOP in a dose-dependent manner.

6.1 Introduction

The regulation of eye growth is understood to be a negative feedback homeostasis process, dependent on growth and stop signals within a cell signal transduction pathway network, initiated by optical signals and extracellular biochemical signaling ligands [313]. In experimental lens-induced myopia (LIM) animal models, mounting a negative lens on the animal's eyes causes images to focus behind the retina, known as hyperopic defocus. This hyperopic defocus is the optical signal that activates the biochemical pathway network associated with "Growth," leading to an accelerated axial elongation (AL) [97]. Similarly, when the negative lens is removed during recovery, images focus in front of the retina, known as myopic defocus. This myopic defocus activates the biochemical pathway network associated with "Stop," which results in a decreased rate or even termination of AL [142]. The developmental regulation of AL is closely associated with optical signal elements. It is widely accepted that the axial length-related pathway networking is located in the retina and sclera region of the posterior eye segment. Cell signaling pathways associated with cytoskeleton reconstruction are believed to regulate AL under both normal physiological conditions and pathological conditions, including myopia and hyperopia [314]. However, the detailed biochemical and molecular mechanisms underlying myopia pathogenesis remain unclear. Previously, atropine was the only available drug used for treating myopia, but its side effects were intolerable for many children. Currently, more than 1,200 people in Denmark are using 7-MX (7-methylxanthine) tablets to treat myopia without any adverse effects [315]. Therefore, atropine remains a common treatment, and 7-MX (7-methylxanthine) is an important option for controlling myopia.

AL plays an important role in the development of myopia. The sclera is thought to influence eye size biomechanically, facilitating excessive AL during myopia development [316]. This process involves significant remodeling of the sclera, in which its biomechanical properties undergo changes. One key factor of scleral remodeling is the overexpression of α-smooth muscle actin (α-SMA), which has

been implicated in altering the structural integrity and function of the sclera, contributing to the progression of myopia [317]. RhoA/Rho kinase is an important mechano-transduction pathway in differentiating fibroblasts from myofibroblasts. Targeting RhoA/Rho kinase presents a potential strategy for preventing the induction of α -SMA expression associated with remodeling in myopia. In a study on Guinea Pigs, four weeks of Form-deprivation myopia (FDM) treatment significantly increased scleral RhoA protein and expression in this group [317]. Furthermore, the Rho kinase inhibitors (Y27632) were shown to reduce transforming growth factor-β (TGF-β) induced myofibroblast trans-differentiation in primary human peripapillary sclera fibroblasts. These findings suggest that altered fibroblast activity promoted by Rho kinase inhibitors could modify scleral biomechanics and be relevant to myopia treatment [318]. Consequently, pharmacological testing shows that the ocular growth of animal eyes can be modulated by targeting Rho-associated Protein Kinase. Interestingly, Y-33075 was identified as the hydrochloric salt form of the core chemical structure CID-18754163. Y-33075, in its free base form, is referred to as Y-39983. This compound serves as a tool drug because of its enzymatic inhibitory effects on Rho-associated Protein Kinase.

Several studies demonstrated that Rho kinase and Rho GTPase inhibitors effectively increased aqueous humour drainage in the trabecular meshwork (TM), leading to a reduction in IOP [319, 320]. These inhibitors induce temporary changes in cell morphology and interactions, which lead to increased aqueous outflow and lowered IOP. In human trabecular meshwork (HTM) and Schlemm's canal (SC) cell cultures, the Rho kinase inhibitor Y-27632 (10-100 μM) significantly reduced actin fibres, cell adhesions, and myosin phosphorylation while increasing SC cell permeability by 80%[321]. Animal studies have demonstrated that Rho kinase inhibitors induced morphological changes in TM and juxtacanalicular areas, resulting in rapid and long-lasting IOP reductions, effective within 30 minutes and lasting up to 12 hours [322, 323]. Another animal study found that H-1152P, a potent Rho kinase inhibitor, effectively lowered IOP in both normal and ocular hypertensive rabbits, with maximum effects observed between 60-90 minutes post-application [324]. Similarly,

Y-27632 improved aqueous outflow in bovine eyes by promoting separation between the juxtacanalicular connective tissue and the inner wall of the aqueous plexus [325]. These findings highlight that juxtacanalicular region is a key target for anti-glaucoma therapies. Clinically, Netarsudil (0.02% ophthalmic solution) effectively reduced IOP by 5-6 mmHg by increasing aqueous outflow, and Ripasudil lowered IOP by 20-30% in patients with primary open-angle glaucoma [326, 327]. Considering the critical role of Rho kinase in cytoskeletal regulation and scleral remodeling, we hypothesized that inhibiting Rho kinase would prevent the development of experimental myopia. Therefore, this study aimed to investigate the effects of the Rho kinase inhibitor Y-33075 dihydrochloride on the development of FDM and LIM in the chick model.

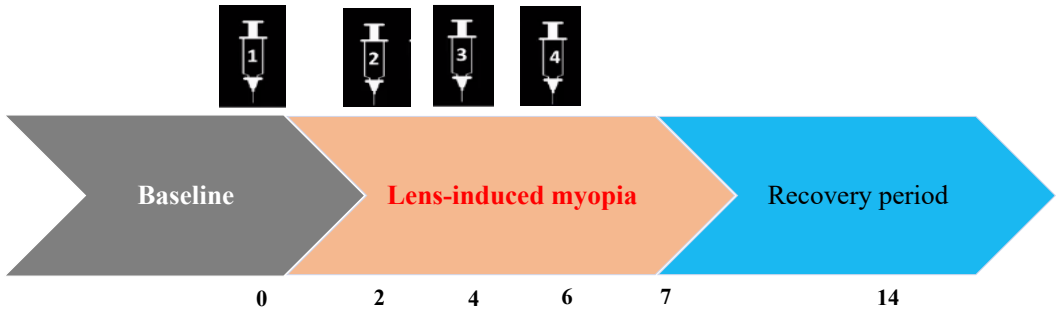
6.2 Methods

6.2.1 Animals

Chapter 2 explains the ethical guidelines and procedures for lens-induced myopia (LIM) and Form-deprivation myopia (FDM), as well as the methods of refractive error and Choroidal thickness determination.

6.2.2 Intraviral Injections

Visual Experience in Lens-induced Myopia (LIM)



Visual Experience in Form-deprivation Myopia (FDM)

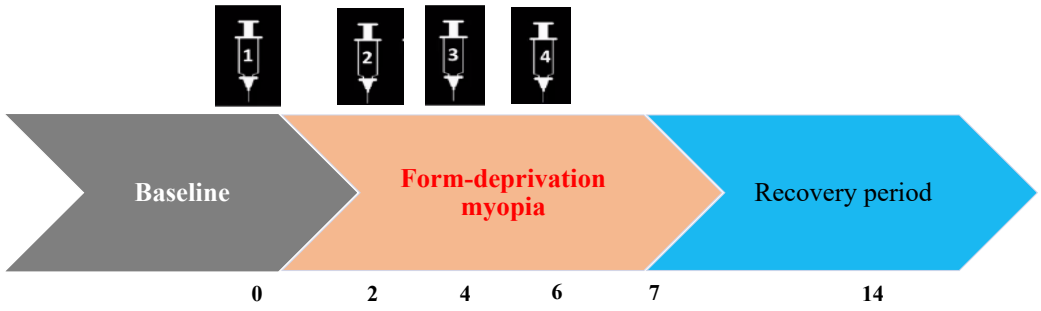


Figure 6.1 A schematic diagram of the overall days of visual experience time points and intravitreal injection procedures for baseline, treatment time points, and recovery time points in both (a) LIM and (b) FDM models.

In experiment I, 40 chicks were randomly divided into four groups. Y-33075 dihydrochloride (FDM) treated groups received doses of 1 nmol (n=10), 10 nmol (n=10), and 100 nmol (n=10), while the control group received (10% DMSO + 90% Corn oil) (n=10). In experiment II, 40 chicks were randomly divided into four groups. Y-33075 dihydrochloride (LIM)- treated groups received doses of 1 nmol (n=10), 10 nmol (n=10), and 100 nmol (n=10), while the control-treated group received 10% DMSO + 90% Corn oil (n=10). During the experiment, we excluded chickens with corneal infection or lens dislocation.

Intravitreal injections were performed every alternative day at the same time between (10:00 am - 12:00 pm), which helps to maintain the circadian rhythm, avoid growth-retardation effects, pain, and ocular infections. Chicks were anesthetized with 0.5% isofluorane in 50% oxygen (O2) and 50% nitrous oxide (N2O). Ethanol was used to clean the surface of the injected eye and lid retractor for disinfection. A 20 µl aliquot of the solution was injected into the dorsal quadrant of the eye using a 30-gauge needle attached to a 25 µl Hamilton Gastight Syringe. The needle was inserted approximately 3 to 4 mm deep into the chick' s eye. The needle was held for three to five seconds and slowly removed to prevent backflow. Different places were used for following intravitreal injections to avoid conjunctival scarring and backflow through the injection holes. On day 7, all lenses were removed, and the eyes were allowed to recover from experimentally induced myopia.

6.2.3 Preparation of the Intravitreal Injections

Drugs were dissolved in 10% dimethyl sulfoxide DMSO (41640-500ml; Sigma-Aldrich; Waltham, MA, USA) and 90% Corn Oil (C8267-500ML; Sigma-Aldrich;) at room temperature and gently vortexed for 30 seconds three times to fully dissolve the higher concentrations of the drug. Fresh solutions were prepared each time for intravitreal injections to prevent contamination and ensure maximum drug efficacy.

The selected tested doses were based on atropine concentrations shown to significantly inhibit FDM in a chick model [135].

6.2.4 Rationale for Drug Selection

Rho kinase inhibitor (Y-33075 dihydrochloride) was chosen because Rho kinase is involved in various cellular processes, including smooth muscle contraction, cell adhesion, and cell migration. Secondly, Y-33075 (the free base form of Y-39983) was used as it is derived from Y-27632, the first Rho kinase inhibitor [328]. Its inhibitory effect on Rho kinase is 30 times stronger than that of Y-27632 and has higher stability [329]. The higher potency is beneficial as a lower concentration of the drug can exert the desired magnitude of effect, thus lowering the chance of toxicity. Thirdly, Rho kinase inhibitors regulate the contractility of cells in the trabecular meshwork, which results in increased outflow of aqueous humor, reduces resistance in the trabecular meshwork, and leads to lower intraocular pressure (IOP).

6.2.5 Intraocular Pressure (IOP) Measurement

Intraocular pressure (IOP) was assessed using an iCare tonometer (Model: TV02), chosen for its accuracy and ease of use. Measurements were taken early in the day, between 9 and 10 am, to account for diurnal variations in IOP. Baseline IOP measurements were recorded before the initiation of induced myopia. Following the treatment period, IOP was measured again one day after the final injection, just before the removal of the lenses. This ensured a clear understanding of the effects of the treatments administered. IOP was measured three times for each eye, and the average of these measurements was used for statistical analysis.

6.2.6 Ocular biometry measurement

Ocular biometry was assessed using high-frequency A-scan ultrasonography employing a 20 MHz probe (custom-made) on days 0 (Baseline), 7, and 14, respectively. Each measurement involved capturing three traces of each eye, which were subsequently analyzed offline. The reported values were calculated as the sum of anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL).

6.3 Statistics

Graph Pad Prism (8) software was used for the data analysis. All measurements were expressed as the means of interocular difference (IOD), calculated as the values for the (treated eye minus untreated eye in each chick, and presented as the mean ± SD). One-way ANOVA was used to compare the different drug dose groups, and Tukey’ s post hoc test was applied to determine the least significant difference between the groups when p was less than 0.05. For the recovery of data, the IOD was calculated as the values for the (IOD, treated eye -recovery eye) and presented as the mean ± SD, using a paired t-test. The strength of the association between the refractive error versus vitreous chamber depth and refractive error versus axial length, IOP, was assessed using Pearson's correlation coefficient (R2).

6.4 Results

6.4.1 Effect of Y-33075 dihydrochloride on Lens-induced Myopia

Baseline data indicated no significant differences in refractive error and ocular biometry outcomes between the treated and control eyes, as summarized in Table 6.1. After seven days of treatment, the effect of Rho kinase inhibitor (Y-33075 dihydrochloride) on the LIM and FDM myopia models was dose-dependent. The different doses demonstrated a clear relationship between the dose and treatment effectiveness. This treatment significantly inhibited both induced myopia and changes in ocular parameters. IOD in refractive errors (Diopter, mean ± SD) at day 7, when compared to the control group (DMSO + Corn oil), were as follows: -8.56 ± 2.70D versus (Y-33075 dihydrochloride at 1 nmol) -7.29 ± 2.82D, p = 0.72; (Y-33075 dihydrochloride at 10 nmol) -2.72 ± 2.53D p = 0.001; and (Y-33075 dihydrochloride at 100 nmol) -3.87 ± 2.69D, p = 0.01; See Table 6.2 and Figures 6.2.a. The administered doses of Y-33075 dihydrochloride at 10 and 100 nmol significantly inhibited shorter ACD induced by LIM compared with the control group (DMSO + Corn oil), whereas 1 nmol had no effect (Table 6.2 and Figure 6.2.b). However, there was no significant difference in lens thickness between the treated and control groups induced by LIM (Table 6.2 and Figure 6.2.c). In addition, the findings showed that higher doses of Y-33075 dihydrochloride (10 and 100 nmol) significantly decreased VCD elongation compared to the control (IOD of VCD, DMSO + Corn oil) 0.64 ± 0.25 mm versus (Y-33075 dihydrochloride at 10 nmol) 0.23 ± 0.25 mm p = 0.004; and (Y-33075 dihydrochloride at 100 nmol) 0.29 ± 0.20 mm, p = 0.01; Table 6.2 and Figures 6.2.d. In contrast, Y-33075 dihydrochloride at 10 and 100 nmol significantly inhibited AL in the treated chicks compared to the controls (IOD of AL, DMSO + Corn oil): 0.76 ± 0.24 mm versus (Y-33075 dihydrochloride at 10 nmol) 0.26 ± 0.23 mm, p=0.001; and (Y-33075 dihydrochloride at 100 nmol) 0.37 ± 0.23 mm, p = 0.01; Table 6.2 and Figures 6.2. e. Although choroidal thickness markedly increased at all tested doses of Y-33075 dihydrochloride, it was statistically significant only for Y-

33075 dihydrochloride at 10 nmol doses when compared to the control group (Table 6.2 and Figures 6.2.f).

6.4.2 Recovery From Lens-induced Myopia

The recovery effects of Y-33075 dihydrochloride from LIM showed that all treated groups demonstrated signs of recovery in refractive error and ocular biometry after lens removal and cessation of Y-33075 dihydrochloride injections. As shown in Figure 6.3, myopia recovery differed significantly across groups at the end of the 7-day recovery period. The IOD in refractive errors (Diopter, mean ± SD) at day 14 compared with the control (DMSO + Corn oil) was 7.31 ± 2.18D versus 5.87 ± 2.62D (1 nmol), $p > 0.54$; 1.88 ± 2.64D (10 nmol), $p < 0.001$; and 2.439 ± 2.37D (100 nmol), $p < 0.01$ respectively (Table 6.3 and Figure 6.3.a). There were no significant differences in lens thickness between the treated and control groups during the recovery period (Table 6.3 and Figure 6.3.b). However, lower doses of Y-33075 dihydrochloride resulted in slightly increased lens thickness compared to the DMSO + Corn oil (IOD of LT: -0.02 ± 0.07 mm; versus 1 nmol 0.04 ± 0.10 mm, $p = 0.05$); Table 6.3 and Figure 6.3.c. Only the higher doses of Y-33075 dihydrochloride (10 and 100 nmol) showed significantly reduced elongation of VCD (IOD of VCD: -0.56 ± 0.23 mm versus 10 nmol: -0.16 ± 0.13 mm, $p = 0.001$; and 100 nmol: -0.17 ± 0.15 mm, $p = 0.01$ (Table 6.3 and Figure 6.3.d). Furthermore, higher doses of Y-33075 dihydrochloride (10 and 100 nmol) showed markedly reduced elongation of AL compared to control (DMSO + Corn oil: -0.64 ± 0.14 mm versus 10 nmol: -0.16 ± 0.22 mm, $p = 0.001$; and 100 nmol: -0.23 ± 0.18 mm, $p = 0.01$), (Table 6.3 and Figure 6.3.e). Choroidal thickness of 10 and 100 nmol Y-33075 dihydrochloride-treated chicks was significantly increased compared to the control (IOD of ChT: 23.77 ± 10.18 μm versus 10 nmol: 7.02 ± 9.48 μm, $p = 0.01$, 100 nmol: 6.20 ± 7.19 μm, $p < 0.001$); Table 6.3 and Figure 6.3.f.

6.4.3 Effect of Y-33075 dihydrochloride on Form-deprivation Myopia

FDM (Baseline) data indicated no significant differences in refractive error and ocular biometry outcomes between treated eyes and control eyes, as summarized in Table 6.1. IOD in refractive errors (Diopter, mean ± SD) at day 7 induced by FDM compared to the control (DMSO + Corn oil) were as follows: -7.51 ± 3.95D versus (Y-33075 dihydrochloride at 1 nmol) -6.66 ± 1.90D, p = 0.72; (Y-33075 dihydrochloride at 10 nmol) -3.09 ± 2.39D p = 0.01; and (Y-33075 dihydrochloride at 100 nmol) -2.94 ± 2.42D, p = 0.01; Table 6.2 and Figures 6.4.a. As expected, all tested doses of Y-33075 dihydrochloride at 1, 10, and 100 nmol reduced the ACD length, but only the marked inhibition by Y-33075 dihydrochloride at 10 nmol was statistically significant (Table 6.2 and Figure 6.4.b). In accordance, only Y-33075 dihydrochloride at 10 nmol significantly altered lens thickness compared to the control group induced by FDM (Table 6.2 and Figures 6.4.c). Although both intravitreal administration of Y-33075 dihydrochloride at 10 and 100 nmol significantly decreased VCD elongation compared to the control group (IOD of VCD, DMSO + Corn oil - were 0.60 ± 0.21 mm versus (Y-33075 dihydrochloride at 10 nmol) 0.12 ± 0.21 mm p = 0.004; and (Y-33075 dihydrochloride at 100 nmol) 0.26 ± 0.19 mm, p = 0.01; Table 6.2 and Figures 6.4.d. Intravitreal injection of doses of Y-33075 dihydrochloride at 10 and 100 nmol also significantly inhibited the excessive AL to the control group (IOD of AL, DMSO + Corn oil - were 0.67 ± 0.32 mm versus (Y-33075 dihydrochloride at 10 nmol) 0.26 ± 0.21 mm p = 0.001; and (Y-33075 dihydrochloride at100 nmol) 0.30 ± 0.24 mm, p = 0.01; Table 6.2 and Figures 6.4.e. Similarly, only Y-33075 dihydrochloride at 10 and 100 nmol significantly inhibited the trend of reduced ChT, while the control group shows an excessive decreasing trend (IOD of AL, DMSO + Corn oil - were -31.00 ± 8.41 μm versus (Y-33075 dihydrochloride at 10 nmol) -7.62 ± 8.40 μm p = 0.001; and (Y-33075 dihydrochloride at 100 nmol) -4.66 ± 5.68 μm, p = 0.01; Table 6.2 and Figures 6.4.f.

6.4.4 Recovery From Form-deprivation Myopia

The recovery of Y-33075 dihydrochloride treated chicks from FDM showed that all demonstrated signs of recovery in refractive error and ocular biometry after lens removal and cessation of Y-33075 dihydrochloride injections. Their eyes became emmetropic, similar to the control eyes. As shown in Figure 6.5, myopia recovery differed significantly across groups at the end of the 7-day recovery period. The IOD in refractive errors (Diopter, mean ± SD) at day 14 compared with the control DMSO + Corn oil was 6.41 ± 4.29D versus 5.07 ± 3.09D (1 nmol), $p > 0.63$; 1.71 ± 2.55D (10 nmol), $p < 0.05$; and 2.11 ± 2.46D (100 nmol), $p < 0.05$ respectively (Table 6.3 and Figure 6.5.a). There were no significant differences in ACD between the treated and control groups during the recovery period (Table 6.3 and Figure 6.5.b). However, lens thickness only slightly increased with lower tested doses of Y-33075 dihydrochloride compared to the control (IOD of LT: -0.03 ± 0.04 mm; versus 1 nmol 0.06 ± 0.11 mm, $p = 0.05$; Table 6.3 and Figure 6.5.c). Only higher doses (10 and 100 nmol) led to significantly decreased VCD elongation (IOD of VCD: -0.51 ± 0.22 mm versus 10 nmol: -0.07 ± 0.08 mm, $p = 0.001$; and 100 nmol: -0.20 ± 0.18 mm, $p = 0.001$ (Table 6.3 and Figure 6.5.d). Similarly, only the higher doses significantly decreased the AL (DMSO + Corn oil: -0.50 ± 0.27 mm versus 10 nmol: -0.09 ± 0.23 mm, $p = 0.001$; and 100 nmol: -0.21 ± 0.27 mm, $p = 0.05$ (Table 6.3 and Figure 6.5.e). In contrast, only the 10 and 100 nmol doses showed significantly increased choroidal thickness compared to the control: 22.57 ± 7.06 μm versus 10 nmol: 7.79 ± 4.29 μm, $p = 0.0001$, 100 nmol: 8.45 ± 4.22 μm, $p < 0.0001$) table 6.3 and figure 6.5.f.).

6.4.5 Effect of Y-33075 dihydrochloride on IOP

All concentrations of intravitreal Y-33075 dihydrochloride effectively reduced IOP in both LIM and FDM-treated groups of chicks. However, chicks receiving the lower doses of Y-33075 dihydrochloride had a higher IOP in both LIM and FDM-treated groups of chicks, similar to those in the control group (DMSO + Corn oil). In the LIM groups, the change in IOP (IOD) from baseline to day 7 when compared to controls was as follows: (DMSO + Corn oil), 0.62 ± 1.49 mm Hg and 5.74 ± 4.14

mm Hg versus (Y-33075 dihydrochloride at 1 nmol), 0.37 ± 1.79 mm Hg and 5.37 ± 4.06 mm Hg, p = 0.99; (Y-33075 dihydrochloride at 10 nmol), 0.12 ± 1.53 mm Hg and 3.0 ± 3.53 mm Hg, p = 0.48; and (Y-33075 dihydrochloride at 100 nmol), 0.5 ± 2.06 mm Hg and -0.62 ± 5.04 mm Hg, p = 0.004; (Table 6.4, Figures 6.8.a). Similarly, in the FDM groups, IOD from baseline to 7 days post-treatment compared to controls was: (DMSO + Corn oil), 0.37 ± 1.57 mm Hg and 5.37 ± 2.39 mm Hg versus (Y-33075 dihydrochloride at 1 nmol), 0.50 ± 1.93 mm Hg and 5.5 ± 1.65 mm Hg, p = 0.99; (Y-33075 dihydrochloride at 10 nmol), 0.12 ± 1.83 mm Hg and 3.0 ± 3.53 mm Hg, p = 0.48; and (Y-33075 dihydrochloride at 100 nmol), 0.25 ± 1.78 mm Hg and -1.37 ± 5.04 mm Hg p = 0.001; (Table 6.4 and Figures 6.8.b). In contrast, the higher dose of Y-33075 dihydrochloride (100 nmol) shows a significant reduction in IOP in both LIM group and FDM group compared to controls. However, the change in IOP from baseline to the end of the treatment period in the high-dose LIM and FDM groups was not statistically significant (p = 0.50, Table 6.4).

6.4.6 Coefficient of Determination Between Refractive Error Versus Vitreous Chamber Depth and Axial Length

A strong correlation was observed between changes in IOD refractive errors and VCD in the LIM group of chicks (R^2 = 0.78; p<0.001; Figure 6.6.a). As well as between changes in IOD refractive errors and axial length (R^2 = 0.82; p<0.001); Figure 6.6.b). Similar trends were also observed in the FDM chicks, where the Pearson correlations between changes in IOD refractive errors and VCD (R^2 = 0.78; p<0.001; Figure 6.7.a) and between changes in IOD refractive errors and axial length (R^2 = 0.84; p<0.001; Figure 6.7.b).

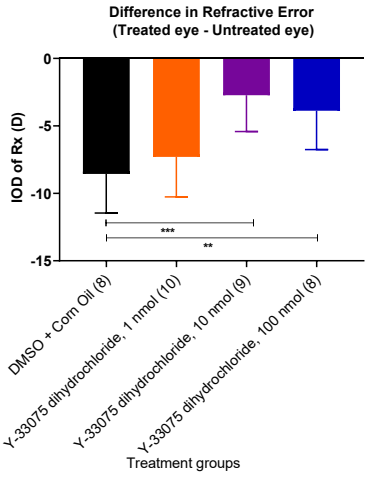
6.4.7 Coefficient of Determination Between Axial Length and IOP

This study found a moderate correlation between changes in IOD axial length (AL) and IOP in response to different doses (1, 10, and 100 nmol) of Y-33075 dihydrochloride. In both the LIM and FDM groups, the correlation coefficient (R^2 = 0.41) indicated a mild relationship between AL and IOP (Figure 6.9a and b). These findings demonstrate that higher doses of Y-33075 dihydrochloride can effectively modulate IOP, subsequently impacting AL in both LIM and FDM models. However, a lower correlation was observed between interocular differences in refractive error and IOP. In LIM group, the coefficient of determination was R^2 = 0.30 (Figure 10a), while in FDM group, the correlation was R^2 =0.27 (Figure 10b). These findings suggest that changes in IOP are moderately associated with refractive errors in both FDM and LIM models.

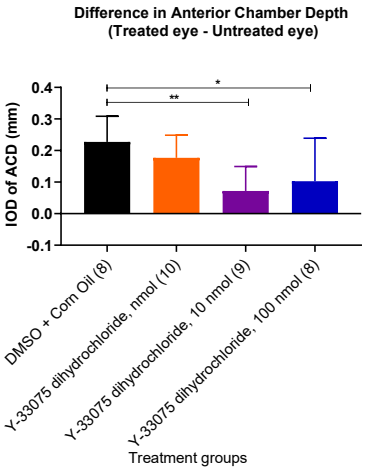
6.4.8 Body Weight

No significant differences in body weight were observed between the treatment and control groups at any time point (Table 6.5). All groups of chicken showed a normal weight gain over time, which is consistent with normal chick development. There was no association between body weight and changes in refractive error or axial length elongation.

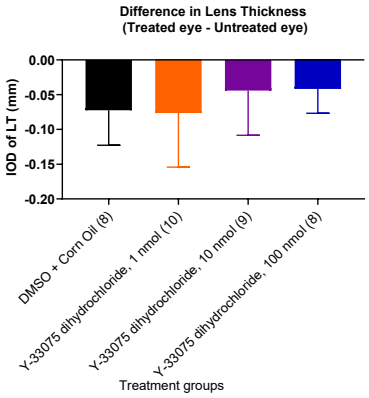
(a)


Difference in Refractive Error
(Treated eye - Untreated eye)
IOD of Rx (D)
0
-5
-10
-15
***
**
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, 1 nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

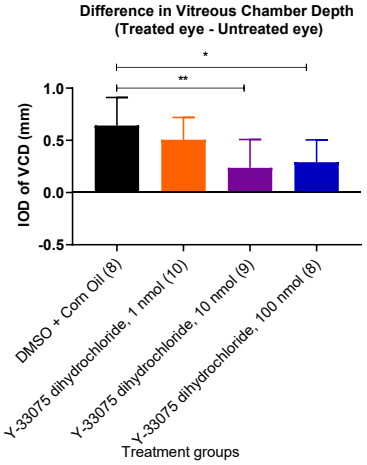
(b)


Difference in Anterior Chamber Depth
(Treated eye - Untreated eye)
IOD of ACD (mm)
0.4
0.3
0.2
0.1
0.0
-0.1
*
**
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

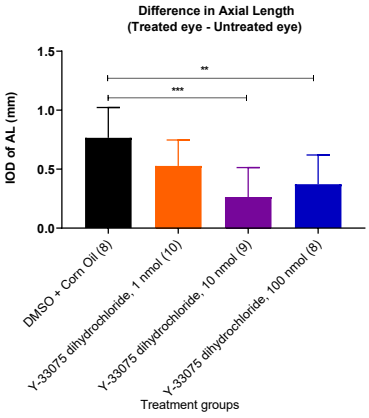
(c)


Difference in Lens Thickness
(Treated eye - Untreated eye)
IOD of LT (mm)
0.00
-0.05
-0.10
-0.15
-0.20
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, 1 nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

(d)


Difference in Vitreous Chamber Depth
(Treated eye - Untreated eye)
IOD of VCD (mm)
1.0
0.5
0.0
-0.5
*
**
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, 1 nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

(e)


Difference in Axial Length
(Treated eye - Untreated eye)
IOD of AL (mm)
1.5
1.0
0.5
0.0
**
***
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, 1 nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

(f)

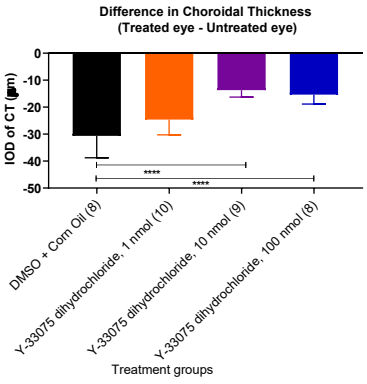
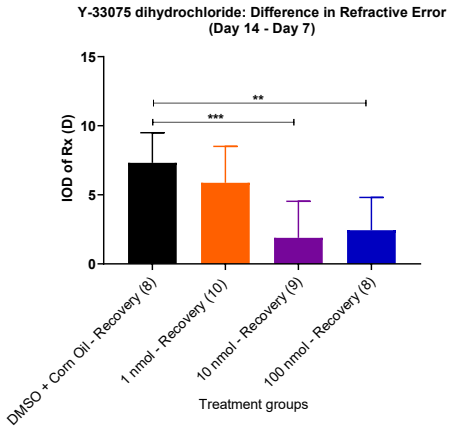
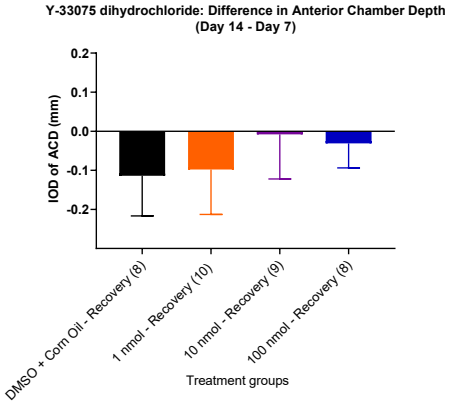

Difference in Choroidal Thickness
(Treated eye - Untreated eye)
IOD of CT (μm)
0
-10
-20
-30
-40
-50
****
****
DMSO + Corn Oil (8)
Y-33075 dihydrochloride, 1 nmol (10)
Y-33075 dihydrochloride, 10 nmol (9)
Y-33075 dihydrochloride, 100 nmol (8)
Treatment groups

Figure 6.2 The interocular differences (IOD, treated eye minus untreated eye) in (a) myopic refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL and (f) ChT after seven days of treatment with Y-33075 dihydrochloride (1, 10 and 100 nmol) on LIM chicks. Only higher doses of (10 nmol and 100 nmol) Y-33075 dihydrochloride significantly inhibited the induced myopic refractive error (a). However, (10 and 100 nmol) doses of Y-33075 dihydrochloride significantly inhibited reduction of ACD (b), VCD (d), and (e) AL. Furthermore, only higher doses of (10 and 100 nmol) Y-33075 dihydrochloride significantly increased the choroidal thickness (f). No significant changes were observed in lens thickness (c). Statistics: One-way ANOVA followed by Tukey's post-hoc test. The error bar denotes standard deviation (SD); the sample size is shown in brackets (n). * $p < 0.05$, **$p < 0.01$ and *** $p < 0.001$.**** $p < 0.0001$.

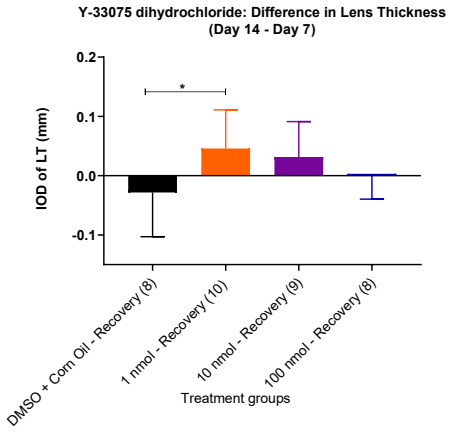
(a)


Y-33075 dihydrochloride: Difference in Refractive Error
(Day 14 - Day 7)
IOD of Rx (D)
15
10
5
0
**
***
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

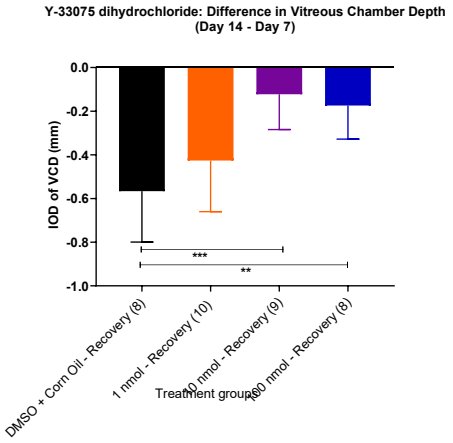
(b)


Y-33075 dihydrochloride: Difference in Anterior Chamber Depth
(Day 14 - Day 7)
IOD of ACD (mm)
0.2
0.1
0.0
-0.1
-0.2
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

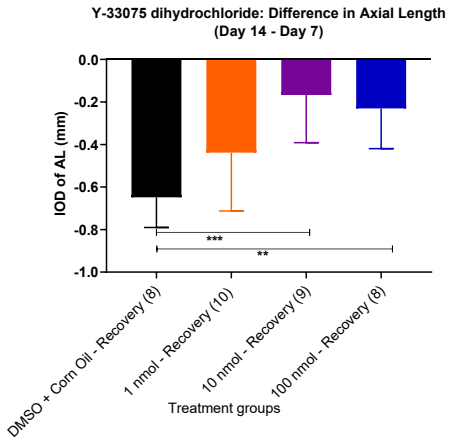
(c)


Y-33075 dihydrochloride: Difference in Lens Thickness
(Day 14 - Day 7)
IOD of LT (mm)
0.2
0.1
0.0
-0.1
*
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

(d)


Y-33075 dihydrochloride: Difference in Vitreous Chamber Depth
(Day 14 - Day 7)
IOD of VCD (mm)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
***
**
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

(e)


Y-33075 dihydrochloride: Difference in Axial Length
(Day 14 - Day 7)
IOD of AL (mm)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
***
**
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

(f)

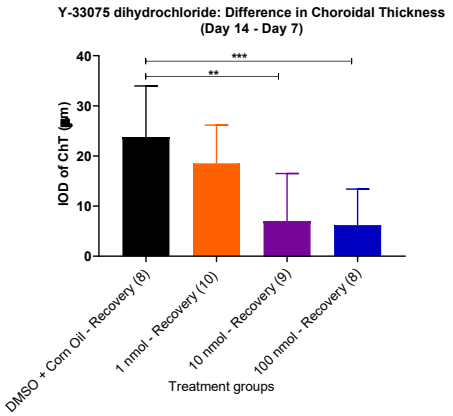
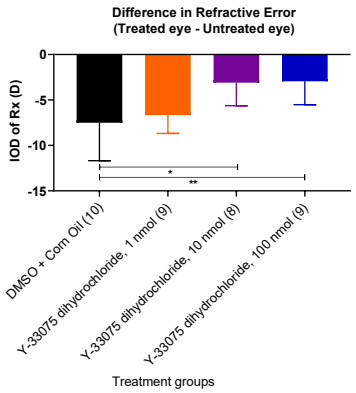
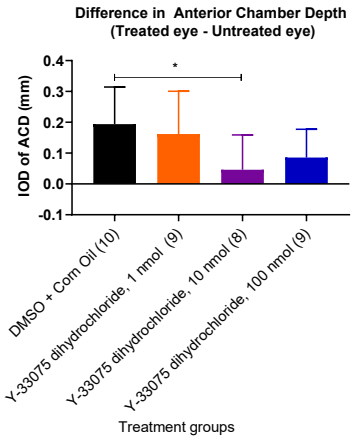

Y-33075 dihydrochloride: Difference in Choroidal Thickness
(Day 14 - Day 7)
IOD of ChT (µm)
40
30
20
10
0
***
**
DMSO + Corn Oil - Recovery (8)
1 nmol - Recovery (10)
10 nmol - Recovery (9)
100 nmol - Recovery (8)
Treatment groups

Figure 6.3 Recovery from lens-induced myopia. The interocular differences in (IOD, recovery eye minus treated eye) in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT seven days after lens removal and cessation of injections of Y-33075 dihydrochloride. Only higher doses of (10 and 100 nmol) Y-33075 dihydrochloride significantly increased induced myopia (a). There were no differences in ACD (b). However, the lower tested dose of Y-33075 dihydrochloride (1 nmol) significantly increased lens thickness (c). Higher tested doses (10 and 100 nmol) significantly reduced the reduction of VCD (d) and AL (e). In contrast, higher tested doses of (10 and 100 nmol) Y-33075 dihydrochloride significantly increased choroidal thickness (f). Statistics - One-Way ANOVA of unpaired t-test. Error bars denote standard deviation (SD) and the Number in brackets shows sample size (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

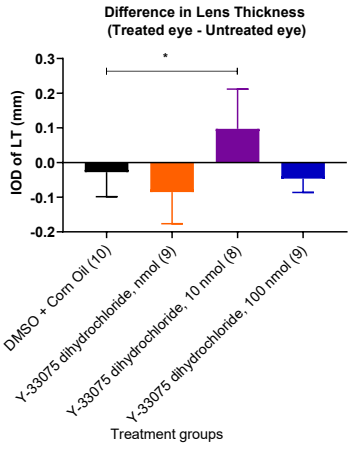
(a)


Difference in Refractive Error
(Treated eye - Untreated eye)
IOD of Rx (D)
0
-5
-10
-15
*
**
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, 1 nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

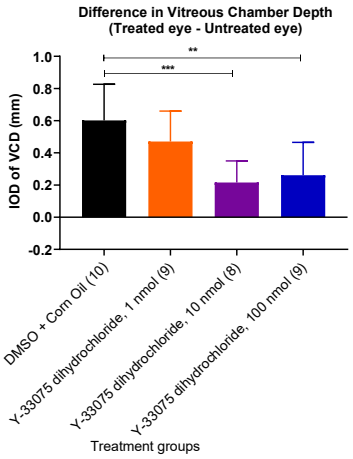
(b)


Difference in Anterior Chamber Depth
(Treated eye - Untreated eye)
IOD of ACD (mm)
0.4
0.3
0.2
0.1
0.0
-0.1
*
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, 1 nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

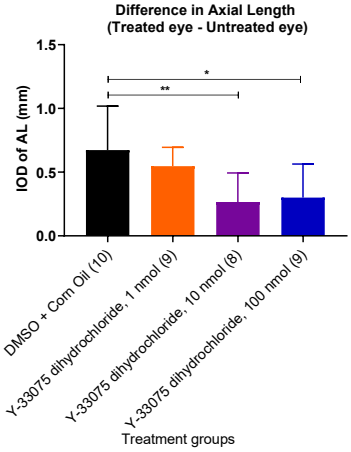
(a)


Difference in Lens Thickness
(Treated eye - Untreated eye)
IOD of LT (mm)
0.3
0.2
0.1
0.0
-0.1
-0.2
*
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

(b)


Difference in Vitreous Chamber Depth
(Treated eye - Untreated eye)
IOD of VCD (mm)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
**
***
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, 1 nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

(c)


Difference in Axial Length
(Treated eye - Untreated eye)
IOD of AL (mm)
1.5
1.0
0.5
0.0
*
**
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, 1 nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

(d)

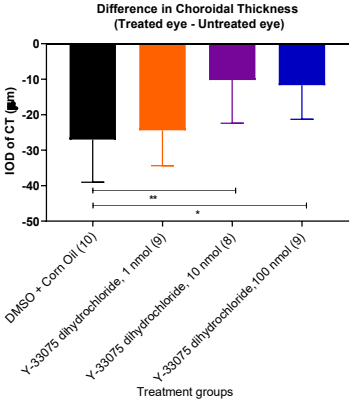
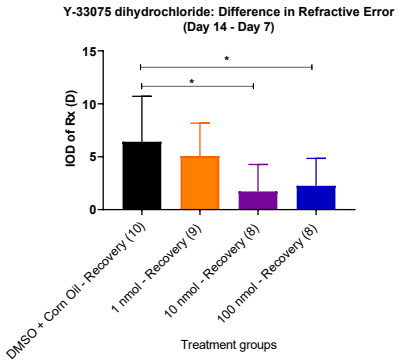
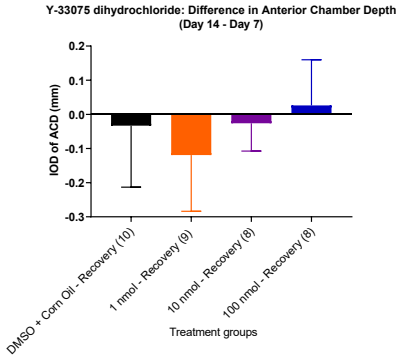

Difference in Choroidal Thickness
(Treated eye - Untreated eye)
IOD of CT (μm)
0
-10
-20
-30
-40
-50
**
*
DMSO + Corn Oil (10)
Y-33075 dihydrochloride, 1 nmol (9)
Y-33075 dihydrochloride, 10 nmol (8)
Y-33075 dihydrochloride, 100 nmol (9)
Treatment groups

Figure 6.4 The interocular differences (IOD, treated eye minus untreated eye) in (a) myopic refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL and (f) ChT measured after 7 days of treatment with Y-33075 dihydrochloride (1, 10 and 100 nmol) of FDM chicks. Only higher doses (10 nmol and 100 nmol) significantly inhibited the induced myopic refractive error (a). However, only the 10 nmol concentration significantly decreased ACD (b) and LT (c). Furthermore, the higher doses (10 and 100 nmol) significantly affected the reduction of VCD (d) and (e) AL. In addition, only the higher doses (10 and 100 nmol) significantly increased the choroidal thickness (f). Statistics: One-way ANOVA followed by Tukey's post-hoc test. Error bar denotes standard deviation (SD); sample sizes are shown in brackets (n). * $p < 0.05$, **$p < 0.01$ and *** $p < 0.001$.

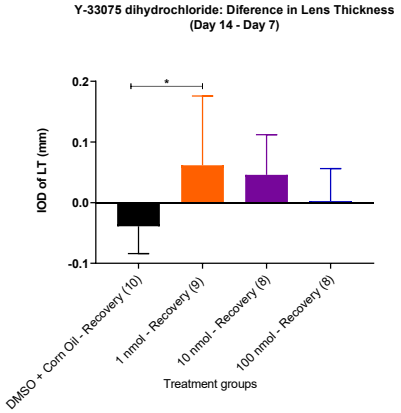
(a)


Y-33075 dihydrochloride: Difference in Refractive Error
(Day 14 - Day 7)
IOD of Rx (D)
15
10
5
0
*
*
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Treatment groups

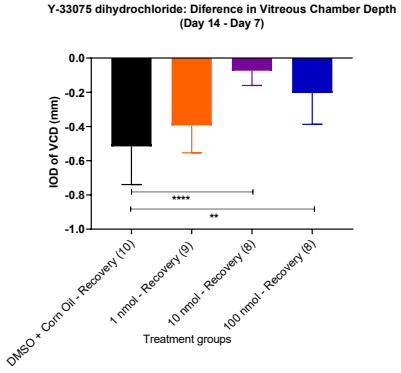
(b)


Y-33075 dihydrochloride: Difference in Anterior Chamber Depth
(Day 14 - Day 7)
IOD of ACD (mm)
0.2
0.1
0.0
-0.1
-0.2
-0.3
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Treatment groups

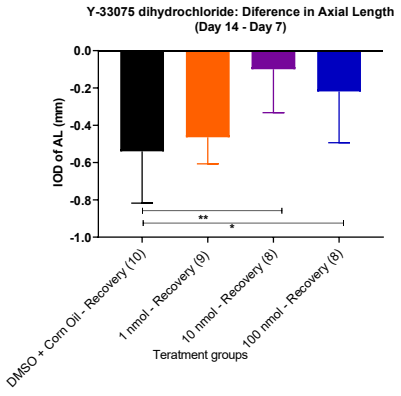
(c)


Y-33075 dihydrochloride: Diference in Lens Thickness
(Day 14 - Day 7)
IOD of LT (mm)
0.2
0.1
0.0
-0.1
*
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Treatment groups

(d)


Y-33075 dihydrochloride: Diference in Vitreous Chamber Depth
(Day 14 - Day 7)
IOD of VCD (mm)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
****
**
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Treatment groups

(e)


Y-33075 dihydrochloride: Diference in Axial Length
(Day 14 - Day 7)
IOD of AL (mm)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
**
*
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Teratment groups

(f)

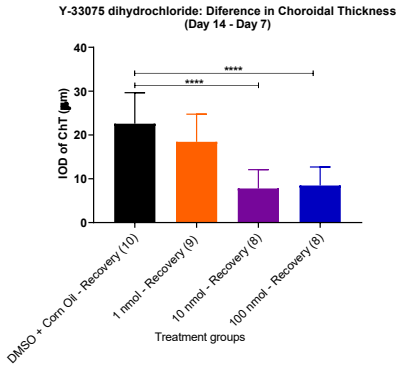
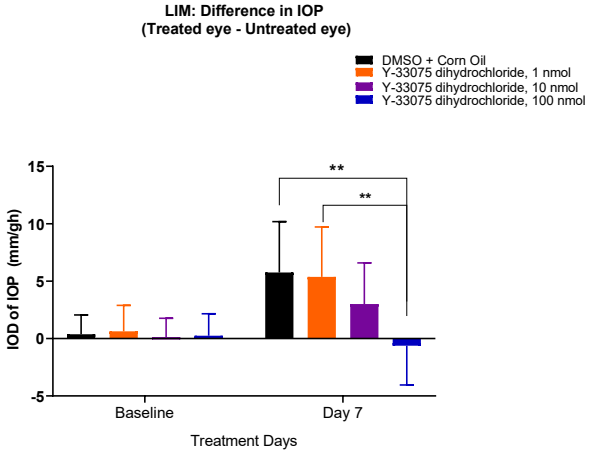

Y-33075 dihydrochloride: Diference in Choroidal Thickness
(Day 14 - Day 7)
IOD of ChT (μm)
40
30
20
10
0
****
****
DMSO + Corn Oil - Recovery (10)
1 nmol - Recovery (9)
10 nmol - Recovery (8)
100 nmol - Recovery (8)
Treatment groups

Figure 6.5 Recovery from form-deprivation myopia. The interocular differences in (IOD, recovery eye minus treated eye) in (a) refractive error, (b) ACD, (c) LT, (d) VCD, (e) AL, and (f) ChT seven days after lens removal and cessation of injections of Y-33075 dihydrochloride. Only higher doses (10 and 100 nmol) of Y-33075 dihydrochloride significantly increased induced myopia (a). However, the lower dose (1 nmol) significantly increased lens thickness (c). Higher tested doses of (10 and 100 nmol) significantly prevented reduction of VCD (d) and AL (e). In contrast, higher tested doses of (10 and 100 nmol) significantly increased choroidal thickness (f). Statistics - One-Way ANOVA of unpaired t-test. Error bars denote standard deviation (SD) and the number in brackets shows the sample size (n). * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

(a) Interocular difference (IOD) of IOP between different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on LIM



(b) Interocular differences (IODs) of IOP between different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on FDM

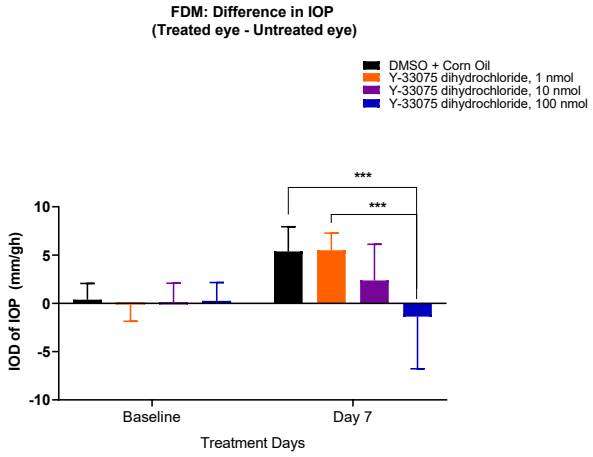
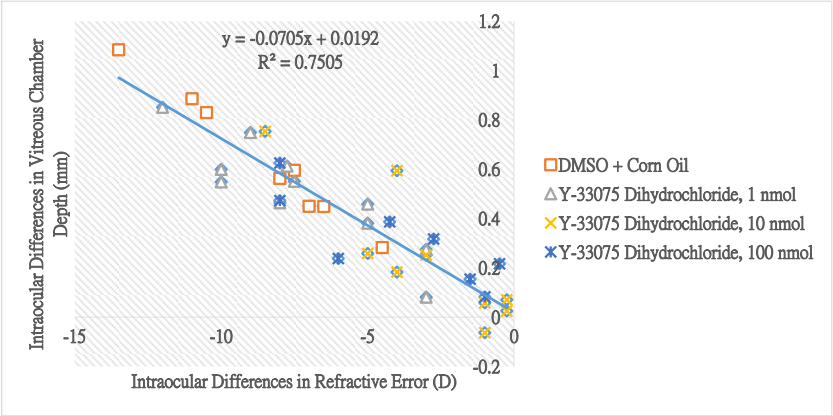


Figure 6.6 Line graph of the intraocular pressure (IOP, mmHg) on FDM and LIM. The interocular differences in IOP were recorded as (mean ± SEM, mm Hg) from baseline to end of the treatment period with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride (a) LIM group and (b) FDM group. Both treated groups showed significantly lower IOP at the higher tested doses (10 and 100 nmol) * $p < 0.05$, **$p < 0.01$ and*** $p < 0.001$.

(a) Correlation of interocular differences in refractive errors with vitreous chamber depth in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on LIM



(b) Correlation of interocular differences in refractive errors and axial length in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on LIM

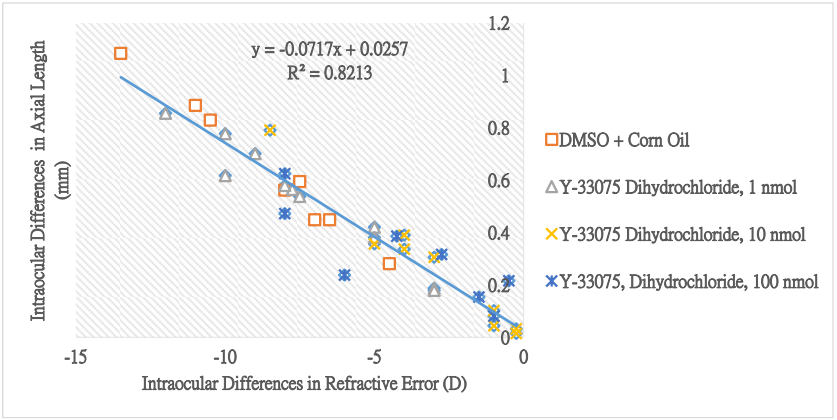
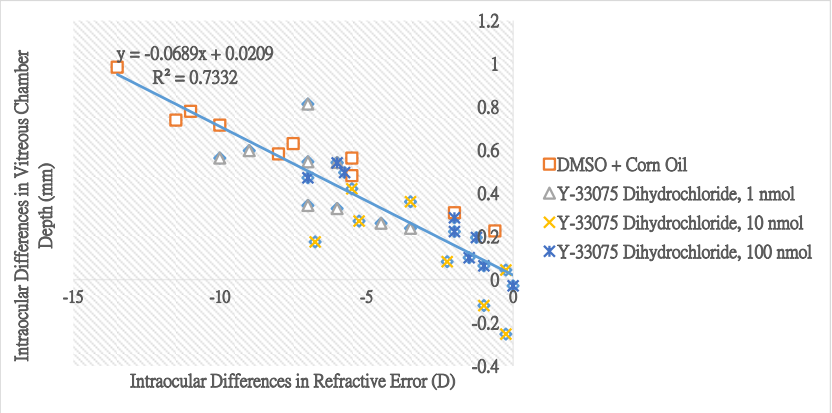


Figure 6.7 Coefficient of determination between (a) interocular differences in refractive errors and vitreous chamber depth and (b) between refractive errors and axial length on day 7 in the LIM group (R^2 = 0.75; $p<0.001$) and (0.82, $p<0.001$).

(a) Correlation of interocular differences in refractive errors with vitreous chamber depth in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on FDM



(b) Correlation of interocular differences in refractive errors and axial length in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on FDM

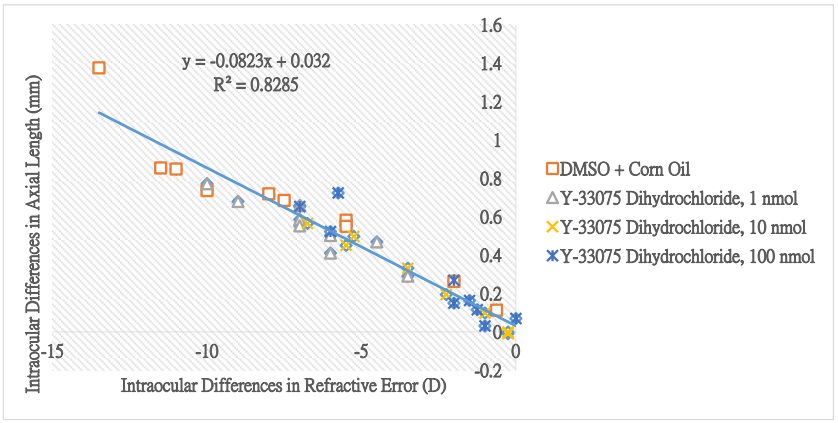
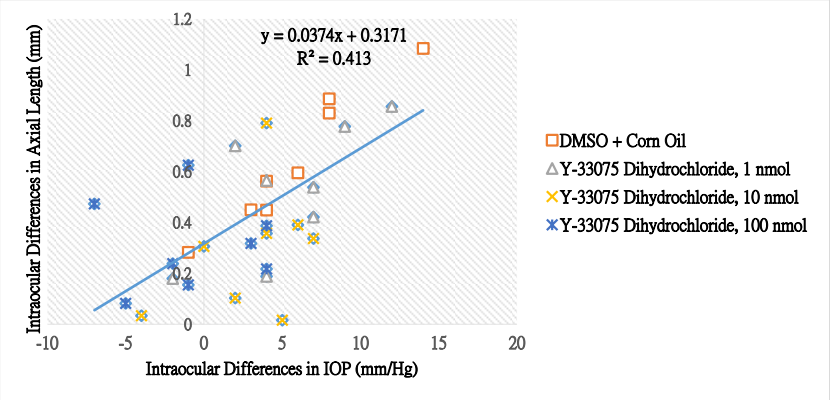


Figure 6.8 Coefficient of determination between (a) interocular differences in refractive errors and vitreous chamber depth and (b) between refractive errors and axial length on day 7 in the FDM group (R^2 = 0.73; $p<0.001$) and (0.82; $p<0.001$).

(a) Correlation of interocular differences in axial length and intraocular pressure (IOP) in chicks treated with different doses of (1, 10, and 100 nmol) on LIM



(b) Correlation of interocular differences in axial length and intraocular pressure (IOP) in chicks treated with different doses of (1, 10, and 100 nmol) on FDM

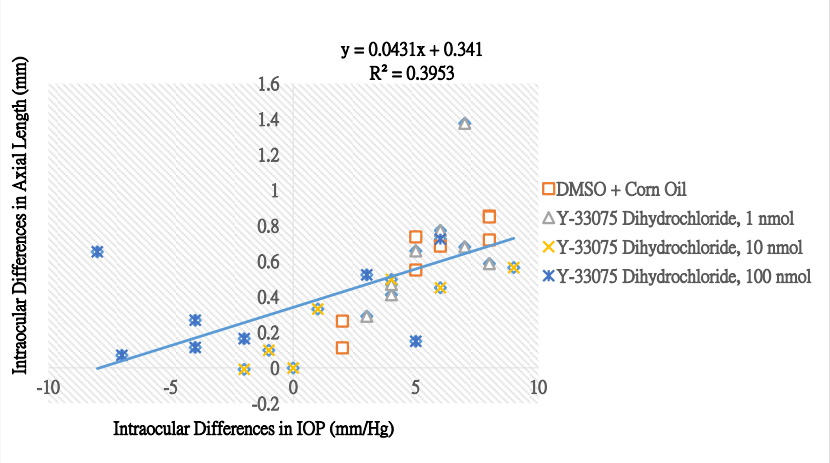
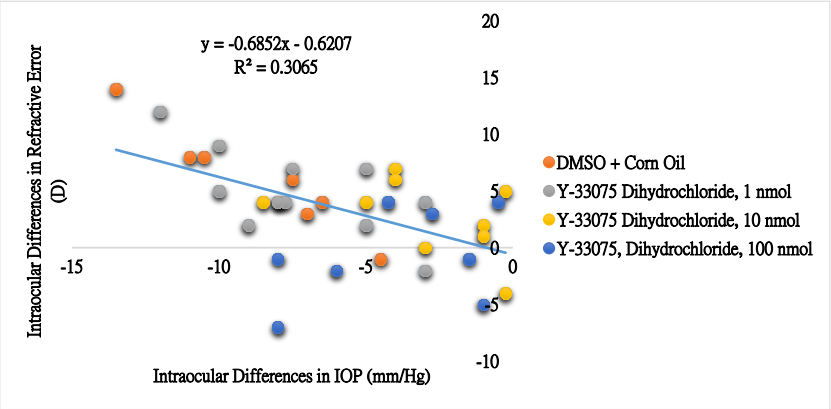


Figure 6.9 The changes in intraocular pressure (IOP) over a 7-day treatment period with Y-33075 dihydrochloride in both LIM and FDM groups of chicks. Coefficient of determination between interocular differences in AL and IOP on LIM group (a); (R^2 = 0.41 and $p<0.001$) and FDM group (b); (R^2 = 0.39 and $p<0.001$).

(a) Correlation of interocular differences in refractive error and intraocular pressure (IOP) in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride on LIM



(b) Correlation of interocular differences in refractive error and intraocular pressure (IOP) in chicks treated with different tested doses of (1, 10, and 100 nmol) Y-33075 dihydrochloride in FDM

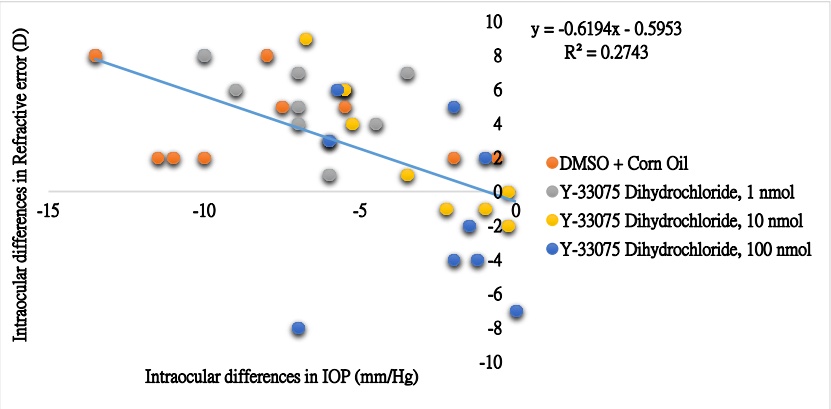


Figure 6.10 shows the changes in intraocular pressure (IOP) over the 7-day treatment period with Y-33075 dihydrochloride in chicks from both the LIM and FDM groups. Coefficient of determination between interocular differences in refractive error and IOP for the LIM group (a); ($R^2 = 0.30$ and $p<0.001$) and FDM group (b); ($R^2 = 0.27$ and $p<0.01$).

Table 6.1 Summarized baseline data interocular difference (IOD) in refractive error (D) and ocular parameters (mm) at day 0 (Baseline)

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
Lens-induced Myopia (LIM)						
DMSO + Corn Oil	-0.02 (0.25)	-0.01 (0.01)	0.02 (0.02)	0.01 (0.04)	0.02 (0.04)	1.16 (5.28)
1 nmol, Y-33075 dihydrochloride	-0.01 (0.03)	-0.04 (0.11)	0.00 (0.02)	-0.01 (0.04)	-0.05 (0.13)	1.04 (5.01)
10 nmol, Y-33075 dihydrochloride	-0.12 (0.27)	-0.02 (0.03)	-0.05 (0.07)	0.01 (0.05)	0.04 (0.02)	-1.82 (7.95)
100 nmol, Y-33075 dihydrochloride	0.02 (0.09)	-0.03 (0.10)	0.00 (0.02)	-0.00 (0.04)	- 0.03 (0.12)	2.04 (8.48)
Form-deprivation Myopia (FDM)						
DMSO + Corn Oil	-0.02 (0.37)	0.00 (0.01)	0.01 (0.01)	0.03 (0.04)	0.06 (0.05)	-3.08 (5.40)
1 nmol, Y-33075 dihydrochloride	0.04 (0.20)	-0.01 (0.01)	0.02 (0.02)	0.00 (0.01)	0.01 (0.02)	1.93 (16.96)
10 nmol, Y-33075 dihydrochloride	-0.07 (0.18)	-0.00 (0.19)	0.00 (0.01)	-0.00 (0.03)	-0.00 (0.04)	-1.27 (8.254)
100 nmol, Y-33075 dihydrochloride	-0.17 (0.29)	-0.00 (0.01)	0.00 (0.02)	0.04 (0.05)	0.04 (0.05)	-2.25 (4.83)

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 6.2 Summary of data treatment effects on Y-33075 dihydrochloride on day 7, interocular difference (IOD) in refractive error (D), and ocular parameters (mm).

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
Lens-induced Myopia (LIM)						
DMSO + Corn Oil	-8.56 (2.70)	0.22 (0.07)	-0.07 (0.04)	0.64 (0.25)	0.76 (0.24)	-33.16 (12.35)
1 nmol, Y-33075 dihydrochloride	-7.29 (2.82)	0.17 (0.06)	-0.07 (0.07)	0.50 (0.20)	0.52 (0.20)	-22.33 (12.54)
10 nmol, Y-33075 dihydrochloride	-2.72 (2.53)	0.07 (0.07)	-0.04 (0.06)	0.23 (0.25)	0.26 (0.23)	-11.81 (9.00)
100 nmol, Y-33075 dihydrochloride	-3.87 (2.69)	0.10 (0.12)	-0.04 (0.03)	0.29 (0.20)	0.37 (0.23)	-15.47 (11.52)
Form-deprivation Myopia (FDM)						
DMSO + Corn Oil	-7.51 (3.95)	0.19 (0.11)	-0.02 (0.06)	0.60 (0.21)	0.67 (0.32)	-31.00 (8.41)
1 nmol, Y-33075 dihydrochloride	-6.66 (1.90)	0.16 (0.13)	-0.08 (0.08)	0.47 (0.17)	0.54 (0.13)	-29.96 (8.70)
10 nmol, Y-33075 dihydrochloride	-3.09 (2.39)	0.04 (0.10)	0.09 (0.10)	0.12 (0.21)	0.26 (0.21)	- 7.62 (8.40)
100 nmol, Y-33075 dihydrochloride	-2.94 (2.42)	0.08 (0.08)	-0.04 (0.03)	0.26 (0.19)	0.30 (0.24)	-4.66 (5.68)

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 6.3 Summary data for recovery effects of Y-33075 dihydrochloride from FDM and LIM on day 14, interocular difference (IOD) in refractive error (D), and ocular parameters (mm).

Category	IOD of Rx (D)	IOD of ACD (mm)	IOD of LT (mm)	IOD of VCD (mm)	IOD of AL (mm)	IOD of ChT (μm)
Lens-induced Myopia (LIM)						
DMSO + Corn Oil	7.31 ± 2.18	-0.11 ± 0.10	-0.02 ± 0.07	-0.56 ± 0.23	-0.64 ± 0.14	23.79 ± 10.18
1 nmol, Y-33075 dihydrochloride	5.87 ± 2.62	-0.09 ± 0.11	0.04 ± 0.06	-0.42 ± 0.23	-0.43 ± 0.27	18.52 ± 7.64
10 nmol, Y-33075 dihydrochloride	1.88 ± 2.64	-0.00 ± 0.11	0.03 ± 0.05	-0.12 ± 0.16	-0.16 ± 0.22	7.02 ± 9.48
100 nmol, Y-33075 dihydrochloride	2.43 ± 2.37	-0.03 ± 0.06	-0.00 ± 0.03	-0.17 ± 0.15	-0.23 ± 0.18	6.20 ± 7.19
From-deprivation Myopia (FDM)						
DMSO + Corn Oil	6.41 ± 4.29	-0.03 ± 0.17	-0.03 ± 0.04	-0.51 ± 0.22	-0.50 ± 0.27	22.57 ± 7.06
1 nmol, Y-33075 dihydrochloride	5.07 ± 3.09	-0.11 ± 0.16	0.06 ± 0.11	-0.39 ± 0.16	-0.46 ± 0.14	18.44 ± 6.29
10 nmol, Y-33075 dihydrochloride	1.71 ± 2.55	-0.02 ± 0.08	0.04 ± 0.06	-0.07 ± 0.08	-0.09 ± 0.23	7.79 ± 4.29
100 nmol, Y-33075 dihydrochloride	2.11 ± 2.46	-0.02 ± 0.13	0.00 ± 0.05	-0.20 ± 0.18	-0.21 ± 0.27	8.45 ± 4.22

IOD: Inter-ocular differences; Rx: Refractive Power; ACD: Anterior chamber depth; LT: Lens Thickness; VCD: Vitreous chamber depth; AL: Axial length; CT: Choroidal thickness.

Table 6.4 Summary of IOD in Intraocular Pressure (IOP) effects before and after treatment

Category	Baseline (mean±SD)	Day 7 (mean±SD)	Statistics (P Value)
Lens-induced Myopia (LIM)			
DMSO + Corn Oil	0.62±1.49	5.74±4.14	<0.02
1 nmol, Y-33075 dihydrochloride	0.37±1.79	5.37±4.06	<0.001
10 nmol, Y-33075 dihydrochloride	0.12±1.53	3.0 ±3.35	<0.04
100 nmol, Y-33075 dihydrochloride	0.5 ±2.06	-0.62±5.04	> 0.50
Form-deprivation Myopia (FDM)			
DMSO + Corn Oil	0.37±1.57	5.37±2.39	<0.001
1 nmol, Y-33075 dihydrochloride	0.50±1.93	5.5±1.65	<0.001
10 nmol, Y-33075 dihydrochloride	0.12±1.83	2.37±3.49	>0.18
100 nmol, Y-33075 dihydrochloride	0.25 ±1.78	-1.37±5.04	> 0.47

LIM: Lens-induced Myopia; FDM: Form-deprivation Myopia; DMSO: Dimethyl sulfoxide

Table 6.5 Average body weight (mean±SD) of chickens at baseline, days 7 and 14

Weight of chickens			
Groups	Baseline/g	Day 7/g	Day 14/g
Lens-induced Myopia (LIM)			
DMSO + Corn Oil	48.26 ± 1.97	90.39 ± 8.31	143.94 ± 19.12
1 nmol, Y-33075 dihydrochloride	43.68 ± 3.69	94.11 ± 4.58	169.41 ± 12.50
10 nmol, Y-33075 dihydrochloride	57.83 ± 3.62	95.72 ± 5.09	174.23 ± 11.62
100 nmol, Y-33075 dihydrochloride	47.89 ± 1.90	98.35 ± 5.30	189.39 ± 16.31
Form-deprivation Myopia (FDM)			
DMSO + Corn Oil	49.44 ± 3.02	97.11 ± 5.55	160.52 ± 7.05
1 nmol, Y-33075 dihydrochloride	49.66 ± 4.87	94.87 ± 4.12	162.75 ± 11.40
10 nmol, Y-33075 dihydrochloride	57.30 ± 3.86	105.60 ± 9.87	174.37 ± 13.50
100 nmol, Y-33075 dihydrochloride	43.36 ± 3.27	111.08 ± 6.10	180.84 ± 11.50

6.5 Discussion

Our findings present the first evidence that Y-33075 dihydrochloride significantly inhibited myopia progression and abnormal ocular growth in both FDM and LIM models in a dose-dependent manner. Interestingly, treatment with Y-33075 dihydrochloride slowed changes in ACD, VCD, AL, and ChT over the 7 days of the treatment period, while changes in lens thickness were minimal and not statistically significant. Previous studies have shown that ocular hypertensive drugs are effective in treating myopia in different animal models. Timolol eye drops have been shown to lower IOP in myopic chick eyes but not affect myopia progression [175]. Similarly, clinical trials in children found that timolol reduced IOP but was not effective in controlling myopia progression [181]. However, brimonidine, an alpha2-adrenoceptor agonist, has demonstrated greater efficacy against both FDM and LIM in guinea pigs by reducing aqueous humor secretion and increasing uveoscleral outflow [176, 183, 186]. Latanoprost, a prostaglandin F2α analogue, has also been shown to inhibit LIM in rhesus monkeys and FDM in guinea pigs by increasing non-pressure-dependent uveoscleral outflow. In our study, we demonstrated a clear dose-dependent effect of Y-33075 dihydrochloride, with 10 and 100 nmol significantly inhibited myopia in both LIM and FDM models, whereas 1 nmol was ineffective. However, high doses of Rho kinase inhibitors may causes off-target effects as these enzymes share homology with other serine/threonine kinases, including protein kinase A and C. Comparable dose-dependent effects were reported with brimonidine (200 nmol) and atropine (250 mg), both of which were required to control FDM in chicks [156]. Brimonidine showed a similar effect to atropine, but the required concentration was high, and it bound to receptors [330]. In addition, co-administration of atropine with nitric oxide inhibitors or muscarinic antagonists further reduced experimental myopia, highlighting the complexity of pharmacological intervention [331, 332].

Over the past two decades, the development of pharmacological treatment for various ocular diseases using Rho kinase inhibitors has increased interest. ROCK inhibitors such as Y27632 and Fasudil specifically targeted the ATP-dependent protein kinase domain of ROCK1 and ROCK2, which shared significant structural similarity [333]. A recent study showed that Rho kinase activation increased α-smooth muscle actin (α-SMA) expression in the sclera, thereby contributing to myofibroblast differentiation and scleral remodeling in myopia [317, 334, 335]. While the biomechanical properties of the sclera were altered in myopic eyes, this did not explain how biochemical signals were delivered to act as biomechanical signals during myopia development [334, 336, 337]. In addition, finite element models showed a positive correlation between IOP-related stress and axial elongation, but this correlation was not associated with scleral thickness [338]. Rho kinase mediated the conversion of mechanical signals into cellular responses, which led to increased cytoskeletal organization and enhanced protein production [339]. Pharmacological inhibition of Rho kinase with Y27632 modulated these effects, leading to increased phosphorylation of the myosin phosphatase subunits and altered sclera fibroblast activity [340]. Y-27632 dihydrochloride inhibits the Rho kinase pathway, reduces α-SMA expression, and increases myofibroblast activation [318]. Myofibroblast differentiation in myopic eyes is driven by mechanical stress, tissue stiffness, and cytokine signaling [341]. Rho kinase inhibitors are considered potent therapeutic interventions for myopia treatment, leading to changes in the biomechanical and microarchitectural properties of the sclera. Scleral prostaglandin treatment increased scleral permeability and decreased collagen types I and III [342]. The study also found that Rho kinase inhibitors modulate fibronectin deposition and cytoskeletal organization in human trabecular meshwork cells, whereas latanoprost primarily influences extracellular matrix degradation [343]. Rho Kinase inhibitors have been shown to upregulate matrix metalloproteinase 2 (MMP2), leading to extracellular

matrix remodeling [344]. Combined treatment with Y27632 and TGF-ß2 can normalize transcellular pressure and increase outflow facility and pan-Rho kinase inhibitors, such as ripasudil, up-regulate MMP2, MMP9, and MMP14 [345, 346]. Clinical trials have investigated Rho kinase inhibitors such as Y-27632 and netarsudil, which are approved for lowering IOP in glaucoma patients [347]. The combination of netarsudil and latanoprost has demonstrated enhanced efficacy in reducing IOP compared to either drug alone [348].

Our results showed that all tested doses of Y-33075 dihydrochloride effectively reduced IOP compared to controls. Rho kinase inhibitors increase aqueous outflow facility through the trabecular meshwork, resulting in reduced IOP, similar to the effect of prostaglandin analogues. However, prostaglandin analogues primarily increase uveoscleral outflow facility, while Rho kinase inhibitors also reduce aqueous humor production and episcleral venous pressure [349, 350]. Rho kinase pathway inhibition can occur in different ways, such as with Netarsudil, which inhibits norepinephrine transport, further decreasing aqueous production and episcleral venous pressure [198]. Topical administration of Y-39983, a related Rho kinase inhibitor, has also demonstrated dose-dependent reductions in IOP and increased outflow facility in rabbit eyes [351]. In our study, control groups treated with dimethyl sulfoxide and corn oil showed increased IOP and greater myopia progression, whereas Y-33075 dihydrochloride treatment reduced both IOP and axial elongation. These findings are similar to previous reports of higher IOP in myopia compared with emmetropia [352]. Previous studies have also demonstrated that elevated IOP is associated with high myopia, while others report no significant differences between refractive groups [299-301]. A recent randomized controlled trial suggested that for every 1 mm Hg increase in IOP, spherical equivalent refractive error increases by approximately 0.05 to 0.09 diopters [45]. This evidence supports the idea that higher

IOP may be associated with increased progression of myopia. The current study found that Y-33075 dihydrochloride effectively reduced IOP, thereby controlling AL elongation and refractive error changes. Conversely, control groups with higher IOP showed higher myopia and AL elongation. Previous animal studies showed that ocular hypotensive drugs can affect myopia progression in different ways. Timolol did not affect AL elongation in guinea pigs, whereas prostaglandin analogues, such as latanoprost, significantly inhibited myopia and AL elongation by altering scleral collagen remodeling and normalizing scleral microstructures [178, 303]. Brimonidine also significantly reduced myopia progression and reduced AL in animal models [176, 183]. In children, greater axial elongation has been observed in those with higher myopia, although the correlation between IOP and AL is not always consistent across studies [162].

In addition, the present study observed that higher doses of Y-33075 dihydrochloride were effective in preserving choroid thinning in both LIM and FDM. Preservation of choroidal thickness may help stabilize axial length and limit ocular elongation, which are key contributors to myopia progression. ROCK inhibition may control ocular perfusion and scleral biomechanics by reducing IOP. Similarly, previous clinical and animal studies have shown that glaucoma drugs such as latanoprost can significantly increase choroidal thickness in humans and young guinea pigs [177, 294]. In contrast, the lowest doses of Y-33075 dihydrochloride did not significantly affect choroidal thinning, suggesting a threshold effect for biochemical and biomechanical processes. Although studies have investigated that both FDM and LIM lead to the development of myopic refractive error, their mechanisms differ [79]. Our findings show that Y-33075 dihydrochloride effectively inhibited myopia progression in both models, indicating that Rho kinase inhibition targets shared downstream pathways involved in axial elongation. Similar intravitreal injections of atropine were found to be

effective in both FDM and LIM in chick models [202]. Previous studies have reported differential drug responses between FDM and LIM, such as dopamine agonists and muscarinic antagonists, but Y-33075 dihydrochloride demonstrated efficacy in both paradigms [102, 290].

6.6 Conclusions

The results of this study provide novel evidence that a Rho-kinase inhibitor (Y-33075 dihydrochloride) exhibits a similar inhibitory effect on both experimentally induced myopia models in chickens. This implies that this agent may be a potential pharmacological agent for myopia therapy. Intravitreal injection of Y-33075 dihydrochloride effectively inhibited abnormal eye growth and myopia development in both LIM and FDM in a dose-dependent manner. The treatment significantly slowed the progression of axial components, including ACD, VCD, AL and choroidal thinning in both models, with higher concentrations showing maximum efficacy.

These findings align with the effects of other ocular hypotensive drugs, including brimonidine and latanoprost, which have shown varying degrees of effectiveness in various animal models. Notably, higher doses of Y-33075 dihydrochloride did not exhibit adverse effects associated with non-specific kinase inhibition, suggesting a potential for safe therapeutic application. Furthermore, Y-33075 dihydrochloride significantly reduced IOP, contributing to myopia control by increasing aqueous outflow and reducing episcleral venous pressure. These results underscore the potential of Rho-kinase inhibitors, particularly Y-33075 dihydrochloride, as a promising pharmacological intervention for myopia management by targeting both ocular growth and IOP regulation mechanisms.

Chapter 7

Overall Conclusions of a Thesis

7.1 Key findings are as follows

7.1.1 Overview

This study investigated the effectiveness and mechanisms of several pharmacological agents, such as atropine, an alpha-adrenergic receptor agonist, latanoprost, and a Rho-kinase inhibitor, which control experimentally induced myopia using chick models. Myopia is a common cause of correctable vision loss, and atropine has proven effective. However, atropine is limited to use in myopia treatment due to adverse side effects. This study aimed to identify an alternative pharmaceutical candidate with comparable efficacy, lower toxicity, and fewer adverse effects.

Our findings indicate that all IOP-lowering drugs cannot inhibit both FDM and LIM in chicks. Latanoprost and the Rho-kinase inhibitor Y-33075 dihydrochloride were effective in slowing myopia progression and controlling ocular elongation in both the FDM and LIM models, and in reducing intraocular pressure (IOP). However, we

did not examine IOP changes for every tested drug, such as atropine and alpha-adrenergic agonists. Therefore, we cannot conclude that all IOP-lowering drugs are effective in both types of animal models of myopia.

Secondly, the anti-myopia effects of latanoprost and Rho-kinase inhibitors are strongly associated with their IOP-lowering properties. By reducing IOP, these agents may decrease mechanical stress on the sclera, promote extracellular matrix remodelling and limit excessive ocular elongation. However, latanoprost not only lowered IOP but also prevented scleral thinning and upregulated Egr1 protein expression, indicating a complex mechanism involving both biomechanical and molecular pathways. Although alpha-adrenergic agonists also inhibited myopia progression, whether this effect is primarily mediated through IOP reduction or alternative mechanisms remains unclear. Overall, IOP-lowering plays a significant role in the observed anti-myopia effects observed but it is not the only contributing factor.

Current literature presents inconsistent results regarding changes in IOP during myopia development. Different experimental factors may contribute to this inconsistency. The age of the animals is important, as younger and older chicks can show different physiological responses during myopia development. The lens type or deprivation method used, including variations in lens power, material and technique, may affect experimental outcomes. Treatment duration and the timing of IOP measurement relative to the intervention may affect the results. Differences in measurement methods, particularly in the types of tonometry and protocols applied, may produce inconsistent IOP readings. Pharmacological interventions, including the administration of drugs or vehicle injections, may confound IOP measurements. Finally, environmental factors, such as light exposure, handling, and housing conditions, may also influence experimental outcomes. While changes in IOP are associated with myopia progression, current evidence suggests that IOP alone may

not be the primary causal factor. While some studies report increased IOP during myopia development, others find no significant change. The effectiveness of IOP-lowering drugs in reducing myopia progression suggests that lowering IOP may promote scleral remodelling and help to limit ocular elongation. However, other additional factors, including molecular signalling pathways and extracellular matrix dynamics, are also involved. Therefore, IOP changes may represent one of the several interacting mechanisms that control myopia development.

7.1.2 Future Research Directions

This study has demonstrated that pharmacological agents such as latanoprost and Rho-kinase inhibitor Y-33075 could significantly inhibit the progression of form-deprivation myopia (FDM) and lens-induced myopia (LIM) in the chick model. These drugs are usually used for glaucoma and to lower IOP.

Based on our findings, latanoprost-induced upregulation of retinal EGR1 and Y-33075 preserved the choroidal thickness, which indicates that their therapeutic effects may extend to extracellular matrix remodeling and retinal or choroidal signaling pathways. Therefore, future studies should focus on molecular or cellular approaches to identify the downstream targets and signaling cascades. Additionally, α1A, α2A, and non-selective adrenergic agonists effectively inhibited LIM and showed partial effects on FDM. Future investigations could include detailed receptor-binding studies, use of selective antagonists and gene expression analyses to differentiate the roles of individual α-adrenoceptor subtypes.

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Appendix

Appendix: Table 3.1 Summarized refractive power (Rx), anterior chamber depth (ACD), and lens thickness (LT) baseline data for all tested concentrations of atropine.

	Rx		ACD		LT	
Category	Treated eye (D)	Control eye (D)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Atropine 5%	4.62±0.20	4.6±0.16	1.31±0.05	1.32±0.04	1.86±0.05	1.84±0.04
Atropine 3%	4.60±0.12	4.65±0.2	1.30±0.02	1.30±0.02	1.85±0.04	1.83±0.04
Atropine 1%	4.51±0.07	4.57±0.18	1.32±0.03	1.32±0.03	1.78±0.05	1.76±0.04
Atropine 0.1%	4.52±0.07	4.62±0.17	1.33±0.03	1.34±0.02	1.75±0.05	1.74±0.04
Atropine 0.01%	4.55±0.10	4.7±0.19	1.33±0.02	1.33±0.02	1.75±0.04	1.75±0.04
PBS	4.5±0.19	4.52±0.20	1.33±0.02	1.32±0.02	1.73±0.05	1.74±0.04

Appendix: Table 3.2 Summarized vitreous chamber depth (VCD), axial length (AL) and Choroidal thickness (ChT) baseline data for all tested concentrations of atropine.

	VCD		AL		ChT	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (μm)	Control eye (μm)
Atropine 5%	5.13±0.13	5.13±0.12	8.32±0.15	8.29±0.14	170.43±5.72	170.93±9.29
Atropine 3%	5.12±0.10	5.10±0.08	8.27±0.09	8.24±0.08	167.06±6.47	164.86±4.33
Atropine 1%	5.47±0.35	5.42±0.35	8.57±0.34	8.51±0.34	168.61±6.89	166.23±4.62
Atropine 0.1%	5.45±0.29	5.38±0.25	8.57±0.30	8.46±0.25	169.90±7.19	171.46±8.78
Atropine 0.01%	5.33±0.27	5.29±0.30	8.45±0.29	8.38±0.29	171.6±7.05	170.43±9.83
PBS	5.61±0.24	5.64±0.24	8.70±0.27	8.71±0.22	169±4.14	168±5.23

Appendix: Table 3.3 Summarized refractive power (Rx) data for the outcome of different concentrations of atropine on days 4, 8, and 12.

Category	Rx of Day 4		Rx of Day 8		Rx of Day 12	
	Treated eye (D)	Control eye D)	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)
Atropine 5%	0.05 ± 0.95	4.25 ± 0.07	-1.57 ± 1.65	3.57 ± 0.11	-4.30 ± 2.67	2.92 ± 0.16
Atropine 3%	0.15 ± 1.67	4.1 ± 0.32	-2.37 ± 1.15	3.25 ± 0.25	-3.35 ± 1.72	2.95 ± 0.18
Atropine 1%	-0.37 ± 0.53	3.5 ± 0.11	-1.60 ± 0.76	3.50 ± 0.11	-3.95 ± 1.25	2.95 ± 0.15
Atropine 0.1%	-1.4 ± 0.41	4.15 ± 0.21	-3.72 ± 0.65	3.27 ± 0.24	-5.55 ± 1.05	2.92 ± 0.11
Atropine 0.01%	-1.60 ± 0.68	4.0 ± 0.23	-4.45 ± 0.84	3.22 ± 0.28	-6.10 ± 1.17	2.87 ± 0.16
PBS	-2.3 ± 0.71	4.0 ± 0.25	-4.22 ± 0.97	3.45 ± 0.24	-8.10 ± 0.68	2.94 ± 0.15

Appendix: Table 3.4 Summarized anterior chamber depth (ACD) data for the outcome of different concentrations of atropine on days 4, 8 and 12.

Category	ACD of Day 4		ACD of Day 8		ACD of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Atropine 5%	1.40 ± 0.07	1.39 ± 0.03	1.57 ± 0.10	1.52 ± 0.11	1.67 ± 0.13	1.55 ± 0.07
Atropine 3%	1.47 ± 0.06	1.44 ± 0.03	1.62 ± 0.09	1.53 ± 0.04	1.65 ± 0.09	1.57 ± 0.03
Atropine 1%	1.41 ± 0.02	1.38 ± 0.02	1.53 ± 0.04	1.44 ± 0.03	1.65 ± 0.07	1.51 ± 0.04
Atropine 0.1%	1.45 ± 0.05	1.39 ± 0.02	1.58 ± 0.08	1.47 ± 0.05	1.69 ± 0.1	1.53 ± 0.06
Atropine 0.01%	1.44 ± 0.03	1.38 ± 0.02	1.57 ± 0.04	1.46 ± 0.03	1.68 ± 0.03	1.52 ± 0.03
PBS	1.43 ± 0.04	1.34 ± 0.03	1.57 ± 0.08	1.41 ± 0.03	1.77 ± 0.12	1.49 ± 0.06

Appendix: Table 3.5 Summarized lens thickness (LT) data for the outcome of different concentrations of atropine on day 4, 8 and 12.

Category	LT of Day 4		LT of Day 8		LT of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Atropine 5%	2.10 ± 0.08	2.07 ± 0.07	2.16 ± 0.06	2.14 ± 0.04	2.19 ± 0.05	2.23 ± 0.04
Atropine 3%	2.17 ± 0.05	2.13 ± 0.05	2.26 ± 0.05	2.26 ± 0.05	2.46 ± 0.08	2.41 ± 0.05
Atropine 1%	1.97 ± 0.1	1.95 ± 0.02	2.09 ± 0.03	2.11 ± 0.05	2.17 ± 0.04	2.21 ± 0.05
Atropine 0.1%	1.94 ± 0.05	1.96 ± 0.05	2.08 ± 0.07	2.10 ± 0.06	2.16 ± 0.06	2.20 ± 0.04
Atropine 0.01%	1.98 ± 0.03	1.98 ± 0.03	2.11 ± 0.03	2.13 ± 0.02	2.19 ± 0.04	2.25 ± 0.03
PBS	1.92 ± 0.04	1.93 ± 0.04	2.05 ± 0.05	2.08 ± 0.04	2.16 ± 0.05	2.21 ± 0.04

Appendix: Table 3.6 Summarized vitreous chamber depth (VCD) data for the outcome of different concentrations of atropine on days 4, 8, and 12.

	VCD of Day 4		VCD of Day 8		VCD of Day 12	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Atropine 5%	5.48 ± 0.16	5.25 ± 0.10	5.77 ± 0.27	5.40 ± 0.25	6.12 ± 0.29	5.66 ± 0.16
Atropine 3%	5.42 ± 0.13	5.17 ± 0.13	5.73 ± 0.13	5.30 ± 0.10	5.99 ± 0.12	5.52 ± 0.10
Atropine 1%	5.45 ± 0.11	5.20 ± 0.12	5.75 ± 0.18	5.35 ± 0.16	5.96 ± 0.27	5.57 ± 0.20
Atropine 0.1%	5.62 ± 0.15	5.25 ± 0.14	5.88 ± 0.19	5.44 ± 0.17	6.13 ± 0.20	5.58 ± 0.17
Atropine 0.01%	5.57 ± 0.17	5.16 ± 0.11	5.82 ± 0.23	5.34 ± 0.13	6.15 ± 0.20	5.58 ± 0.12
PBS	5.52 ± 0.15	5.05 ± 0.17	5.83 ± 0.18	5.22 ± 0.15	6.15 ± 0.13	5.39 ± 0.15

Appendix: Table 3.7 Summarized axial length (AL) data for the outcome of different concentrations of atropine on days 4, 8, and 12.

	AL of Day 4		AL of Day 8		AL of Day 12	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Atropine 5%	8.99 ± 0.20	8.66 ± 0.17	9.46 ± 0.34	9.01 ± 0.26	9.99 ± 0.38	9.42 ± 0.18
Atropine 3%	9.07 ± 0.18	8.72 ± 0.14	9.62 ± 0.20	9.10 ± 0.11	10.10 ± 0.22	9.51 ± 0.10
Atropine 1%	8.92 ± 0.14	8.53 ± 0.15	9.39 ± 0.21	8.91 ± 0.18	9.79 ± 0.33	9.28 ± 0.25
Atropine 0.1%	9.02 ± 0.15	8.62 ± 0.15	9.54 ± 0.22	8.98 ± 0.19	10.0 ± 0.26	9.35 ± 0.19
Atropine 0.01%	9.00 ± 0.15	8.53 ± 0.13	9.51 ± 0.23	8.94 ± 0.14	10.03 ± 0.19	9.36 ± 0.15
PBS	8.80 ± 0.13	8.26 ± 0.18	9.38 ± 0.31	8.67 ± 0.27	9.98 ± 0.17	9.10 ± 0.16

Appendix: Table 3.8 Summarized Choroidal thickness (ChT) data for the outcome of different concentrations of atropine on day 4, 8, and 12.

	ChT of Day 4		ChT of Day 8		ChTof Day 12	
Category	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)
Atropine 5%	155.26±5.27	166.01±5.82	151.2±9.20	170.7±2.89	149.13±6.23	172.96±5.59
Atropine 3%	158.7±6.44	170.56±5.65	158±4.60	170.73±2.88	151.83±5.95	170.06±3.74
Atropine 1%	158.96±6.51	170.56±5.65	158.9±6.05	172.3±7.99	155.76±7.13	173.96±6.18
Atropine 0.1%	159.3±6.28	172.53±7.40	157.52±6.79	173.63±5.94	145.8±8.26	169.93±6.17
Atropine 0.01%	155.36±4.59	173.2±5.03	144.6±10.11	172.86±5.16	138.21±5.71	171±4.28
PBS	149.28±6.00	169.03±7.91	135.88±3.51	171.77±7.80	117.66±6.01	176.56±3.89

Appendix: Table 4.1 Summarized refractive power (Rx) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

Category	Rx of Baseline		Rx of Day 7		Rx of Day 14	
	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)	Recovery eye (D)	Treated eye (D)
PBS	4.15±0.31	4.07±0.26	-5.38±0.91	3.19±0.41	7.59±0.770	-8.57±1.08
3 nmol	4.22±0.28	4.35±0.29	-3.77±1.00	3.68±0.82	6.61±1.09	-7.46±1.20
30 nmol	4.25±0.32	4.53±0.34	-0.32±1.80	3.10±0.23	3.09±2.90	-3.43±2.76
300 nmol	4.18±0.48	4.11±0.43	0.35±.37	2.97±0.26	2.15±1.92	-2.61±1.84

Appendix: Table 4.2 Summarized anterior chamber depth (ACD) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

Category	ACD of Baseline		ACD of Day 7		ACD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	1.37±0.02	1.36±0.02	1.74±0.07	1.55±0.04	-0.15±0.05	0.19±0.05
3 nmol	1.38±0.03	1.37±0.03	1.67±0.04	1.55±0.01	-0.06±0.05	0.11±0.05
30 nmol	1.40±0.03	1.38±0.03	1.53±0.08	1.53±0.04	0.01±0.06	-0.00±0.06
300 nmol	1.38±0.04	1.39±0.03	1.58±0.08	1.56±0.08	0.02±0.11	0.01±0.06

Appendix: Table 4.3 Summarized lens thickness (LT) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

Category	LT of Baseline		LT of Day 7		LT of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	2.00±0.04	2.01±0.05	2.28±0.07	2.31±0.06	-0.04±0.05	-0.02±0.04
3 nmol	2.01 ±0.04	2.02±0.02	2.29±0.05	2.32±0.04	-0.01±0.03	-0.03±0.02
30 nmol	2.02±0.04	2.02±0.05	2.28±0.06	2.29±0.06	0.01±0.06	-0.01±0.04
300 nmol	2.04±0.06	2.03±0.05	2.20±0.06	2.23±0.07	-0.00±0.06	-0.03±0.04

Appendix: Table 4.4 Summarized vitreous chamber depth (VCD) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

	VCD of Baseline		VCD of Day 7		VCD of Day 14	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	5.39±0.14	5.33±0.13	6.26±0.20	5.71±0.18	-0.41±0.09	0.55± 0.09
3 nmol	5.29 ±0.13	5.22±0.11	6.20±0.10	5.73±0.11	-0.36±0.10	0.47± 0.08
30 nmol	5.43±0.08	5.37±0.09	6.00±0.27	5.64±0.35	-0.29±0.21	0.35 ±0.18
300 nmol	5.30±0.15	5.28±0.13	6.11±0.10	5.83±0.08	-0.19±0.15	0.28 ±0.15

Appendix: Table 4.5 Summarized axial length (AL) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

	AL of Baseline		AL of Day 7		AL of Day 14	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	8.78±0.18	8.71±0.18	10.21±0.24	9.58±0.18	-0.46±0.09	0.62 ± 0.09
3 nmol	8.70 ±0.16	8.62±0.14	10.17±0.11	9.62±0.13	-0.39±0.09	0.54 ±0.10
30 nmol	8.86±0.09	8.79±0.09	9.81±0.32	9.47±0.18	-0.27±0.23	0.34 ±0.22
300 nmol	8.73±0.18	8.71±0.15	9.96±0.15	9.67±0.13	-0.16±0.22	0.28 ±0.19

Appendix: Table 4.6 Summarized Choroidal thickness (ChT) data for the outcome of different doses of intravitreal injection of Metaraminol on baseline, Days 7 and 14.

	ChT of Baseline		ChT of Day 7		ChT of Day 14	
Category	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Recovery eye (μm)	Treated eye (μm)
PBS	171.83±10.20	168.13±12.41	131.09±6.84	184.39±6.42	50.41±14.25	-53.58± 9.70
3 nmol	160.7±10.52	159.23±10.22	140.5±10.13	185.0±9.15	38.38±12.18	-44.48± 14.36
30 nmol	160.637.83	12.03±7.30	163.36±16.8	183.8±15.46	9.24±7.95	-20.00± 08.27
300 nmol	167.46±10.47	166.63±9.28	171.35±15.8	188.28±11.23	8.85±6.14	-16.95± 10.00

Appendix: Table 4.7 Summarized refractive power (Rx) data for the outcome of different doses of intravitreal injection of lofexidine on baseline, Days 7 and 14.

Category	Rx of Baseline		Rx of Day 7		Rx of Day 14	
	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)	Recovery eye (D)	Treated eye (D)
PBS	4.57±0.11	4.72±0.20	-4.90±1.41	4.30±0.26	8.45±1.53	-9.17±1.44
3 nmol	4.60±0.12	4.72±0.23	-1.76±2.50	3.91±0.30	5.19±2.62	-5.67±2.74
30 nmol	5.33±0.25	5.51±0.39	-0.03±1.65	4.03±0.30	3.44±2.07	-4.07±1.98
300 nmol	4.90±0.20	4.92±0.18	0.55±2.30	3.78±0.48	2.39±2.53	-3.23±2.67

Appendix: Table 4.8 Summarized anterior chamber depth (ACD) data for the outcome of different doses of intravitreal injection of lofexidine on baseline, Days 7 and 14.

Category	ACD of Baseline		ACD of Day 7		ACD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	1.33±0.02	1.34±0.02	1.77±0.06	1.57±0.04	-0.15±0.05	0.19 ±0.07
3 nmol	1.30±0.03	1.31±0.02	1.66±0.10	1.59±0.06	0.01±0.10	0.06± 0.08
30 nmol	1.40±0.04	1.40±0.03	1.53±0.07	1.55±0.05	0.03±0.06	0.00± 0.04
300 nmol	1.34±0.04	1.33±0.03	1.44±0.07	1.51±0.05	0.12±0.10	-0.07± 0.06

Appendix: Table 4.9 Summarized lens thickness (LT) data for the outcome of different doses of intravitreal injection of lofexidine on baseline, Days 7 and 14.

Category	LT of Baseline		LT of Day 7		LT of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	1.88±0.04	1.86±0.04	2.27±0.05	2.30±0.04	0.03±0.04	-0.03 ±0.02
3 nmol	1.86±0.03	1.85±0.03	2.29±0.03	2.32±0.03	0.01±0.08	-0.02 ±0.02
30 nmol	2.00±0.04	2.00±0.05	2.27±0.05	2.28±0.05	0.01±0.06	-0.01±0.03
300 nmol	1.84±0.05	1.83±0.03	2.24±0.02	2.23±0.02	-0.02±0.05	0.00± 0.01

Appendix: Table 4.10 Summarized vitreous chamber depth (VCD) data for the outcome of different doses of intravitreal injection of lofexidine on baseline, Days 7 and 14.

Category	VCD of Baseline		VCD of Day 7		VCD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	5.20±0.08	5.16±0.08	6.42±0.17	5.83±0.14	-0.47±0.12	0.59±0.11
3 nmol	5.09±0.10	5.06±0.08	6.21±0.25	5.85±0.17	-0.26±0.23	0.36±0.16
30 nmol	5.36±0.16	5.29±0.16	6.02±0.18	5.72±0.22	-0.23±0.10	0.31± 0.09
300 nmol	5.20±0.12	5.16±0.10	5.95±0.25	5.63±0.13	-0.25±0.17	0.31± 0.09

Appendix: Table 4.11 axial length (AL) data for the outcome of different doses of intravitreal injection of lofexidinel on baseline, Days 7 and 14.

Category	AL of Baseline		AL of Day 7		AL of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	8.42±0.09	8.37±0.09	10.44±0.23	9.71±0.20	-0.57±0.16	0.72± 0.14
3 nmol	8.27±0.14	8.23±0.10	10.17±0.31	9.77±0.20	-0.28±0.23	0.40± 0.17
30 nmol	8.77±0.18	8.69±0.18	9.93±0.20	9.56±0.24	-0.26±0.11	0.36± 0.10
300 nmol	8.39±0.14	8.33±0.12	9.64±0.29	9.38±0.16	-0.14±0.17	0.25± 0.19

Appendix: Table 4.12 Summarized Choroidal thickness (ChT) data for the outcome of different doses of intravitreal injection of lofexidine on baseline, Days 7 and 14.

Category	ChT of Baseline		ChT of Day 7		ChT of Day 14	
	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Recovery eye (μm)	Treated eye (μm)
PBS	163.79±11.76	167.53±12.24	110.42±10.21	158.33±10.74	41.76±14.19	-47.27±14.23
3 nmol	167.33±8.88	168.50±13.81	110.11±8.36	147.66±14.88	33.11±11.01	-38.64±11.80
30 nmol	168.80±8.09	171.30±10.68	122.46±11.33	144.30±8.59	19.12±13.05	-21.74±12.51
300 nmol	169.33±7.97	167.26±8.31	137.42±12.78	149.26±8.19	6.84±7.65	-11.77±6.32

Appendix: Table 4.13 Summarized refractive power (Rx) data for the outcome of different doses of intravitreal injection of cirazoline on baseline, Days 7 and 14.

Category	Rx of Baseline		Rx of Day 7		Rx of Day 14	
	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)	Recovery eye (D)	Treated eye (D)
PBS	4.15±0.33	4.05±0.26	-3.31±0.47	3.76-±0.22	5.93±0.71	-7.08±0.58
3 nmol	4.42±0.41	4.45±0.41	-3.11±1.01	3.30-±0.36	5.31±1.40	-6.42±1.31
30 nmol	4.45±0.37	4.45±0.5	-2.22±1.05	3.17-±0.22	4.06±1.19	-5.31±1.39
300 nmol	4.56±0.40	4.55±0.44	-0.48±0.86	3.75±0.36	2.98±1.02	-4.09±1.18

Appendix: Table 4.14 Summarized anterior chamber depth (ACD) data for the outcome of different doses of intravitreal injection of cirazoline on baseline, Days 7 and 14.

Category	ACD of Baseline		ACD of Day 7		ACD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	1.37±0.03	1.36±0.03	1.79±0.13	1.57±0.03	-0.21±0.14	0.22 ±0.06
3 nmol	1.38±0.02	1.37±0.03	1.71±0.14	1.54±0.04	-0.09±0.12	0.16 ±0.08
30 nmol	1.39±0.02	1.37±0.02	1.65±0.05	1.54±0.04	-0.04±0.05	0.10± 0.04
300 nmol	1.38±0.03	1.39±0.03	1.62±0.06	1.55±0.02	-0.03±0.05	0.07 ±0.04

Appendix: Table 4.15 Summarized lens thickness (LT) data for the outcome of different doses of intravitreal injection of cirazoline on baseline, Days 7 and 14.

Category	LT of Baseline		LT of Day 7		LT of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	2.00±0.04	2.02±0.05	2.34±0.06	2.43±0.06	0.11±0.12	-0.09± 0.05
3 nmol	2.03±0.03	2.02±0.05	2.24±0.07	2.26±0.06	-0.00±0.09	-0.04± 0.05
30 nmol	2.01±0.04	2.01±0.04	2.24±0.05	2.28±0.06	0.00±0.09	-0.01± 0.05
300 nmol	2.05±0.05	2.03±0.05	2.22±0.04	2.26±0.05	0.03±0.06	-0.03± 0.03

Appendix: Table 4.16 Summarized vitreous chamber depth (VCD) data for the outcome of different doses of intravitreal injection of cirazolinel on baseline, Days 7 and 14.

	VCD of Baseline		VCD of Day 7		VCD of Day 14	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	5.38±0.15	5.32±0.14	6.32±0.24	5.66±0.23	-0.52±0.16	0.65± 0.09
3 nmol	5.32±0.16	5.29±0.15	6.03±0.20	5.64±0.17	-0.33±0.11	0.44±0.12
30 nmol	5.40±0.14	5.33±0.14	6.03±0.26	5.61±0.18	-0.28±0.08	0.36± 0.18
300 nmol	5.28±0.13	5.27±0.12	6.02±0.15	5.67±0.15	-0.21±0.11	0.34± 0.11

Appendix: Table 4.17 axial length (AL) data for the outcome of different doses of intravitreal injection of cirazoline on baseline, Days 7 and 14.

	AL of Baseline		AL of Day 7		AL of Day 14	
Category	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
PBS	8.75±0.18	8.71±0.19	10.25±0.23	9.54±0.23	-0.58±0.13	0.72± 0.08
3 nmol	8.73±0.17	8.69±0.16	9.98±0.28	9.45±0.23	-0.40±0.11	0.53 ±0.44
30 nmol	8.74±0.19	8.68±0.17	9.93±0.22	9.48±0.22	-0.35±0.19	0.44 ±0.17
300 nmol	8.71±0.17	8.70±0.15	9.88±0.20	9.45±0.22	-0.25±0.10	0.39± 0.11

Appendix: Table 4.18 Summarized Choroidal thickness (ChT) data for the outcome of different doses of intravitreal injection of cirazoline on baseline, Days 7 and 14.

	ChT of Baseline		ChT of Day 7		ChT of Day 14	
Category	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Recovery eye (μm)	Treated eye (μm)
PBS	154.13±3.83	149.07±3.45	134.38±6.11	181.35±12.85	44.12±19.98	-48.79±20.24
3 nmol	159.06±9.87	164.10±7.99	136.45±8.15	174.23±12.05	32.05±13.17	-37.78±14.48
30 nmol	156.40±11.75	160.10±8.73	152.31±11.16	176.20±11.48	17.30±17.28	-23.56±18.46
300 nmol	160.63±5.69	159.56±6.85	152.31±11.16	176.20±11.48	15.00±9.80	-22.90±9.40

Appendix: Table 5.1 Summarized baseline data on the effect of topical latanoprost on FDM and LIM chicks for refractive power (Rx), anterior chamber depth (ACD), and lens thickness (LT)

Category	Rx		ACD		LT	
	Treated eye (D)	Control eye (D)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
FDM + Vehicle	5.10±0.31	5.14±0.31	1.35±0.03	1.35±0.03	1.84±0.04	1.84±0.03
FDM+Latanoprost	5.13±0.30	5.2±0.33	1.35±0.03	1.35±0.03	1.85±0.05	1.85±0.04
LIM + Vehicle	4.76±0.23	4.83±0.25	1.34±0.03	1.36±0.02	1.82±0.05	1.80±0.03
LIM+ Latanoprost	5.00±0.21	5.12±0.30	1.31±0.04	1.32±0.04	1.83±0.05	1.82±0.04

Appendix: Table 5.2 Summarized baseline data on the effect of topical latanoprost on FDM and LIM chicks for vitreous chamber depth (VCD), axial length (AL) and Choroidal thickness (ChT)

Category	VCD		AL		ChT	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (μm)	Control eye (μm)
FDM + Vehicle	5.20±0.12	5.17±0.11	8.41±0.14	8.37±0.12	170.15±7.70	169.79±6.51
FDM+Latanoprost	5.21±0.13	5.18±0.13	8.43±0.13	8.39±0.14	176.7±12.4	174.83±13.31
LIM + Vehicle	5.19±0.11	5.22±0.09	8.37±0.10	8.39±0.07	179.93±7.95	178.7±9.30
LIM+ Latanoprost	5.17±0.10	5.15±0.10	8.33±0.10	8.30±0.11	173.86±7.46	171.76±8.28

Appendix: Table 5.3 Summarized refractive power (Rx) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	Rx of Day 4		Rx of Day 8		Rx of Day 12	
	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)
FDM + Vehicle	-4.75±2.24	3.72±0.17	-9.82±1.21	3.12±0.20	-12.98±3.03	3.03±0.23
FDM+Latanoprost	-0.75±2.10	3.85±0.32	-4.50±2.04	3.85±0.22	-7.12±2.14	3.25±0.75
LIM + Vehicle	-1.32±1.11	3.90±0.20	-4.03±1.89	3.63±0.19	-7.90±1.24	3.10±0.2
LIM+ Latanoprost	0.87±1.52	3.97±0.07	-2.47±1.22	3.67±0.19	-4.17±0.96	3.00±0.22

Appendix: Table 5.4 Summarized anterior chamber depth (ACD) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	ACD of Day 4		ACD of Day 8		ACD of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
FDM + Vehicle	1.54±0.09	1.41±0.03	1.78±0.10	1.52±0.06	2.06±0.21	1.55±0.08
FDM+Latanoprost	1.51±0.07	1.46±0.03	1.73±0.12	1.54±0.04	1.87±0.17	1.59±0.03
LIM + Vehicle	1.47±0.04	1.41±0.02	1.53±0.06	1.47±0.02	1.80±0.12	1.55±0.07
LIM+ Latanoprost	1.40±0.03	1.36±0.02	1.61±0.09	1.53±0.05	1.73±0.07	1.56±0.03

Appendix: Table 5.5 Summarized lens thickness (LT) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	LT of Day 4		LT of Day 8		LT of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
FDM + Vehicle	2.06±0.03	2.05±0.04	2.25±0.06	2.25±0.06	2.33±0.05	2.35±0.04
FDM+Latanoprost	2.15±0.05	2.12±0.05	2.24±0.05	2.25±0.04	2.28±0.06	2.31±0.05
LIM + Vehicle	1.96±0.02	2.00±0.03	2.19±0.06	2.18±0.04	2.30±0.04	2.32±0.03
LIM+ Latanoprost	2.09±0.05	2.09±0.03	2.25±0.05	2.24±0.03	2.28±0.09	2.33±0.05

Appendix: Table 5.6 Summarized vitreous chamber depth (VCD) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	VCD of Day 4		VCD of Day 8		VCD of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
FDM + Vehicle	5.79±0.23	5.16±0.12	6.45±0.32	5.56±0.25	6.93±0.21	5.74±0.09
FDM+Latanoprost	5.74±0.22	5.35±0.11	6.24±0.24	5.57±0.12	6.53±0.24	5.71±0.10
LIM + Vehicle	5.66±0.12	5.30±0.11	5.90±0.17	5.45±0.12	6.41±0.41	5.74±0.11
LIM+ Latanoprost	5.36±0.14	5.09±0.11	5.81±0.20	5.43±0.14	6.10±0.19	5.59±0.18

Appendix: Table 5.7 Summarized axial length (AL) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	AL of Day 4		AL of Day 8		AL of Day 12	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
FDM + Vehicle	9.39±0.31	8.64±0.21	10.60±0.36	9.47±0.32	11.21±0.32	9.69±0.15
FDM+Latanoprost	9.41±0.26	8.93±0.13	10.22±0.30	9.37±0.14	10.58±0.25	9.61±0.13
LIM + Vehicle	9.11±0.12	8.72±0.10	9.61±0.18	9.11±0.11	10.52±0.18	9.65±0.11
LIM+ Latanoprost	8.86±0.17	8.55±0.10	9.65±0.26	9.18±0.18	10.15±0.20	9.49±0.19

Appendix: Table 5.8 Summarized Choroidal thickness (ChT) data for the outcome of topical latanoprost on FDM and LIM on days 4, 8, and 12.

Category	ChT of Day 4		ChT of Day 8		ChT of Day 12	
	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)
FDM + Vehicle	141.43±14.69	164.23±14.25	135.87±6.14	178.60±8.92	120.63±13.06	184.9±9.75
FDM+Latanoprost	150.16±4.06	166.66±3.11	154.36±10.14	179.86±5.95	151.93±14.85	182.23±8.63
LIM + Vehicle	147.96±8.27	167.73±9.74	139±10.87	169.8±11.59	129.1±11.35	183±12.59
LIM+ Latanoprost	153.46±11.38	166.1±11.20	147.16±6.51	169.63±6.03	136.8±6.82	163.96±10.9

Appendix: Table 5.9 Summarized data for the outcome of topical latanoprost at baseline, on days 4, 8, and 12.

Day	Rx		ACD		LT	
	Treated eye (D)	Control eye (D)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Baseline	5.00±0.25	5.25±0.41	1.29±0.04	1.30±0.04	1.80±0.03	1.80±0.03
Day 4	4.47±0.32	4.55±0.24	1.36±0.04	1.37±0.03	2.01±0.05	1.98±0.04
Day 8	3.80±0.20	3.95±0.25	1.46±0.04	1.45±0.04	2.12±0.04	2.12±0.04
Day 12	3.12±0.43	3.22±0.23	1.53±0.04	1.53±0.04	2.25±0.04	2.24±0.03

Appendix: Table 5.10 Summarized data for the outcome of topical latanoprost at baseline, on days 4, 8, and 12.

Day	VCD		AL		ChT	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (μm)	Control eye (μm)
Baseline	5.16±0.18	5.09±0.18	8.26±0.21	8.20±0.21	161.3±9.44	163.06±10.67
Day 4	5.17±0.18	5.15±0.18	8.54±0.22	8.52±0.22	169.0±7.52	166.83±11.41
Day 8	5.38±0.19	5.35±0.20	8.97±0.24	8.92±0.24	168.46±6.22	168.93±6.69
Day 12	5.71±0.20	5.70±0.22	9.50±0.25	9.48±0.26	167.1±13.39	166.86±11.77

Appendix: Table 5.10 Summarized data for the outcome of topical PBS at baseline, on days 4, 8, and 12.

Day	Rx		ACD		LT	
	Treated eye (D)	Control eye (D)	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)
Baseline	4.50±0.12	4.5±0.23	1.30±0.03	1.31±0.02	1.86±0.03	1.85±0.03
Day 4	4.00±0.29	4.00±0.27	1.36±0.03	1.35±0.03	2.03±0.04	2.03±0.04
Day 8	3.50±0.41	3.75±0.25	1.44±0.03	1.43±0.03	2.15±0.06	2.15±0.04
Day 12	3.00±0.47	3.25±0.19	1.53±0.02	1.52±0.02	2.28±0.06	2.28±0.05

Appendix: Table 5.10 Summarized data for the outcome of PBS at baseline, on days 4, 8, and 12

Day	VCD		AL		ChT	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Treated eye (μm)	Control eye (μm)
Baseline	5.09±0.10	5.06±0.08	8.27±0.14	8.23±0.10	164.8±7.38	165.86±8.74
Day 4	5.10±0.11	5.07±0.09	8.50±0.14	8.46±0.11	165.5±9.16	164.7±10.22
Day 8	5.24±0.15	5.22±0.13	8.84±0.18	8.81±0.14	162.6±8.18	165.26±8.72
Day 12	5.56±0.13	5.56±0.12	9.38±0.17	9.36±0.14	164.13±8.15	164.36±9.57

Appendix: Table 5.11 Summary of Intraocular Pressure (IOP) data for the effects of topical latanoprost before and after treatment

Category	Baseline (mean±SD)		
	TE	CE	Statistics (P Value)
FDM + Vehicle	17.20±2.16	19.93±1.28	> 0.69
FDM + Latanoprost	16.40±1.42	16.60±1.35	> 0.76
LIM + Vehicle	18.60±2.44	18.13±1.78	> 0.56
LIM+ Latanoprost	16.86±3.15	16.6±2.93	> 0.81
Category	Day 4		
	TE	CE	Statistics (P Value)
FDM + Vehicle	20.53±2.52	18.6±1.58	<0.03
FDM + Latanoprost	16.46±2.57	18.2±1.72	>0.10
LIM + Vehicle	19.00±3.09	18.06±1.43	>0.13
LIM+ Latanoprost	16.20±2.68	17.33±2.05	>0.08
Category	Day 8 (mean±SD)		
	TE	CE	Statistics (P Value)
FDM + Vehicle	21.33±3.23	18.6±2.91	<0.002
FDM + Latanoprost	15.86±2.62	19±1.41	<0.002
LIM + Vehicle	20.33±3.96	17.8±2.4	<0.006
LIM+ Latanoprost	15.91±3.25	17.82±2.73	<0.007
Category	Day 12 (mean±SD)		
	TE	CE	Statistics (P Value)
FDM + Vehicle	22±3.14	19.1±2.98	<0.001
FDM + Latanoprost	16.2±2.56	18.66±1.19	<0.001
LIM + Vehicle	21.33±3.06	18.13±2.52	<0.009
LIM+ Latanoprost	15.73±1.76	18.4±1.89	<0.006

Appendix: Table 6.1 Summarized refractive power (Rx) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	Rx of Baseline		Rx of Day 7		Rx of Day 14	
	Treated eye (D)	Control eye (D)	Treated eye (D)	Control eye (D)	Recovery eye (D)	Treated eye (D)
Lens-induced Myopia (LIM)						
1 nmol	5.06 ± 0.14	5.08 ± 0.18	-3.88 ± 2.58	3.40 ± 0.32	5.87 ± 2.49	-7.22 ± 2.95
10 nmol	4.55 ± 0.15	4.56 ± 0.15	0.61 ± 2.28	3.33 ± 0.31	1.88 ± 2.44	-2.72 ± 2.49
100 nmol	4.85 ± 0.36	4.97 ± 0.29	-0.56 ± 2.58	3.31± 0.34	2.43 2.21	-3.87 ± 2.69
DMSO + Corn Oil	4.58 ± 0.20	4.56 ± 0.13	-5.56 ± 2.57	3.0 ± 0.25	7.31 ± 2.04	-8.56 ± 0.84
Form-deprivation Myopia (FDM)						
1 nmol	5.12 ± 0.21	5.07 ± 0.08	-3.02±2.20	3.63±0.37	5.07±2.93	-6.66±1.90
10 nmol	5.05 ± 0.09	5.14 ± 0.19	0.18±1.02	3.27±0.22	1.71±2.38	-3.09±2.37
100 nmol	5.0±0.09	5.07±0.16	0.88±2.22	3.83±0.26	2.11±2.32	-2.94±2.42
DMSO + Corn Oil	5.00±0.11	5.17±0.22	-3.81±3.79	3.7±0.55	6.41±4.07	-7.51±3.95

Appendix: Table 6.2 Summarized anterior chamber depth (ACD) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	ACD of Baseline		ACD of Day 7		ACD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
Lens-induced Myopia (LIM)						
1 nmol	1.39 ± 0.03	1.40 ± 0.03	1.73 ± 0.07	1.56 ± 0.04	-0.10±0.11	0.18±0.07
10 nmol	1.45 ± 0.02	1.49 ± 0.10	1.63 ± 0.06	1.56 ± 0.02	-0.00±0.10	0.06±0.07
100 nmol	1.34 ± 0.04	1.36 ± 0.04	1.64 ± 0.15	1.53 ± 0.03	-0.03±0.05	0.10±0.12
DMSO + Corn Oil	1.44 ± 0.03	1.48 ± 0.09	1.69 ±0.03	1.56 ± 0.04	-0.11±0.09	0.22±0.07
Form-deprivation Myopia (FDM)						
1 nmol	1.40 ± 0.02	1.41 ± 0.03	1.69±0.07	1.52±0.07	-0.11±0.15	0.17±0.07
10 nmol	1.36 ± 0.01	1.37 ± 0.01	1.63±0.10	1.58±0.10	-0.02±0.07	0.07±0.07
100 nmol	1.33±0.04	1.34±0.02	1.56±0.12	1.48±0.06	0.02±0.12	0.11±0.11
DMSO + Corn Oil	1.39±0.03	1.39±0.03	1.68±0.11	1.48±0.06	-0.03±0.17	0.11±0.11

Appendix: Table 6.3 Summarized lens thickness (LT) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	LT of Baseline		LT of Day 7		LT of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
Lens-induced Myopia (LIM)						
1 nmol	1.96 ± 0.04	1.93 ± 0.05	2.26 ± 0.07	2.34 ± 0.04	0.04±0.06	-0.08±0.05
10 nmol	2.00 ± 0.04	1.99 ± 0.02	2.32 ± 0.09	2.36 ± 0.06	0.03±0.05	-0.05±0.06
100 nmol	2.12 ± 0.08	2.07 ± 0.05	2.25 ± 0.05	2.29 ± 0.03	-0.00±0.03	-0.04±0.03
DMSO + Corn Oil	2.00 ± 0.04	1.99 ± 0.03	2.29 ± 0.05	2.36 ± 0.03	-0.02±0.06	-0.01±0.04
Form-deprivation Myopia (FDM)						
1 nmol	1.95 ± 0.04	1.92 ± 0.04	2.23±0.08	2.31±0.12	0.07±0.11	-0.09±0.05
10 nmol	1.97 ± 0.03	1.95 ± 0.03	2.42±0.05	2.33±0.03	0.03±0.06	-0.04±0.06
100 nmol	1.96±0.04	1.96±0.04	2.19±0.07	2.23±0.04	-0.00±0.05	-0.04±0.03
DMSO + Corn Oil	2.10±0.04	2.10±0.03	2.25±0.04	2.27±0.04	-0.04±0.04	0.00±0.03

Appendix: Table 6.4 Summarized vitreous chamber depth (VCD) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	VCD of Baseline		VCD of Day 7		VCD of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
Lens-induced Myopia (LIM)						
1 nmol	5.25 ± 0.10	5.23 ± 0.09	6.13 ± 0.17	5.63 ± 0.11	-0.42±0.22	0.51±0.21
10 nmol	5.39 ± 0.10	5.40 ± 0.09	5.84 ± 0.15	5.60 ± 0.25	-0.12±0.15	0.25±0.24
100 nmol	5.28 ± 0.09	5.27 ± 0.12	5.93 ± 0.17	5.62 ± 0.11	-0.17±0.14	0.31±0.16
DMSO + Corn Oil	5.35 ± 0.12	5.36 ± 0.12	6.22 ± 0.21	5.57 ± 0.11	-0.56±0.21	0.64±0.25
Form-deprivation Myopia (FDM)						
1 nmol	5.25 ± 0.10	5.25 ± 0.09	6.16±0.17	5.68±0.16	-0.39±0.15	0.47±0.17
10 nmol	5.18 ± 0.11	5.14 ± 0.11	5.77±0.18	5.66±0.11	-0.07±0.08	0.21±0.12
100 nmol	5.08±0.16	5.08±0.13	5.62±0.31	5.36±0.16	-0.20±0.17	0.28±0.19
DMSO + Corn Oil	5.21±0.14	5.16±0.14	5.93±0.28	5.33±0.19	-0.51±0.21	0.61±0.23

Appendix: Table 6.5 Summarized axial length (AL) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	AL of Baseline		AL of Day 7		AL of Day 14	
	Treated eye (mm)	Control eye (mm)	Treated eye (mm)	Control eye (mm)	Recovery eye (mm)	Treated eye (mm)
Lens-induced Myopia (LIM)						
1 nmol	8.60 ± 0.13	8.57 ± 0.10	10.06 ± 0.20	9.53 ± 0.16	-0.43±0.26	0.52±0.21
10 nmol	8.84 ± 0.12	8.90 ± 0.16	9.79 ± 0.16	9.53 ± 0.25	-0.16±0.21	0.26±0.23
100 nmol	8.76 ± 0.13	8.72 ± 0.14	9.83 ± 0.27	9.46 ± 0.16	-0.23±0.17	0.37±0.23
DMSO + Corn Oil	8.80 ± 0.16	8.83 ± 0.19	10.19 ± 0.18	9.43 ± 0.13	-0.64±0.13	0.76±0.14
Form-deprivation Myopia (FDM)						
1 nmol	8.61 ± 0.12	8.59 ± 0.11	10.00±0.25	9.45±0.15	-0.46±0.13	0.55±0.14
10 nmol	8.53 ± 0.14	8.47 ± 0.12	9.75±0.20	9.48±0.18	-0.14±0.19	0.27±0.21
100 nmol	8.38±0.19	8.39±0.16	9.38±0.39	9.08±0.21	-0.21±0.25	0.33±0.24
DMSO + Corn Oil	8.70±0.14	8.66±0.14	9.77±0.37	9.09±0.25	-0.62±0.34	0.67±0.33

Appendix: Table 6.6 Summarized Choroidal thickness (ChT) data for the outcome of different doses of Y-33075 dihydrochloride on baseline, Days 7 and 14.

Category	ChT of Baseline		ChT of Day 7		ChT of Day 14	
	Treated eye (μm)	Control eye (μm)	Treated eye (μm)	Control eye (μm)	Recovery eye (μm)	Treated eye (μm)
Lens-induced Myopia (LIM)						
1 nmol	154.1±10.56	153.1±9.62	146.33±7.34	166±9.06	18.51±7.20	-24.4±9.40
10 nmol	161.77±10.40	163.88±12.45	133.66±8.95	145.05±8.32	7.02±8.86	-10.3±11.37
100 nmol	162.41±9.37	160.37±10.16	155.31±18.77	159.20±10.33	6.19±6.78	-11.7±9.07
DMSO + Corn Oil	157.05±7.96	155.88±8.77	131.69±2.25	164.85±10.48	23.79±9.52	-31±9.11
Form-deprivation Myopia (FDM)						
1 nmol	173.2±19.86	171.24±19.36	148.33±14.23	177.62±13.40	18.44±5.93	-26.3±3.02
10 nmol	159.07±22.55	160.35±25.21	136.6±4.44	144.19±5.66	6.88±4.56	-13.7±2.42
100 nmol	141.89±7.12	144.14±7.03	153.7±20.35	158.3±14.05	8.45±3.95	-15.37±3.26
DMSO + Corn Oil	142.62±6.58	145.70±7.67	114.06±12.09	145±15.18	22.56±6.70	-29.2±7.37

Appendix: Table 6.7 Summary of Intraocular Pressure (IOP) data for the effects of different doses of Y-33075 dihydrochloride before treatment

Lens-induced Myopia (LIM)	Baseline (mean±SD)		
Category	TE	CE	Statistics (P Value)
DMSO + Corn Oil	18.12±2.61	17.75±1.71	> 0.75
1 nmol, Y-33075 dihydrochloride	17.75±2.63	17.37±1.21	> 0.73
10 nmol, Y-33075 dihydrochloride	17.01±0.86	16.87 ±1.45	> 0.84
100 nmol, Y-33075 dihydrochloride	17.00 ±1.50	16.65±1.39	> 0.75
Form-deprivation Myopia (FDM)	Baseline (mean±SD)		
Category	TE	CE	Statistics (P Value)
DMSO + Corn Oil	19.37±1.99	19±2.59	> 0.76
1 nmol, Y-33075 dihydrochloride	18.12±1.26	18.25±1.39	> 0.84
10 nmol, Y-33075 dihydrochloride	19.38±1.57	19.5±1.93	> 0.89
100 nmol, Y-33075 dihydrochloride	19.12±2.47	18.87±1.89	> 0.83

Appendix: Table 6.8 Summary of Intraocular Pressure (IOP) data for the effects of different doses of Y-33075 dihydrochloride after treatment

Lens-induced Myopia (LIM)	Day 7 (mean±SD)		
Category	TE	CE	Statistics (P Value)
DMSO + Corn Oil	23.87±3.58	18.12±2.61	< 0.007
1 nmol, Y-33075 dihydrochloride	23.75±3.26	18.37±1.93	< 0.009
10 nmol, Y-33075 dihydrochloride	21.5±1.93	18.5 ±2.06	< 0.04
100 nmol, Y-33075 dihydrochloride	17.5 ±2.82	18.12±1.05	> 0.62
Form-deprivation Myopia (FDM)	Day 7 (mean±SD)		
Category	TE	CE	Statistics (P Value)
DMSO + Corn Oil	23.5±4.18	18.12±3.55	< 0.005
1 nmol, Y-33075 dihydrochloride	22.87±2.80	17.25±1.56	< 0.005
10 nmol, Y-33075 dihydrochloride	21±3.12	18.62±2.11	> 0.11
100 nmol, Y-33075 dihydrochloride	17.12±4.93	19.25±1.39	> 0.27