THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學
Pao Yue-kong Library
包玉剛圖書館

Copyright Undertaking

This thesis is protected by copyright, with all rights reserved.

By reading and using the thesis, the reader understands and agrees to the following terms:

1. The reader will abide by the rules and legal ordinances governing copyright regarding the use of the thesis.
2. The reader will use the thesis for the purpose of research or private study only and not for distribution or further reproduction or any other purpose.
3. The reader agrees to indemnify and hold the University harmless from and against any loss, damage, cost, liability or expenses arising from copyright infringement or unauthorized usage.

IMPORTANT

If you have reasons to believe that any materials in this thesis are deemed not suitable to be distributed in this form, or a copyright owner having difficulty with the material being included in our database, please contact lbsys@polyu.edu.hk providing details. The Library will look into your claim and consider taking remedial action upon receipt of the written requests.

Pao Yue-kong Library, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

http://www.lib.polyu.edu.hk

DEVELOPMENT OF A NOVEL NASAL SPRAY FOR RAPID PREVENTION OF NAUSEA AND VOMITING INDUCED BY CHEMOTHERAPY OR RADIOTHERAPY

XU LIANG

PhD

The Hong Kong Polytechnic University

2020

The Hong Kong Polytechnic University

Department of Applied Biology and Chemical Technology

Development of a Novel Nasal Spray for Rapid Prevention of Nausea and Vomiting Induced by Chemotherapy or Radiotherapy

Xu Liang

A thesis submitted in partial fulfilment of the requirements

for the degree of Doctor of Philosophy

July 2019

Certificate of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

Xu Liang (Name of student)

Abstract

Background

Nausea and vomiting are commonly and severely debilitating adverse events in cytotoxic chemotherapy and radiotherapy. The management of chemotherapy-induced and radiotherapy-induced nausea and vomiting (CINV and RINV) has improved greatly since the discovery of 5-HT3 receptor antagonists (5-HT3 RAs). The 5-HT3 RAs are regarded as highly effective antiemetic agents and recommended as the first choice to control CINV and RINV.

Granisetron is a potent and highly selective 5-HT3 RA, and is effective and well-tolerated for preventing CINV and RINV. It is currently marketed in various dosage forms including oral tablet/solution, transdermal patch, intravenous injection and subcutaneous injection. The onset of antiemetic effects of oral and transdermal dosage forms of granisetron is relatively slow (> 1 hour for oral granisetron and 24 hours for transdermal granisetron). In addition, oral administration of granisetron tablets may be extremely difficult for patients with compromised swallowing capacity or nausea symptoms. Granisetron injection is an invasive dosage form, so patients will suffer unnecessary pains and potential infection due to injection, which is an important issue in those immune-compromised patients. Therefore, it is imperative to develop an alternative dosage form administered via noninvasive route and with rapid onset for better management of nausea and vomiting.

Intranasal administration is a noninvasive route for both local and systemic drug delivery. The nasal mucosa is highly vascularized and permeable, which enables drugs to quickly and completely transport across the mucosa and directly enter into the systemic circulation without first-pass metabolism. Granisetron is a small and lipophilic

molecule with high water solubility and stability. Therefore it is an ideal drug candidate for intranasal delivery.

Purpose

This project aims to develop novel granisetron intranasal spray for rapid prevention of CINV/RINV. Various granisetron bioadhesive formulations will be prepared and optimized, followed by the pharmacokinetics and brain targeting study. Finally, a Phase I clinical trial will be conducted to confirm the safety and efficacy of granisetron nasal sprays as compared to granisetron intravenous injection and granisetron tablet in healthy volunteers.

Methodology

In order to develop granisetron nasal spray, several research objectives have to be achieved: (1) to evaluate the feasibility of delivering granisetron via nasal route; (2) to prepare various formulations utilizing bioadhesive technologies and screen the mucoadhesive capabilities basing on the animal studies; (3) to study the pharmacokinetics and brain distribution of granisetron nasal spray solution in SD rats; (4) to evaluate the pharmacokinetics of granisetron and its major metabolite (7-OH granisetron) in Beagle dogs following intranasal administration; (5) to investigate the pharmacokinetics, safety and tolerability of granisetron nasal spray in healthy volunteers.

Results

Various bioadhesive formulations were prepared and screened basing on nasal residence time and bioavailability in rats. After intranasal administration of granisetron formulations containing HPMC at different concentrations, the highest bioavailability was achieved when the concentration of HPMC was 0.25%. The C_{max} and $AUC_{0-\infty}$

increased in a dose-proportional manner over the dose range of 0.4 to 1.6 mg/kg in rats and 0.5 to 2.0 mg/dog in Beagle dogs after intranasal administration of granisetron formulations. As compared to oral administration, higher C_{max} and shorter t_{max}, as well as improved bioavailability were observed after intranasal administration of bioadhesive formulations. In the brain distribution study in rats, only limited direct nose-to-brain transport was observed following intranasal administration of granisetron formulation. In a pharmacokinetic study in Beagle dogs, rapid and complete absorption of granisetron was achieved after intranasal administration of bioadhesive nasal spray. Meanwhile, the systemic exposure of the metabolite 7-OH granisetron after intranasal administration was approximate 50% lower than that after oral administration.

In Phase I clinical study (open-label and parallel-group), the absolute bioavailability of intranasal granisetron at the doses of 0.5, 1.0, and 2.0 mg were 50.4%, 75.5%, and 64.0%, respectively. The intranasal administration of granisetron bioadhesive spray presented more rapid absorption rate in comparison with oral administration of granisetron tablet (Kytril®). A dose-proportional increase in AUCs was observed in subjects after intranasal administration of granisetron nasal spray over the dose range of 0.5 to 2.0 mg.

Conclusion

A rapid absorption of granisetron was achieved through nasal route. The granisetron nasal sprays (0.5 mg, 1.0 mg and 2.0 mg) are generally safe and well tolerated in comparison with intravenous and oral administrations of Kytril® 1.0 mg.

Acknowledgements

I would like to express my sincere gratitude to the following persons who made this tough journey possible:

First and foremost, my deep appreciation goes to my supervisor Prof. Wong Wing-tak, for giving me this precious chance to pursue my doctor's degree. I still remember how nervous and stressed I was in my first acquaintance with Prof. Wong. However, I was so impressed at his kindness, generosity and patience during the talking. His incisiveness, wisdom and flexibility are worthy of my gratitude and respect forever.

I would like to offer my sincere gratitude to my co-supervisor Prof. Chow Ming-cheung. He has been helping me with comprehensive supports in academic, technical and spiritual aspects. His passion and enthusiasm towards academic research are impressive to me and set an example on rigorous attitude to science.

I have to give my utmost gratitude to Dr. Johnson Y.N. Lau, the Chief Executive Officer and Board Chairman of Athenex, Inc. He is the most experienced person I've ever seen both in scientific field and business management. He is one of the few people with "reality distortion field", which means the ability to convince and encourage others to believe almost all the tasks at hand are possible. He always cared about my academic project and provided comprehensive support, such as resource, creativity, guidance and encouragement. I'm always impressed by his personality charm and deep insight into pharmacy. I will remember his generous help and careful guidance forever.

Also, I would like to express my appreciation to Dr. Benjamin Lee, the head of Athenex HK R&D, who is always enthusiastic in pharmaceutical industrialization. He gave me a lot of suggestions and guidance, and made my progress on schedule. He made a strong impression to me with his passion and easy-going personality, and set a

good model to follow in my future academic and industrial career. The name of Dr. Wang Yanfeng, Associate Director of Maxinase Life Science Limited, is never forgotten. I feel much privileged to be his team member and learned a lot from him during the past five years. He supported me with his wealth of experience in pharmacy and taught me with his great patience. It's my fortune to have such a guider in my academic career.

I would also like to extend my thanks to the technicians and colleagues in my company, for the project would not run smoothly without their help. I have to thank Dr. Yoon Wengli, Senior Research Director of Athenex HK R&D, who is a patient and knowledgeable scientist. She always encouraged me when I was frustrated in work and made me refreshed. My heartiest thanks also go to other fellows in our lab for their technical support and constructive advice on my project. I would like to deliver my heartful thanks to Dr. Kuo Ming Yu, Dr. Liam Lee, Dr. Terence Kwan, Dr. Tsz Cheung Chong and Mr. Jiahao Li. Working with those optimistic and enthusiastic young people was the happiest experience in my past years. I'm so delighted to have all of you in my life.

Last but not the least, my deepest gratitude goes to my beloved parents, wife and lovely daughter. Thanks to their endless support and understanding through my life. Without the support from my family, I would not achieve anything. I love you all.

List of Abbreviations

ACN	Acetonitrile
API	Active pharmaceutical ingredient
$AUC_{0\text{-last}}$	The area under the plasma concentration versus time profile from time 0 to the last sampling time point
$AUC_{0\text{-}\infty}$	The area under the plasma concentration versus time profile from time 0 to infinity
BBB	Blood-brain barrier
CINV	Chemotherapy-induced nausea and vomiting
CL	Total body clearance
CL/F	Apparent total body clearance
C_{max}	Peak plasma concentration
CNS	Central nervous system
CTZ	Chemoreceptor trigger zone
CYP 450	Cytochrome P450
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
F_{abs}	Absolute bioavailability
GI tract	Gastrointestinal tract
Granisetron	GNT
HPLC	High-performance liquid chromatography
5-HT3 RAs	5-HT3 receptor antagonists
IS	Internal standard
k_{el}	Elimination rate constant
LLOQ	Lower limit of quantification
MTT	Mucociliary transport time
NOAEL	No observed adverse effect level

ORNs	Olfactory receptor neurons
PBS	Phosphate buffered saline
P-gp	P-glycoprotein
RE	Relative error
RINV	Radiotherapy-induced nausea and vomiting
RSD	Relative standard deviation
SNES	Simulated nasal electrolyte solution
SSE	Error sum of squares
$t_{1/2}$	Elimination half-life
TEAE	Treatment-emergent adverse event
TK	Toxicokinetics
t_{max}	Time to achieve maximum plasma concentration
$T_{sol\text{-}gel}$	Solution to gel transition temperature
VAP	Vagal afferent pathway

Table of Contents

CHAPTER 2

DEVELOPMENT AND CHARACTERIZATION OF GRANISETRON BIOADHESIVE SYSTEM FOR INTRANASAL DELIVERY 50

Chapter 1

Introduction: Overview of CINV prophylaxis and intranasal drug delivery system

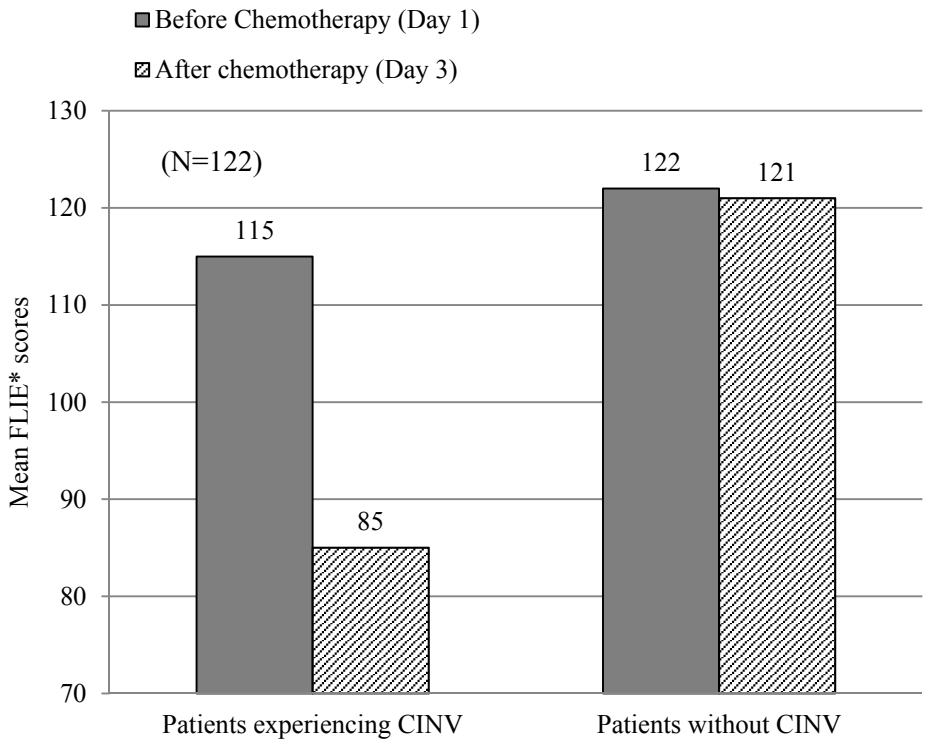
1.1 Management of Chemotherapy-Induced Nausea and Vomiting

1.1.1 The impact of CINV on patients

Chemotherapy-induced nausea and vomiting (CINV) are the most distressing symptoms in patients receiving antineoplastic treatment [1-3]. Figure 1.1 shows the change of patients' life quality immediately prior to and after chemotherapy. The rate of the quality of life decreases over 30% if they experienced emesis [4]. Besides, patients also experience an unpleasant sensation in the upper abdomen and the back of the throat during the nausea and vomiting episode, which may induce the discharge of stomach contents, and further deteriorate the emesis [5, 6]. In spite of the distinct symptoms of these distressing side effects, they usually occur simultaneously [7]. As compared to vomiting, nausea is a symptom which is hard to be objectively quantified, considering the nausea is a relatively slight emetogenic symptom without triggering vomiting. Clinical therapeutic effects showed that treatments for vomiting symptoms were often effective for nausea [8]. Therefore, all the mechanism and treatments of vomiting involved in this chapter can be also applied to nausea as well.

The symptoms of nausea and vomiting can limit patients' ability to eat and drink, remarkably reduce the quality of life, even threaten the success of therapy [9] [10]. Frequent vomiting usually leads to loss of appetite and food intake, consequently resulting in malnutrition, decreased immunity, electrolyte imbalances, mental deterioration, social isolation, inability to daily activities, etc. [11] CINV can further

influence the patient's compliance to chemotherapy and result in failure of therapy. It has been reported that up to 20% of cancer patients were forced to postpone or refuse potentially curative treatment because of the severely debilitating adverse events [12].



*FLIE means Functional Living Index-Emesis.

Figure 1.1 CINV: Impact on Quality of Life

1.1.2 The category of CINV

According to the time of onset, CINV is classified as acute, delayed and anticipatory symptoms [13] [14, 15]. The acute CINV occurs within 24 hours after chemotherapy. The intensity of the symptom reaches peak after 5 to 7 hours. The delayed CINV often occurs between 24 hours and 7 days after treatment. The peak intensity occurs 48 to 72 hours after chemotherapy. The anticipatory CINV may also occur before a chemotherapy treatment as an adverse memory of emesis in previous treatment cycles, which can be triggered by some elements during chemotherapy, such

as taste, odor, memories, visions, or anxiety [16] [17] [18]. As the most incident emesis, about 30% of patients experience anticipatory CINV when receiving chemotherapy. The incidence of the anticipatory CINV has decreased in recent years owing to the improvement of prophylaxis treatments for emesis [19].

1.1.3 Emetic responses caused by chemotherapy agents

Triggered by vomiting center, the CINV involves central and peripheral neural systems, the chemoreceptor trigger zone (CTZ), the vagal nerve afferents, neurotransmitters and various receptors. The vomiting center is the central area responsible for emesis [20], which is believed to be a collection of neurons distributed within the medulla oblongata located in the brainstem, rather than existing in specific locations in the brain [21]. It consists of reticular formation, solitary nucleus, and multiple afferent nerve fibers [22] [23] [24]. After the stimulation of the vomiting center, the output impulses transmit via motor pathways and then trigger vomiting.

The chemoreceptor trigger zone (CTZ), which contains muscarinic, dopamine (D2), serotonin, neurokinin-1 (NK-1), histamine (H1), substance P, opioid, and acetylcholine receptors, is an area located between the medulla oblongata and the floor of the fourth ventricle [25]. The CTZ is not protected by the blood-brain barrier (BBB), and the endothelium of its capillaries is permeable to the substances in systemic circulation. The CTZ can be easily stimulated by the cytotoxic agents in both the bloodstream and the cerebrospinal fluid [26-28].

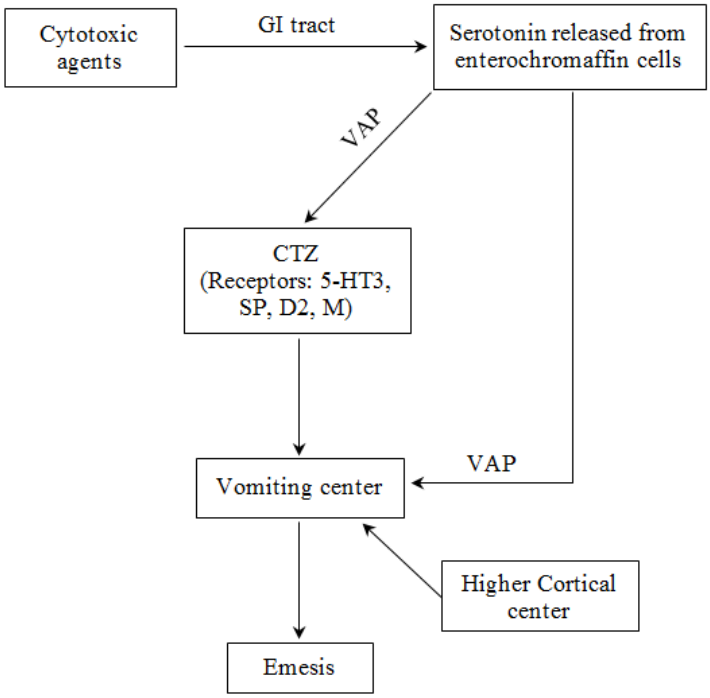


Figure 1.2 Activation of emetic pathway induced by cytotoxic agents

CTZ: chemoreceptor trigger zone. GI tract: Gastrointestinal tract. 5-HT3: 5-Hydroxytriptamin3. SP: Substance P. D2: Dopamine. M: Muscarinic. VAP: vagal afferent pathway.

The activation of emetic pathway induced by cytotoxic drugs is illustrated in Figure 1.2. The antineoplastic drugs can cause vomiting through several pathways [23]: (1) stimulating the vomiting center via vagal afferent pathway, (2) stimulating the vomiting center by activating the chemoreceptor trigger zone, and (3) directly activating the vomiting center. Various neurotransmitters, such as serotonin, substance P and dopamine, play a great important role in induction of nausea and vomiting [29] [30, 31]. After chemotherapy, antineoplastic drugs may cause the damage of enterochromaffin cells near abdominal vagal afferents, and result in the release of serotonin (also known as 5-HT3). The released serotonin could bind to the 5-HT3 receptors on the adjacent fibers of vagal afferents. Then neural impulses take place and transmit through vagal nerve system to stimulate the CTZ in the brain stem. The stimulation of CTZ will subsequently activate the vomiting center, then the vomiting reflex is initiated, followed

by the coordination of different muscles involved in vomiting, e.g., the relaxation of lower esophageal sphincter, the contraction of stomach, diaphragm and abdominal muscles.

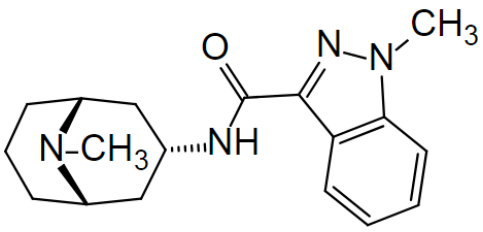
1.1.4 5-Hydroxytryptamine 3 receptor antagonists (5-HT3RAs)

The application of 5-Hydroxytryptamine 3 receptor antagonists (or 5-HT3RAs) in early 1990s was a breakthrough in the management of chemotherapy-induced nausea and vomiting. 5-HT3RAs are antiemetic drugs with high therapeutic index for both prevention and treatment of CINV in the patients receiving moderately to highly emetogenic antineoplastic agents. The 5- HT3RAs exert the therapeutic effect by selectively and competitively binding to 5-HT3 receptors and terminating the transmission of emetogenic signals to the CTZ [32].

To date, there are seven 5-HT3 antagonists approved for CINV: Ondansetron (ZOFRAN®, Glaxo SmithKline), Tropisetron (Navoban®, Novartis), Granisetron (Kytril®, Roche), Dolasetron (Anzemet®, Sanofi-Aventis), Palonosetron (Aloxi®, MGI Pharma), Azasetron (Serotone®, Torii Pharmaceutical) and Ramosetron (Irribow®, Astellas Pharma). These 5-HT3RAs are highly effective in both prophylactic therapy and treatment for patients receiving highly to moderately emetogenic agents. 5-HT3RAs can be administered alone or combined with a glucocorticoid, such as dexamethasone, to achieve the synergetic effects [33]. Granisetron, dolasetron, ondansetron and tropisetron have been demonstrated to be equivalent in efficacy and toxicities when used at the recommended doses [34] [35] [36]. The most common side effects of the 5-HT3RAs are transient elevation of hepatic aminotransferase levels, mild headache and constipation.

1.1.5 Overview of Granisetron

Granisetron is a 5-HT_3 receptor antagonist and used as an antiemetic agent for managing CINV. It is a white-to-off-white powder, which is readily soluble in water at 20°C. Its molecular formula is $C_{18}H_{24}N_4O$ with a molecular weight of 312.409 (free base). The structure of granisetron is shown as Figure 1.3. Granisetron is mainly metabolized through liver, with slow metabolic rate. The half-life of granisetron is about 9 hours, longer than other 5-HT3 receptor antagonists (except palonosetron). The oral absorption of granisetron is rapid and complete, but the oral bioavailability is only 60% due to the first pass metabolism. The absorption of granisetron is further affected by food. The plasma protein binding is approximately 65% and the drug is mainly excreted via urine (48%) and feces (38%).



$C_{18}H_{24}N_4O$, Molecular weight: 312.409

1-methyl-N-[(1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl]-1H-indazole-3-carboxamide.

Figure 1.3 Chemical structure of granisetron

1.1.5.1 Pharmacology of granisetron

After cytotoxic agents enter into the gastrointestinal tract, the enterochromaffin cells are damaged and then release serotonin (5-hydroxytryptamine, or 5-HT). The released serotonin binds to the 5-HT3 receptors, which are mainly located on vagal nerve terminals and the chemoreceptor trigger zone in the brainstem. Then the vagal afferent discharge is stimulated to induce vomiting. Granisetron exerts antiemetic

effects by preventing the serotonin from binding to the 5-HT3 receptors in the brain stem and the vagal afferent nerves in the gastrointestinal tract [37]. Granisetron has high and selective affinity to 5-HT3 receptor, but no affinity with other subtypes of 5-HT receptors. This is different from ondansetron, which is found to have affinity for 5-HT1B, 5-HT1C, α1-adrenergic, and μ-opioid receptors. The high selectivity of granisetron causes the minimal adverse events as compared with other 5-HT3 receptor antagonists [38].

The most frequently reported adverse events of granisetron are constipation, headache, asthenia and diarrhea.

1.1.5.2 Pharmacokinetic profiles of granisetron

Food can impact the absorption of oral granisetron both on rate and extent by delaying the gastric emptying, changing the acidity of the proximal small intestine, stimulating bile flow and increasing hepatic blood flow [39]. When healthy volunteers received a 1 mg granisetron tablet with food, the area under the curve decreased to 95% of that under fast state and the peak plasma concentration increased by 30%. The metabolism of granisetron involves N-demethylation and aromatic ring oxidation followed by conjugation. In vitro studies imply that the major route of metabolism involves the cytochrome P-450 3A subfamily. The clearance of granisetron and its metabolites is predominantly by hepatic metabolism. The impact of gender on the pharmacokinetics is not significant.

1.1.5.3 Efficacy in acute CINV

The total control and complete control are generally used as the primary end points to evaluate the efficacy of anti-emetic drugs in the management of CINV [40]. The total

control means no vomiting, no nausea and no need to use antiemetic rescue medication. The complete control is defined as no vomiting, no worse than mild nausea and no use of rescue medication.

Several clinical trials were conducted to estimate the dose-efficacy relationship of intravenously administered granisetron in controlling the acute CINV. The 24-hour complete response rates and the dosage of intravenously administered granisetron were shown in Table 1.1. These studies showed that the intravenously administered granisetron was effective in preventing acute CINV within the first 24 hours after chemotherapy treatment at the dose of 10-40 μg/kg. No evidence indicated continuously increasing the dosage of granisetron to more than 40 μg/kg could bring additional therapeutic benefits.

Table 1.1 The efficacy of intravenous granisetron at different doses

Study	Doses	24-hour complete response rates
Kamanabrou et al. [41]	Study 1 (2, 10 and 40 μg/kg)	30.8% (2 μg/kg), 61.5% (10 μg/kg), 67.9% (40 μg/kg)
	Study 2 (40 and 160 μg/kg)	56.5% (40 μg/kg), 58.5% (160 μg/kg)

The antiemetic efficacy of granisetron oral tablet (Kytril®) has been demonstrated in patients receiving moderately or highly emetogenic chemotherapy, with the regimen of 2 mg granisetron tablets once daily or 1 mg granisetron tablets twice daily. A double-blind, randomized, parallel clinical trial was conducted to compare the antiemetic efficacy of both regimens: a single oral dose of 2 mg granisetron given one hour prior to chemotherapy, or a divided dose of 1 mg which is given 1 mg granisetron one hour prior to chemotherapy and twelve hours after the initiation of chemotherapy

[42]. The complete response rate and the incidence of emesis and nausea were used as the primary endpoints in assessing the efficacy of the both regimens. The results of the two regimens are not significantly different between the two groups in respects to all endpoints, since the percentage of patients achieving complete response in the 1-mg twice daily group and the 2-mg once daily group are 51% and 50% respectively. Furthermore, the incidences of adverse events attributed to both treatments are similar, indicating oral granisetron is well tolerated.

The combination of granisetron and a corticosteroid is also suggested for CINV management. A clinical trial involving 39 patients with Hodgkin lymphoma was conducted to assess the antiemetic efficacy of granisetron in combination with dexamethasone for controlling CINV [43]. The complete control rate of acute CINV significantly increased in the group treated with combined therapy, as compared to the group without dexamethasone.

1.1.5.4 Efficacy in delayed CINV

The treatment by 5-HT3 receptor antagonists in prevention of delayed CINV is recommended by antiemetic guidelines [44]. The efficacy of granisetron in delayed CINV is also confirmed by previous clinical trials [45]. In a randomized clinical study, the efficacy of single dose of granisetron was compared with other 5-HT3 receptor antagonists in prevention of both acute and delayed CINV [46]. Fifty-four patients were enrolled in and assigned into three groups: 19 patients received 3 mg of intravenous granisetron, 18 patients received 8 mg of intravenous ondansetron, and 17 patients received 5 mg of intravenous tropisetron. The complete control rates for delayed CINV were 73.7% in granisetron group, 38.8% in ondansetron group, and 52.9% in tropisetron group respectively. It was demonstrated that the efficacy of granisetron was superior to

the other two serotonin antagonists in the prevention of delayed emesis. An explanation for the higher control rate for granisetron is the relatively higher specificity and affinity of granisetron for 5-HT3 receptors and the prolonged half-life.

1.1.5.5 Dosage forms of granisetron

1.1.5.5.1 Immediate release dosage forms of granisetron

The immediate release dosage forms of granisetron are marketed under the brand name Kytril®. To date, the immediate release dosage forms of granisetron include immediate release tablets, oral solution and intravenous injection. Each granisetron tablet (Kytril®) contains 1 mg of granisetron free base. For Kytril® oral solution, 2 mg of granisetron free base is dissolved to 10 ml solution. Kytril® intravenous injection solution (1 mg/ml) is available in 1 ml single-use and 4 ml multi-use vials, respectively. Kytril® injection solution at the concentration of 0.1 mg/ml is also available in a 1 ml single-use vial.

The oral dose of 2 mg once daily or 1 mg twice daily are recommended dose of granisetron (Kytril®) taken one hour before the emetogenic therapy. For Kytril® intravenous injection, the recommended dosage is 10 μg/kg, which is administered intravenously within 30 minutes before chemotherapy. Both oral and intravenous dosage forms are administered only on the chemotherapy days.

All those granisetron products (Kytril®) are indicated for the prevention of nausea and vomiting associated with initial and repeat courses of emetogenic antineoplastic therapy. Granisetron injection is also indicated for the prevention and treatment of postoperative nausea and vomiting in adults. Although Kytril® injection is not a recommended drug for routine prophylaxis with low expectation of postoperative

nausea and vomiting, it can still be used where nausea and vomiting must be avoided during the postoperative period.

1.1.5.5.2 Extended-release dosage forms of granisetron

Besides the immediate release dosage forms, granisetron is also available as transdermal patch (Sancuso®, Kyowa Kirin Inc.). Sancuso® was approved by US FDA in 2008 to prevent nausea and vomiting in cancer patients receiving moderately or highly emetogenic chemotherapy for three to five days. It is a 52 cm^2 clear plastic-backed patch with an adhesive layer containing 34.3 mg of granisetron dissolved in a thin adhesive layer. When patients stick the patch to the skin, granisetron is released from the adhesive layer at the release rate of 3.1 mg/day. Then the drug is continuously delivered into the bloodstream via transdermal route. Sancuso® is applied on skin from a minimum of 24 hours to a maximum of 48 hours before scheduled chemotherapy treatment, and can be applied up to 7 days. Unlike other antiemetic medications, Sancuso® can protect the patients from nausea and vomiting for five consecutive days.

Sustol® (Heron Therapeutics, Inc.) is an extended-release (ER) injectable dosage form of granisetron, which was approved by US FDA in 2016, and indicated for preventing acute and delayed CINV associated with the moderately or highly emetogenic chemotherapy, or anthracycline and cyclophosphamide combination chemotherapy regimens. Sustol® is a sterile, clear, colorless to slightly yellow, viscous liquid supplied in a single-dose, pre-filled syringe. It is administered as an extended-release subcutaneous injection at the concentration of 10 mg of granisetron per 0.4 ml of injection solution. The recommended dose for adults is 10 mg as a single subcutaneous injection 30 minutes prior to the initiation of emetogenic chemotherapy on day 1. The dosing frequency of Sustol® is not more than once a week due to the

extended-release kinetics of the formulation. An erosion-controlled technology called Biochronomer™ was utilized in the formula to achieve sustained release up to 7 days. Biochronomer™ technology (Figure 1.4) includes a bio-erodible matrix consisting of triethylene glycol poly(orthoester) polymer and polyethylene glycol monomethyl ether. Granisetron is formulated into the viscous matrix with slow release of drug and long-acting therapeutic effects over 5 days. The drug peak plasma concentration (C_{max}) reached approximately in 24 hours after receiving a 10 mg of granisetron subcutaneous injection, and the concentration of therapeutic level can be maintained for up to seven days. In a metabolic fate study conducted in healthy volunteers, the breakdown products of biodegradable polymer in the subcutaneous injection were detected in urine, although the recovery was incomplete till the 10^{th} day from the initiation of study. The accumulation of metabolites of the polymers in injection was not detected in plasma.

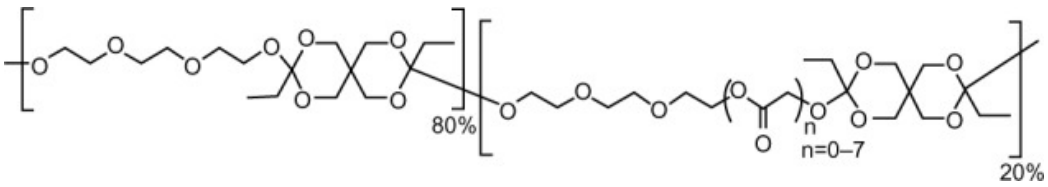


Figure 1.4 Structure of Biochronomer™ used in Sustol®

1.1.5.5.3 Potential administration routes for granisetron

Recently, several innovative drug delivery routes have been explored for granisetron. The buccal drug delivery has been studied aiming at prolonged effects and enhancing the bioavailability of granisetron [47]. The provesicular carriers with nonionic surfactants (Span® 80 or Span® 20) and cholesterol were selected to formulate the granisetron buccoadhesive tablets. Once contacting the saliva on buccal surface, granisetron is entrapped in the reconstituted niosomes, which is transformed from provesicular carriers, and then the drug is absorbed via buccal mucosa. Compared to the

conventional granisetron oral tablets, improved bioavailability and absorption rate were achieved via the buccal delivery system. Meanwhile, part of the drug in the tablets is swallowed with saliva to GI tract, and the antiemetic effects may be compromised. Another disadvantage for buccal delivery is the unpleasant taste and the long time use at the buccal site may affect the compliance of patients. In addition, cancer patients receiving chemotherapy or radiotherapy are at high risk for oral mucositis, which limits the application of buccal drug delivery in cancer patients [48]. Briefly, buccal drug delivery system may be not a suitable administration route for granisetron.

Intranasal administration is another non-invasive route for both local and systemic drug delivery. The nasal mucosa is highly vascularized and permeable, thus drugs can quickly transport across the mucosa and then directly enter into the systemic circulation with a rapid onset of action. The gastrointestinal and hepatic first-pass effects can be completely avoided by intranasal administration, therefore the high bioavailability of granisetron can be achieved. Granisetron is a small and lipophilic molecule with acceptable water solubility and stability at ambient condition. Compared to other serotonin receptor antagonists (i.e., ondensetron), the dose of granisetron is much lower (1-2 mg/person/day). Therefore, it is an ideal drug candidate for intranasal delivery.

1.2 Overview of intranasal drug delivery

1.2.1 Introduction of intranasal drug delivery system

Intranasal drug delivery system has been considered as a promising route to achieve rapid onset of action and high bioavailability. The nasal mucosa is highly vascularized and more permeable than the gastrointestinal tract because there are fewer enzymes and less dilution effect in nasal cavity [49]. In recent decades, there has been increasing interests in the research and development of intranasal route for local [50],

systemic [51] and brain-targeted [52] drug delivery. It is believed to be an alternative choice to replace invasive administrations, and a direct access to the systemic circulation. Up to date, multiple drugs candidates, including small molecules, peptides proteins and vaccines, have been investigated and shown to achieve faster and better systemic absorption with improved patient compliance via intranasal route [53]. Intranasal drug delivery is painless, non-invasive, without sterile preparation, and easy to self-dose. In view of these advantages, intranasal drug delivery can be explored as an alternative to overcome the unsatisfactory properties of certain drugs or prolong the commercial life of the marketed products. The advantages and the limitations of intranasal drug delivery system are summarized in Table 1.2 [54].

Table 1.2 Advantages and limitations of intranasal drug delivery

Advantages	Limitations
➢ A non-invasive drug delivery. ➢ Bypass hepatic first-pass metabolism. ➢ Rapid absorption and quick onset of action. ➢ Satisfactory absorption of small molecular drugs. ➢ The bioavailability of large molecular drugs can be improved by increasing the permeability of nasal mucosa. ➢ Intranasal route is worth a shot for those drugs with poor oral absorption. ➢ Maximize patients' convenience, comfort and compliance, especially when long term therapy with parenteral medication is needed.	➢ The absorption area of nasal cavity is smaller comparing with gastrointestinal tract. ➢ The absorption enhancer is likely to result in potential histological toxicity of nasal mucosa. ➢ Irreversible damage of the nasal cilia may occur, resulted from either the drugs or the excipients. ➢ Possibility of nasal irritation, especially for those susceptible people.

1.2.2 Nasal anatomy

The human nasal cavity has the volume of 15-20 ml, with the surface area of 150-200 cm^2. It is divided by the nasal septum into two symmetrical chambers, each of them consists of nasal vestibule, respiratory region and olfactory region [55]. Nasal vestibule is a small dilated space at the opening site of the nostril. Respiratory region is responsible for air exchange from the respiratory system. It is the largest segment of the nasal cavity, which is richly vascularized and covered with respiratory epithelium. The respiratory epithelium consists of mucous cells with or without cilia. The ciliated and non-ciliated cells in the respiratory region are covered by non-motile microvilli, which provide large surface area as an ideal region for drug absorption. The motile cilia are on the surface of those ciliated cells, responsible for mucociliary clearance. The mucociliary clearance is the main obstacle that eliminates the drugs intranasally administered from the absorption site, by the mucociliary movement from the anterior to the posterior region of the nasal cavity. The olfactory region is at the apex of nasal cavity and is lined by olfactory epitheliums, where the olfactory receptors are found. Over 90% of the nasal mucosa is the respiratory region, while the olfactory mucosa is less than 10 %. Meanwhile, the olfactory receptor cells connect to the central nerve system (CNS), which is a potential pathway directly delivering drugs from nose to brain [56].

1.2.3 The pathways of nasal absorption

There are two pathways for drugs administered via intranasal route. The first pathway is the paracellular route for the hydrophilic drugs [57]. The paracellular transport is slow and passive. The drugs with molecular weight >1000 Daltons show poor bioavailability and are absorbed via paracellular route [58]. The second pathway

called as transcellular route is related to lipophilic drugs [59]. The transport rate of drugs via the transcellular route is dependent on drug lipophilicity.

1.2.4 Nose-to-Brain Pathways

Blood-brain barrier (BBB) is a tight border with highly selective semipermeable properties which could separate the brain from systemic blood circulation. The lipophilic drugs, glucose as well as amino acids that are vital to neural function are allowed to penetrate the BBB by passive diffusion. However, lots of therapeutic treatments for brain disorders are not effective due to the failures of drugs from passing through the BBB. Intranasal drug delivery system has gained mounting interest these years as a non-invasive administration route that could provide directly nose-to-brain pathways. Two pathways are involved in nose-to-brain delivery: trigeminal and olfactory pathways [60]. Additionally, the drugs could also penetrate the BBB through systemic circulation pathway after absorption via nasal route, which is an indirect pathway from the nose to the brain. The entrance of drugs from nose to brain is usually by combined pathways illustrated as Figure 1.5 [61].

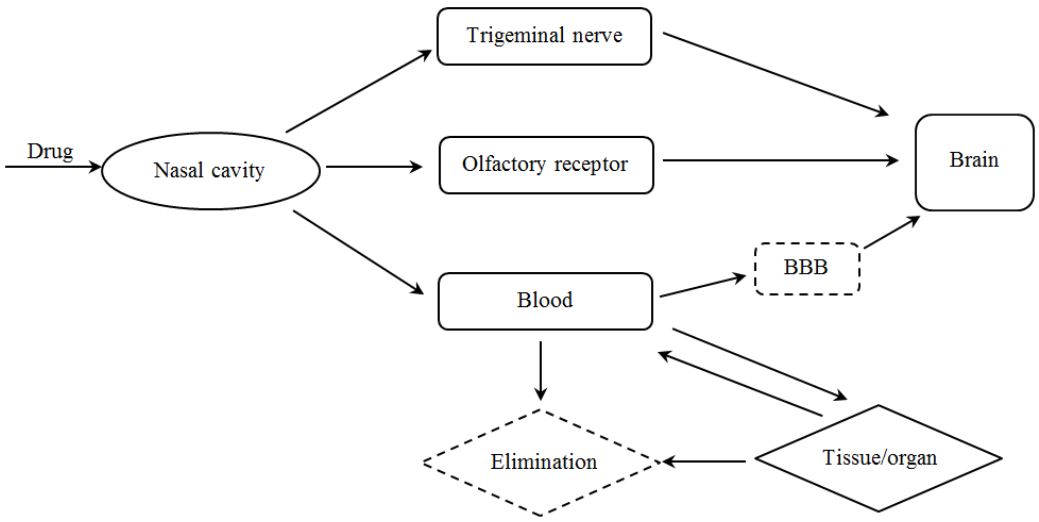


Figure 1.5 Possible drug pathways from the nose cavity to the brain

1.2.4.1 Trigeminal pathway

The trigeminal nerve enters into the brain via the pons at the brain stem, and accounts for the innervation of the olfactory and the respiratory epithelium in the nasal passages. It provides a special direct nose-to-brain pathway for nasally administered drugs. As shown in Figure 1.6, the trigeminal nerve consists of three divisions: ophthalmic branch, maxillary branch and mandibular branch [62] [63]. Unlike the mandibular branch that is responsible for both sensory and motor functions, the ophthalmic and maxillary branches have only sensory function [60]. The latter two branches are the main pathways for nose-to-brain delivery of drugs, since the neurons of the branches directly pass through the nasal mucosa. Moreover, the trigeminal nerves also supply a connection between respiratory epithelium in the nasal cavity and the pons in the brain stem, which offers a unique drug delivery pathway from nose to both anterior and posterior brain areas [64].

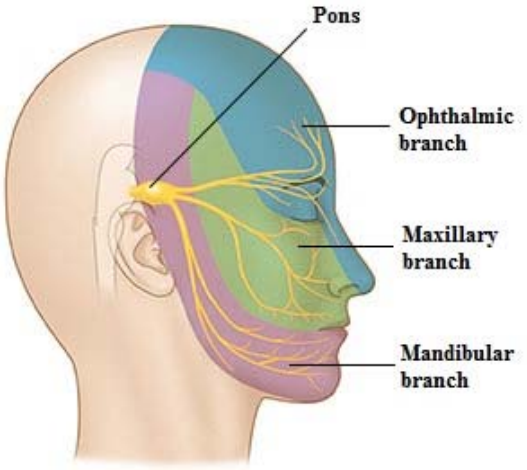


Figure 1.6 Distribution of the trigeminal nerve

1.2.4.2 Olfactory pathway

The surface area of olfactory region is less than 10% of nasal epithelium area in human [65]. The olfactory nerve is the shortest cranial nerve and carries olfactory information from the nose to the brain. The olfactory nerve enters into the cranial cavity through the cribriform plate and connects the olfactory receptors of the nasal mucosa with the forebrain [66]. It offers another direct drug delivery pathway to the brain from the nasal cavity circumventing the BBB. There are two ways involved in the drug delivery from nose to brain through the olfactory nerves [60]: (1) the olfactory nerves take the drug by pinocytosis and endocytosis effects. The drug is transported along the neuraxis to the olfactory, and finally into the brain; (2) the drug could enter into the brain by the extracellular or intracellular pathway, passing through the basal cells of olfactory epithelium. The drug could reach the olfactory bulb in several to 30 minutes by the extracellular pathway, which is the most likely olfactory absorption route of drugs [67].

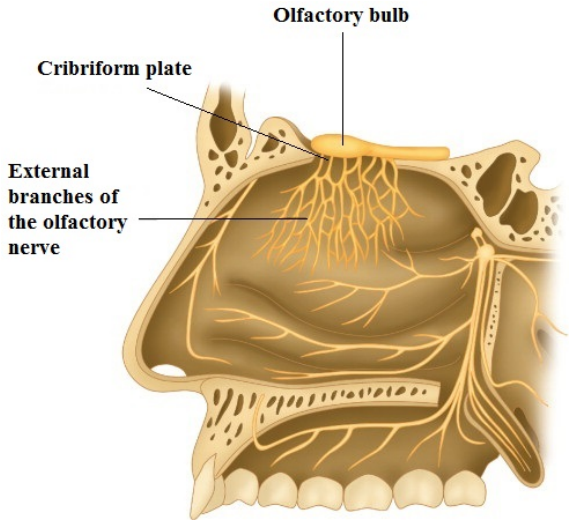


Figure 1.7 The olfactory nerve

1.2.5 Factors influencing nasal drug delivery

The absorption of drugs through intranasal route can be affected by the following factors: physicochemical properties of drugs, nasal effects and formulation effects.

1.2.5.1 Physicochemical properties of drugs

The absorption of drugs through intranasal route can be influenced by the physicochemical properties such as molecular size, lipophilicity and enzymatic degradation.

The lipophilicity of drugs also can greatly affect the drug absorption through intranasal route. The ideal log P range of drug candidates is between 2 and 4 [58]. Within this limit, drug permeability is increased with the lipophilicity [68]. Molecular weight is also a factor for intranasal absorption, especially for drugs with molecular weight over 300 Daltons. The lipophilic drugs with small molecular weight result in rapid nasal permeation [69]. However, hydrophilic drugs with large molecular weight, such as peptide and protein drugs, have poor permeability across nasal mucosa. Those water soluble drugs are mainly absorbed by a passive diffusion pathway via aqueous pores in the nasal mucosa [70]. For those peptide drugs and protein drugs, enzymatic degradation in nasal cavity and epithelium may also account for the low bioavailability [71].

1.2.5.2 The internal environment of nasal cavity

The environmental factors of the nasal cavity are of importance in absorption of drugs via intranasal route. The pH of nasal fluids and the mucociliary clearance have impact on the drug absorption via intranasal route.

The pH of nasal fluid is in the range of 5.5-7.4 [58]. The pH values can affect the

ionization state (the ratio of nonionized and ionized forms) of drugs, and subsequently influence the drug intranasal absorption. The nonionized form of drugs usually has higher permeability than the ionized form. The ionized drugs transport cross the nasal mucosa though the aqueous paracellular route, whereas the nonionized forms via transcellular route [58].

Another factor affecting the nasal membrane permeability is the multidrug resistance transporters. P-glycoprotein (P-gp) located at the apical surface of ciliary epithelial cells and in the submucosal vessels of the olfactory region, may lower the systemic absorption by drug efflux back to nasal cavity [72].

Mucociliary clearance is a self-cleaning mechanism in upper respiratory tract. The exogenous noxious substances, e.g. toxins, viruses and bacteria, are propelled by the mucosa ciliary movements along nasopharynx and eventually discharged into the gastrointestinal tract [73]. The drugs in the nasal cavity may also be cleared up into the nasopharynx by mucociliary clearance mechanism, at a speed of 6 mm/min. Any factor impacting the mucociliary clearance speed can subsequently alter drug absorption profiles.

1.2.5.3 Formulation effects

1.2.5.3.1 pH and osmolality

The intranasal absorption of ionized drugs is pH-dependent. Generally, the pH of formulations should be optimized to maximize the proportion of nonionized drug molecules, since the permeability of the ionized drugs across the nasal mucosa is quite limited. To avoid the irritation induced by extremely low or high pH, the formulation pH should be adjusted to the range of physiological values for nasal fluids, i.e., pH

5.0-6.5 [74].

Similar to pH, the osmolality of formulation also affects nasal absorption of drugs and should be adjusted to physiological level, i.e., 280 MOsm [75]. Inappropriate osmolality values may cause a damage of nasal mucosa.

1.2.5.3.2 Viscosity and bioadhesive property

Both the viscosity and bioadhesive property of nasal formulations can prolong the residence time of drugs in nasal cavity, and hence increase drug intranasal absorption and systemic bioavailability. Meanwhile, the mucociliary clearance is suppressed by the high viscosity of drug solutions. However, the intranasal absorption rate also gets slower due to the decreased drug diffusion rate caused by the increased viscosity of solution [76].

1.2.5.3.3 Drug distribution in nasal cavity

Drugs depositing in posterior area are eliminated faster by mucociliary clearance as compared with drugs in anterior part [73]. The distribution of drugs in nasal cavity is significantly dependent on the dosage forms and delivery devices. The drugs delivered by nasal sprays deposit more anteriorly than nasal drops, leading to a slower mucociliary clearance for nasal sprayed dosage forms. The particle size of nebulized droplets should be also taken into consideration, since the droplets with an aerodynamic diameter of 10-20 μm are readily deposited on the nasal mucosa. In contrast, droplets with aerodynamic size greater than 50 μm mainly distribute at exterior nasal cavity.

1.2.5.3.4 Solubilizers

Both solubility and stability of drugs can be improved with the solubilizers. For

example, cyclodextrin can form inclusion complexes with drug molecules due to its unique bucket-like structure including a hydrophobic inner cavity and a hydrophilic outer surface, and improve water solubility as well as bioavailability of drugs [77].

1.2.5.3.5 Enzyme inhibitors

For drugs susceptible to nasal enzymes, the peptidase and protease inhibitors are applied to inhibit the enzymatic degradation. The absorption of drugs as the substrates of P450 cytochromes or P-glycoprotein can be effectively improved by related inhibitors [78]. In addition, certain salts and derivatives of fusidic acid can inhibit enzymes in nasal mucosa as well, and thus enhance the intranasal bioavailability of drugs [79].

1.2.5.3.6 Permeation enhancers

An ideal permeation enhancer only induces transient and reversible modifications on structure of nasal mucosal epithelium and enhances its permeability, without any permanent mucosal impairment. Besides, the enhancement of absorption should be highly specific to the administered drugs, without excess absorption of any exogenous toxic substances. The permeation enhancers should not have any irritating effects to nasal mucosa or incompatibility with other ingredients in drug products. The mechanisms for permeability enhancement include [80]: a) improve the stability and the solubility of drugs; b) opening the tight junctions between mucosal cells; c) decrease mucociliary clearance; d) reduce the viscosity and elasticity of nasal mucus. A variety of permeation enhancers have been evaluated for enhancing drug penetration across nasal mucosa, including bile salts, fatty acid salts, cyclodextrins, surfactants and bioadhesive materials (Table 1.3) [81].

Table 1.3 Permeation enhancers for nasal drug delivery

Classification	Ingredients	Mechanisms
Bile salts	Fusidic acid derivatives (STDHF), Trihydroxy salts	Open tight junctions, enzyme inhibition, mucolytic activity
Fatty acid salts	Oleic acid, Caprate, Caprylate, Laurate	Membrane disruption
Cyclodextrins	α, β, and γ- cyclodextrins, the derivatives of cyclodextrins	Open tight junctions, membrane disruption.
Surfactants	Saponin, Polyozyethylene-9-lauryl ether (Laureth-9)	Membrane disruption
Bioadhesive materials	Carbopol, starch microspheres, chitosan	Prolong nasal residence time, open tight junctions

1.2.6 Current marketed nasal products

Thus far, the nasal delivery technology has been applied as a local treatment of nasal diseases, such as nasal congestion, rhinitis, allergy and sinusitis. Meanwhile, it is also used to mitigate the issues of drugs with properties of degradation by enzymes in gastrointestinal tract or hepatic first-pass metabolism, slow oral absorption and poor bioavailability. In recent years, the nasal drug delivery market has been driven by a mounting demand in alternative administration routes for pediatric and geriatric patients, the rise in needs of products with better efficacy, and the growth of self-medication at home. With the growing number of applications, the global market size of nasal drug delivery technology was around 44.0 billion US dollars in the year 2016 and is projected to expand at a compound annual growth rate of 6.5% over the following forecast years. The nasal drug delivery market is expected to be driven by both the increasing preference of alternative administration mode and the growing incidence of chronic disease over the upcoming years. The predominant dosage forms applied in nasal drug delivery are nasal spray, nasal solution, nasal ointment, nasal gel, nasal

inhaler and nasal aerosol. Nowadays, a growing interest has been gained in the use of nasal drug delivery for drugs such as small polar molecules, vitamins, vaccines, hormones and peptides [82]. The commercial nasal products available on the US market are listed as Table 1.4.

Table 1.4 Current nasal products on the market

Brand name	API	Indication	Dosage form
Atrovent®	Ipratropium bromide	Symptomatic relief of rhinorrhea associated with the common cold or seasonal allergic rhinitis	Nasal Spray
Dymista®	Azelastine hydrochloride; Fluticasone propionate	Allergic rhinitis	Nasal spray
Bactroban®	Mupirocin calcium	Nasal colonization with methicillin-resistant Staphylococcus aureus (MRSA)	Nasal ointment
CaloMist®	Cyanocobalamin	Vitamin B12 Deficiency	Nasal spray
DDAVP®	Desmopressin acetate	Central cranial diabetes insipidus	Nasal spray
Nasarel®	Flunisolide	Seasonal or perennial rhinitis	Nasal solution
IMITREX®	Sumatriptan	Acute treatment of migraine attacks	Nasal spray
Influenza A (H1N1) 2009 Monovalent Vaccine Live	Influenza vaccine	Influenza disease caused by pandemic (H1N1) 2009 virus	Nasal spray
NARCAN®	Naloxone hydrochloride	The known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression.	Nasal spray
NASALCROM®	Cromolyn sodium	Allergy symptom	Nasal spray
PATANASE®	Olopatadine hydrochloride	Seasonal allergic rhinitis	Nasal spray
ZOMIG®	Zolmitriptan	Migraine	Nasal spray
AllerNaze®	Triamcinolone acetonide	Seasonal and perennial allergic rhinitis	Nasal spray
ASTEPRO®	Azelastine hydrochloride	Allergic rhinitis	Nasal spray

Beconase®	Beclomethasone dipropionate	Seasonal or perennial allergic and nonallergic (vasomotor) rhinitis	Nasal inhaler; Nasal spray
FLONASE®	Fluticasone propionate	Perennial nonallergic rhinitis	Nasal spray
FluMist® Quadrivalent	Four vaccine virus strains: an A/H1N1 strain, an A/H3N2 strain and two B strains. B strains from both the B/Yamagata/16/88 and the B/Victoria/2/87 lineages.	Active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine	Nasal spray
GOPRELTO®	Cocaine hydrochloride	The induction of local anesthesia of the mucous membranes	Nasal solution
KOVANAZE®	Tetracaine HCl and oxymetazoline HCl	Regional anesthesia	Nasal spray
LAZANDA®	Fentanyl citrate	Breakthrough pain in cancer patients	Nasal spray
Minirin®	Desmopressin acetate	Central diabetes insipidus	Nasal spray
NASONEX®	Mometasone furoate monohydrate	Seasonal allergic and perennial allergic rhinitis	Nasal spray
Natesto®	Testosterone	Replacement therapy in adult males for conditions associated with a deficiency or absence of endogenous testosterone	Nasal gel
Nicotrol® NS	Nicotine	The relief of nicotine withdrawal symptoms	Nasal spray
NOCTIVA™	Desmopressin acetate	Nocturia due to nocturnal polyuria in adults	Nasal spray
OMNARIS®	Ciclesonide	Seasonal allergic rhinitis	Nasal spray
ONZETRA™ Xsail™	Sumatriptan	Migraine	Nasal powder
QNASL®	Beclomethasone dipropionate	Allergic rhinitis	Nasal aerosol
SPRAVATO™	Esketamine hydrochloride	Treatment-resistant depression (TRD)	Nasal spray
SPRIX®	Ketorolac tromethamine	Moderate to moderately severe pain	Nasal spray
Stimate®	Desmopressin acetate	Hemophilia A; Von Willebrand's Disease (Type I)	Nasal spray
TOSYMRA®	Sumatriptan	Migraine	Nasal spray

XHANCE™	Fluticasone propionate	Nasal polyps	Nasal spray
ZETONNA®	Ciclesonide	Seasonal and perennial allergic rhinitis	Nasal aerosol
Stadol®	Butorphanol tartrate	Pain	Nasal spray
FORTICAL®	Calcitonin-salmon [rDNA origin]	Postmenopausal osteoporosis	Nasal spray
RHINOCORT AQUA	Budesonide	Seasonal or perennial allergic rhinitis	Nasal spray
MIGRANAL®	Dihydroergotamine mesylate	Migraine headaches	Nasal spray
VERAMYST®	Fluticasone furoate	Seasonal and perennial allergic rhinitis	Nasal spray
ASTELIN®	Azelastine hydrochloride	Seasonal allergic rhinitis	Nasal spray
Nasacort® AQ	Triamcinolone acetonide	Seasonal and perennial allergic rhinitis	Nasal spray
BECONASE AQ®	Beclomethasone dipropionate, monohydrate	Seasonal or perennial allergic and nonallergic (vasomotor) rhinitis	Nasal spray
DYMISTA®	Azelastine hydrochloride and fluticasone propionate	Seasonal allergic rhinitis	Nasal spray
Nascobal®	Cyanocobalamin	Pernicious anemia; Vitamin B12 deficiencies	Nasal spray
Synarel®	Nafarelin acetate	Central precocious puberty	Nasal solution

1.2.7 Intranasal dosage forms

1.2.7.1 Nasal drops

Nasal drops are drug solutions for nasal instillation, applied by a dropper with a flexible rubber, or directly from a squeezable plastic container. Nasal drops deposit completely in nasal cavity with immediate absorption. However, the drug solution depositing on the ciliated mucosal regions may be quickly eliminated by mucociliary clearance, giving rise to a fraction of drug swallowed into GI tract, as well as the

variable systemic absorption [83]. Besides, nasal drops are inconvenient for patient use and associated with the risk of cross contamination.

1.2.7.2 Nasal sprays

The nasal spray solution or suspension is dispensed in a non-pressurized dispenser with a pre-metered dose spray pump. The spray pump consists of a chamber, a piston and an actuator. The nasal spray pump can be designed for unit dose or multiple doses. The pumps can deliver precise volume of drug solution or suspension (25-200 μL) per actuation. The droplet size and plume geometry of sprays are pertinent to the nasal distribution of drugs and influenced by several formulation factors, such as viscosity, surface tension and thixotropy [84] [85]. Other factors from spray pumps, e.g. applied force, pump types and orifice size, also impact the plume geometry of sprays and then influence the nasal drug deposition [86].

1.2.7.3 Nasal gels

The physical states of gels can range from viscous liquid to brittle gels, depending on polymer types, concentration and environmental factors (iron strength, temperature, pH, etc.). Some polymers with bioadhesive properties could suppress mucociliary clearance, and thus prolong the residence time of drugs at absorption site. Due to the relatively high viscosity, the nasal application of gels is not convenient, and the delivered dose is not accurate. In situ gel for nasal delivery is an attractive alternative to overcome above disadvantages [87] [88]. The in situ gel is in a liquid form with low viscosity before use. After intranasal administration, it immediately transforms into gel form. Basing on the mechanisms of gelation, they can be further classified as pH sensitive gel, thermosensitive gel, ion-sensitive gel and enzyme sensitive gel. Polymers

with in situ gelation properties, such as gellan gum, xanthan, and poloxamers, are applied in intranasal drug delivery [89] [90] [91]. The nasal solution containing in situ gelling polymers is easily sprayed to form a viscous gel layer on nasal mucosa, with good biocompatibility, prolonged residence time and improved bioavailability.

1.2.7.4 Particulate drug delivery system

Encapsulation of drug in particulate carriers is a viable approach for nasal administration, especially for drugs with poor water solubility and stability, or potential toxicity. Nano-emulsions, microspheres and liposomes have been used as carriers to encapsulate poorly water-soluble drugs for nasal delivery. The intranasal delivery of nano-emulsion with cyclosporine can achieve increased drug concentration in brain, as well as decreased peripheral exposure [92]. The microspheres with bioadhesive property can also enhance drug bioavailability via intranasal route by prolonging the nasal residence time. Liposome with the amphiphilic structure can be also considered as a carrier to enhance the intranasal transport of both hydrophobic and hydrophilic drugs. The anionic liposome is better tolerated as compared to cationic liposome [93].

1.2.7.5 Nasal powders

The nasal powder can be applied for the drugs with stability or irritation issues. The dry powder formulations consist of APIs and bioadhesive polymers as carriers for intranasal delivery. It is more stable than liquid dosage forms and free of preservatives. The deposition of the nasal powder relies on the particle size and the aerodynamic properties of the powders. Several nasal powder products have been marketed for various indications, such as allergic rhinitis (QNASL®, beclomethasone dipropionate nasal aerosol) and migraine (ONZETRA® Xsail®, sumatriptan nasal powder). Apart

from the small molecular drugs, the nasal powder was also applied in peptides delivery. The glucagon nasal powder for treatment of hypoglycemia was evaluated and proved to be effective in a clinical trial involving 48 youth with type I diabetes [94].

1.3 Significances of developing intranasal delivery for granisetron

At present, granisetron is available on the market as oral tablet (Kytril®), oral solution (Kytril®), intravenous injection (Kytril®), transdermal patch (Sancuso®) and extended-release subcutaneous injection (Sustol®). The current granisetron dosage forms have various disadvantages respectively.

For oral granisetron tablet or solution, the bioavailability of the drug is limited (about 60%) due to the first pass metabolism [95]. The onset of oral granisetron is relatively slow, therefore the Kytril® tablets or solution are usually given 1 hour prior to chemotherapy. Moreover, oral administration of tablets may be extremely difficult, especially when patients are suffering from nausea and vomiting and the swallowing capacity is compromised. Patients receiving chemotherapy or radiotherapy may also develop oral mucositis causing difficulty of food or drink intake.

Granisetron intravenous injection can achieve relatively rapid pharmacological effect. However, it is invasive and patients will undergo unnecessary pains and potential infection after injection, which is an important issue in those immune-compromised patients. Furthermore, the extra time and cost for medical staffs in giving the injectable medication should also be considered.

The onset of the granisetron transdermal patch is quite delayed. For the patients receiving transdermal patch, a minimum of 24 hours to a maximum of 48 hours is required before the scheduled treatment. The patch has to be applied on skin for several days, and is inconvenient when taking shower. Also, the patients should avoid strenuous exercise or sweating, which may cause the detachment of the patch and the failure of treatment. Above inconveniences may result in poor compliances of patients using the granisetron transdermal patch.

For recently marketed granisetron extended-release subcutaneous injection (Sustol®), besides the time and costs on medical staff, the adverse reactions at injection site may also occur, including infection, bleeding, pain, tenderness, nodules, swelling, and induration. Therefore, it is imperative to develop an alternative dosage form that is ease of use, non-invasive and safe, with rapid onset for CINV.

Intranasal drug delivery system is widely applied to deliver (a) small molecular drugs for local or systemic actions, (b) the drugs direct from nose to brain, (c) peptide drugs for systemic absorption, (d) diagnostic drugs, and (e) vaccines. It is a convenient alternative to conventional oral and parenteral routes. An ideal drug candidate for intranasal delivery should have the following properties [58]: (1) good aqueous solubility to deliver sufficient dose in small spray volume (50 to 150 µl per nostril); (2) small and lipophilic molecule; (3) no nasal ciliotoxicity; (4) small dose, i.e. < 20 mg; (5) no unpleasant smell; and (6) acceptable stability.

Granisetron is a small and lipophilic compound with high water solubility, and low clinical dose (oral dose of 2 mg daily). Thus, it may be a desirable candidate for intranasal delivery. Preliminary animal pharmacokinetic studies showed the absorption of granisetron was improved after intranasal administration in rabbits, as compared with that of oral route [96]. In that study, the granisetron drop was prepared for intranasal instillation, which was not convenient and the drug solution could be easily swallowed to GI tract. Moreover, the nasal absorption of granisetron in that study was slow (t_{max} = 90 min ± 5.52 min), which might be attributed to the delayed drug release from nasal drop and a considerable portion of drug absorbed from GI tract.

In our studies, granisetron bioadhesive nasal spray will be developed for improved absorption, rapid onset of anti-emetic effect, and improved patient compliance. If

successfully developed, our new granisetron product will offer a safe and convenient alternative to the oral, intravenous and transdermal granisetron dosage forms on the market, with similar onset of action as intravenous injection but administered through a noninvasive route.

1.4 Study challenges

We aim at developing a novel granisetron nasal spray, which is safe, convenient, noninvasive and rapid-onset for better management of CINV and RINV. Various polymers with bioadhesive properties will be used to enhance the bioavailability of nasal absorption. In this study, HPMC-based solution, thermosensitive in situ gel and ion-sensitive in situ gel will be investigated in both in vitro and in vivo studies.

To successfully develop novel granisetron nasal spray for rapid prevention of CINV and RINV, we have to overcome the following challenges:

(1) The gelation of in situ gels under physiological conditions.

(2) The compatibility and stability of gel system containing granisetron.

(3) The acute and chronic toxicity and local irritation of drug product.

(4) The scaled-up production from small batches to continuous GMP production.

(5) The anti-emetic effects of granisetron nasal spray as compared with marketed oral and injectable products.

1.5 Research objectives and study methods

Objective 1: To explore the feasibility of systemic delivery of granisetron via intranasal route.

Objective 2: To prepare several dosage forms for granisetron nasal spray and

evaluate the pharmacokinetics of the formulations.

Objective 3: To evaluate the pharmacokinetics, safety and tolerability of granisetron nasal spray in healthy volunteers.

To achieve Objective 1, we will:

(1) Establish an in vitro analytical method for quantifying concentration of granisetron.

(2) Conduct preformulation studies for developing granisetron nasal spray solution.

To achieve Objective 2, we will:

(1) Develop and validate analytical method to determine granisetron in rat plasma.

(2) Develop and optimize several granisetron nasal spray formulations, such as HPMC-based solution, thermosensitive in situ gel and ion-sensitive in situ gel. The physicochemical characteristics of the spray solutions will also be evaluated.

(3) Evaluate the in vivo pharmacokinetics and the brain pharmacokinetics of the optimized nasal spray formulations in rats.

(4) Study the pharmacokinetics of granisetron and its metabolite in Beagle dogs.

To achieve Objective 3, we will:

(1) Evaluate the toxicity of granisetron nasal spray by non-clinical safety study.

(2) Conduct clinical studies on granisetron hydrochloride intranasal spray in healthy volunteers.

The intranasal dosage form of granisetron may possess many distinctive advantages, such as non-invasive administration, rapid onset of action, ease of use and brain targeting. The new dosage form of granisetron can fill the gap between

conventional anti-emetic products and the clinical needs, especially when oral or intravenous route is not available or inconvenient. It is believed that the new intranasal granisetron product could offer a convenient, safe and noninvasive alternative choice to the present granisetron products with similar onset of action as intravenous injection but less potential infection for those immuno-compromised patients.

Chapter 2

Development and characterization of granisetron bioadhesive system for intranasal delivery

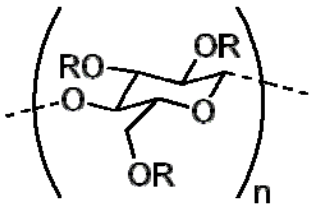
2.1 Introduction

Granisetron ($C_{18}H_{24}N_4O$) is a 5-HT3 receptor antagonist indicated for prevention of chemotherapy-induced and radiotherapy-induced nausea and vomiting (CINV and RINV). It exerts therapeutic effects by selectively and competitively binding to 5-HT3 receptors on the vagal afferent nerves in the gastrointestinal tract and the brainstem, stopping the stimulus to the vomiting center [97]. The marketed granisetron products include oral solution and tablets (Kytril®), intravenous injection (Kytril®), transdermal patch (Sancuso®) and extended-release subcutaneous injection (Sustol®). The relatively slow absorption of oral or transdermal granisetron, and the patients' low compliance of granisetron injections have propelled a growing interest of delivering granisetron through epithelial tissues, especially via intranasal route.

Nasal mucosa is a vascularized and highly permeable membrane, which enables the rapid and complete absorption of small molecular drugs with moderate to high lipophilicity. The lipophilic drugs with the molecular weight less than 1000 Da could rapidly and efficiently penetrate the nasal membrane via a transcellular way [98]. However, the nasal mucociliary clearance is considered as one of the major factors that lead to limited bioavailability of drugs absorbed through intranasal route [99] [73]. The nasal mucus and the exogenous substances, including drugs, are propelled towards to the nasopharynx under the force of ciliary beating, resulting in the loss of drugs in nasal cavity and the decreased bioavailability. Various approaches were applied to suppress

the effect of nasal mucociliary clearance and prolong the residence time of drugs administered nasally. In recent decades, there has been a rising interest on the application of bioadhesion technologies in the nasal drug delivery system [100] [101]. It was found that the increase of viscosity or bioadhesion of nasal formulations could prolong the residence time and improve the bioavailability of the nasally administered drugs. The rate of nasal mucociliary clearance could be influenced by the rheological properties of the polymers in the nasal formulations [102]. Various studies assessed the intranasal absorption enhancement by application of hydrophilic polymers, including methylcellulose [103], hydroxypropyl methylcellulose [104], hydroxypropyl cellulose [105], hyaluronan [106], poloxamer [107] [91], and gellan gum [108] [109] [110]. In this study, several strategies, such as bioadhesive technology and in situ gels based on thermosensitive and ion-sensitive polymers, were applied to increase the residence time of nasally administered granisetron and improve its bioavailability.

Hydroxypropyl methylcellulose (HPMC, Figure 2.1) is a semisynthetic modification of natural cellulose, which is tasteless and odorless, white to off-white powder. It is available in several grades, varying in the content of methoxy group and the hydroxypropoxy group. HPMC has good solubility in aqueous solution, excellent compatibility with drugs and other excipients, no unpleasant taste or odor, and high stability in the manufacturing process. It is widely used as binder, thickener, suspending agent, wetting agent, emulsifier and adhesive in oral, nasal, ophthalmic and topical pharmaceutical formulations [111] [112] [113] [114]. Furthermore, its strong mucoadhesive capacity could prolong the contact time of drug solutions at the application site and enhance the bioavailability of drugs. The HPMC concentration used in marketed nasal product is around 0.1% [115]. In a study by Pennington et al., the clearance half-life of the nasal preparations increased with the HPMC concentration in the formulations [116].



R = H or CH_3 or $CH_2CH(OH)CH_3$

Figure 2.1 Chemical structure of hydroxypropyl methylcellulose (HPMC)

Gellan gum (Figure 2.2) is a linear polymer chain consisting of repeating tetrasaccharide units of L-rhamnose, D-glucose and D-glucuronate, in the molar ratios of 1:2:1. Gellan gum can form gel matrix in the presence of cationic ions. The mechanism of cation activated gelation is related to the formation of double-helical junction zones, followed by the combination of the inter-helical structures to form a 3-Dimension network structure through cations and hydrogen binding with water [117]. Nasal mucosa is covered with about 100 μL of mucus containing calcium, sodium and potassium ions, so the solution-gel transition instantaneously occurs after gellan gum solution contacting the nasal mucus. Several studies indicated that gellan gum could improve bioavailability of drugs after intranasal administration by prolonging the nasal residence time [118] [119] [120].

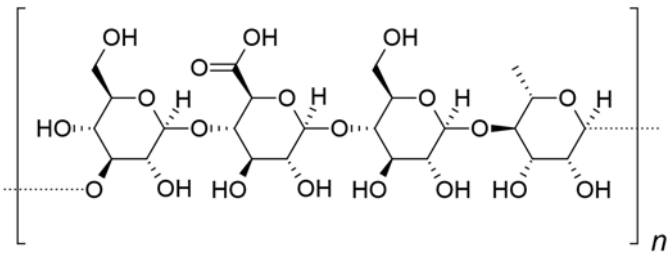


Figure 2.2 Chemical structure of low-acyl form of gellan gum

Poloxamers are copolymers consisting of polyoxyethylene (PEO) and polyoxypropylene (PPO) chains (Figure 2.3). It is a nonionic surfactant and can be

applied as solubilizer, emulsifier, wetting agent and drug delivery vehicles. At certain temperature, poloxamer forms micelles with hydrophobic core and hydrophilic shell above the critical micellar concentration (CMC). However, the hydrophilic chains (polyoxyethylene) are desolvated above the sol-gel transition temperature, because of the rupture of the hydrogen bonds between the solvent and the PEO chains [121]. The thermosensitive gel can be obtained by heating the poloxamer solutions above the sol-gel transition temperature. The unique property of thermosensitive gelation enables the formation of in situ gel at physiological temperature. Therefore, Poloxamer 407 solutions can promptly transform into in situ gel after reaching the phase-transition temperature in nasal cavity, and can be used for intranasal drug delivery [122] [123].

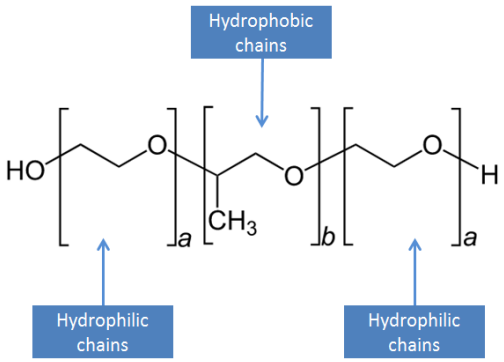


Figure 2.3 Chemical structure of Poloxamer

In this chapter, a HPLC-UV method will be developed and validated for quantification of granisetron for preformulation studies and formulation development. The granisetron nasal formulations with bioadhesive properties will be developed by following technologies: thermosensitive in situ gel, ion-activated in situ gel and HPMC based bioadhesive solution. The physicochemical properties of granisetron nasal formulations, such as sol-gel transition temperature, dissolution behavior and rheological property, will be investigated and optimized. Also, the mucociliary transport time (MTT) will be evaluated for formulation optimization.

2.2 Materials

Granisetron hydrochloride (purity: >99.5%, Lot#: 207005GJ) was purchased from Qilu Pharmaceutical Co., Ltd. Poloxamer 407, Poloxamer 188, polyethylene glycol 6000 (PEG 6000), sodium hydroxide, sodium chloride, 1-octanol and indigo carmine were products of Sigma-Aldrich Co., USA. Gellan gum (Kelcogel®, CG-LA) was a gift from CPKelco Co. China. Potassium chloride and calcium chloride were obtained from Sinoreagent Co., China. Sodium hyaluronate (M.W. 200K Da ~ 400K Da) was purchased from Bloomage Freda Biopharm Co., Ltd., Jinan, China. Hydroxypropyl methylcellulose (METHOCEL™, HPMC, K100 LV) was obtained from Colorcon Co., Shanghai. Formic acid, trimethylamine and ammonium formate were purchased from Sigma-Aldrich Co., USA. Acetonitrile (HPLC grade) was manufactured by Merck Millipore. Deionized water was produced in-house and used for preparing all solutions. Spectra/Por® dialysis bags (cut off molecular weight: 12000-14000) were ordered from Spectrum Laboratories Inc., CA, USA. Centriprep filters were obtained from Millipore, MA, USA (size: 0.5 ml, MWCO: 30 kDa).

2.3 Methods

2.3.1 Validation of assay method for granisetron hydrochloride

2.3.1.1 HPLC methods

The HPLC-UV system consists of Shimadzu SPD-M20A Photodiode Array detector, Shimadzu LC-20AD pump and Shimadzu SIL-20A HT auto sampler and. Data was collected by a Shimadzu LC Solution data system. Chromatographic separation was achieved by a Thermo BDS C18 Hypersil column (250×4.6 mm i.d.; 5 μm particle size) equipped with a guard column (Thermo C18 Guard-Pak). The mobile phase was 80%

eluent A [5% acetonitrile + 95% aqueous solution, containing 0.5% triethylamine and 50 mM ammonium formate solution adjusted to pH 4.0 by formic acid] and 20% eluent B [acetonitrile]. An isocratic elution was performed at 1 ml/min. The detection wavelength was 301 nm. Both the autosampler and the column were at ambient temperature. The sample injection volume was 20 µL.

2.3.1.2 Preparation of stock, working standard and QC solutions

The stock solution was prepared by dissolving granisetron hydrochloride in acetonitrile-water (v/v, 1:1) solution at the concentration of about 1.0 mg/ml (granisetron free base). The working standard solutions were prepared by serial dilutions of stock solution with acetonitrile-water (v/v, 1:1) solution. The blank solutions were spiked with granisetron hydrochloride solution at known concentration to prepare QC solutions. The QC solutions were at low, medium and high concentrations within the linearity range.

2.3.1.3 Linearity

The working standard solutions for linearity test were prepared by serial dilutions of the granisetron hydrochloride stock solution with acetonitrile-water (v/v, 1:1) solution. The concentration of the working standard solutions for linearity was from 5 µg/ml to 200 µg/ml of granisetron. At least seven working standard solutions were injected and analyzed with the HPLC-UV method. Each working standard solution was prepared once and then analyzed by triplicate injections to assess the linearity.

2.3.1.4 Precision

- *Repeatability*

Five granisetron hydrochloride solutions at the concentrations of 10, 50 and 100 μg/ml were prepared. Each sample solution was analyzed by single injection. The relative standard deviations (RSD %) of the samples in each group were calculated.

- *Intermediate precision*

The assessment of intermediate precision was performed in two different days. The solutions of granisetron hydrochloride were obtained according to the preparation method for repeatability test, with three samples for each group. All the samples were injected once, and then the RSD % of each group was calculated.

2.3.1.5 Accuracy

Granisetron hydrochloride solutions at the concentrations of 10, 50 and 100 μg/ml were prepared, with five samples for each concentration group. The samples were injected once. The recovery of each group was calculated by the following equation:

Recovery % = C_d / C_n × 100%

Where C_d is determined concentration;

C_n is nominal concentration.

2.3.2 Physicochemical properties of granisetron

2.3.2.1 Solubility of granisetron hydrochloride

The solubility of granisetron hydrochloride in various mediums was determined at room temperature. Firstly, granisetron hydrochloride was dissolved in deionized (DI) water, normal saline (0.9% NaCl), and 50 mM PBS solutions with various pHs (PBS 4.0, PBS 5.5 and PBS 7.0). Then an excess amount of granisetron hydrochloride was added into above vehicles and shaken at 25 ^oC for more than 72 hours. The suspension

solutions were centrifuged at 10000 rpm for 5 minutes. The supernatant was withdrawn and diluted for concentration determination by HPLC.

2.3.2.2 Stability of granisetron hydrochloride

22.4 mg/ml granisetron hydrochloride (equivalent to 20 mg/ml granisetron base form) solutions were prepared in various mediums (DI water, normal saline and PBS solutions), and placed into stability chamber at 25 ^{o}C and 40 ^{o}C respectively. Drug stability in these solutions was then evaluated by determining granisetron hydrochloride concentration at 0, 2, 5, 10, and 20 days with HPLC-UV method.

2.3.2.3 Distribution Coefficient (Log D) of granisetron hydrochloride

The equilibrium between 1-Octanol and aqueous solutions with different pHs were completed before partition experiment. The mixture of 1-octanol and excess solution was shaken at room temperature (25 ^{o}C) for 24h. After separation of the two phases, the aqueous solutions saturated with octanol were withdrawn to prepare granisetron solutions for Log D determination. The aqueous solutions used to prepare granisetron solutions included buffer solutions (pH 4.0, pH 5.5 and pH 7.0), normal saline solution and distilled water. Exactly 3 ml of octanol saturated with buffer solution was transferred into a screw-cap glass tube, and then mixed with the 3ml of granisetron hydrochloride aqueous solution with the concentration of 1 mg/ml of granisetron free base at room temperature. The two phases were continuously equilibrated in a shaking water bath at 37 ^{o}C. After 48 hours, the mixture suspending solutions were left to separate with each other, and the concentrations of granisetron in aqueous phase and organic phase were determined by a HPLC-UV method respectively. The distribution coefficient was calculated according to the following equation:

$$\text{Log D} = \text{Log}\left(\frac{[\text{solute}]_{\text{octanol}} \times V_{\text{aqueous}}}{[\text{solute}]_{\text{aqueous}} \times V_{\text{octanol}}}\right)$$

Where $[\text{solute}]_{\text{octanol}}$ and $[\text{solute}]_{\text{aqueous}}$ are granisetron equilibrium concentration in octanol and aqueous medium after partition experiment, respectively. V_{aqueous} and V_{octanol} are the volume of aqueous phase and octanol phase, respectively.

2.3.3 Preparation and characterization of granisetron dosage forms

2.3.3.1 Preparation of thermosensitive in situ gel

The thermosensitive in situ gel based on poloxamers was prepared by the method described by Schmolka et al [124]. Briefly, granisetron hydrochloride and additives were added into the solutions and dissolved at room temperature. Then the poloxamer was gradually added into the solutions and dissolved in an ice bath with continuously stirring. The final solutions were stored in the refrigerator at 4 ºC for further studies.

2.3.3.2 Preparation of ion-sensitive in situ gel

The gellan gum (CG-LA) was dissolved in deionized water and dissolved by heating to 95 ºC with gently stirring. The solution was then cooled to below 40 ºC. Granisetron hydrochloride was then added and mixed till dissolved. The solutions were kept at 4 ºC.

2.3.3.3 Preparation of bioadhesive solution

The HPMC (K100 LV) was added into hot water (80 ºC-90 ºC) and dispersed by moderate stirring. Then the solution was cooled to room temperature with continuously stirring. Granisetron hydrochloride was dissolved in the HPMC solution by stirring at room temperature. The final solution was kept at 4 ºC.

2.3.3.4 Visual determination of sol-gel transition temperature

The sol-gel transition temperature of the poloxamer in situ gel solutions was determined by the method developed by Gilbert et al. with minor modifications [125]. Briefly, approximate 3.0 ml of the in situ gel solution was added into a glass vial and sealed with a screw cap. The sample was incubated in a water bath at 10 ^{o}C. After equilibrium for 10 minutes, the water bath was slowly heated at the rate of 2-3 ^{o}C per minute to 18 ^{o}C, and then at the rate of 0.2 -0.5 ^{o}C per minute until sol-gel transition. At each temperature plateau, the samples were kept in the water bath for at least 8 minutes for equilibrium. After equilibrium, the vial was pulled out and quickly placed upside down. The formation of the in situ gel was confirmed if there was no liquid flowing by visual inspection. Each sample was measured in triplicate.

2.3.3.5 Critical concentration of gellan gum solution

Critical concentration for phase transition is the minimal concentration of gellan gum at which the sol-gel transition is triggered instantaneously in the simulated nasal electrolyte solution (SNES) for ion-activated in situ gel. SNES consists of 0.24 mg/ml of $CaCl_2$, 7.45 mg/ml of NaCl and 1.29 mg/ml of KCl, with pH of 5.7. The critical concentration for phase transition was determined by mixing gellan gum solutions (1.0 ml) at different concentrations with SNES in glass vials [126]. At fixed time point (30 seconds), the vials were placed upside down to check if the in situ gels adhered to the bottom of the vials. If the gel sticks to the bottom of the vials, the in situ gel formation was confirmed, with the mark "+". Conversely, the mark "-" was recorded if the gel slides down from the vial bottom and indicated the incomplete formation of the in situ gel. The critical concentration of gellan gum solution to form in situ gel is the minimal concentration with "+".

2.3.3.6 In vitro drug release from granisetron formulations

- ***Membrane model***

The drug release behavior of the in situ gel system was evaluated in 900 ml of SNES [127]. USP II dissolution method (paddle) was used at 32 ºC ± 0.5 ºC, and the paddle speed was set at 50 rpm. 2 ml of drug solution was sealed in a dialysis bag and then immersed into the dissolution medium. An aliquot of sample (5 ml) was withdrawn from each dissolution vessel and passed through a 0.22 μm syringe filter at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 and 240 minutes. The aliquot of collected sample was replaced by 5 ml of fresh SNES after each sample collection. The samples were analyzed using the validated HPLC-UV method. As a control group, Drug-SNES solution was prepared by dissolving granisetron hydrochloride in SNES directly. The release behavior of the Drug-SNES solution was determined by the same dissolution method. All in vitro release tests were repeated in triplicate.

- ***Membraneless model***

In order to study the release of drug from ion-activated in situ gel, the membraneless dissolution method was used with slight modifications [128] [129]. The drug in situ gel solution was spread in a round mold with diameter of 1 cm and immersed into 500 ml of SNES in each dissolution vessel at 32 ºC ± 0.5 ºC. The dissolution of granisetron ion-activated in situ gel was also conducted according to USP II method with paddle speed setting at 50 rpm. At each time point, 2 ml of sample was collected from each dissolution vessel and filtered through a 0.22 μm membrane. The 2 ml of SNES was added after each sample collection to maintain the volume of dissolution medium.

2.3.3.7 Rheological characterization of the in situ gels

The Brookfield rotational viscometer (DV-II+) was used to monitor the rheological properties of the in situ gel formulations. The relationship between shear rate (γ) and shear stress (τ) of the in situ gel solution was determined at different temperature. The spindles were immersed in the in situ gel solutions and rotated with the spindle speed increased from 0.3 rpm to 100 rpm. The parameters, including shear rate (γ), shear stress (τ) and viscosity (η), were determined in triplicate.

2.3.3.8 Water-holding capacity study

The granisetron-gellan gum in situ gel was mixed with SNES with a ratio of 2:1 in test tube, and placed for 2 minutes. Then, 0.4 g of formed gel was accurately weighted and transferred into the ultrafiltration tube with the centriprep filter (total weight W_0), followed by centrifugation at 300 rpm for 1, 5, 10, 20 and 30 minutes, respectively. The gel with centriprep filter device was then weighted (W_t) and the water-holding capacity of the gel is calculated as: $W_t/W_0 \times 100\%$ [109].

2.3.3.9 Expansion coefficient of the in situ gel

The volume of the in situ gel solution may increase when the solution transforms into a gel, and subsequently cause discomfortable sensation in nasal cavity. Thus, the expansion of the in situ gels during drug administration should be taken into account in formulation development and optimization.

The expansion coefficient of the in situ gels was determined by incubating 1 ml of drug in situ gel (10 mg/ml) with 0.25 ml SNES in a graduated test tube and equilibrating in water bath at 32 °C. The total volume after equilibrium was recorded as V_M. The expansion of the in situ gels after adding 2 ml of SNES was also investigated,

and the final total volume was determined as V_T. The volume of the gel after transition (V_G) was calculated as $V_G = V_T - 2.0$. Finally, the expansion coefficient (S %) was calculated by the equation [109]: $S\% = (V_G - V_M)/V_M \times 100\%$.

2.3.3.10 The determination of mucociliary transport time (MTT) of the in situ gel

The mucociliary transport time (MTT) was used to assess the effects of mucoadhesive effects of various formulations. The method for determination of MTT was previously described by Lale et al. [130]. Male Sprague-Dawley (SD) rats were anesthetized by intraperitoneal injection of urethane solution (i.p. injection volume/body weight: 1ml/100 g, concentration of urethane injection: 80 mg/ml). The indigo carmine was dissolved in the formulations at the concentration of 5 mg/ml and used as the indicator. 10 μL of in situ gel solution containing indigo carmine was instilled into the right nostrils of anesthetized rats by a micropipette. The micropipette tips were inserted into the nostrils (approximate 5 mm), and the solutions was slowly injected. Cotton-tipped applicators were used to swab the mucosal regions in oral cavity every 20-30 seconds post dosing. The time when the blue dye was spotted in the pharyngeal and nasopalatine was recorded to assess the mucoadhesive effects of formulations. The normal saline containing 5 mg/ml of indigo carmine was used as the control group.

2.3.3.11 Data analysis

- **The drug release model**

Different kinetic models, e.g., zero-order equation, first-order equation [131] [132], Higuchi equation [133] and Korsmeyer-Peppas equation [134], were used to analyze the kinetics of drug releasing from the nasal formulations. The equations are listed in Table

2.1, where “Q” indicates the accumulated release amount of granisetron at time point of “t”; “k” with different subscripts is the corresponding release constant. In Korsmeyer-Peppas equation, “n” is used to determine the mechanism of granisetron releasing from the nasal formulations, which indicates: (1) zero order release when n is 1; (2) release through diffusion mechanism when n is 0.5; (3) Quasi-Fickian diffusion when n is less than 0.5; (4) anomalous diffusion or non-Fickian diffusion when n is in the range of 0.5 to 1, implying both diffusion and erosion occur during the release.

Table 2.1 Equations of drug release kinetic models

Model	Equation
Zero-order	$F = k_0 \cdot t$
First-order	$F = 100 \cdot (1-e^{(-k_1 t)})$
Higuchi	$F = k_H \cdot t^{0.5}$
Korsmeyer-Peppas	$F = k_{KP} \cdot t^n$

- **Rheological model**

The rheological data in the diagram were fitted by Power Law model listed as below [135]. The flow index “n” indicates the degree of deviation between the test system and Newtonian fluid. Regarding the linear relationship between shear stress and shear rate in Newtonian fluid (n =1), the flow index indicates the degree of “non-Newtonian” in nature for the tested system. When n is less than 1, the tested system is regarded to be pseudoplastic or shear-thinning, which means the viscosity decreases as shear rate increases. Conversely, when n is greater than 1, the fluid is regarded to be dilatant or shear-thickening, and the viscosity increases with the increase of the shear rate.

The Power Law equation: $\tau = K \cdot \gamma^n$

τ: shear stress; K: consistency index; γ: shear rate; n: flow index

2.4 Results

2.4.1 Optimization of HPLC method for quantifying granisetron

Granisetron is a weakly basic compound with pKa of 9.4, therefore both ionized forms and free base forms of granisetron exist in aqueous solution. The disassociation of granisetron can influence the polarity of the drug as well as the retention time in HPLC system. The pH values of the mobile phase was adjusted and optimized to achieve desirable resolution and peak shape. The theoretical plates and the peak shape of granisetron were significantly influenced by the pH of the mobile phase. Increased peak tailing factor and broaden peak shape of granisetron were observed when increasing the pH of mobile phase from 5.0 to 7.0. Ammonium formate was the buffering agent, combined with triethylamine to suppress the tailing factor. The final mobile phase was obtained by mixing the aqueous phase with acetonitrile at the ratio of 80:20 (*v*/*v*). The Thermo BDS C18 Hypersil column (250×4.6 mm i.d.; 5 μm particle size) with a guard column (Thermo C18 Guard-Pak) was applied to achieve desirable peak shape.

2.4.2 HPLC-UV assay validation

The representative UV spectrum and HPLC-UV chromatograms of granisetron were shown in Figure 2.4 and Figure 2.5, respectively. The maximum absorbance occurred at the wavelength of 301 nm, which was chosen for HPLC analysis.

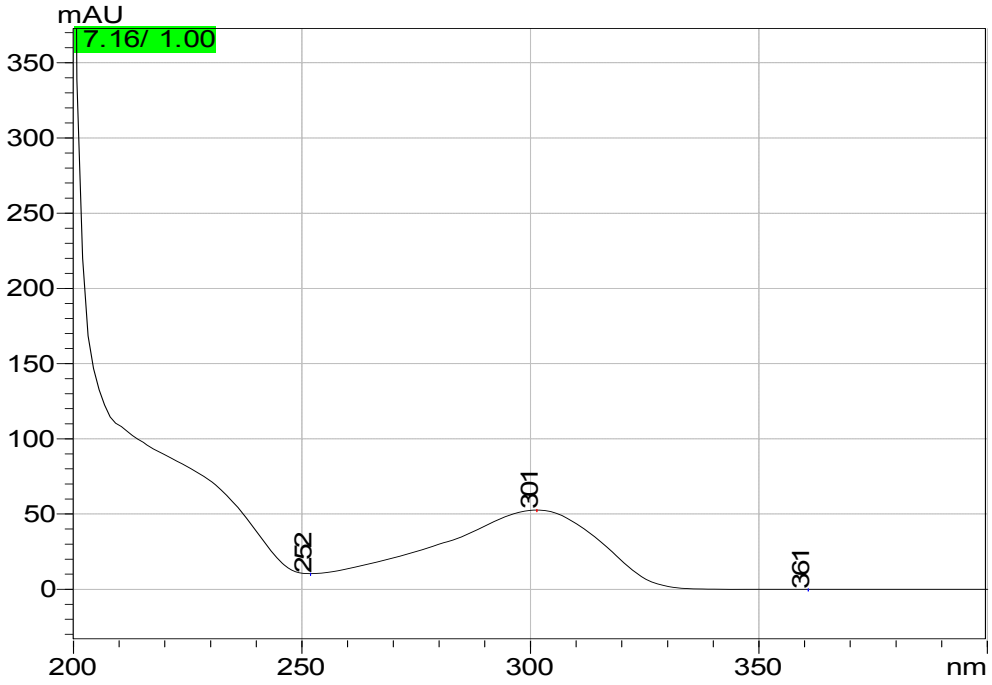


Figure 2.4 UV spectrum of granisetron hydrochloride

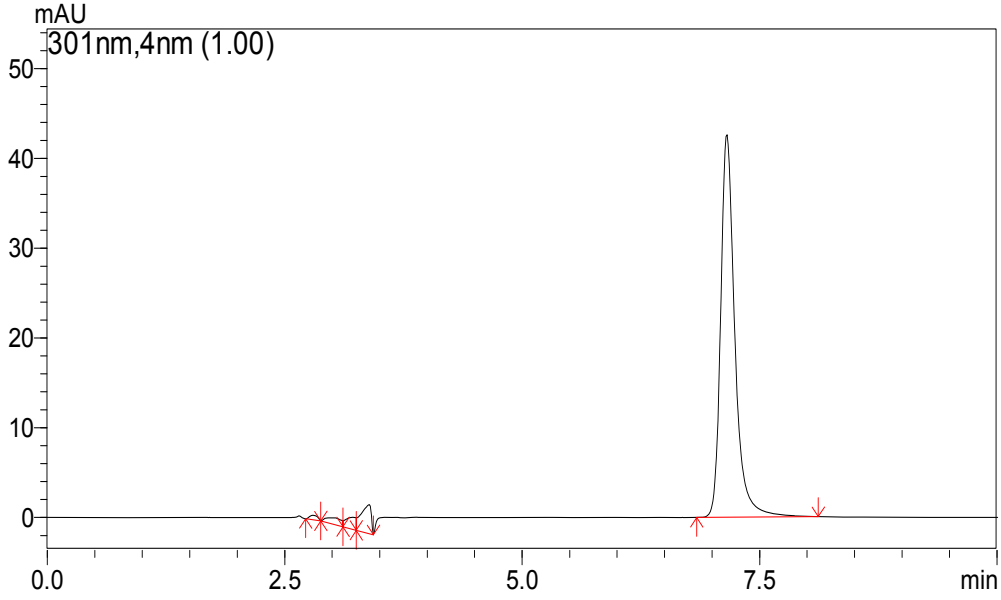


Figure 2.5 Representative HPLC/UV chromatogram of granisetron hydrochloride (20 µg/ml)

Under current chromatographic conditions, the retention time of granisetron was found to be 7.2 min (Figure 2.5). The calibration curves of the peak area of granisetron hydrochloride were linear over the concentration range of 5~200 µg/ml. The intra-day,

inter-day accuracy and precision were shown in Table 2.2. For all the QC samples at three concentration levels (low, intermediate and high), the RSD% of both intra-day and inter-day precision was below 2%, and the accuracy (relative error, RE) was within the range of -3.17% to 2.97%. These results indicated that the current HPLC method for quantifying granisetron is sensitive, accurate and repeatable. Thus, it is suitable for the sample analysis in the studies.

Table 2.2 Linearity, intra-day and inter-day precision and accuracy of the HPLC assays for granisetron

Nominal	Intra-day (*n*=5)			Inter-day (*n*=3)			Linearity	
Conc. (μg/ml)	Determined Conc. (μg/ml)	Precision (%RSD)	Accuracy (%RE)	Determined Conc. (μg/ml)	Precision (RSD, %)	Accuracy (RE, %)	Range (μg/ml)	r^2
10.1	10.3	1.48	1.98	10.4	0.62	2.97		
50.5	49.4	0.21	-2.18	48.9	0.31	-3.17	5-200	0.9994
101.0	102.5	0.62	1.49	101.7	0.14	0.69		

2.4.3 Solubility of granisetron hydrochloride

Aqueous solubility is an important factor of drug candidates for intranasal delivery due to the limited dosing volume. To deliver 2 mg of granisetron in 200 μL of spray solution (100 μL for each nostril), the solubility of granisetron in water should be higher than 10 mg/ml. Granisetron hydrochloride is a highly soluble compound. At 25 ^{o}C, 150 mg of granisetron hydrochloride could quickly dissolve in 0.5 ml of all solutions tested (PBS 4.0, PBS 5.5, PBS 7.0, normal saline and distilled water) by vortex or sonication. No precipitation was observed when stored at 4 ^{o}C overnight. The solubility of granisetron hydrochloride in the tested solutions are higher than 300 mg/ml at 4 ^{o}C and 25 ^{o}C, thus sufficient dose of granisetron can be delivered by nasal spray. Therefore, solubility enhancement in the formulation development is not required.

2.4.4 Stability tests of granisetron hydrochloride in aqueous solutions

To evaluate the effects of pH and temperature on stability of granisetron solutions, stability tests were performed at 25 ^{o}C and 40 ^{o}C. Results showed that granisetron hydrochloride were stable at least up to 20 days at 25 ^{o}C and 40 ^{o}C, when dissolved in DI water, normal saline, and PBS solutions with pH between 4.0, 5.5 and 7.0. The remaining granisetron hydrochloride in all solutions was higher than 96% after the testing period, indicating desirable stability of granisetron for future research.

2.4.5 Distribution coefficient (Log D) of granisetron hydrochloride

The distribution coefficient (Log D) is obtained by calculating the ratio of drug concentration between two immiscible phases at equilibrium. It reflects the hydrophilic-lipophilic properties of the drug and can provide useful information on the

capability of the drug penetrating across biological barriers. The ideal drug Log D for nasal delivery is between 2 and 4. The Log D of granisetron hydrochloride in various media is listed in Table 2.3. Granisetron is a weakly basic compound with pKa of 9.4 [136]. Therefore, pH is the critical factor to affect the Log D. Since the proportion of free base form of granisetron increased at higher pH, and the granisetron in the molecular form was more likely to distribute in organic phase, the experimental Log D values increased at higher pH.

Table 2.3 Distribution coefficients of granisetron hydrochloride in various solutions (25 ºC)

Solutions	PBS 4.0	PBS 5.5	PBS 7.0	DI Water	Normal saline
Log D	-1.47	-1.02	0.27	-1.68	-0.59

2.4.6 Determination of sol-gel transition temperature

The solution to gel transition temperature of thermosensitive solutions with different concentrations of Poloxamer 407 was determined and shown as Figure 2.6. The solution to gel transition temperature ($T_{sol-gel}$) of the system decreased from 38.6 ºC to 19.7 ºC with the concentration of Poloxamer 407 increasing from 15% to 24%. In previous studies, APIs or other hydrophilic ingredients may also affect the $T_{sol-gel}$ of the thermosensitive system [127, 137]. In our study, the $T_{sol-gel}$ of solutions containing 18% of Poloxamer 407 and other ingredients (granisetron hydrochloride, sodium hyaluronate, Poloxamer 188 and PEG 6000) was determined as well. All above ingredients could increase the $T_{sol-gel}$ of the thermosensitive systems except sodium hyalunate, which resulted in slightly decreased $T_{sol-gel}$. The temperature in human nasal cavity was reported to be in the range of 32 ºC and 33.5 ºC [138], thus the $T_{sol-gel}$ of the formulations

should be adjusted in the range from 27 ºC to 30 ºC. Since the $T_{sol-gel}$ of 18% Poloxamer solution containing 1% granisetron hydrochloride was 27.5 ºC, both Poloxamer 188 and PEG 6000 were selected to adjust the gelation temperature close to 30 ºC, at which the nasal spray solutions with decreased viscosity at room temperature can be also achieved. The granisetron formulations, containing Poloxamer 407, Poloxamer 188, PEG 6000 and sodium hyaluronate, were prepared, and the $T_{sol-gel}$ was shown in Table 2.4. Formulations F1, F2, F5 and F6 had the favorable $T_{sol-gel}$ around 30 ºC. Although both Poloxamer 188 and PEG 6000 could increase the $T_{sol-gel}$ of formulations, Poloxamer 188 is more advantageous than PEG 6000, because of its ability to counteract the diluent effects caused by secretion of nasal fluid [139]. Therefore, Poloxamer 188 was selected in final formulations (F1 and F2) for further studies.

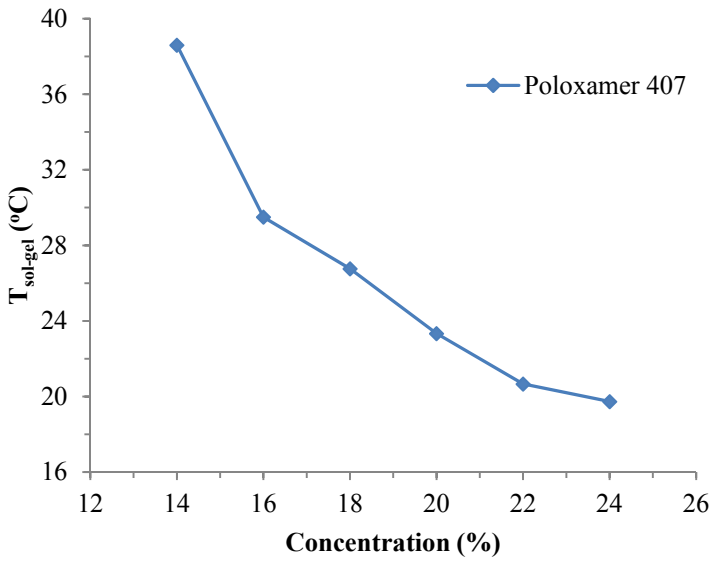
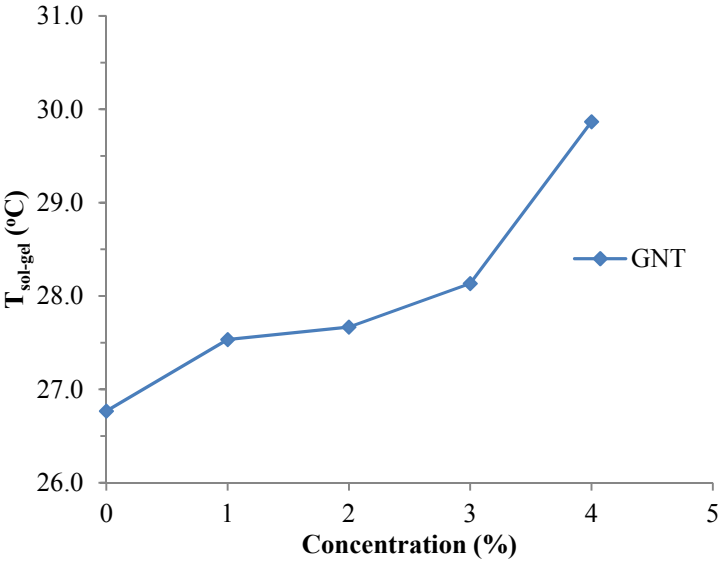
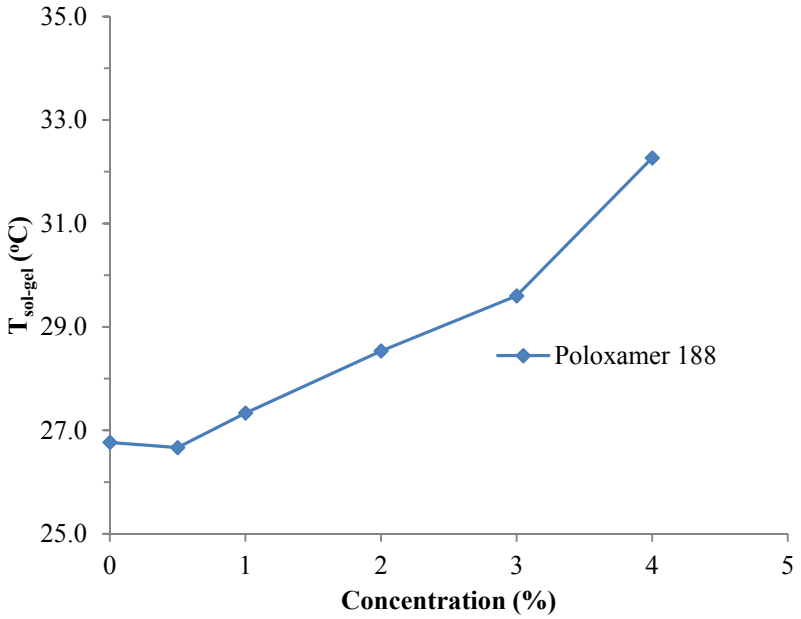


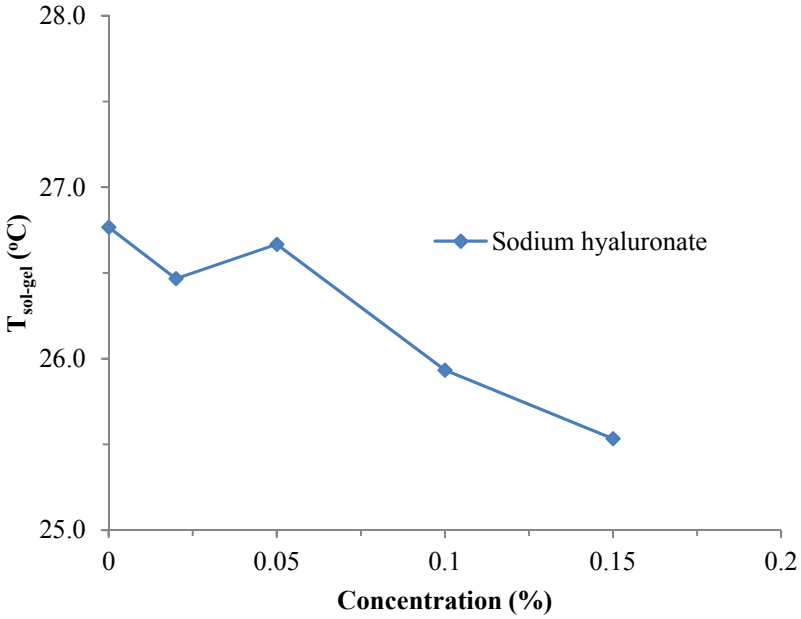
Figure 2.6 The relationship of sol-gel transition temperature and concentration of Poloxamer 407


31.0
30.0
29.0
28.0
27.0
26.0
$T_{sol-gel}$ (°C)
0
1
2
3
4
5
Concentration (%)
GNT

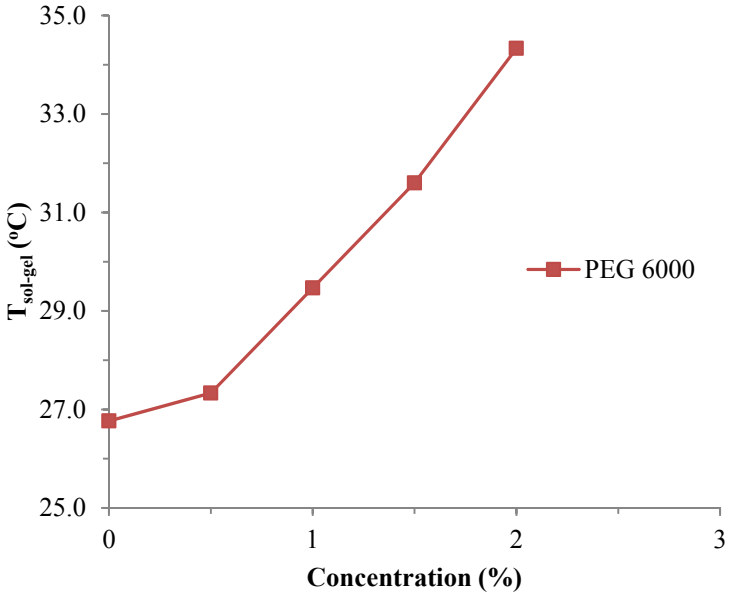
(A)


35.0
33.0
31.0
29.0
27.0
25.0
$T_{sol-gel}$ (°C)
0
1
2
3
4
5
Concentration (%)
Poloxamer 188

(B)



(C)



(D)

Figure 2.7 The effects of granisetron hydrochloride (A), Poloxamer 188 (B), sodium hyaluronate (C) and PEG 6000 (D) on the $T_{sol-gel}$ of thermosensitive solutions containing 18% Poloxamer 407

Table 2.4 In situ gel formulations and sol-gel transition temperature determination

Ingredients (%, *w/v*)	Formulations							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
GNT	1	1	1	1	1	1	1	1
Poloxamer 407	18	18	18	18	18	18	18	18
Poloxamer 188	1	1	2	2	-	-	-	-
Sodium hyaluronate	-	0.05	-	0.05	-	0.05	-	0.05
PEG 6000	-	-	-	-	0.5	0.5	1	1
$T_{sol\text{-}gel}$ (^oC) Mean (SD)	29.1 (0.2)	28.3 (0.2)	31.4 (0.2)	30.5 (0.3)	29.3 (0.5)	29.8 (0.2)	31.4 (0.2)	32.2 (0.2)

2.4.7 In vitro release of granisetron from in situ gels – membrane model

The membrane dissolution model was used to determine the in vitro release of granisetron from Poloxamer in situ gels. The results are shown in Figure 2.8. In comparison with the drug release from simulated nasal electrolyte solution, the in situ gel formulations (F1 and F2) showed delayed release profile, with less than 60% of drug release in the first hour. In comparison of formulation F1, F2 showed slightly slower drug release rate, indicating the sodium hyaluronate in F2 may retard drug release from gel matrix. Different release models were applied to investigate the mechanism of drug release from the in situ gels. The model fitting and parameters are presented in Table 2.5. Based on the goodness of fit, the in situ formulations (F1 and F2) have different best-fitting models. The drug releasing from F1 fits well with Higuchi model, while the Korsmeyer-Peppas model fits well to the release data of F2 with the n value of 0.6181. With the n value of 0.4975, the release data of F1 could be also well fitted by the Korsmeyer-Peppas equation with the lowest error sum of squares (SSE)

and the highest R-square. The significant differences in the n values of F1 and F2 indicate different release mechanisms for the two in situ gels. According to the description in Section 2.3.3.11, the release mechanism of F1 is diffusion, while the drug in F2 released following both diffusion and erosion mechanisms.

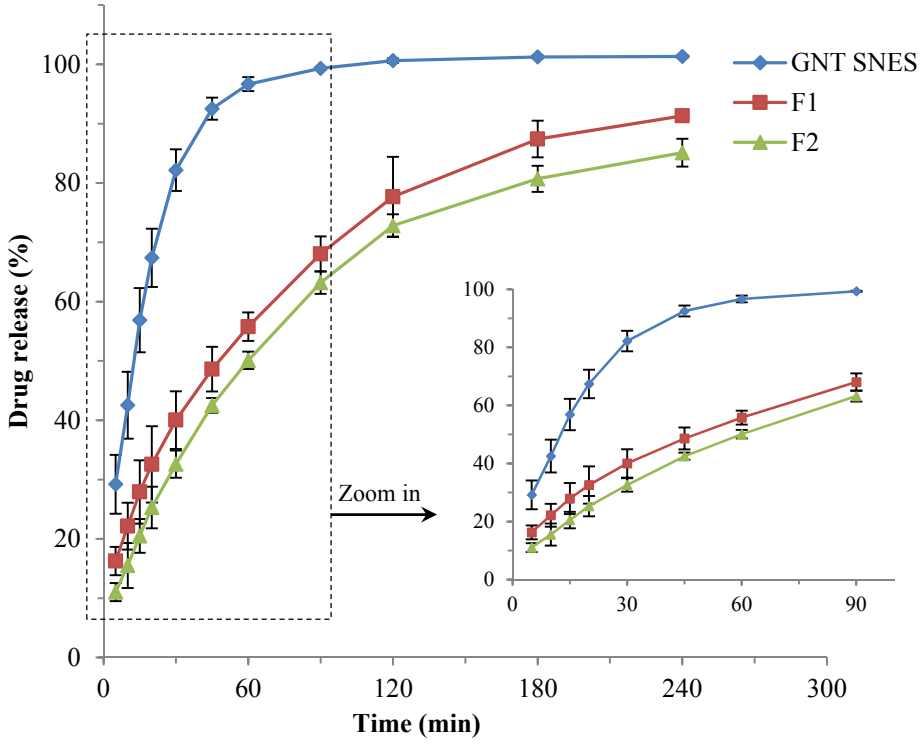


Figure 2.8 Release of granisetron from simulated nasal electrolyte solution (GNT SNES) and in situ gels (F1, F2) (n=3)

Table 2.5 Models for granisetron release from in situ gels (F1 and F2)

		Equations	Parameters	Goodness of fitting	
				r^2	SSE
F1	Zero-order	$F = k_0 \cdot t$	k_0=0.9257	0.4838	1127.45
	First-order	$F = 100 \cdot (1-e^{(-k_1 t)})$	k_1=0.0156	0.8585	309.05
	Higuchi	$F = k_H \cdot t^{0.5}$	k_H=7.2113	0.9995	1.02
	Korsmeyer-Peppas	$F = k_{KP} \cdot t^n$	k_{KP}=7.2815, n=0.4975	0.9996	0.98
F2	Zero-order	$F = k_0 \cdot t$	k_0= 0.8201	0.8012	456.66
	First-order	$F = 100 \cdot (1-e^{(-k_1 t)})$	k_1= 0.0124	0.9617	88.07
	Higuchi	$F = k_H \cdot t^{0.5}$	k_H= 6.2458	0.9702	68.53
	Korsmeyer-Peppas	$F = k_{KP} \cdot t^n$	k_{KP}= 3.9571, n= 0.6181	0.9988	2.73

SSE: Error sum of squares.

2.4.8 Rheological properties of thermosensitive in situ gels

The rheological properties of formulations F1 and F2 were characterized. As shown in Figure 2.9, the viscosity of both formulations decreased slightly when temperature increased from 4 ^{o}C to 10 ^{o}C. However, an exponential increase in viscosity of both formulations was observed around the sol-gel transition point (28 ^{o}C). In addition, the shear rate (γ) and shear stress (τ) at 10 ^{o}C and 20 ^{o}C were well fitted with Power-Law equation ($\tau = K \cdot \gamma^{n}$), with n value of 1 (Figure 2.10). Therefore, both F1 and F2 formulations can be categorized as Newtonian fluid, indicating that the fluids have the same viscosity over the range of shear rates tested.

The viscosity of nasal liquid formulations should be well controlled and the solution can be easily sprayed and nebulized using the nasal spray pumps. Once the sprayed solution contacts the nasal cavity, sol-gel transition occurs instantaneously. Compared to F2, F1 has lower viscosity and can be easily sprayed and atomized using marketed nasal spray pumps, with better pump delivery uniformity. Finally, the formulation F1 was selected for further studies.

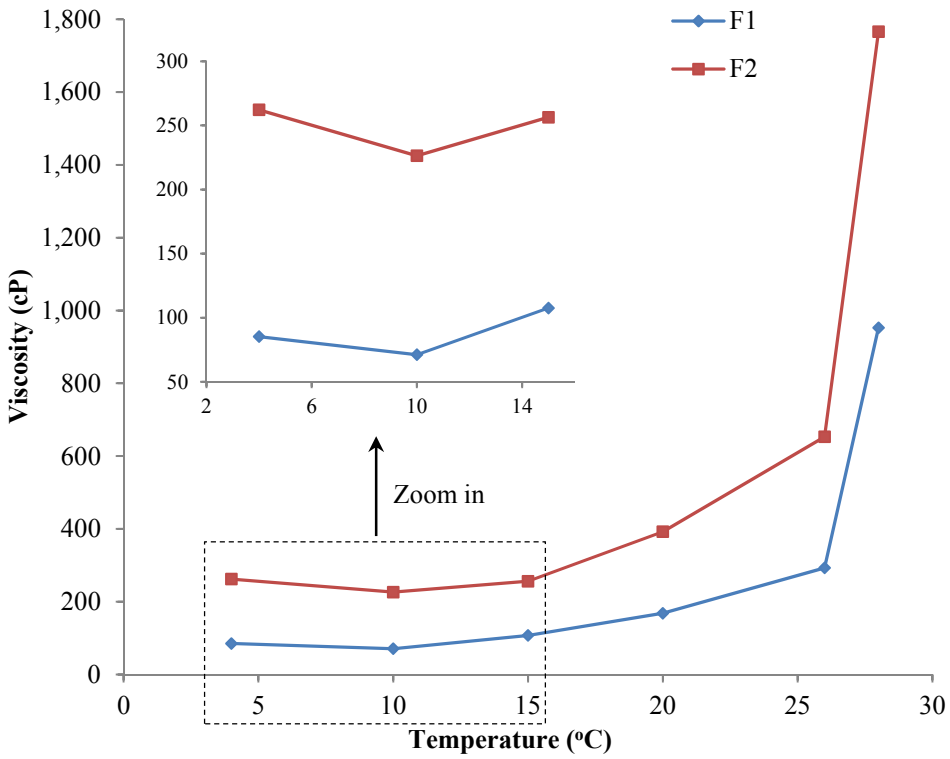


Figure 2.9 The viscosity of F1 and F2 at different temperatures below the sol-gel transition point

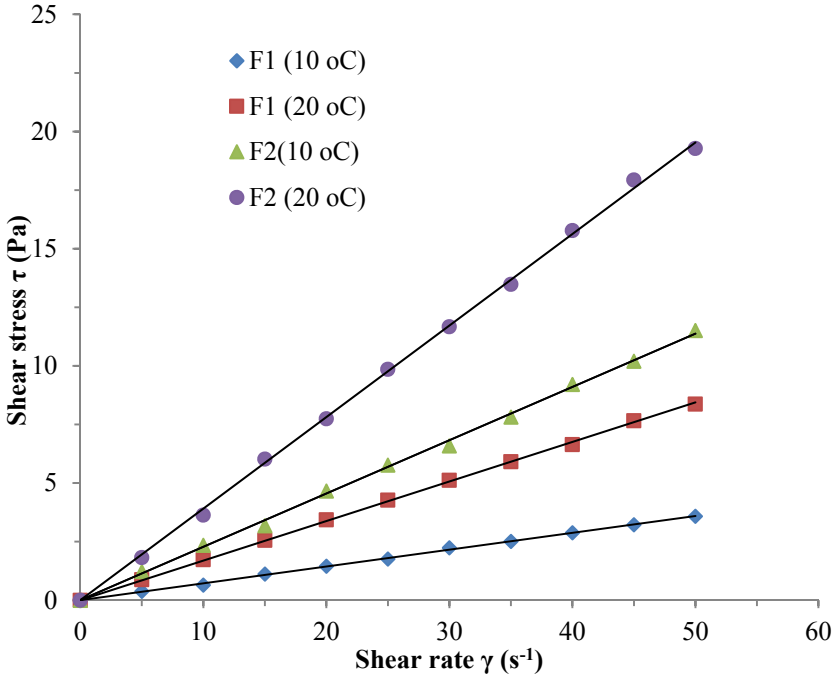


Figure 2.10 Rheological properties and fitting lines at 10 °C, 20 °C

Table 2.6 Rheological parameters of in situ gels at 10 °C and 20 °C

Fitted equation	Formulation	Temperature (°C)	K	r^2
$\tau = K \cdot \gamma$	F1	10	0.0719	0.9989
		20	0.1688	0.9996
	F2	10	0.2274	0.9986
		20	0.3907	0.9991

2.4.9 Determination of critical concentration of gellan gum for phase transition

1.0 ml of gellan gum solutions at different concentrations was mixed with SNES to determine the critical concentrations of sol-gel transition. As shown in Table 2.7, gellan gum solutions below critical concentration could not form gels after mixing with various amounts of SNES. Once the concentration of gellan gum was above the critical concentration, the formation of transparent, odorless and colorless gel was instantaneously triggered by adding various amounts of SNES. In brief, the gellan gum solutions with higher concentrations need less SNES to form gels. However, the gellan gum solutions with the concentration exceeding 0.6% cannot be completely atomized because of the high viscosity. Therefore, the optimal concentration of gellan gum for nasal spray should be below 0.6%.

Table 2.7 Gelation behavior of gellan gum solutions with SNES

Concentration of gellan gum (%)	Volume of SNES (µL)					
	50	100	150	200	250	300
0.1	-	-	-	-	-	-
0.2	-	-	-	-	+	+
0.3	-	-	-	-	+	+
0.4	-	-	-	+	+	+
0.5	-	-	+	+	+	+
0.6	-	-	+	+	+	+
0.7	-	+	+	+	+	+

Notes: (-) Slide. (+) Not slide.

2.4.10 In vitro drug release from in situ gel system - Membraneless model

The in vitro release test of granisetron in situ gels prepared by gellan gum was carried out with the membraneless model described in Section 2.3.3.6. 0.5 g of granisetron-gellan gum solution was spread in a round mode with the diameter of 1 cm and then immersed into 500 ml of SNES. The in vitro release profiles are shown as Figure 2.11. All granisetron in situ gels with different concentrations of gellan gum showed slow release of granisetron with around 60% released in the first 2 hours. There was no significant difference among the in situ gels, suggesting the similar robustness of the formulations with different concentrations of gellan gum. The drug release profiles of all formulations fits well with Korsmeyer-Peppas equation, and the modeling parameters are listed in Table 2.8. The n value is around 0.5, indicating the drug released from gellan gum in situ gels by diffusion. Considering the critical concentration of phase transition, the formulation containing 0.50% gellan gum was chosen for further studies.

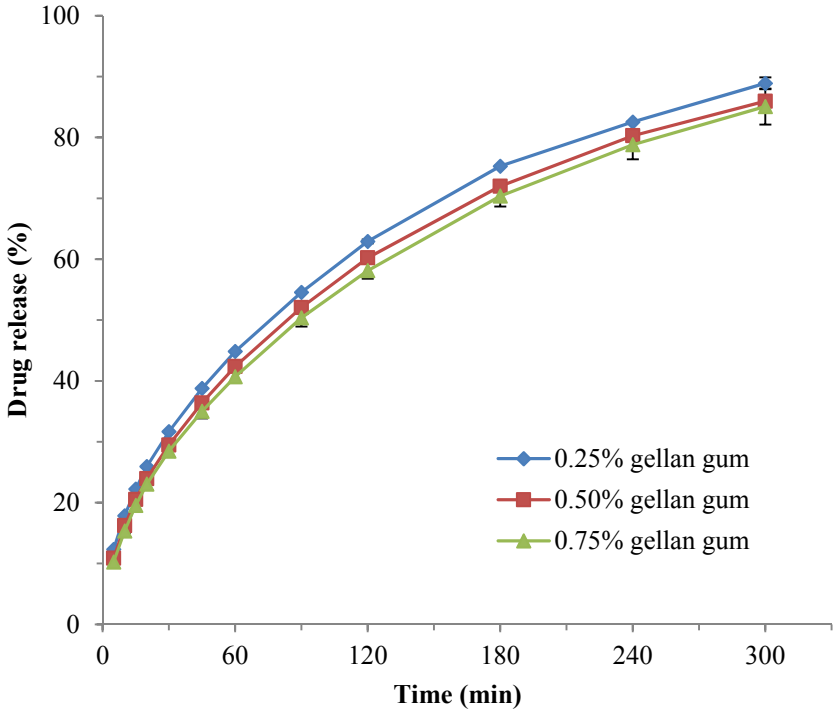


Figure 2.11 In vitro release profiles of granisetron in situ gels

Table 2.8 Model fitting of granisetron release from gellan gum based in situ gels

Model	Equation	Formulation	Parameter	Goodness of fit	
				r^2	SSE
Korsmeyer-Peppas	$F = k_{KP} \cdot t^n$	0.25% gellan gum	k_{KP}=5.7026, n=0.5022	0.9998	0.57
		0.50% gellan gum	k_{KP}=4.9524, n=0.5228	0.9997	0.70
		0.75% gellan gum	k_{KP}=4.6670, n=0.5280	0.9995	1.04

2.4.11 Water-holding capacity of ion-activated in situ gels

The water-holding capacity refers to the capability of the in situ gel holding water in the gel matrix, which could be obtained by measuring the water retained in the gels under external force. The water-holding capacity is a key parameter to assess the

physical stability of gel matrix. The in situ gels containing 0.5% of gellan gum and 10 mg/ml of granisetron were used to determine the water-holding capacity. The granisetron in situ gels formed by mixing 0.5% gellan gum and SNES at a ratio of 2:1 exhibited a water-holding capacity above 96.0 % (Figure 2.12) after centrifugation (300 rpm) for 30min, indicating that the in situ gel matrix was stable under low mechanical force.

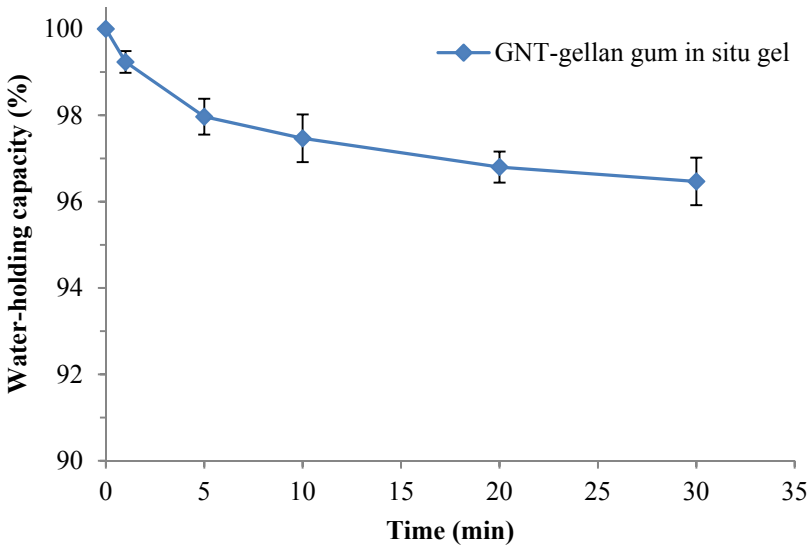


Figure 2.12 Water-holding capacity of granisetron-gellan gum in situ gel at different centrifugation time

2.4.12 Expansion coefficient of GNT-gellan gum in situ gel

In our study, no obvious volume expansion was observed when the test solution containing 0.5% gellan gum and 10 mg/ml of granisetron transformed into transparent gels. The observed expansion coefficient was only about 4.1%. Therefore, the patients' compliance may not be affected by the slight expansion of the in situ gels in nasal cavity.

2.4.13 Rheological properties of granisetron-gellan gum in situ gel

As shown in Figure 2.13, the viscosity of in situ gel containing 0.5% gellan gum and 10 mg/ml granisetron (GNT) increased after mixing with SNES, and the maximum viscosity was obtained when drug solution and SNES mixed at a ratio of 4:1. Since the viscosity of the mixture decreased with the increased shear rate, the tested samples demonstrated pseudoplastic fluid behavior. The viscosity at a shear rate of 3.96 s^{-1} was summarized in Table 2.9. After mixing the drug solution with SNES at 4:1, the viscosity of in situ gel is10-fold higher than that of the drug solution, which may facilitate the drug solution prolong its residence time and enhance the nasal absorption.

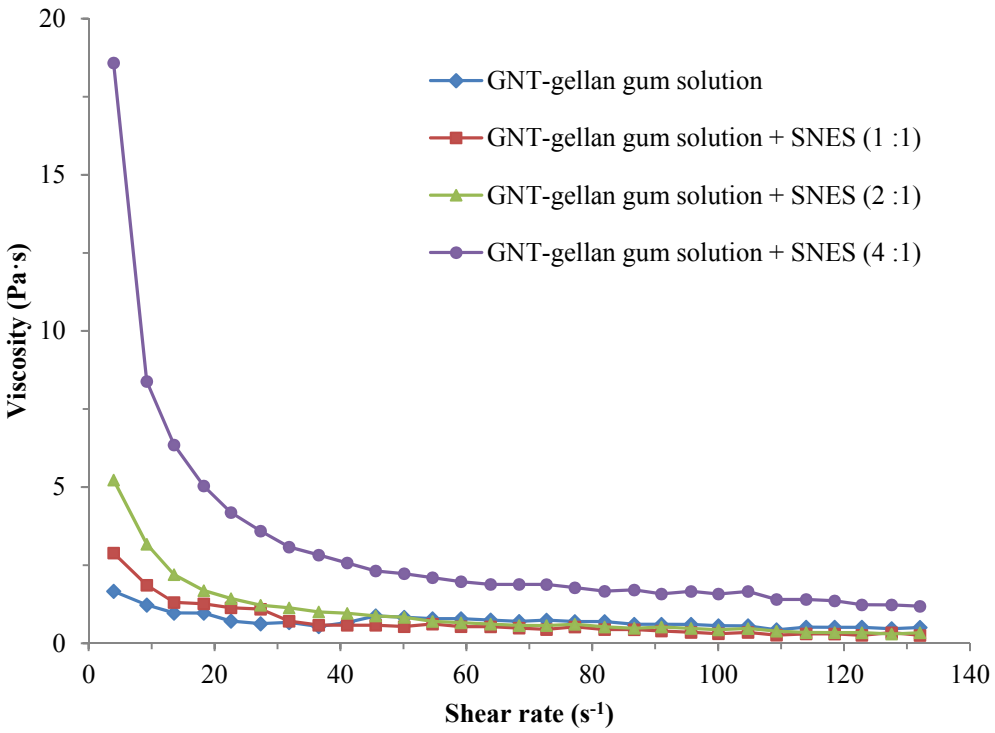


Figure 2.13 Viscosity of GNT-gellan gum solution mixed with SNES at different ratios

Table 2.9 Viscosity of granisetron-gellan gum solution absence or in presence of SNES

	Viscosity (Pa·s)
GNT-gellan gum solution	1.57 ± 0.21
GNT-gellan gum solution + SNES (1:1)	2.53 ± 0.59
GNT-gellan gum solution + SNES (2:1)	4.81 ± 1.02
GNT-gellan gum solution + SNES (4:1)	17.48 ± 3.24

2.4.14 Drug release profile of granisetron-HPMC solution

The drug release profile of granisetron-HPMC solutions was measured using the membrane dissolution model described in Section 2.3.3.6. The results in Figure 2.14 indicate the fast drug release in all tested solutions, with about 80% of drug release in the first 30 minutes. The drug release rate was not affected by HPMC concentration over the range of 0.25% to 0.75%. Interestingly, granisetron solutions containing the HPMC (0.25% - 0.75%) have similar release rate with that from SNES, indicating drug could be released rapidly from the HPMC solutions while keeping the mucoadhesive capability.

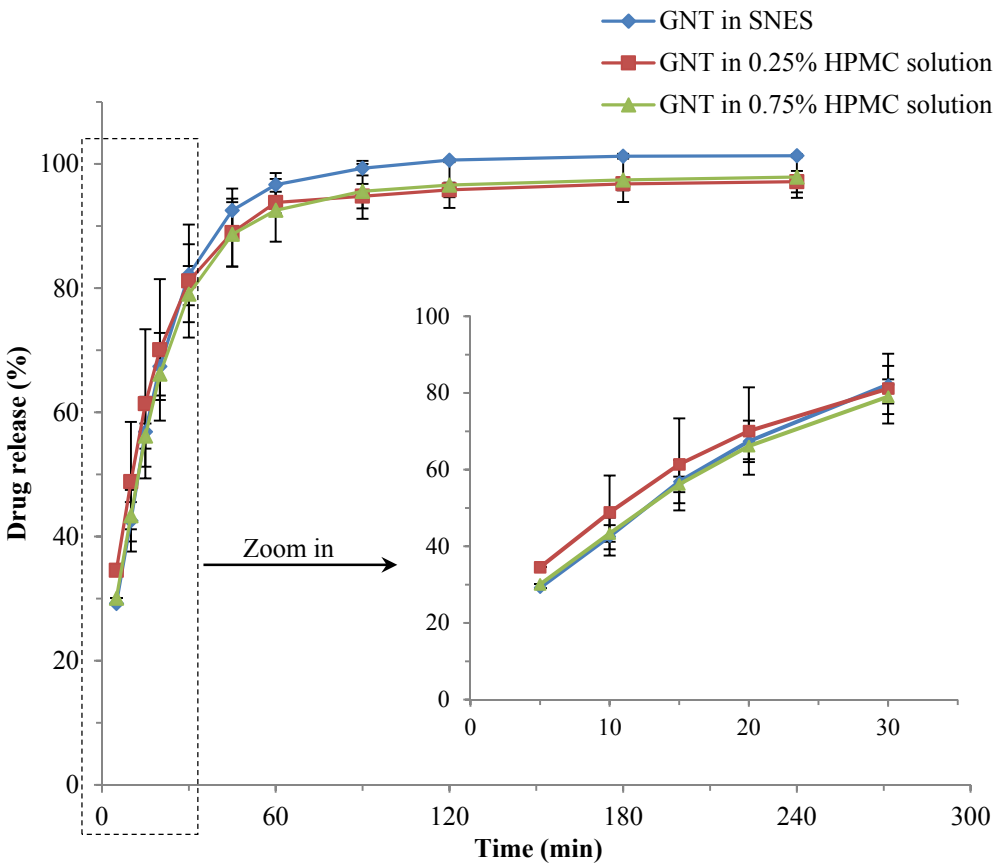


Figure 2.14 The in vitro release of granisetron from HPMC solutions

2.4.15 The mucociliary transport time (MTT) of the formulations

The Poloxamer 407, Poloxamer 188, gellan gum and HPMC were used to prepared mucoadhesive solution. The solution was spiked by indigo carmine (5 mg/ml) as an indicator. As shown in Table 2.10, after nasally dosing the normal saline, the blue dye was detected at 1.5 minutes and 4.6 minutes in nasopalatine and pharyngeal of rats respectively, which are two joints between the nose and the oral cavity. The mucociliary transport time of HMPC solutions increased remarkably as compared with that of normal saline group. Furthermore, the solution with higher HPMC concentration had relatively longer mucociliary transport time. For in situ gel solutions, the Poloxamer 407/Poloxamer 188 solution showed longer MTT than the 0.5% gellan gum solution.

All the tested formulations had longer mucociliary transport time (both to nasopalatine and pharyngeal) than the normal saline solution. Briefly, all the polymers in the formulations had positive effects on prolonging the nasal mucociliary transport time of drugs.

Table 2.10 Nasal mucociliary transport time of in situ gel and mucoadhesive solutions containing 5 mg/ml indigo carmine (n=4)

Formulation	Transport time to nasopalatine (min)	Transport time to pharyngeal (min)
Normal saline	1.5 ± 0.7	4.6 ± 2.4
0.75% HPMC	5.4 ± 2.5	16.1 ± 5.0*
0.25% HPMC	3.1 ± 0.8	11.8 ± 5.1
0.5% gellan gum	4.3 ± 1.9	18.0 ± 8.5
18% poloxamer 407, 1% poloxamer 188	17.6 ± 7.3*	> 60*

* Significantly different from control group (Normal saline): *P<0.05*.

2.5 Discussion

Poloxamer 407 and Poloxamer 188 were used to prepare thermosensitive in situ gel formulation. Poloxamer polyols are more easily to dissolve in cold water than in hot water due to the increased solvation and hydrogen bonding at low temperature [140]. Therefore, the cold method was applied in preparation of thermosensitive in situ gel formulations. The gelation mechanism is related to the formation of micelle and the dehydration-induced entanglements of the poloxamer chains at the critical temperature and the critical concentration [141]. The poloxamer micelles can pack together above critical temperature and critical concentration, and then a highly ordered structure is formed, which is similar to cubic crystalline phase or crystal lattices. The dehydration of the hydrophobic polyoxypropylene (PPO) blocks in the structure of poloxamer is believed to trigger the sol-gel transition. Therefore, higher sol-gel transition temperature can be achieved by increasing the ratio of hydrophilic polyoxyethylene (PEO) blocks to hydrophobic polyoxypropylene (PPO) blocks with more extensive hydrogen binding. Poloxamer 188 contains higher ratio of PEO/PPO (80/27) as compared to the ratio of PEO/PPO in poloxamer 407 (101/56). Thus, the addition of poloxamer 188 resulted in the increase of the sol-gel transition temperature of in situ gel system. Such effect is also concentration dependent (Figure 2.7-B). The ratio of PEO/PPO of the in situ gel system could also be increased by adding PEG 6000 with oxyethylene groups, leading to increased sol-gel transition temperature (Figure 2.7-D). Apart from Poloxamer 188 and PEG 6000, granisetron hydrochloride was also found to increase the $T_{sol\text{-}gel}$ of the in situ gel systems, which may attribute to the change of micellar formation of Poloxamer 407 induced by hydrophilic granisetron hydrochloride. Similar results were also found in previous studies on other hydrophilic drugs in Poloxamer systems, such as venlafaxine hydrochloride [142] and geniposide [143]. In addition, sodium hyaluronate at

low concentrations could slightly decrease the sol-gel transition temperature, which is supposed to be the dehydration of the hydrophilic chains of poloxamer polyols caused by the entanglement of the sodium hyaluronate. However, sodium hyaluronate could increase the viscosity of Poloxamer solution, and impact the nebulization by nasal spray pump.

There are microscale aqueous pores in Poloxamer-based in situ gel above the sol-gel transition temperature. The incorporated drug in the in situ gels can be released via those aqueous pores in spite of the rigid structure of the gel [144]. Previous studies showed that the water soluble drugs released from the Poloxamer gels following zero-order kinetics, suggesting the drug release was controlled by the dissolution of the gel [145]. In our in vitro release studies, the Poloxamer-granisetron solution was sealed in dialysis bags and the thermosensitive gels swelled after absorbing water from dissolution medium. Granisetron released from micropores in the gel matrix following diffusion mechanism. Meanwhile, the swelling gels were eroded during water uptake, resulting in the accelerated drug release. The drug release from formulations F1 and F2 follows Korsmeyer-Peppas equation with the n value of 0.4975 (F1) and 0.6181 (F2), suggesting that the drug was released from the gels by both diffusion and erosion. Specifically, the model-fitting results indicated drug was released from F1 by diffusion mechanism, while both diffusion and erosion mechanisms were involved in the drug release from Poloxamer gels containing sodium hyaluronate (F2). In comparison with F1, slower release rate was observed in F2, which may be attributed to the high viscosity caused by sodium hyaluronate in the aqueous channels of the gel matrix. Sodium hyaluronate may reduce the rigidity of the gel matrix, resulting in the erosion of the gels during release process. Similar results in previous studies showed that sodium hyaluronate in Poloxamer system could reduce the rigidity of the in situ gels and

decrease the diffusion rate [146].

The viscosity of sodium hyaluronate was shown in the rheological characterization in Section 2.4.8. Newtonian behavior was exhibited in both F1 and F2 solutions in the dynamic mechanical test. The viscosity of F2 was around 3 times higher than the formulation F1. When the solutions were gradually heated under the sol-gel transition temperature, a slight decrease in viscosity was observed in both F1 and F2 (Figure 2.9), which may be induced by the increase in micellization during heating process. Similar results were also obtained in previous studies [147].

As an ion-sensitive polymer, gellan gum can form odorless, colorless and transparent in situ gel when mixing with SNES. A visual inspection was used to determine the critical concentration for phase transition. In consideration of the sol-gel phase transition and the viscosity of the solution, 0.5% of gellan gum solution was selected for the further studies. Besides visual inspection, the stirring method was also used in previous researches [148]. Briefly, when the rotation of magnetic stir bar under a controlled torque is stopped by the resistance from the in situ gel solution, the initiation of sol-gel phase transition is confirmed. However, the stirring method has several disadvantages. For instance, it is difficult to accurately control the torque imposed on the stir bar. Furthermore, the gel with such high rigidity is not suitable for nasal spray to deliver the drug. In our current method, whether the formed gel-like stuff could stick to the bottle bottom or not was visually observed to evaluate the formation of the in situ gels. The visual inspection method could evaluate both adhesive property of the formed gels and the sol-gel phase transition.

Membraneless dissolution method was applied in evaluation of drug release profile from gellan gum prepared in situ gels. Unlike the conventional membrane method using

dialysis bag to measure drug release, the membraneless method allows the in situ gels directly contact the dissolution medium (SNES), which is more similar to the in vivo condition [129]. The curve fitting results indicated that drug was released from gellan gum by diffusion mechanism primarily, as well as the erosion mechanism when gellan gum content was above 0.5%. The brittleness of the low acyl gellan gum in the formulation may contribute to the erosion of in situ gels during the release process. Similar results were also reported previously [143].

The in situ gels and the bioadhesive formulations were developed to prolong the drug nasal residence time of nasally administered drug. Various in vivo or in vitro methods were reported to evaluate the change of mucociliary clearance by bioadhesive formulations, such as saccharin test [149], detecting radioactive particle transport [150] and measuring the excised frog palate ciliary beat frequency [151]. These methods, however, are inconvenient (e.g., detecting radioactive particle transport) or inaccurate (e.g., frog palate ciliary beat frequency). In this study, the transport time for the formulations containing indigo carmine (blue dye) from nasal cavity to nasopalatine and pharyngeal was measured, as the indicator of the mucociliary clearance for different formulations. The blue dye detected in the nasopalatine was much earlier than that in the pharyngeal. It is attributed to the anatomical structure in rat nasal cavity: nasopalatine was closer to the nostrils than pharyngeal. All tested formulations achieved prolonged nasal clearance time in rats, indicating the bioadhesive effects of the polymers.

2.6 Conclusion

In this chapter, different granisetron nasal formulations, including thermosensitive in situ gel, ion-activated in situ gel and HPMC-based bioadhesive solution, were prepared and the properties were evaluated by sol-gel transition temperature, critical concentration for phase transition, in vitro drug release, rheological characterization, etc. All the formulations showed prolonged nasal mucociliary transport time. The granisetron in situ gel containing 18% Poloxamer 407 & 1% Poloxamer 188, the formulation with 0.5% gellan gum and the formulation using 0.5% HPMC as bioadhesive vehicle were chosen for further studies.

Chapter 3

Pharmacokinetic studies of granisetron formulations

3.1 Introduction

Nausea and vomiting are commonly and severely debilitating adverse events of cytotoxic chemotherapy, radiotherapy and certain types of surgeries [10] [9]. These symptoms of chemotherapy induced nausea and vomiting (CINV) limit patients' ability to eat and drink, remarkably reduce quality of life, threaten the success of therapy. It has been reported that up to 20% of patients were forced to postpone or refuse potentially curative treatment because of the severe nausea and vomiting induced by chemotherapy [12].

The management of CINV has been improved greatly since the introduction of 5-HT3 receptor antagonists (5-HT3 RAs). The 5-HT3 RAs can prevent serotonin from binding to the corresponding receptors and possess high therapeutic index for controlling CINV of patients receiving moderately to highly emetogenic chemotherapy [32]. Granisetron ($C_{18}H_{24}N_4O$, M.W.: 312.409) is the first generation of 5-HT3 RA and exerts therapeutic effects by selectively and competitively binding to the 5-HT3 receptors to block the nervous impulse for stimulation of CTZ in the brain stem [152]. The marketed products of granisetron include Kytril® (oral tablets, oral solution and IV injection), Sancuso® (transdermal patch) and Sustol® (extended-release subcutaneous injection). Those current granisetron dosage forms have their own unsatisfactory properties respectively. For instance, oral absorption of granisetron is affected by food, which implies the therapeutic effects may be different between the fasted patients and non-fasted patients. The oral dosing of granisetron may not have very good compliance

especially when patients are suffering severe oral mucositis and the swallowing capacity is compromised. As to granisetron IV injection, patients will undergo unnecessary pains and potential infection which is an important issue in the immune-compromised patients. Neither granisetron transdermal patch nor extended-release subcutaneous injection is designed for rapid onset of action. To date, there is still not a non-invasive and quickly effective granisetron product on the market.

Nasal cavity is covered by highly vascularized mucosa, with surface area of 150-200 cm^2 [153]. The drugs can pass through the thin epithelial cell layer to the systemic circulation rapidly, circumventing first pass metabolism and the degradation caused by the gastrointestinal enzymes [154]. Nasal drug delivery is a convenient, non-invasive and painless administration route, and is promising to be an alternative to current granisetron dosage forms for rapid onset of action. Granisetron is a water soluble antiemetic drug with the oral dose of 2 mg per day, and its molecular weight is less than 500 Dalton. In our previous studies, three granisetron bioadhesive formulations basing on poloxamer, gellan gum and HPMC were developed and evaluated by mucociliary transport time (MTT). According to the characteristics of granisetron formulations, (1) 18% Poloxamer 407 and 1% Poloxamer 188 solution, (2) 0.5% gellan gum solution, and (3) 0.5% HPMC solution were chosen for further studies. In this chapter, a HPLC-FLD method for quantification of granisetron in rat plasma will be developed and validated. The in vivo studies of granisetron formulations will also be conducted in rats to compare the nasal absorption among different rat models (conscious or short-term anesthetized rats) and granisetron dosage forms for further optimization.

3.2 Materials and annimals

3.2.1 Materials

Granisetron hydrochloride (purity: >99.5%, Lot#: 207005GJ) was purchased from Qilu Pharmaceutical Co., Ltd. Zolpidem tartrate was obtained from Lu Nan Better Pharmaceutical Co., Ltd, (Shan Dong, China). Poloxamer 407 and Poloxamer 188 were purchased from Sigma-Aldrich Co., USA. Gellan gum (Kelcogel®, CG-LA) was gifted from CPKelco Co. China. Hydroxypropyl methylcellulose (METHOCEL™, HPMC, K100 LV) was produced by Colorcon Co., Shanghai. Ethyl acetate, formic acid, trimethylamine, ammonium formate, sodium hydroxide, sodium chloride and diethyl ether were products of Sigma-Aldrich Co., USA. Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate were purchased form Sinopharm Chemical Reagent Co., Ltd. Acetonitrile (HPLC grade) was manufactured by Merck Millipore. All solutions were prepared with deionized water.

3.2.2 Animals

Male Sprague-Dawley (SD) rats were provided by Laboratory Animal Services Center of The Chinese University of Hong Kong (Hong Kong, China). The weight of rats was 230-250 g. All the rats were fed with tap water and standard animal food daily. The animal experiments were approved by the Department of Health of Hong Kong and Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.

3.3 Methods

3.3.1 Development and validation of analytical method to determine granisetron in rat plasma

A HPLC-FLD method was developed to determine the granisetron in rat plasma. The method was validated with respect to specificity, linearity, sensitivity, accuracy, precision and stability [155].

3.3.1.1 Preparation of stock and working standard solutions, calibration standards and quality control (QC) samples

- Granisetron stock solution:

Granisetron hydrochloride was dissolved in purified water to obtain a stock solution with the concentration of 1.0 mg/ml (calculated on granisetron free base). The stock solution was stored in glass vial at -20 ^{o}C. The glass vial was covered with aluminum foil to protect the solution from light.

- Zolpidem tartrate stock solution:

Zolpidem tartrate was dissolved in purified water to obtain internal standard (IS) stock solution with the concentration of 1.0 mg/m. The solution was stored in glass vial at -20 ^{o}C and protected from light.

- Working Standard Solution:

Working standard solutions at specific concentrations were prepared by serial dilution of the stock solutions with purified water.

- Calibration standard solutions:

100 µL of blank plasma was spiked with 10 µL of each of working standard solutions and 10 µL of IS working solution (0.3 µg/ml). Then the sample was treated

following the procedure described in Section 3.3.1.2. The concentration range of the calibration standard solutions was 2-400 ng/ml. The calibration curve was obtained by plotting the peak area ratio of granisetron/IS versus the granisetron concentration in plasma.

- Quality control samples:

The drug solutions at low, intermediate and high concentrations were added into the blank plasma samples to prepare QC samples at the concentrations of 3.0 ng/ml, 120.8 ng/ml and 302.0 ng/ml respectively.

3.3.1.2 Plasma sample treatment

10 µL of IS working solution was added into 100 µL of plasma sample, followed by addition of 10 µL of 1 M sodium hydroxide solution and 1 ml of ethyl acetate. After mixing with vortex for 2 minutes, the mixture was centrifuged at 10,000 rpm for 5 minutes. The organic phase was transferred into centrifuge tubes, followed by evaporation at 37 ^{o}C with gentle nitrogen stream. The residue was reconstituted with 150 µL of ammonium formate solution (50 mM) containing 0.5% triethylamine (adjusted to pH4.0 by formic acid) : ACN, 4:1 (v/v). After centrifugation at 10,000 rpm for 5 min at 4 ^{o}C, an aliquot of 50 µL supernatant was analyzed by the HPLC-FLD system.

3.3.1.3 Instruments and HPLC methods

The HPLC/FLD system consists of a Shimadzu LC-20AD pump, a Shimadzu SIL-20A HT auto sampler and a Shimadzu SPD-M20A Photodiode Array detector. Data was collected by a Shimadzu LC Solution data system. Chromatographic separation was obtained by a Thermo BDS C18 Hypersil column (250 mm × 4.6 mm i.d., 5 µm particle

size) equipped with a guard column (Delta-Pak C18 Guard-Pak, Waters). The mobile phase consists of Phase A [5% acetonitrile + 95% aqueous solution, containing 0.5% triethylamine and 50 mM ammonium formate solution adjusted to pH 4.0 by formic acid] and Phase B [acetonitrile]. An isocratic elution was conducted at the flow rate of 1 ml/min (80% Phase A and 20% Phase B). Fluorescence detection was performed with an emission wavelength of 360 nm and an excitation wavelength of 305 nm [156]. All samples were analyzed at room temperature.

3.3.1.4 Specificity

For investigating if there is any endogenous substance in rat plasma interfering the peaks of granisetron and IS, the specificity of the method was conducted by analyzing six blank samples.

3.3.1.5 Sensitivity

Lower Limit of Quantitation (LLOQ) was obtained from drug concentration where the signal-to-noise ratio (S/N) of analyte peak is greater than 5. Five replicates of the samples at concentration of LLOQ were injected and the relative standard deviation (RSD) was calculated. The RSD of the five replicates should be not more than 20%, and the relative error (RE) should be not more than 20%.

3.3.1.6 Precision and accuracy

- Intra-day precision and accuracy

The intraday precision and accuracy of the method were assessed by analyzing five replicates of quality control samples at low (3.0 ng/ml), intermediate (120.8 ng/ml) and high (302.0 ng/ml) concentrations in one day. The relative standard deviation (RSD) of

the five replicates should be not more than 15%, and the relative error (RE) of the five replicates should be not more than 15%.

- Inter-day precision and accuracy

The inter-day precision and accuracy of the method were assessed by analyzing five replicates of quality control samples at low, intermediate and high concentrations in in three separate days. The relative standard deviation (RSD, %) of the five replicates should be not more than 15%, and the relative error (RE) of the five replicates should be not more than 15%.

3.3.1.7 Recovery

The recovery was obtained by comparing the peak area of quality control samples with that of the samples prepared by dissolving the reference standards in reconstituted solvent.

3.3.1.8 Stability

Quality control samples (low, intermediate and high) were used for stability test.

- Freeze-thaw stability test

The quality control samples with low, intermediate and high concentrations were exposed to three cycles of freeze (-80 ^{o}C) - thaw (room temperature) processes, and then analyzed by HPLC.

- Room temperature stability test

The quality control samples with low, intermediate and high concentrations were exposed to ambient temperature for three hours, and then analyzed by HPLC.

- Stability in automatic sampler

Quality control samples with low, intermediate and high concentrations were extracted and reconstituted, and then placed in the automatic sampler for 24 hours. The automatic sampler was set at ambient temperature.

3.3.2 Sample preparation for intranasal administration in rats

The granisetron formulations were prepared according to the Table 3.1.

Table 3.1 Granisetron formulations for intranasal administration

Formulation No.	Composition	Description
GNS-01	Granisetron hydrochloride 0.56% (w/v), equivalent to Granisetron 0.5% (w/v)	Colorless and clear solution.
NF-01	Granisetron hydrochloride 1.116% (w/v), equivalent to Granisetron 1% (w/v) Poloxamer 407 18% (w/v) Poloxamer 188 1% (w/v)	Colorless, clear and viscous solution at room temperature.
NF-02	Granisetron hydrochloride 1.116% (w/v), equivalent to Granisetron 1% (w/v) Gellan gum 0.5% (w/v)	Translucent and light opalescent solution.
NF-03	Granisetron hydrochloride 1.116% (w/v), equivalent to Granisetron 1% (w/v) HPMC 0.5% (w/v)	Colorless and clear solution

- *Granisetron solution (GNS-01):*

5 mg/ml of granisetron nasal solution was prepared by dissolving 11.2 mg granisetron hydrochloride in 2 ml normal saline (5.6 mg/ml granisetron hydrochloride, or equivalent to 5.0 mg/ml granisetron free base). The pH value of the solution was 6.89.

- *Thermosensitive in situ gel (NF-01):*

10 mg/ml of granisetron thermosensitive in situ gel was prepared by dissolving 112 mg of granisetron hydrochloride in 10 ml Poloxamer solution containing 18% Poloxamer 407 and 1% Poloxamer 188 (w/v). The Poloxamer solution was prepared by continuously stirring in an ice bath.

- *Ion-sensitive situ gel (NF-02):*

The 0.5% gellan gum (w/v) was dissolved in deionized water by heating to 95 ^{o}C with gently stirring. 112 mg of granisetron hydrochloride was added into 10 ml of gellan gum solution after cooling below 40 ^{o}C.

- *Bioadhesive solution (NF-03):*

The HPMC solution (K100 LV, 0.5%, w/v) was prepared by dispersing the HPMC in hot water (80-90 ^{o}C) and mixing. After cooling the HPMC solution to room temperature, 112 mg of granisetron hydrochloride was dissolved in 10 ml of 0.5% HPMC solution.

3.3.3 Pharmacokinetics of granisetron intranasal formulations in rats

3.3.3.1 Intranasal absorption of granisetron in conscious rats and anesthetized rats

10 rats were divided into two groups (5 rats in each group) to evaluate the influences of short-term anesthesia in intranasal absorption of granisetron. The rats were dosed with granisetron solution (GNS-01, 5 mg/ml) under conscious state and short-term anesthetized state respectively. The conscious rats were hand-restrained and placed in an upright position with the head in the vertical position. 20 µL of granisetron solution (GNS-01, 5 mg/ml, equivalent to 0.4 mg/kg) was administered by nasal instillation using a micropipette, with 10 µL of granisetron solution in each nostril [157]. For the anesthesia group, rats were placed in a covered cylinder jar containing a gauze

sponge saturated with diethyl ether (about 50 ml) prior to intranasal drug administration. Rats were retained in the jar for 3 minutes to achieve short-term anesthesia. Then the anesthetized rats were placed in a supine position for intranasal administration. 20 μL of granisetron solution was instilled into the nostrils by a micropipette, with 10 μL of solution for each nostril [158]. The dosing schedule is listed in Table 3.2. The systemic absorption of nasal granisetron in both groups was compared to evaluate the impact of ethyl ether induced short-term anesthesia on pharmacokinetics of the drug.

Table 3.2 The dosing schedule for preliminary pharmacokinetic study in rats

Group No. (Route)	Model	Animal No.	Test Article	Position when/after dosing	Dose Level (Granisetron Base)		
					Dose (mg/kg)	Dose Volume (ml/kg)	Concentration (mg/ml)
1 (IN)	Short-term anesthesia	5	GNS-01	Supine/normal	0.4	0.08	5
2 (IN)	Conscious	3	GNS-01	Upright/normal	0.4	0.08	5

3.3.3.2 Pharmacokinetics of granisetron nasal formulations

The short-term anesthetized rat model was applied to study the pharmacokinetics of granisetron nasal dosage forms. The rats were randomly assigned to three groups, with 6 rats in each group. The granisetron nasal dosage forms (NF-01, NF-02 and NF-03, refer to Table 3.1) were nasally administered to the short-term anesthetized rats induced by diethyl ether. 20 μL of nasal solutions (10 mg/ml of granisetron, equivalent to 0.8 mg/kg/dose) was administered by nasal instillation using a micropipette, with 10 μL for each nostril. The anesthetized rats were kept in a supine position during the nasal administration and allowed to access water after dosing.

3.3.4 Blood collection and processing

At 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes post dose, around 300 μL blood samples were taken from the tail vein and collected into heparinized tubes. Plasma samples were obtained by centrifugation at 10,000 rpm for 3 min and stored at -80 ^{o}C until analysis. Plasma concentration of granisetron was analyzed by a validated HPLC-FLD method.

3.3.5 Pharmacokinetic modeling and data analysis

Pharmacokinetic parameters, including C_{max}, t_{max}, $t_{1/2}$, $AUC_{0\text{-}last}$ and $AUC_{0\text{-}\infty}$, were generated using non-compartmental approaches by assistance of WinNonlin (version 4.0, Pharsight, Mountain View, CA). The maximum plasma concentration (C_{max}) and the time at which this occurred (t_{max}) were noted directly. The elimination rate constant (k_{el}) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated by use of the formula: $t_{1/2}=0.639/ k_{el}$. The area under the plasma concentration-time curve to the last measurable plasma concentration ($AUC_{0\text{-}last}$) was calculated by use of the linear trapezoidal rule.

SPSS software (version 17, SPSS Inc, Chicago, IL, USA) and One-way ANOVA with multiple comparisons of the means were used for the statistical analysis of the pharmacokinetic parameters. Probability level of $p < 0.05$ was adopted as the significant criteria.

3.4 Results

3.4.1 Validation of analytical method to determine granisetron in rat plasma

3.4.1.1 Selectivity, sensitivity and linearity

Under the current analytical conditions, granisetron and zolpidem (internal standard, IS) can be separated without interferences caused by endogenous substances in plasma (Figure 3.1). The retention time for granisetron and IS were 7.2 and 10.2 min, respectively. The LLOQ for granisetron was 1.0 ng/ml. The accuracy (%, RE) and precision (%, RSD) of granisetron at LLOQ were less than 14.0% and 3.5%, respectively. Good linearity ($r^2 > 0.995$) was achieved within the linearity range (2~400 ng/ml) (Table 3.3).

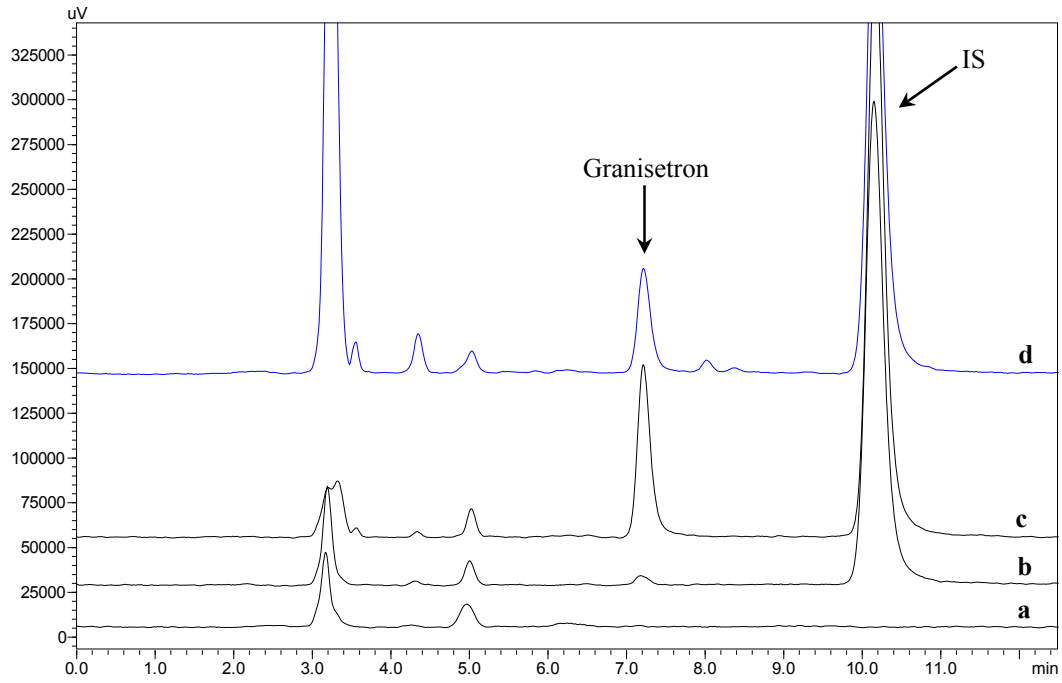


Figure 3.1 Representative chromatograms of: blank plasma (a), plasma spiked with granisetron at 1ng/ml (LLOQ) (b), plasma spiked with granisetron at 15 ng/ml (c), plasma sample at 30 min after intranasal administration of 0.4 mg/kg granisetron solution in rat (d)

IS: Internal standard (zolpidem tartrate)

Table 3.3 Method validation for granisetron quantification in rat plasma

Linear range (ng/ml)	r^2	Spiked Conc. (ng/ml)	Recovery (%, n = 5)	Intra-day (n = 5)			Inter-day (n = 5)		
				Determined (ng/ml)	Precision (% RSD)	Accuracy (% RE)	Determined (ng/ml)	Precision (% RSD)	Accuracy (% RE)
2-400	0.9974	1.0 (LLOQ)	100.3 ± 6.3	1.14 ± 0.04	3.5	14.0	1.10 ± 0.13	11.8	10.0
		3.0	94.2 ± 6.6	3.11 ± 0.14	4.5	3.7	2.99 ± 0.25	8.4	-0.3
		120.8	97.2 ± 5.0	117.3 ± 2.41	2.1	-2.9	118.1 ± 3.17	2.7	-2.2
		302.0	91.2 ± 4.9	305.2 ± 2.83	0.93	1.1	308.6 ± 3.46	1.1	2.2

Recovery of IS: 92.8 ± 3.8%.

3.4.1.2 Precision and accuracy

The accuracy, intra-day precision and inter-day precision for granisetron quantification in rat plasma are shown in Table 3.3. The %RSD of intra-day precision and inter-day precision of QC samples at different concentrations was below 8.4%. The relative error of accuracy was within the range from -2.9 to 3.7%.

3.4.1.3 Stability and recovery

After bench top storage for 3 hours, three freeze-thaw cycles, and storage in auto-sampler for 24 hours, the granisetron was still stable in plasma (See Table 3.4). The average extraction recovery of granisetron in rat plasma QC samples spiked with different granisetron concentrations was from 91.2 to 100.3% with RSD value less than 10%. The average recovery for IS was 92.8%, which is comparable to that by Jiang et al. using ethyl acetate (97.9%) [159], and is higher than the method using methyl t-butyl ether (75.3%) [160] and diethyl-ether / dichloromethane mixture (62.5%) [161]. The results suggested that the liquid-liquid extraction method by ethyl acetate is effective and reproducible.

Table 3.4 Stability of granisetron hydrochloride in rat plasma

Spiked level (ng/ml)	Determined concentration (n = 4)		
	Bench top 3 h [a] (ng/ml)	Freeze-thaw three cycles [b] (ng/ml)	Autosampler 24 h [c] (ng/ml)
3.0	2.94 ± 0.33	3.12 ± 0.38	2.84 ± 0.08
120.8	122.8 ± 2.7	121.6 ± 3.1	118.6 ± 3.8
302.0	299.5 ± 3.8	297.8 ± 2.5	296.1 ± 4.2

[a] bench at ambient temperature; [b] Freeze-thaw at -80 ^{o}C and room temperature;
[c] autosampler at ambient temperature.

3.4.2 Pharmacokinetics of granisetron after intranasal administration in conscious rats and short-term anesthetized rats

Figure 3.2 presents the plasma concentration profiles of granisetron after intranasal administration of granisetron solution (GNS-01) at a single dose of 0.4 mg/kg in both conscious rats and anesthetized rats. The main pharmacokinetic parameters are listed in Table 3.5.

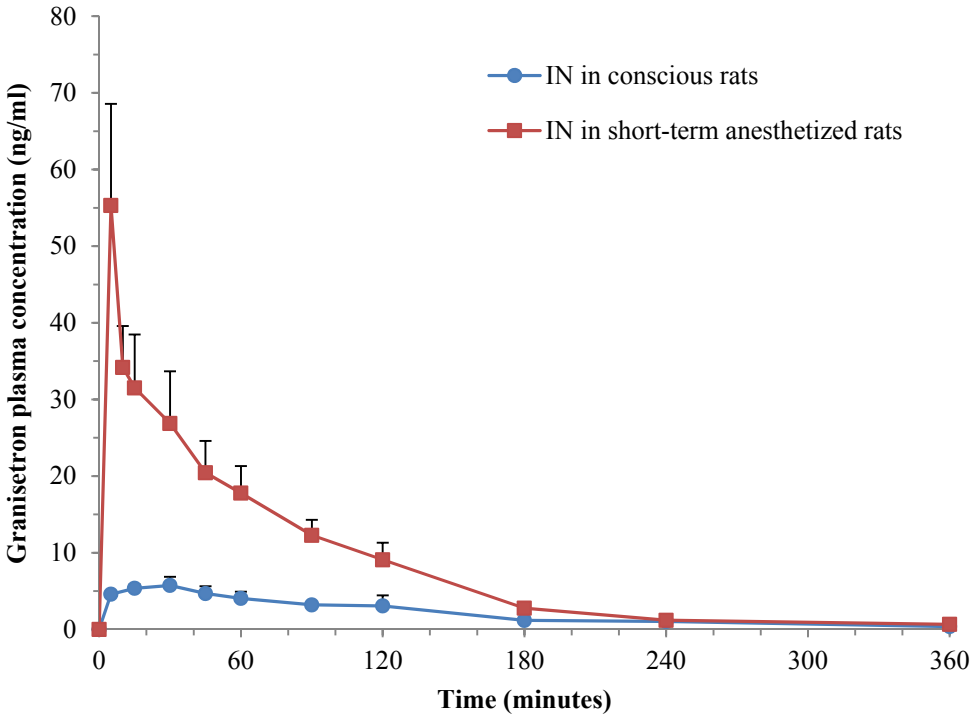


Figure 3.2 Plasma concentration versus time profiles of granisetron in conscious rats (n=3, closed circle, ●) and ethyl ether-induced short-term anesthetized rats (n=5, closed square, ■) after intranasal administration of granisetron solution (GNS-01) at a single dose of 0.4 mg/kg (each point represents mean ± s.d.)

Table 3.5 Pharmacokinetic parameters of granisetron after intranasal administration of granisetron solutions (GNS-01) in conscious rats (n=3) and short-term anesthetized rats (n=5) at a single dose of 0.4 mg/kg

PK parameters	Nasal administration (GNS-01)	
	Conscious rats	Anesthetized rats
t_{max} (min)	25.0±8.7 *	5.0±0.0
C_{max} (ng/ml)	5.9±0.9 *	55.3±13.3
AUC_{0-6h} (ng·min/ml)	770.9±189.8 *	3002.8±553.7
$AUC_{0-\infty}$ (ng·min/ml)	831.0±176.9 *	3061.0±593.4
$t_{1/2}$ (min)	111.9±39.3 *	53.0±11.7

*: $p < 0.05$.

Compared with conscious rats, the anesthetized rats had higher exposure of granisetron after nasal administration at a single dose of 0.4 mg/kg. The mean AUC_{0-6h} of anesthetized rat group was approximate 2.9 times higher than that of the conscious rat group. The C_{max} of granisetron in conscious rat group was 5.9 ng/ml, and achieved in 0.5 hour. Meanwhile, the C_{max} of granisetron in anesthetized rat group was 55.3 ng/ml and achieved within 5 minutes, and then declined quickly with a mean half-life of 60 minutes. In comparison with the pharmacokinetic behavior of granisetron in the conscious rat group, the short-term anesthetized rats had a more rapid and complete absorption after intranasal administration of granisetron solution.

3.4.3 Pharmacokinetics of granisetron nasal formulations

The short-term anesthetized rats were nasally dosed with selected formulations (NF-01, NF-02 and NF-03, in Table 3.1) at the dose of 0.8 mg/kg. The plasma concentration versus time profiles of granisetron (GNT) after intranasal administration is shown in Figure 3.3. The pharmacokinetic parameters are listed in Table 3.6.

All formulations showed rapid absorption after intranasal administration, with the t_{max} from 5 min to 11.7 min. In all tested formulations, the HPMC-based bioadhesive solution (NF-03) achieved remarkably higher maximum plasma concentration (C_{max}) than in situ gel formulations NF-01 and NF-02 ($p<0.05$). In comparison with NF-01 and NF-02, NF-03 had a significantly higher $AUC_{0\text{-}inf}$ (about 4-fold higher), indicating that HPMC is a superior vehicle for intranasal absorption than in situ gels. No significant difference was found in $AUC_{0\text{-}inf}$ and elimination half-life ($t_{1/2}$) between two in situ gels ($p>0.05$). Granisetron in situ gels showed comparable pharmacokinetic profiles irrespective of the different gelling mechanisms.

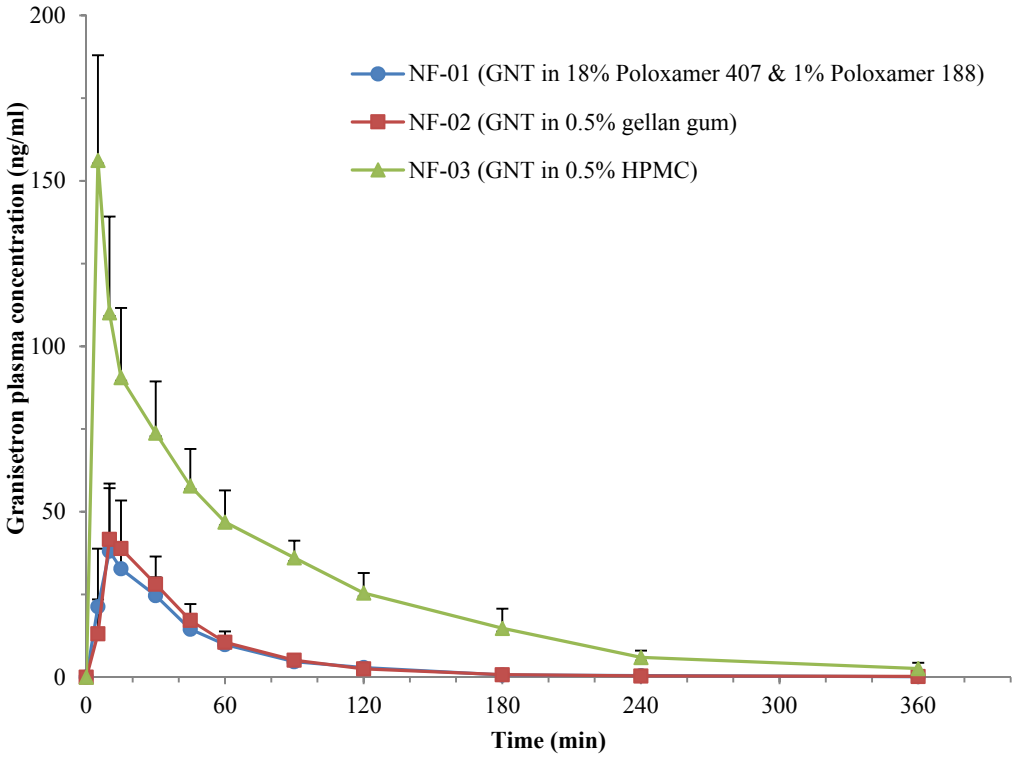


Figure 3.3 Plasma concentration versus time profiles of granisetron in rats after intranasal administration of granisetron formulations NF-01 (circle), NF-02 (square) and NF-03 (triangle) at a single dose of 0.8 mg/kg (n=6)

Table 3.6 Pharmacokinetic parameters of granisetron in rats following intranasal administration of granisetron formulations (NF-01, NF-02 and NF-03) at the single dose of 0.8 mg/kg

PK Parameter	Statistics	Formulation		
		NF-01 (n=6)	**NF-02 (n=6)**	**NF-03 (n=6)**
t_{max} (min)	Mean (SD)	10.8 (3.8)	11. 7 (2.6)	5 (0)
C_{max} (ng/ml)	Mean (SD)	44.6 (17.7)	43. 7 (14.1)	156.2 (31.8)
AUC_{0-t} (ng·min/ml)	Mean (SD)	1794.1 (288.8)	1939.0 (562.9)	9046.5 (1750.3)
AUC_{0-inf} (ng·min/ml)	Mean (SD)	1803.1 (290.7)	1953.1 (563.0)	9317.2 (1841.0)
$t_{1/2}$ (min)	Mean (SD)	43.1 (3.8)	46.6 (4.5)	65.3 (12.1)

3.5 Discussion

The anesthetized rats had higher exposure of granisetron after nasal administration in comparison with conscious rats. Technically, it is difficult to intranasally administer drug solution to the conscious rats, since the sneeze or rhinorrhea often occurs during nasal dosing procedure.[162]. The sneeze could significantly impact the nasal dosing accuracy and reproducibility, and some rats cannot receive the full dose intranasally. In addition, animals were always moving during the intranasal dosing process, so it was difficult to insert the tip of the micropipette deeply into the naris of the conscious rats. Meanwhile, the administered solution was mostly distributed in anterior part of the nostrils, where the drug might be easily eliminated by mucociliary clearance before absorption.

Granisetron is extensively metabolized by the cytochrome P450 (CYP450) monooxygenase system. The activities of various CYP450 enzymes in liver, such as CYP1A and CYP2B, could be partially inhibited (up to 65%) by ethyl ether used in short-term anesthesia [163]. The inhibition effect induced by ethyl ether is reversible, so the activities of the enzymes could recover to normal level in a short time after the elimination of ethyl ether [164] [165]. The recovery time of the enzymatic activities is positively related to the exposure duration time to ethyl ether. In our study, the rats were retained in a covered glass cylinder jar full of ethyl ether gas for 3 minutes to achieve short-term anesthesia. All those rats could recover from anesthesia within 6 minutes. On the other hand, hypothermia caused by anesthesia may further influence the activities of the enzymes. In general, the activities of most enzymes decrease at low body temperature. The in vitro activity of CYP3A4 was reported to decrease to 69% at 32 ^{o}C, indicating a strong temperature dependence of enzymatic activities [166]. CYP1A1 and

CYP3A4 are involved in metabolism of granisetron by N-demethylation and aromatic ring oxidation, followed by conjugation. In the hypothermic animals, the reduced functions or activities in multiple organs were found, such as the function of kidney and liver, systemic circulation and metabolism [167] [168]. Therefore, the anesthesia procedure should be carefully controlled in future studies.

The pharmacokinetic study in rats suggests that the higher exposure of granisetron after nasal administration of HPMC-based solution as compared to in situ gels. The delayed drug absorption after nasal administration of in situ gels (thermosensitive and ion-activated in situ gels) may be attributed to the slow drug release from the viscous gel matrix. The Poloxamer-based in situ gel showed low $AUC_{0\text{-}inf}$ in comparison with the HPMC-based solution, in spite of the longer mucociliary transport time (refer to Chapter 2). The low $AUC_{0\text{-}inf}$ of in situ gels reflected the slow release rate of the drug from gel matrix, which was also confirmed by in vitro release tests (refer to Chapter 2). Drug in the in situ gels cannot be completely released before the clearance by the mucociliary movement. Finally, HPMC-based formulation was rapidly and completely absorbed after nasal administration in rats without the release issue and was selected for further studies.

3.6 Conclusion

In this chapter, a sensitive, accurate and reproducible HPLC-FLD method was developed and validated for quantifying granisetron in rat plasma. The short-term anesthetized rats were used to evaluate nasal absorption of different granisetron formulations. Compared to the granisetron thermosensitive gel and ion-sensitive gel, the granisetron HPMC-based bioadhesive formulation (0.5% HPMC, w/v) achieved more rapid and complete absorption and was applied for further studies.

Chapter 4

Systemic pharmacokinetics and brain pharmacokinetics of granisetron bioadhesive solution in rats

4.1 Introduction

Up to date, cancer is still a main public health problem worldwide [169]. Although comprehensive treatments for antineoplastic therapy have been developed considerably in decades, the chemotherapy is still one of the primary anticancer treatments. The side effects of chemotherapy are usually associated with severe nausea and vomiting. In this case, the cancer patients treated with chemotherapy not only have to suffer the pain from the disease, but also are plagued by the intolerable nausea and vomiting induced by the cytotoxic antineoplastic agents. Furthermore, the severe nausea and vomiting could even impede or interrupt the therapeutic schedule and negatively impact the effects of chemotherapy [170] [171].

Granisetron ($C_{18}H_{24}N_4O$, M.W. 312.409) is a 5-HT3 receptor antagonist and used as an antiemetic agent for CINV by selectively and competitively binding to the 5-HT3 receptor to inhibit the afferent stimulation of the vomiting center. In our previous studies, granisetron was developed into bioadhesive solution and delivered via intranasal route. The bioadhesive polymer, hydroxypropyl methylcellulose (HPMC), was selected as the main vehicle for further development due to the rapid and complete intranasal absorption of granisetron in rat pharmacokinetic studies. HPMC is a synthesized, inert and viscoelastic polymer and widely used in oral, nasal, ophthalmic and topical pharmaceutical preparations [172] [173] [174] [175]. The bioadhesive property enables the application of HPMC in various transmucosal drug delivery systems, such

as nasal, pulmonary, rectal and buccal delivery, to enhance the systemic absorption of drugs with poor or erratic bioavailability [176] [177] [178].

In recent decades, intranasal delivery has been advocated as one of the promising drug administration routes for the patients with compromised swallowing capacity. Intranasal administration is a non-invasive route for both local and systemic drug delivery. The nasal mucosa is highly vascularized and highly permeable with sufficient blood supply from the ophthalmic, maxillary and facial arteries, which contributes to the rapid and complete absorption of drugs via intranasal route. In addition, the direct pathway from nose to brain is another interesting advantage of nasal drug delivery. There are two proposed main pathways to brain after intranasal administration: olfactory and trigeminal pathways. Generally, drugs enter into the brain through a combination of these pathways, rather than merely relying on only one predominant pathway.

In our previous studies, the bioadhesion of HPMC formulation was confirmed by comparing the in vivo mucociliary transport time (MTT) between HPMC-based solution and normal saline (negative control). As compared to the formulations based on Poloxamers and gellan gum, the HMPC-based granisetron nasal formulation can achieve faster absorption as well as higher systemic exposure of granisetron. In this chapter, the effects of HPMC concentration and the granisetron dose on the pharmacokinetics of HPMC-based formulations will be further investigated. The pharmacokinetics of granisetron after oral, intravenous, and intranasal administrations will be studied as well. In addition, the brain PK of granisetron after intranasal administration will be studied and compared with that after intravenous administration.

4.2 Materials, animals and reference products

4.2.1 Materials

Granisetron hydrochloride (purity: > 99.5%, Lot#: 207005GJ) was purchased from Qilu Pharmaceutical Co., Ltd. Kytril® (Granisetron tablet, 1 mg/tablet) and Kytril® (Granisetron intravenous infusion, 3 mg/3 ml) were manufactured by F. Hoffmann-La Roche Ltd Basel, Switzerl. Zolpidem tartrate was obtained from Lu Nan Better Pharmaceutical Co., Ltd, (Shan Dong, China). Tolbutamide was purchased from Sigma. Hydroxypropyl methylcellulose (METHOCEL™, HPMC, K100 LV) was obtained from Colorcon Co., Shanghai. Carboxymethylcellulose sodium (CMC-Na) was purchased from Sigma-Aldrich Corporation. Ethyl acetate, formic acid, trimethylamine, sodium chloride, sodium hydroxide, EDTA and ammonium formate were products of Sigma-Aldrich Co., USA. Acetonitrile (HPLC grade) was manufactured by Merck Millipore (Germany). All other reagents were analytical grade and used without further purification. Deionized water was produced in-house and used for the preparation of all solutions.

4.2.2 Animals

Adult male Sprague-Dawley (SD) rats were supplied by Laboratory Animal Services Center of The Chinese University of Hong Kong. The weight of rats was 230−250 g. The animal study was approved by the Department of Health of Hong Kong and Animal Experimentation Ethics Committee at the Chinese University of Hong Kong.

4.2.3 Reference products

I. Kytril® Granisetron tablets (1 mg)

Composition	Each film-coated tablet contains 1 mg granisetron present as the hydrochloride. Inactive ingredients: hydroxypropyl methylcellulose, lactose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide
Description	Film-coated tablet
Manufacturer	F. Hoffmann-La Roche Ltd Basel, Switzerland
Storage condition	Store between 15°C and 30°C

II. Kytril® Granisetron (1 mg/ml) for IV injection

Composition	Each 1 ml contains 1.12 mg granisetron hydrochloride equivalent to granisetron, 1 mg; citric acid, 2 mg; sodium chloride, 9 mg; and benzyl alcohol, 10 mg.
Description	Colorless and clear solution
Manufacturer	F. Hoffmann-La Roche Ltd Basel, Switzerland
Storage condition	Store at 25°C, excursions permitted to 15°C to 30°C

4.3 Methods

4.3.1 Preparation of test articles

The formulations of granisetron hydrochloride solutions for intranasal administration were listed in Table 4.1. The products were prepared by the methods described below.

Table 4.1 Granisetron hydrochloride solution (GNS) formulations for intranasal administration

Product Code	Composition
GNS-S01	Granisetron hydrochloride 1.117% (*w/v*), equivalent to granisetron free base 10 mg/ml, and normal saline
GNS-B01-1	Granisetron hydrochloride 1.117% (*w/v*), equivalent to granisetron free base 10 mg/ml, and 0.125% HPMC
GNS-B01-2	Granisetron hydrochloride 1.117% (*w/v*), equivalent to granisetron free base 10 mg/ml, and 0.25% HPMC
GNS-B01-3	Granisetron hydrochloride 1.117% (*w/v*), equivalent to granisetron free base 10 mg/ml, and 0.5% HPMC
GNS-B01-4	Granisetron hydrochloride 0.559% (*w/v*), equivalent to granisetron free base 5 mg/ml, and 0.25% HPMC
GNS-B01-5	Granisetron hydrochloride 2.234% (*w/v*), equivalent to granisetron free base 20 mg/ml, and 0.25% HPMC

- Granisetron hydrochloride Nasal Solution

111.7 mg of granisetron hydrochloride was accurately weighed and dissolved in 10 ml of normal saline with stirring at room temperature. The content of granisetron (free base) in the solution was 10 mg/ml. The solution was placed at 4ºC before use.

- Granisetron hydrochloride nasal bioadhesive solution

The HPMC (K100 LV) was added into hot water (80 ºC-90 ºC) and dispersed with agitation. Then the solution was cooled to room temperature gradually with continuously stirring. Granisetron hydrochloride was dissolved in the HPMC solution by stirring at room temperature. The final solution was stored at 4 ºC before use.

- Granisetron hydrochloride oral suspension solution

The granisetron oral suspension solution was prepared by dissolving two granisetron tablet (Kytril®, strength: 1 mg/tablet) into 10 ml of 0.5% CMC-Na solution. The suspension solution was stored at 4ºC and mixed for 30 seconds with a vortex

before use.

4.3.2 Systemic pharmacokinetic studies of granisetron in rats after intranasal, intravenous and oral administrations

4.3.2.1 Design of PK studies

Male SD rats were randomly assigned into eight dosing groups (6 intranasal groups, 1 intravenous group and 1 oral group) with 5-7 rats in each group. Prior to intranasal drug administration, rats were anesthetized by being placed in a covered glass cylinder jar containing medical cotton saturated with diethyl ether for around 3 minutes. Anesthesia was achieved when rats lost righting reflex within two minutes. According to our observations, the short-term anesthetized rats could recover within 6 minutes after being removed from the glass cylinder jar. After anesthesia, drug administrations were conducted according to Table 4.2.

Table 4.2 Group assignment and dosing design

Group No. (Route)	Number of animals	Test Article	Dose level (Granisetron free base)*		
			Dose (mg/kg)	Dose Volume (ml/kg)	Concentration (mg/ml)
1 (IN)	6	GNS-S01	0.8	0.08	10
2 (IN)	6	GNS-B01-1	0.8	0.08	10
3 (IN)	6	GNS-B01-2	0.8	0.08	10
4 (IN)	6	GNS-B01-3	0.8	0.08	10
5 (IN)	6	GNS-B01-4	0.4	0.08	5
6 (IN)	6	GNS-B01-5	1.6	0.08	20
7 (PO)	7	Kytril® Tablet (1 mg/tablet)	0.8	4	0.2
8 (IV)	5	Kytril® IV injection	0.8	0.8	1

*Doses are calculated basing on a mean weight/rat of ~250 g.

For intravenous administration (IV), 0.2 ml of Kytril® IV injection (1 mg/ml) was injected to short-term anesthetized rats via tail veins. For intranasal administration (IN), 20 μL of drug nasal formulation (10 μL for each nostril) was administered to short-term anesthetized rats (5 mm depth into the nostril) by the aid of a micropipette. Rats were intranasally administered with a supine position. For oral administration, 1 ml of 0.2 mg/ml granisetron oral suspension solution, which was prepared by dissolving two Kytril® tablets (1 mg/tablet) into 10 ml of 0.5% CMC-Na solution, was given to each short-term anesthetized rat by gavage.

4.3.2.2 Blood collection and processing

At pre-dose (0 minute) and at 2, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes post-dose, 300 μL blood samples were taken from the tail vein and collected into heparinized tubes. Plasma samples were obtained by centrifugation at 10,000 rpm for 5 minutes and stored at -80 ^{o}C until analysis.

10 μL of internal standard (IS) working solution (zolpidem solution, 0.3μg/ml) was added to 100 μL of plasma sample. After being basified with 10 μL of 1 M sodium hydroxide solution, the plasma sample-IS mixture was extracted with 1 ml ethyl acetate for 2 minutes by vortex mixing. Then the mixture was centrifuged at 10,000 rpm for 5 min. The organic solvent was transferred to centrifuge tubes by a micropipette, followed by evaporation under a gentle stream of nitrogen at 37 ^{o}C. The residue was reconstituted with 150 μL of ammonium formate solution (50 mM) containing 0.5% triethylamine (adjusted to pH4.0 by formic acid): ACN, 4:1 (*v*/*v*). After centrifugation at 10,000 rpm for 5 min, an aliquot of 50 μL of supernatant was analyzed by HPLC-FLD system.

4.3.2.3 Sample analysis

Granisetron concentration in plasma was analyzed by the validated HPLC-FLD method (refer to Table 4.3).

Table 4.3 Instrument operating parameters

Instrument	Shimadzu LC-20AD pump, Shimadzu SIL-20A HT auto sampler Shimadzu SPD-M20A Photodiode Array detector.
Column	Thermo BDS C18 Hypersil column (250 mm × 4.6 mm i.d., 5 μm particle size), protected by a guard column (Delta-Pak C18 Guard-Pak, Waters)
Excitation wavelength / Emission wavelength	305 nm / 360 nm
Column Temperature	Ambient temperature
Sample temperature	Ambient temperature
Injection volume	20 μL
Mobile phase	Eluent A: 5% acetonitrile + 95% aqueous solution, containing 0.5% triethylamine and 50 mM ammonium formate solution adjusted to pH 4.0 by formic acid Eluent B: Acetonitrile Eluent A : Eluent B = 80 : 20
Flow rate	1 ml/min
Run time	12.5 min

4.3.3 Brain pharmacokinetics of granisetron after intravenous and intranasal administration

4.3.3.1 Study design for brain pharmacokinetics

36 male SD rats were randomly assigned to an intranasal (IN) group and an intravenous (IV) group, with 18 rats in each group. All the treatments were conducted on conscious rats. For the intranasal group, 20 μL of 10 mg/ml granisetron bioadhesive solution (10 μL for each nostril) was administered to rats by the aid of a micropipette (5 mm depth into the nostril). For the rats treated with intravenous administration, 0.2 ml of granisetron injection (1 mg/ml) was injected via tail veins. After drug administrations, the rats were returned to cages and allowed to drink water *ad libitum*. The group assignment and dose schedule are listed in Table 4.4.

Table 4.4 Group assignment and dose schedule for brain targeting study

Group No. (Route)	Number of animals	Test Article	Dose level (Granisetron free base)*		
			Dose (mg/kg)	Dose Volume (ml/kg)	Concentration (mg/ml)
1 (IN)	18	GNS-B01-2	0.8	0.08	10
2 (IV)	18	Kytril® IV Injection	0.8	0.8	1

*Doses are calculated basing on a mean weight/rat of ~250 g.

4.3.3.2 Sample collection (plasma and brain tissue)

At predetermined time intervals (5, 15, 30, 60, 120 and 240 minutes) following drug administration, three rats were executed by cervical dislocation. After the execution, blood sample was taken from the trunk into the heparinized tubes at different time intervals. The plasma was separated by centrifugation at 10,000 rpm for 5 minutes

(4 ºC). The plasma samples were kept at -80 ºC for bioanalysis.

At each time point, the whole brain was collected right after the execution, and then immediately rinsed with ice cooling normal saline. The residual water on the surface was absorbed by tissue gently. The brain was accurately weighed before homogenization. The brain tissues were kept at -80 ºC for analysis.

4.3.3.3 Blood sample processing

A 30μL aliquot of rat plasma was mixed with 30 μL IS solution (100 ng/ml of tolbutamide), 30 μL of 50% ACN solution and 120 μL of ACN. The mixture was mixed by a vortex mixer for 2 minutes, and then centrifuged at 10,000 rpm for 5 min at 4 ºC. 150 μL of supernatant was withdrawn. An aliquot of 10 μL of supernatant was injected into the LC-MS/MS system for analysis.

4.3.3.4 Brain sample processing

At each time point, the brain tissues of three rats were collected and accurately weighed, then homogenized respectively in 50% acetonitrile solution (ACN/water, 50/50, *v*/*v*) at the ratio of 1:5 (tissue weight:50% ACN, *w*/*v*) with a Microson XL-2000 ultrasonic cell disruptor (Misonix, USA) for 30 seconds. The brain tissues were kept in ice bath during the homogenization process. After homogenization, the tissue-solvent mixtures were centrifuged at 10,000 rpm for 5 minutes at 4 ºC. Then the supernatant was collected for analysis.

4.3.3.5 Sample analysis

Granisetron concentration in the biological samples (plasma and brain tissue) was analyzed by a validated LC-MS/MS method with respect to selectivity, standard curves,

lower limit of quantitation (LLOQ), precision, accuracy, recovery, matrix effects and freeze-thaw stability.

- Analytical method

LC conditions are shown as below:

Mobile phase A: 0.1% Formic acid in water

Mobile phase B: 0.1% Formic acid in ACN

Chromatography Column: Phenomenex, Synergi 4μ Hydro-RP 80Å, 30×4.6mm

Column temperature: Room temperature

The gradient elution program is listed in Table 4.5.

Table 4.5 Program of gradient elution

Time (min)	Flow rate (ml/min)	Phase A (%)	Phase B (%)
0.01	0.6	95	5
0.50	0.6	95	5
1.50	0.6	5	95
1.80	0.6	5	95
1.81	0.6	95	5
2.50	0.6	Stop	Stop

- MS conditions

The mass spectrometer was used to detect and quantify the granisetron in blood and brain samples. The MS conditions and ion pair parameters of drugs are listed as below.

Table 4.6 The MS conditions for bioanalysis

Ion source	Electrospray Ionization (ESI)	**Ion source temperature (TEM)**	600 ºC
Scan patterns	Positive	**Interface heater (ihe)**	ON
Curtain gas (CUR)	30 psi	**Collision gas (CAD)**	10 psi
Ion source gas 1 (GS1)	60 psi	**Entrance potential (EP)**	10 V
Ion source gas 2 (GS2)	60 psi	**Collision cell exit potential (CXP)**	14 V
Ionspray voltage (IS)	5500 V		

Table 4.7 The ion pair parameters

Compound	Ion Pair		Declustering Potential (DP) (V)	Collision Energy (CE) (eV)
	Parent Ion (m/z)	Daughter Ion (m/z)		
Granisetron	313.3	138.1	80	31
Tolbutamide	271.3	155.0	45	26

4.3.4 Data analysis

Plasma concentration versus time data was analyzed using non-compartmental model by the WinNonlin software program (version 4.0, Pharsight, Mountain View, CA). The maximum plasma concentration (C_{max}) and the time when the maximum plasma concentration occurred (t_{max}) were determined directly from the plasma concentration versus time profiles. The $AUC_{0\text{-}last}$ and $AUC_{0\text{-}\infty}$ were calculated by the trapezoidal rule without or with extrapolation to time infinity ($AUC_{0\text{-}\infty} = AUC_{0\text{-}last} + C_{last}/ \lambda_z$), respectively. The apparent terminal elimination half-life ($t_{1/2}$) was calculated according to the following formula, where λ_z was the first-order rate constant pertinent

to the terminal (log-linear) phase of the curve: $t_{1/2} = \ln 2/ \lambda_z$. The selection criteria for inclusion of data points in the calculation of λ_z required that at least three data points representing the terminal phase were regressed and that $r^2 \geq 0.85$ when rounded. Half-life ($t_{1/2}$) was defined as not determined (ND) if these criteria were not met.

Two parameters were used to evaluate the brain targeting effect after intranasal administration of granisetron [179] [180].

Brain targeting factor (BTF, %), indicating time average partitioning ratio, is calculated as:

$$BTF\ \% = \{[(AUC_{0\text{-}t\ (brain)}/AUC_{0\text{-}t\ (blood)})_{IN}]/[(AUC_{0\text{-}t\ (brain)}/AUC_{0\text{-}t\ (blood)})_{IV}]\} \times 100\%$$

Nose-to-brain direct transport percentage (DTP, %) is calculated as below:

$$DTP\ \% = [(B_{IN} - B_X)/B_{IN}] \times 100\%$$

Where B_{IN} is $AUC_{0\text{-}t\ (brain)}$ after intranasal administration, B_X is the brain AUC proportion contributed by penetrating the blood-brain barrier (BBB) through systemic circulation following intranasal administration, which is calculated as follows:

$$B_X = \{[AUC_{0\text{-}t\ (brain)}]_{IV}/[AUC_{0\text{-}t\ (blood)}]_{IV}\} \times [AUC_{0\text{-}t\ (blood)}]_{IN}$$

Statistical analyses were performed using SPSS (version 17, SPSS Inc, Chicago, IL, USA). One-way ANOVA with multiple comparisons of the means was applied for comparing pharmacokinetic data. A probability level of $p < 0.05$ was set as the criterion of significance.

4.4 Results

4.4.1 Pharmacokinetics following intravenous, oral and intranasal administration

The male SD rats were randomly assigned to three groups according to Table 4.2. Granisetron was given through IN, IV and PO routes at a single dose of 0.8 mg/kg.

The drug plasma concentration versus time curves after oral, intranasal and intravenous administrations are shown as Figure 4.1. For intravenous administration of granisetron injection (Kytril® IV injection), drug plasma concentration rapidly declined in the first 10 minutes, followed by an elimination phase with a half-life ($t_{1/2}$) of 52.4 minutes. After intranasal administration of granisetron nasal solution (GNS-S01), the maximum drug plasma concentration of 99.3 ng/ml was achieved within 5 minutes. Then the plasma concentration gradually declined with a half-life ($t_{1/2}$) of 55.7 minutes. After oral administration of a single dose of 0.8 mg/kg, the maximum drug plasma concentration was 3.3 ng/ml, with a prolonged t_{max} of 27.9 minutes. The absolute bioavailability of granisetron after oral administration was remarkably lower than that through nasal route.

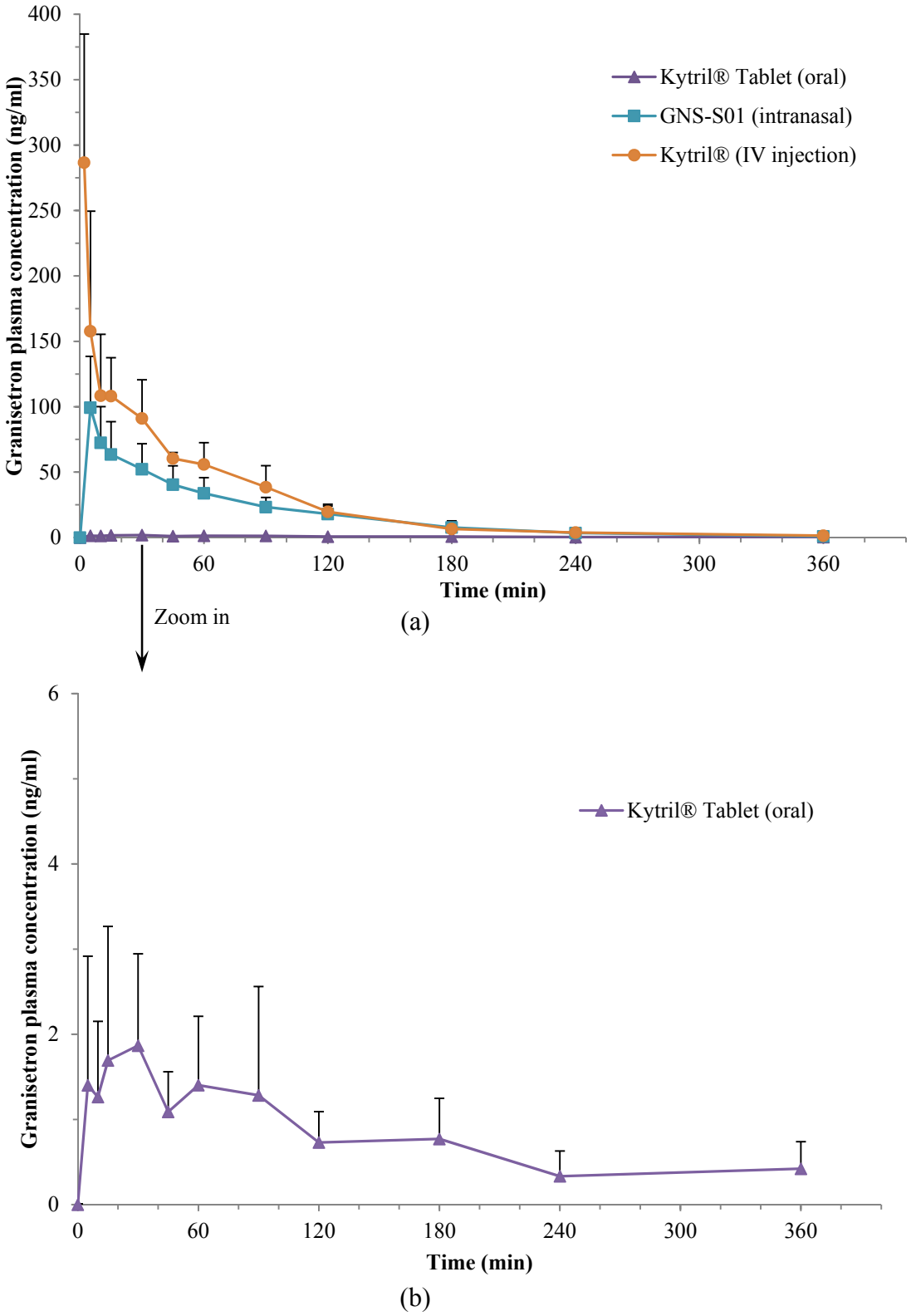


Figure 4.1 Plasma concentration-time curves of granisetron in SD rats following intravenous, intranasal and oral administration of granisetron formulations at a single dose of 0.8 mg/kg (n_{oral}=7, $n_{intranasal}$=6 and $n_{intravenous}$=5)

4.4.2 The effect of HPMC on intranasal bioavailability of granisetron

The granisetron nasal bioadhesive formulations containing various contents of HPMC (0%, 0.125%, 0.25% and 0.5%) were administered to short-term anesthetized rats (n=6) at the single dose of 0.8 mg/kg. The profiles of plasma concentration versus time after nasal administration of various formulations are listed in Figure 4.2. Rapid absorption of granisetron were found in all formulations, with t_{max} ranging from 5 minutes to 5.8 minutes, and the mean terminal half-lives ranging from 55.7 minutes to 76.3 minutes in all the test groups. As shown in Figure 4.3, when HPMC concentration increased from 0% to 0.25%, the mean C_{max} increased from 99.3 ± 39.1 ng/ml to 173.6 ± 14.2 ng/ml, and the mean $AUC_{0-\infty}$ increased from 6065.7 ± 2147.9 min·ng/ml to 10898.4 ± 1190.7 min·ng/ml. However, both the mean C_{max} and $AUC_{0-\infty}$ declined slightly when the HPMC concentration further increased from 0.25% to 0.5%.

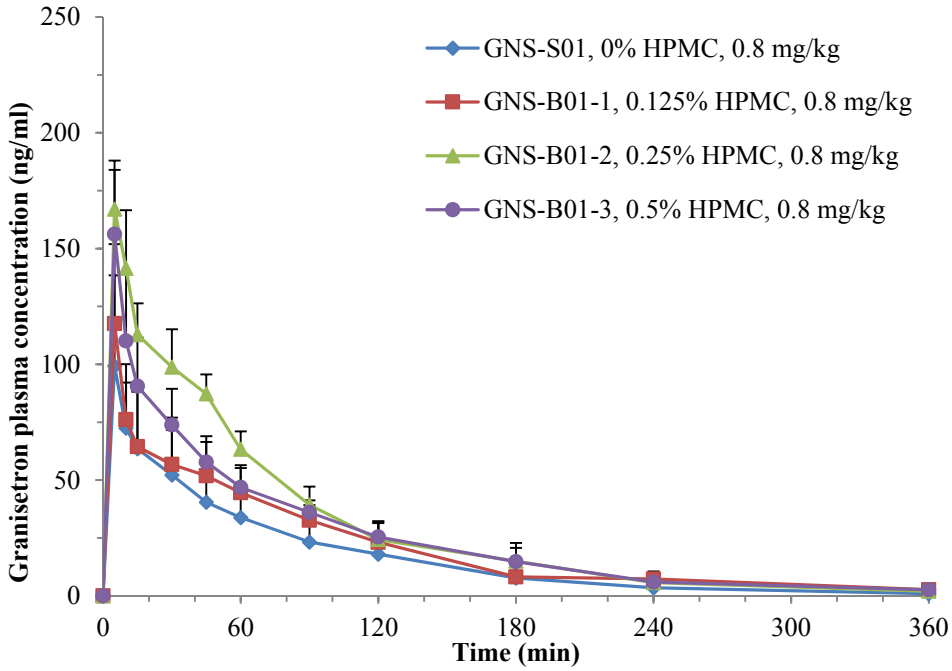
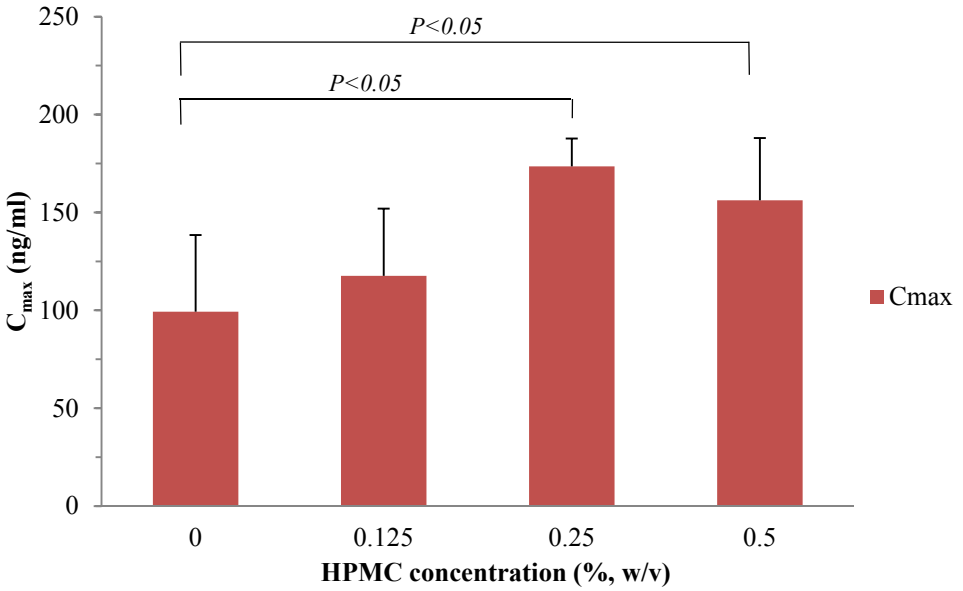
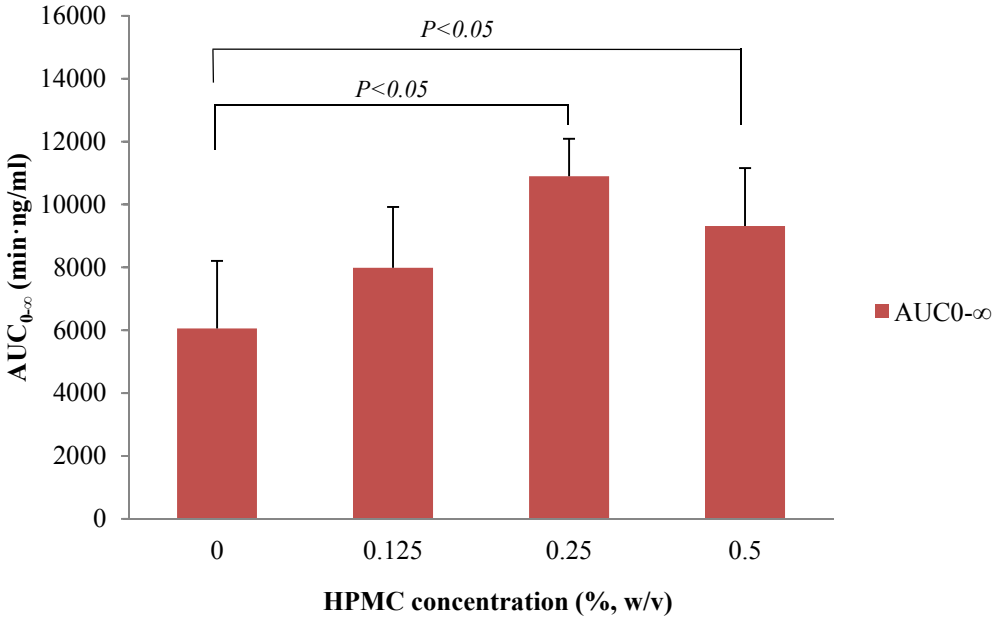


Figure 4.2 Plasma concentration-time curves of granisetron in SD rats following intranasal administration of granisetron nasal bioadhesive formulations containing various concentration of HPMC at a single dose of 0.8 mg/kg (n=6)



(a)



(b)

Figure 4.3 (a) Mean maximum plasma concentration (C_{max}) and (b) area under the curve ($AUC_{0-\infty}$) after intranasal administration of granisetron formulations with various concentration of HPMC (Mean ± SD, n=6)

4.4.3 Dose linearity after intranasal administration of granisetron formulations

A progressive increase in granisetron doses was made to investigate if the dosage can influence the pharmacokinetics of granisetron in SD rats. Rats were randomly assigned to three groups (6 rats in each group) and received single intranasal administration of bioadhesive formulations with the stepwise increased doses of 0.4 mg/kg, 0.8 mg/kg and 1.6 mg/kg, respectively. The granisetron bioadhesive formulations were administered to short-term anesthetized SD rats by a micropipette, and the blood samples were collected and processed as Section 4.3.2.2. The drug plasma concentration-time curves are shown in Figure 4.4. After intranasal administration, the granisetron was absorbed rapidly with the mean t_{max} ranging from 5.0 minutes to 8.3 minutes in all three groups. The maximum plasma concentrations (C_{max}) increased with the dose escalation. After reaching the C_{max}, the drug plasma concentration gradually declined with similar elimination rate in the following 6 hours. The mean terminal half-life ($t_{1/2}$) of granisetron was independent of the doses after intranasal administration (refer to Table 4.8). Following intranasal administration of granisetron bioadhesive formulations over the dose range of 0.4 mg/kg to 1.6 mg/kg, drug exposure ($AUC_{0-\infty}$) increased in a dose-proportional manner, and the maximum plasma concentration (C_{max}) was also dose proportional (Figure 4.5).

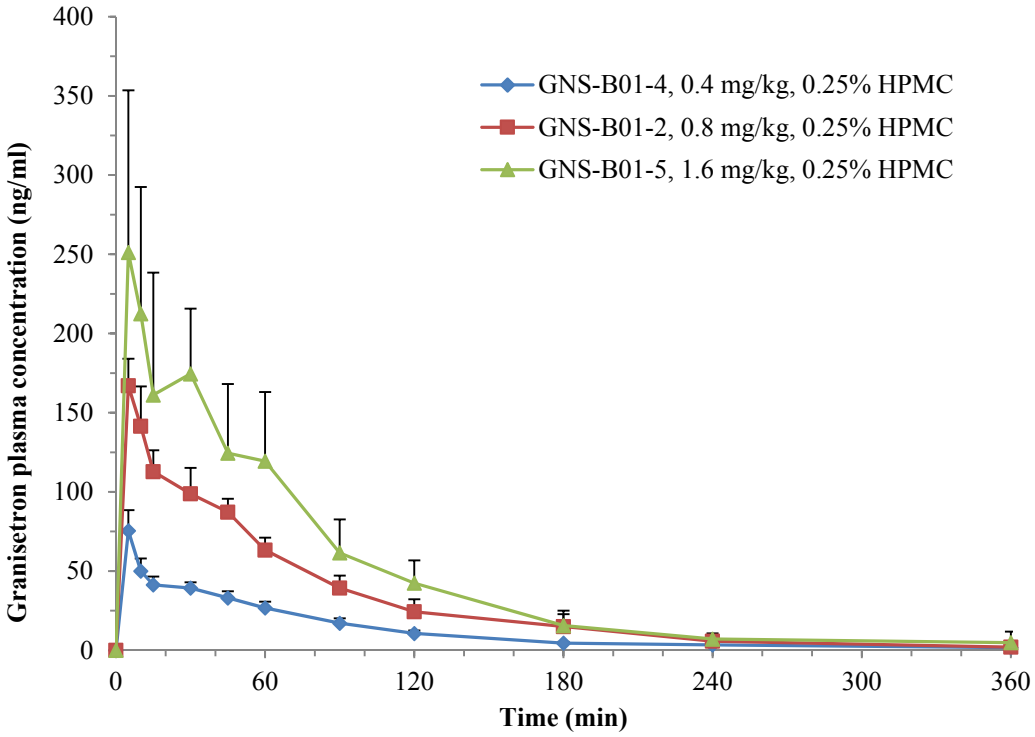
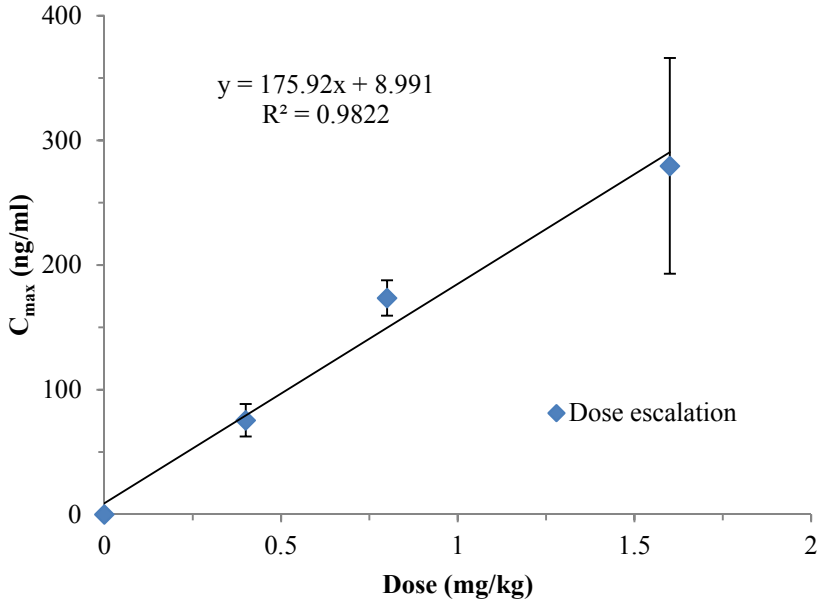
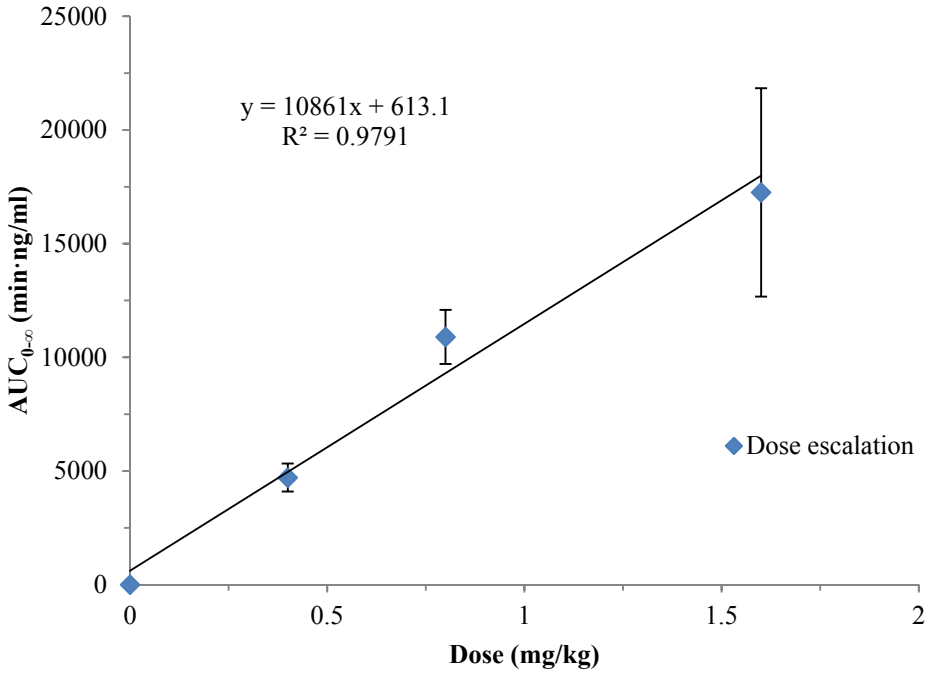


Figure 4.4 Plasma concentration-time curves of granisetron in SD rats (n=6) following intranasal administration of granisetron nasal bioadhesive formulations containing 0.25% HPMC at different doses (0.4 mg/kg, 0.8 mg/kg and 1.6 mg/kg)



(a)



(b)

Figure 4.5 Regression analysis of mean (a) maximum plasma concentration (C_{max}) and (b) area under the curve ($AUC_{0-\infty}$) on the dose of intranasal granisetron (Mean ± SD, n=6)

Table 4.8 Summary of principal pharmacokinetic parameters of granisetron following intravenous, oral and intranasal administration in rats

PK Parameter	Statistics	Intravenous	Oral	Intravenous					
		Kytril® injection, 0.8 mg/kg (n=5)	Kytril® Tab, 0.8 mg/kg (n=7)	GNS-S01, 0.8 mg/kg (n=6)	GNS-B01-1, 0.8 mg/kg (n=6)	GNS-B01-2, 0.8 mg/kg (n=6)	GNS-B01-3, 0.8 mg/kg (n=6)	GNS-B01-4, 0.4 mg/kg (n=6)	GNS-B01-5, 1.6 mg/kg (n=6)
t_{max} (min)	Mean (SD)	—	27.9 (29.0)	5.0 (0.0)	5.0 (0.0)	5.8 (2.0)	5.0 (0.0)	5.0 (0.0)	8.3 (4.1)
C_{max} (ng/ml)	Mean (SD)	290.2 (97.9)	3.3 (1.3)	99.3 (39.1)	117.6 (34.3)	173.6 (14.2)	156.2 (31.8)	75.5 (13.0)	279.5 (86.5)
AUC_{0-t} (min·ng/ml)	Mean (SD)	9513.4 (2462.1)	272.8 (42.7)	5980.4 (2124.8)	7573.0 (1881.1)	10696.6 (1203.9)	9046.5 (1750.3)	4409.1 (400.2)	16728.0 (4086.4)
$AUC_{0-\infty}$ (min·ng/ml)	Mean (SD)	9625.2 (2423.8)	494.4 (298.5)	6056.7 (2147.9)	7988.2 (1931.1)	10898.4 (1190.7)	9317.2 (1841.0)	4710.2 (614.8)	17253.3 (4582.5)
$t_{1/2}$ (min)	Mean (SD)	52.4 (5.8)	133.5 (56.0)	55.7 (5.3)	76.3 (14.4)	65.2 (22.3)	65.3 (12.1)	67.3 (13.5)	62.5 (15.3)
F (%)	Mean	—	5.1	62.9	83.0	113.2	96.8	97.9	89.6

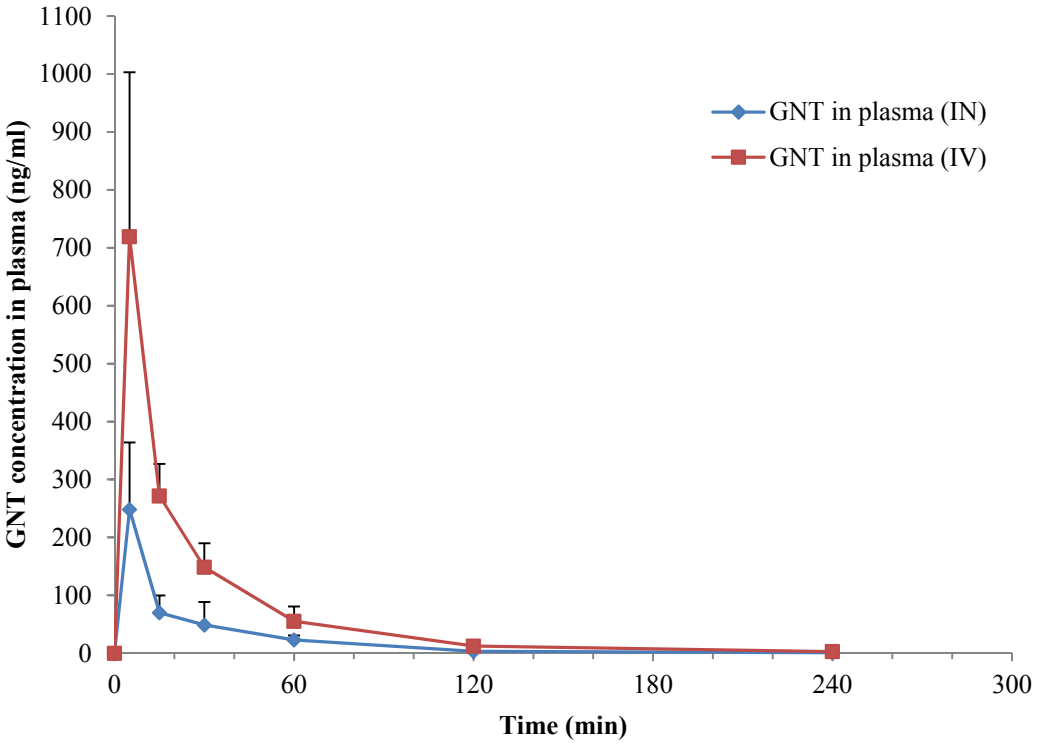
4.4.4 Brain pharmacokinetics of granisetron after intravenous and intranasal administrations

The mean granisetron concentration profiles in plasma and brain tissue after intravenous administration and intranasal administration are shown in Figure 4.6. The pharmacokinetic parameters of granisetron in rats after intravenous administration and intranasal administration are presented in Table 4.9.

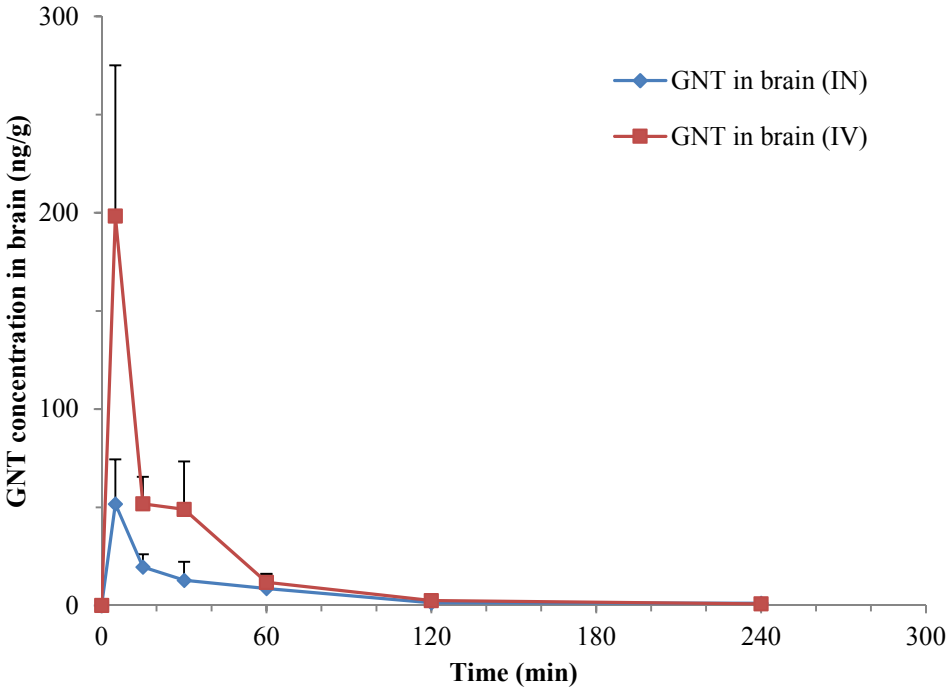
The granisetron concentration versus time profiles (Figure 4.6, a) showed the drug concentration in plasma rapidly declined after intranasal and intravenous administration of granisetron dosage forms, with the terminal elimination half-life ($t_{1/2}$) of 42.2 minutes (IV) and 39.4 minutes (IN). The plasma concentration of 248.3 ng/ml was achieved at 5 minutes after intranasal administration. The absolute bioavailability of granisetron following intranasal administration was 32.9%, as calculated from the ratio of AUC_{0-t} (5236.0 min·ng/ml) after intranasal administration to AUC_{0-t} after intravenous administration (15899.3 min·ng/ml).

The drug concentration in the whole brain after intranasal and intravenous administration of granisetron is shown in Figure 4.6 (b). The maximum drug concentration in brain was achieved within 5 minutes in both intravenous and intranasal groups, indicating a quick transport of granisetron from systemic circulation to brain after drug administrations. The elimination rate of granisetron in brain was comparable to that in blood, with the terminal elimination half-life ($t_{1/2}$) of 36.5 minutes (IV) and 48.0 minutes (IN). The brain targeting factor (BTF) was used to evaluate the partition of granisetron between brain tissue and plasma after intranasal and intravenous administrations. The higher BTF value, the greater degree of drug disposition to the

brain is. The BTF of intranasal administration of granisetron was 113.4%, suggesting that limited brain targeting effect obtained after intranasal administration. Nose-to-brain direct transport percentage (DTP) was to assess the contribution of direct nose-to-brain transport from intranasal delivery in the overall drug transported to brain (including transported from blood circulation). In this study, the DTP of intranasal group was 11.9%, indicating only a small portion of drug absorbed through the nose-to-brain pathway. Most of the nasally administered granisetron entered into the brain via systemic circulation, which was identical to the intravenous granisetron.



(a)



(b)

Figure 4.6 Granisetron concentration in (a) plasma and (b) brain of rats following intranasal administration (IN) of granisetron bioadhesive solution (GNS-B01-2) and intravenous administration (IV) of Kytril® IV Injection

Table 4.9 Pharmacokinetic parameters of granisetron in plasma and brain of rats after intranasal administration of granisetron bioadhesive solution (GNS-B01-2) and intravenous administration of granisetron injection (Kytril® IV Injection) at a single dose of 0.8 mg/kg

PK parameters	Intranasal administration (Mean±SD, n=3)		Intravenous administration (Mean±SD, n=3)	
	Brain	Plasma	Brain	Plasma
t_{max} (min)	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0
C_{max}	(51.8±22.6) ng/g	(248.3±115.7) ng/ml	(198.3±76.8) ng/g	(719.3±283.7) ng/ml
AUC_{0-t}	(1510.9±236.6) min·ng/g	(5236.0±1751.0) min·ng/ml	(4044.0±852.9) min·ng/g	(15899.3±2669.0) min·ng/ml
$AUC_{0-\infty}$	(1595.5±190.3) min·ng/g	(5282.5±1750.1) min·ng/ml	(4088.7±827.1) min·ng/g	(16073.4±2602.8) min·ng/ml
$t_{1/2}$ (min)	48.0±9.5	39.4±3.7	36.5±1.2	42.2±11.2
* BTF (%)	113.4			
** DTP (%)	11.9			

* BTF: Brain targeting factor, calculated as follows:

$$BTF\ \% = \{[(AUC_{0\text{-}t\ (brain)}/AUC_{0\text{-}t\ (blood)})_{IN}]/[(AUC_{0\text{-}t\ (brain)}/AUC_{0\text{-}t\ (blood)})_{IV}]\} \times 100\%$$

** DTP: Nose-to-brain direct transport percentage, calculated as follows:

$$DTP\ \% = [(B_{IN} - B_X)/B_{IN}] \times 100\%$$

Where, B_{IN} is $AUC_{0\text{-}t\ (brain)}$ after intranasal administration

$$B_X = \{[AUC_{0\text{-}t\ (brain)}]_{IV}/[AUC_{0\text{-}t\ (blood)}]_{IV}\} \times [AUC_{0\text{-}t\ (blood)}]_{IN}$$

4.5 Discussion

Granisetron is a potent antiemetic agent and has a highly binding and selective capability to 5-HT3 receptors. In human volunteers, granisetron can be absorbed after oral administration with the maximum plasma concentration achieved at approximate 2 hours. The oral bioavailability of granisetron in human was estimated to be 60%. However, in our study, the absolute oral bioavailability was only 5.1% in rats. Previous studies revealed the complete oral absorption of granisetron but with a limited bioavailability due to the first-pass metabolism in various species. One study by Clarke SE *et al.* [95] compared the metabolism and disposition of granisetron after intravenous and oral administrations in rats and human: in rats, 52%-62% of administered granisetron was excreted in faeces, and 35%-41% was excreted in urine through bile; in human, approximate 36% of administered granisetron was excreted in faeces and 60% was detected in urine, regardless of administration routes. N1-demethylation and 5-hydroxylation were the predominant routes of granisetron biotransformation in rats following both oral and intravenous administrations. The intermediate metabolites are further metabolized to the major metabolites by conjugation in blood, bile and urine in rats. In human, the dominant metabolites of granisetron is 7-hydroxy-granisetron, with a small quantity of 6,7-dihydrodiol and the conjugates. The variation of oral bioavailability of granisetron between rats and human may be attributed to different metabolism pathways among different species.

Compared with the incomplete oral absorption of granisetron oral suspension in rats, the intranasal absorption of granisetron formulations was faster and more complete, with significant earlier and higher plasma concentration which was comparable to that through intravenous route. The nasal cavity is highly vascularized, enabling it as an

effective and attractive route for systemic absorption of drugs with low oral bioavailability. The arterial blood supply of nose comes from the internal carotid arteries through the ophthalmic vessel, and the external carotid arteries through the facial and sphenopalatine vessels. The blood flow irrigates dense capillaries and then converges to capacitance vessels adjacent the turbinate respiratory zone. The venous return consists of the ophthalmic blood, the facial blood, the sphenopalatine blood and the internal jugular blood, and then drains into the right heart chambers. The local blood circulation provides the rationale that the first-pass effect can be completely circumvented via intranasal route, which is also consistent with our study on intranasal granisetron as well as other drugs administered via intranasal route [181] [182].

After intranasal administration of granisetron, the maximum plasma concentration (C_{max}) and the area under the plasma concentration versus time profiles from 0 to infinity ($AUC_{0-\infty}$) increased with the doses in a dose-proportional manner over the dose range of 0.4 mg/kg to 1.6 mg/kg. It was reported that diethyl ether could inhibit the activities of various liver CYP450 enzymes up to 65%. The enzymatic activities could gradually recover to the normal level when removing the exposure of diethyl ether [164] [165]. In our studies, the time of diethyl ether exposure to rats was controlled to about 3 minutes to achieve short-term anesthesia, and all animals could recover from the anesthesia within 6 minutes after moving from the jar with diethyl ether. The diethyl ether induced anesthesia could also lead to hypothermia, which may further affect the activities of liver enzymes. In an in vitro study, Fritz *et al.* [166] demonstrated the activities of CYP3A4 markedly reduced with the decrease of temperature. The activities of CYP3A4 decreased to 48% at 26 ^{o}C and 69% at 32 ^{o}C respectively, using the baseline value at 38 ^{o}C. The hypothermia could further suppress the blood circulation, total metabolism rate and the organ functions, such as kidney and liver [183], and then

result in the decreased drug elimination rate. Therefore, compared with the rats anesthetized by diethyl ether, the shorter elimination half-life ($t_{1/2}$) was observed in the conscious rats in the brain targeting study.

The drugs administered via intranasal route may enter into brain through three pathways: olfactory pathway, trigeminal pathway and blood-brain-barrier (BBB) pathway. Besides the drugs in blood circulation may penetrate across BBB to brain region, it is possible that certain amount of drug transport to brain via direct nose-to-brain pathway. In our brain PK study, the nose to brain direct transport percentage (DTP) was used to estimate the ratio of the drug penetrating into brain through direct nose-to-brain transport to the total drug penetrating to the brain after intranasal administration. DTP (ranging from 0 to 100%) reflects the contribution of the nose-to-brain transport in the overall drug transported into brain. For example, DTP value of 0 indicates the drug was predominantly delivered to the brain from the systemic circulation (via BBB). The DTP value in our study was 11.9%, implying the brain-to-nose pathway may be not the major route for intranasal granisetron. The ratio of AUC_{brain} versus AUC_{plasma} after intranasal administration was close to the AUC ratio after intravenous administration, which also indicated the intranasally administered granisetron may enter into the brain mainly from systemic circulation. A previous study by Merkus *et al.* also revealed the similar result in human volunteers [184]. A clinical study was designed to investigate the possibility of direct nose-to-brain transport from olfactory area to the cerebrospinal fluid (CSF) by collecting blood and CSF samples after intranasal and intravenous administrations of melatonin solution. There was no significant difference about the increase of melatonin concentrations in the CSF whether the drug was administered through intranasal or intravenous pathways, which implied the drug entered into the CSF through the blood-brain barrier from the blood circulation.

The result of the study exhibited no indication for an additional transport mechanism of the drug directly from the nose to the CSF. In our study, following intranasal administration, the granisetron in the brain were eliminated slower than that after intravenous administration, although significant difference was not observed in the half-lives of granisetron in brains.

It is difficult to predict the bioavailability or brain-targeting effects in humans from rat PK results because [185]: (1) The ratio of the nasal epithelium area versus body weight of rats is higher than that of human, thus the drug is more easily absorbed through nasal route in rats. (2) The proportion of olfactory region in the total nasal epithelium of rats (50%) is much larger than that in human (about 3%). (3) The weight of the rat brain is much smaller, resulting in more concentrated drug in the brain. (4) The distance between nose and the olfactory region of brain is shorter in rats in comparison with that in human. Furthermore, the metabolism and distribution of granisetron in rats are also distinct from human. Therefore, further in vivo studies in other animals are imperative.

4.6 Conclusion

In the pharmacokinetic studies of granisetron in rats, all the rats dosed with granisetron through intranasal, oral and intravenous administrations were exposed to the parent drug. Compared to the incomplete oral absorption of granisetron suspension solution, the intranasal absorption of granisetron formulations was rapid and complete, with significant earlier and higher drug plasma concentration which was comparable to intravenous administration. Following intranasal administration of granisetron bioadhesive formulations, the formulation containing 0.25% HPMC presented the highest bioavailability. The C_{max} and $AUC_{0-\infty}$ increased with the doses in a dose-proportional manner over the dose range of 0.4 to 1.6 mg/kg after intranasal administration of granisetron formulations.

After intranasal administration of granisetron, only limited direct nose-to-brain transport was observed in rats. The elimination rate of granisetron in brain after intranasal administration was slower than that after intravenous administrations.

Chapter 5

Pharmacokinetic study of granisetron and its metabolite 7-OH granisetron in Beagle dogs

5.1 Introduction

Nausea and vomiting are consistently regarded as the most distressing side effects by most cancer patients receiving antineoplastic therapies [186] [187]. The serotonin is released from the enterochromaffin cells damaged by cytotoxic agents and can bind to 5-HT3 receptors to trigger CINV [188] [189]. In the 1990s, the discovery of 5-HT3 receptor antagonists that have a similar structure to serotonin ushered in a dramatic improvement of antiemetic prophylaxis. 5-HT3 receptor antagonists could selectively and comparatively bind to the 5-HT3 receptors, and subsequently stop the activation of vomiting center.

Granisetron ($C_{18}H_{24}N_4O$) is a water soluble 5-HT3 receptor antagonist approved by FDA in 1993 for prevention of CINV associated with initial and repeat courses of emetogenic chemotherapy. So far, granisetron can be available on the market as injection (Kytril®), tablets (Kytril®), oral solution (Kytril®), transdermal patch (Sancuso®) and extended-release subcutaneous injection (Sustol®).

Intranasal drug delivery has been regarded as a promising administration route which possesses various advantages, such as non-invasive administration, a rapid onset of action and high bioavailability. It is a potential alternative for drugs restricted to intravenous administration, since the nasal route could circumvent the hepatic first-pass metabolism. The intranasal administration of drugs with systemic effects is attractive in various therapeutic areas where a rapid onset of action is needed, such as migraine

treatment (Imitrex® and Zomig®), severe pain treatment (Instanyl®), flu vaccination (FluMist®) and smoking cessation (Nicorette®) [190]. With the growing number of applications, the global market of nasal delivery technology was 44.0 billion US dollars in 2016 and is expected to exceed more than 64.2 billion US dollars by 2021 at a compound annual growth rate of 6.5% in the given forecast period.

However, nasal mucociliary clearance may limit the drug residence time in nasal cavity and result in an incomplete nasal absorption to the systemic blood circulation. The drug on the surface of nasal mucosa is propelled by the ciliary movement to the nasopharynx and then swallowed into the gastrointestinal tract [191]. The mucoadhesive technology could prolong the contact time between the drug and the mucosa by certain hydrophilic polymers with bioadhesive properties. In our previous studies, granisetron has been developed as a bioadhesive solution formulation containing HPMC as a mucoadhesive vehicle for intranasal administration. Moreover, the intranasal administration of granisetron in rats presented faster absorption and higher bioavailability comparing to the incomplete oral absorption of granisetron suspension. Although granisetron was administered via different routes with different purposes in several experimental studies [192] [193] [95], the pharmacokinetics of intranasal granisetron using the spray device has not been evaluated in big animals. In this chapter, the pharmacokinetic profiles of intranasal granisetron delivered by spray device was evaluated in Beagle dogs and compared with that of oral tablet and intravenous injection. For intranasal administration of granisetron, the dose escalation study was also conducted to evaluate the linear relationship between the doses and drug exposure. The exposure of metabolite 7-hydroxygranisetron (7-OH granisetron) after intranasal administration was also compared with that of oral administration of granisetron.

5.2 Materials and animals

5.2.1 Reagents

Granisetron hydrochloride (purity: >99.5%, Lot#: 207005GJ) was purchased from Qilu Pharmaceutical Co., Ltd. Kytril® (Granisetron tablet, 1 mg/tablet) and Kytril® (Granisetron intravenous infusion, 3 mg/3 ml) were manufactured by F. Hoffmann-La Roche Ltd (Basel, Switzerland). Zolpidem tartrate was obtained from Lu Nan Better Pharmaceutical Co. Ltd (Shan Dong, China). Hydroxypropyl methylcellulose (METHOCEL™, HPMC, K100 LV) was from Colorcon Co., Shanghai. Acetonitrile was purchased from Merck & Co. Ethyl acetate, EDTA-2K and ammonium acetate (AR grade) was ordered from Sigma-Aldrich Corporation. All other reagents were at least analytical grade and used without further purification. Distilled and deionized water was used to prepare the solutions.

5.2.2 Reference standard

The reference standard of granisetron hydrochloride 150 mg (Lot No.: G0K401) was provided by The United States Pharmacopeial Convention, Inc. The reference standard of 7-OH Granisetron was ordered from Nanjing Jinglong Pharmatech, Inc.

5.2.3 Test articles

The test articles listed as Table 5.1 were used for pharmacokinetic evaluation of granisetron in Beagle dogs.

Table 5.1 Test articles for Beagle dog PK study

Granisetron Nasal Spray, 0.5 mg/spray	
Product code	GNS-B01-05
Supplier	Maxinase Life Sciences Limited
Description	Clear and colorless liquid containing 0.5% (w/v) Granisetron base and 0.25% (w/v) HPMC.
Storage condition	Stored at room temperature, avoid direct sunlight
Granisetron Nasal Spray, 1.0 mg/spray	
Product code	GNS-B01-10
Supplier	Maxinase Life Sciences Limited
Description	Clear and colorless liquid containing 1.0% (w/v) Granisetron base and 0.25% (w/v) HPMC.
Storage condition	Stored at room temperature, avoid direct sunlight
Kytril® Granisetron Tablet (1 mg)	
Supplier	F. Hoffmann-La Roche Ltd
Description	Each scored film-coated tablet contains Granisetron 1 mg
Storage condition	Do not store above 30ºC
Kytril® Granisetron (3 mg/3ml) for IV Infusion	
Supplier	F. Hoffmann-La Roche Ltd.
Description	Clear liquid contains Granisetron 3 mg / 3 ml
Storage condition	Do not store above 30ºC, keep ampoule in the outer carton

5.2.4 Animals and acclimatization

Eight Beagle dogs (male/female, 4/4), about 6 months of age, were obtained from Yadong Laboratory animal research center (No. 281 Zhong Shan Bei Road, Nanjing, China). All the dogs were quarantined for about two months prior to the study. No prophylactic or therapeutic treatment was administered during quarantine period. At the dosing period, the animals weighed between 7.36 and 8.55 kg and were approximately 8 months of age.

All animals were housed in individual, stainless steel cages during acclimation. Environmental controls for the animal room were set to maintain a temperature of 22±2^{o}C, a relative humidity of 55±5%, and a 12-hour light/12-hour dark cycle. Food was certified, commercial, dry formula feed. 250 g of food per day and free water was provided *ad libitum*. No contaminants were present in the food or water which could interfere and affect the results of the study.

5.3 Methods

5.3.1 Allocation to treatment groups

Each of the eight Beagle dogs were dosed consciously with granisetron at the doses of 0.5 mg (intranasal administration), 1.0 mg (intranasal administration), 2.0 mg (intranasal administration), 1 mg (oral administration) and 1 mg (intravenous administration), respectively.

5.3.2 Study design

Eight Beagle dogs (4 males and 4 females) were used in the study. In the oral treatment group, the Beagle dogs were dosed with granisetron tablet (1.0 mg/tab) after

an overnight fast. For the Beagle dogs treated with intranasal administration, the granisetron bioadhesive solution was sprayed into the nostrils of dogs from an amber glass vial fitted with a metered multi-dose spray pump. Water was given *ad libitum* throughout the study. Food was provided 4 hours post-dose.

The study was conducted in five cross-over rounds. Table 5.2 showed the treatment schedules of eight Beagle dogs.

Table 5.2 Treatment schedule for Beagle dogs

Round	Dose	Test article	Sample information	Route	Dog No.	Time Point
1	0.5 mg/dog	GNS-B01-05	0.5 mg/spray, 3 ml/bottle	Intranasal administration	01-08	0 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h.
2	1.0 mg/dog	GNS-B01-05	0.5 mg/spray, 3 ml/bottle	Intranasal administration	09-16	
3	2.0 mg/dog	GNS-B01-10	1.0 mg/spray, 3 ml/bottle	Intranasal administration	17-24	
4	1.0 mg/dog	Granisetron Tablet	Kytril® Granisetron Immediate-Release Tablet, 1.0 mg/tab	Oral administration	25-32	
5	1.0 mg/dog	Granisetron IV Infusion	Kytril® Granisetron IV Infusion, 3mg/3ml	Intravenous administration	33-40	

5.3.3 Administration of granisetron dosage forms via different routes

The Beagle dogs were administered with granisetron formulations in Table 5.3.

Intranasal administration: (1) The spray device was primed 4-5 times prior to dose administration in a well ventilated room. (2) Dogs were allowed to stand or in an upright position. (3) The tip of the applicator on the device was placed into the right nostril of the dog pointing in from the center of dog's nose. (4) Granisetron solution was

sprayed into the right nostril by pressing down the spray device evenly once. (5) Similarly, granisetron solution was sprayed into the left nostril by pressing down the spray device evenly once. (6) After dose administration, the spray device was closed with the clear plastic cover and placed in a cool, dry location out of direct sunlight.

Oral administration: dogs were fasted about 16 hours prior to the dosing but had free access to water. Dogs were orally administered with 1.0 mg tablet for the dose of 1.0 mg/dog. Each oral dosing was administered with 20-30 ml of water.

Intravenous administration: the granisetron injection was injected via forearm veins at the dose of 1.0 mg/dog.

Table 5.3 Administration of granisetron via different routes

Administration route	Test articles	Dose
Intranasal administration	GNS-B01-05	0.5 mg/dog (1 spray in right nostril, 1 spray)
	GNS-B01-05	1.0 mg/dog (1 spray per nostril, 2 sprays)
	GNS-B01-10	2.0 mg/dog (1 spray per nostril, 2 sprays)
Oral administration	Kytril® granisetron tablet (1 mg/tablet)	1 tablet/dog (1mg/dog)
Intravenous administration	Kytril® Granisetron IV Infusion (3mg/3ml)	1 ml/dog (1 mg/dog)

5.3.4 Blood sample collection

The blank blood samples were collected before dosing. After administration of granisetron formulations via intranasal, oral, or intravenous routes, blood samples (approximately 2.0 ml) were collected at 5, 10, 15, 30, 45 minutes; and at 1, 2, 3, 4, 6, 8,

12, 24 hours post-dose. All blood samples were placed into EDTA-2K tubes and centrifuged at 10000 rpm for 5 min. The plasma was then transferred into a tube, sealed, and stored at approximately -80 ºC before bioanalysis.

5.3.5 Plasma sample processing

50 μL of NaOH and 50 μL IS solution (5 ng/ml of zolpidem tartrate in acetonitrile) were added into a 100 μL aliquot of plasma sample and mixed by vortex. Then, 500 μL of ethyl acetate was added into the tube. The samples were mixed by vortex for 5 minutes. After centrifugation at 10000 rpm for 5 minutes, 480 μL of the organic phase was transferred to a 96-well plate, and then evaporated to dryness using Eppendorf Concentrator Plus. The residue was reconstituted in 200 μL of solvent consisting of acetonitrile:water (20:80) and mixed by vortex for 5 min. A 10 μL of aliquot was injected and analyzed using the LC-MS/MS system.

5.3.6 Assay method for granisetron in vivo samples

A validated LC-MS/MS method was used for determining granisetron and its major metabolite of 7-OH granisetron in dog plasma. The HPLC system included ternary pumps (model LC-20AD, Shimadzu), a solvent degasser (model DGU-20A5, Shimadzu), an auto-sampler (model SIL-20AC, Shimadzu), a column oven (model CTO-20A) and a system controller for communication (model CBM-20A, Shimadzu). The conditions of HPLC were shown in Table 5.4.

Table 5.4 HPLC method

Column	ACE 5 C18，50 mm×3.0mm		
Mobile Phase	Time (min)	Phase A 10.0 mmol of ammonium acetate	Phase B acetonitrile
	0.01-0.40	80 %	20 %

	0.40-1.40	80-10 %	20-90 %
	1.40-2.40	10 %	90 %
	2.40-3.00	10-80 %	90-20 %
	3.50-4.00	80 %	20 %
	Total flow	0.6 ml/min	
Auto-sampler	Cooler Temp.	4 ºC	
Oven Temp.		35 ºC	
Retention time (min)		Granisetron: 2.14 min; 7-Hydroxy Granisetron: 1.88; IS: 2.24 min	

The analytes were detected and quantified using a triple quadrupole mass spectrometer (API4000, Applied Biosystems) equipped with a Turbo IonsprayTM interface. The mass spectrometer was operated in the positive ionization mode with multiple reactions monitoring (MRM). The parameters of mass spectrometer were shown in Table 5.5. Analyst® 1.5 was used to control the mass spectrometer and to analyze and process data.

Table 5.5 Mass spectrometer parameters

Compound	Granisetron	7-OH granisetron	IS
Ion Source	ESI		
Polarity	Positive		
Scan Type	MRM		
Collision Gas (psi)	6		
Curtain Gas (psi)	20		
Ion Source GS1 (psi)	50		
Ion Source GS1 (psi)	50		
Ionspray Voltage	5000		

Temperature	450		
Entrance Potential (V)	10		
Declustering Potential (V)	90.0	90.0	90.0
Collision Energy (V)	31.0	33.0	53.0
Collision Cell Exit Potential (V)	10.0	8.00	14.0
m/z	313.2/138	329.2/138	411.2/191

5.3.7 Data analysis

The non-compartmental method was used to generate the pharmacokinetic parameters, including C_{max}, t_{max}, $t_{1/2}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}\infty}$, CL/F, V_z/F and MRT. The maximum plasma concentration (C_{max}) and the time at which this occurred (t_{max}) were directly obtained from drug plasma concentration-time curve. The elimination rate constant (k_{el}) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated by use of the formula: $t_{1/2}=0.639/ k_{el}$. The area under the plasma concentration-time curve to the last measurable plasma concentration ($AUC_{0\text{-}last}$) was calculated following the linear trapezoidal rule.

5.4 Results

5.4.1 Pharmacokinetics of granisetron following intranasal, oral and intravenous administration

According to the crossover design in this study with eight dogs in each group, the dogs were randomly assigned to receive granisetron bioadhesive solution (GNS-B01-05, 0.5 mg/spray), granisetron tablet (Kytril®, 1mg/tab) and granisetron intravenous infusion (Kytril®, 3 mg/3 ml) at the dose of 1 mg/dog through intranasal, oral and intravenous routes respectively. The profiles of plasma concentration versus time are shown as Figure 5.1. The pharmacokinetic parameters of granisetron in dogs dosed via different routes are listed in Table 5.6. Bioanalysis results show that the dogs in all administration route groups were exposed to the parent drug.

For intravenous administration of granisetron infusion at a single dose of 1 mg/dog, the plasma concentration of granisetron declined quickly in the first two hours, with an elimination half-life ($t_{1/2}$) of 65.8 minutes. After a single oral dose of 1 mg/dog, the maximum plasma concentration of granisetron was only 1.78 ng/ml with the t_{max} of 43.1 minutes, followed by a gradual decline with an elimination half-life ($t_{1/2}$) of 58.6 minutes. After intranasal administration of granisetron spray solution at the dose of 1 mg/dog, a rapid absorption was observed with the t_{max} of 26.3 minutes, as compared with that after oral administration. The C_{max} of intranasal administration was 3.71 mg/ml, which was 2-fold as compared to that of oral administration. Compared to the low oral bioavailability (10.7%), the significantly higher bioavailability of 25.8% was achieved after intranasal administration.

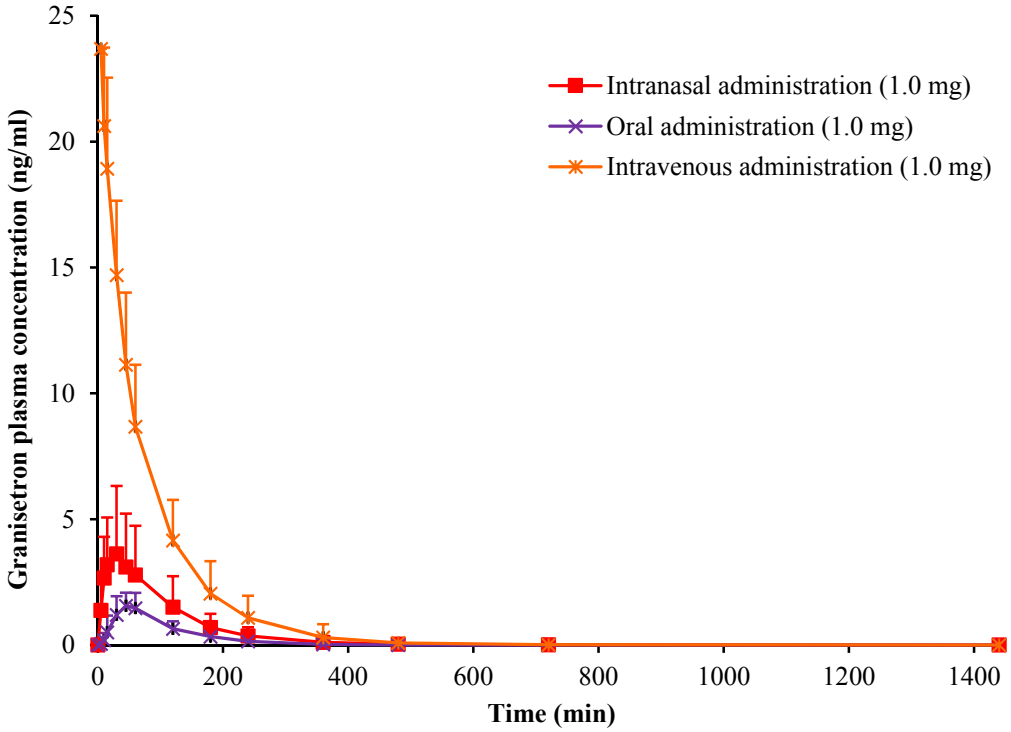


Figure 5.1 Plasma concentration-time curves of granisetron in Beagle dogs (n=8, 4 males and 4 females) following administration of granisetron through intranasal, oral and intravenous routes at a single dose of 1.0 mg/dog

Table 5.6 Pharmacokinetic parameters of granisetron in Beagle dogs

Analyte	Granisetron				
Route	Nasal Administration			Oral	IV
Dosage (mg/dog)	0.5	1.0	2.0	1.0	1.0
PK Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C_{max} (ng/ml)	2.20 ± 1.48	3.71 ± 2.69	8.48 ± 4.21	1.78 ± 0.561	23.8 ± 3.16
t_{max} (min)	26.9 ± 18.1	26.3 ± 10.6	23.8 ± 11.9	43.1 ± 12.5	5.63 ± 1.77
$t_{1/2}$ (min)	59.8 ± 14.7	63.9 ± 15.3	78.3 ± 21.1	58.6 ± 4.12	65.8 ± 10.7
MRT (min)	96.8 ± 33.2	86.9 ± 18.2	101 ± 17.0	90.1 ± 14.6	74.2 ± 12.1
CL/F (L/min)	4.20 ± 4.35	4.26 ± 3.69	2.80 ± 2.18	6.29 ± 2.68	0.608 ± 0.124
Vz/F (L)	348 ± 351	334 ± 217	286 ± 179	531 ± 234	56.9 ± 10.9
$AUC_{0\text{-last}}$ (ng/ml·min)	257 ± 210	436 ± 334	1001 ± 567	177 ± 73.7	1703 ± 386
$AUC_{0\text{-}\infty}$ (ng/ml·min)	264 ± 211	443 ± 334	1012 ± 571	184 ± 71.4	1714 ± 389
F_{abs} (%)	30.8	25.8	29.5	10.7	—

5.4.2 Dose proportionality of granisetron following intranasal administration

An escalation in doses of granisetron nasal spray over the range from 0.5 mg/dog to 2.0 mg/dog was conducted in Beagle dogs. As shown in Figure 5.3, both C_{max} and $AUC_{0-\infty}$ increased proportionally with the escalation of doses after intranasal administration. In all intranasal dosing groups, rapid drug absorption was achieved with t_{max} from 23.8 minutes to 26.9 minutes. No significant differences were observed in the mean terminal half-lives ($t_{1/2}$) of the three groups. As shown in Table 5.6, the absolute bioavailability of granisetron (F %) after intranasal administration was comparable among the three groups with increasing doses.

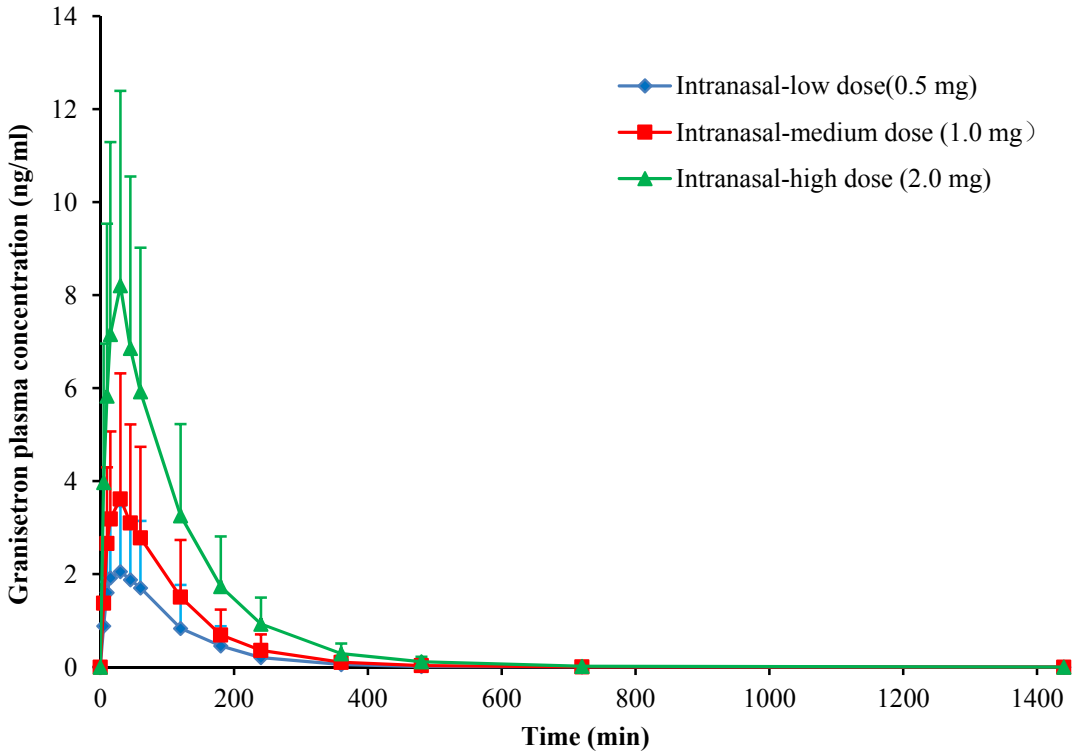
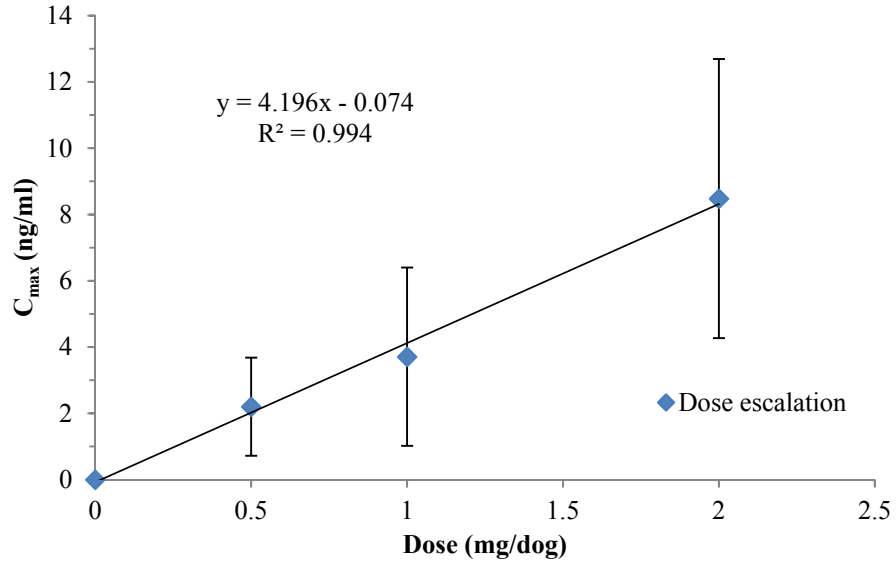
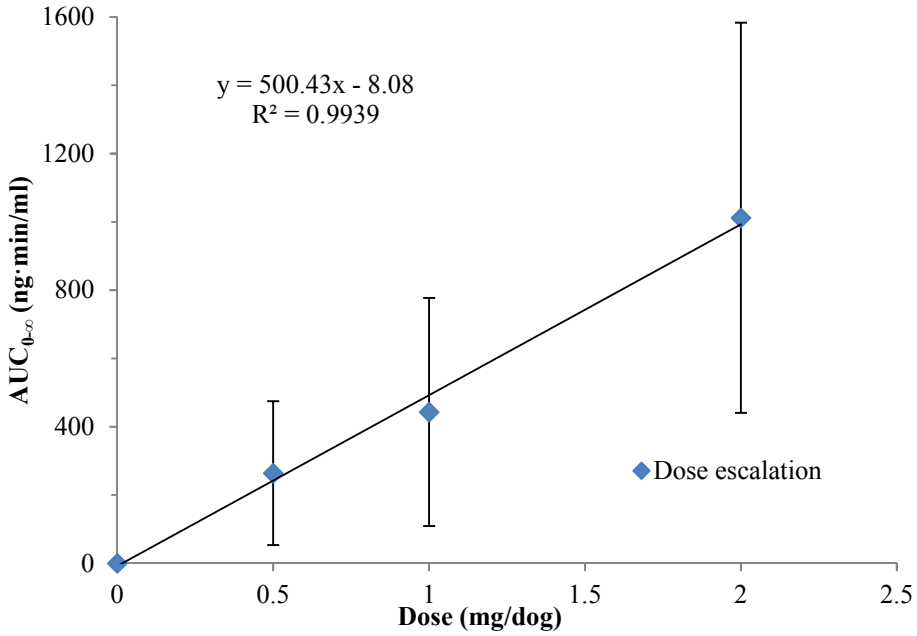


Figure 5.2 Plasma concentration-time curves of granisetron in Beagle dogs (n=8, 4 males and 4 females) following intranasal administration of granisetron nasal sprays at the doses of 0.5 mg/dog, 1.0 mg/dog and 2.0 mg/dog



(a)



(b)

Figure 5.3 Regression analysis of mean (a) maximum plasma concentration (C_{max}) and (b) area under the curve ($AUC_{0-\infty}$) on the dose after intranasal administration (Mean ± SD, n=8, 4 males and 4 females)

5.4.3 Gender differences after intranasal administration of granisetron

The pharmacokinetic parameters of granisetron in male and female Beagle dogs following intranasal, oral and intravenous administration were compared as Table 5.7, 5.8 and 5.9. The ratios of males to females were used to evaluate the gender-specific effects of granisetron administered via different routes. According to the ratios of males to females, a slightly increased drug exposure of granisetron was observed in male Beagle dogs as compared to the female Beagle dogs in all dosing groups. Following administration of granisetron via various routes, the ratios of males to females on C_{max} and $AUC_{0-\infty}$ were ranging from 1.13 to 1.74 and 1.30 to 1.82 respectively. The linear relationship of C_{max} and $AUC_{0-\infty}$ with the increase of the intranasal doses is shown as Figure 5.4. Both the C_{max} and $AUC_{0-\infty}$ increased with the dose in a dose-proportional manner over the dose range of 0.5 mg/dog to 2.0 mg/dog. However, the slopes of the regression lines on C_{max} and $AUC_{0-\infty}$ of male Beagle dogs were larger than that of female Beagle dogs, implying the gender-specific effects of granisetron following intranasal administration increased with the dose escalation.

Table 5.7 Pharmacokinetic parameters of males and females after intranasal administration

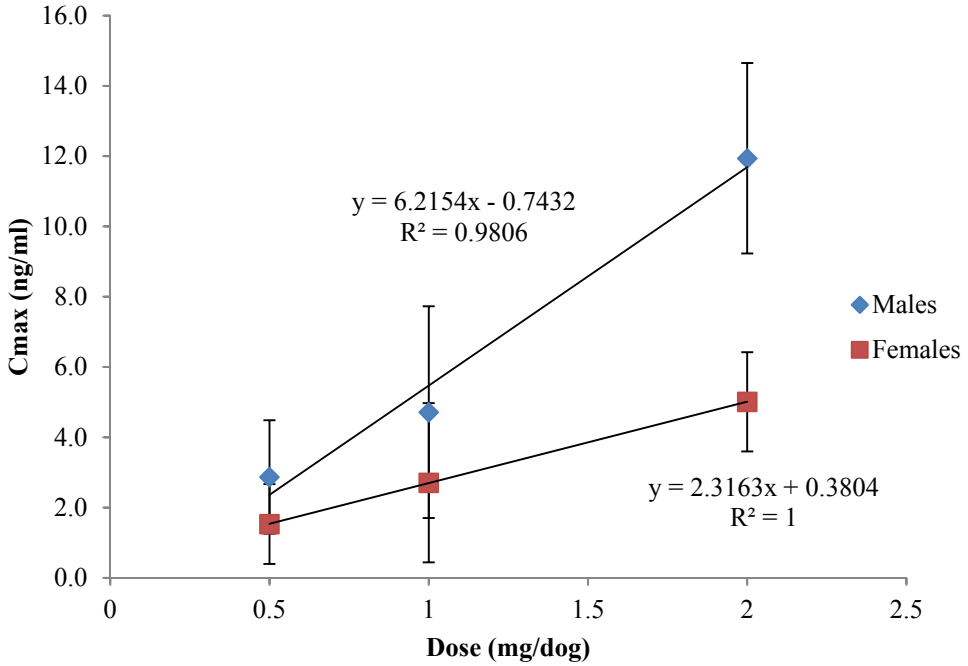
Parameter	Pharmacokinetic parameters after intranasal administration (1.0 mg/dog)				
	Male (n=4)		Female (n=4)		Male/Female
	Mean	SD	Mean	SD	
$t_{1/2}$ (min)	71.5	14.9	56.3	13.1	1.27
t_{max} (min)	30.0	12.2	22.5	8.7	1.33
C_{max} (ng/ml)	4.72	3.01	2.71	2.27	1.74
AUC_{0-t} (ng·min/ml)	543	345	329	333	1.65
$AUC_{0-\infty}$ (ng·min/ml)	549	346	337	333	1.63

Table 5.8 Pharmacokinetic parameters of males and females after oral administration

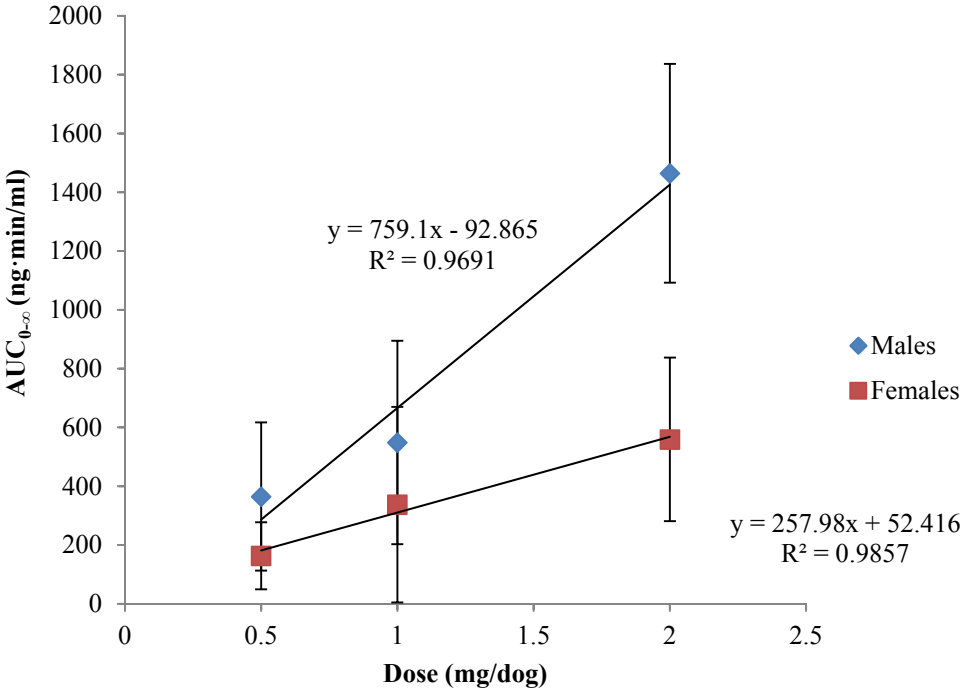
Parameter	Pharmacokinetic parameters after oral administration (1.0 mg/dog)				
	Male (n=4)		Female (n=4)		Male/Female
	Mean	SD	Mean	SD	
$t_{1/2}$ (min)	60.3	3.11	56.9	4.72	1.06
t_{max} (min)	41.3	14.4	45.0	12.2	0.917
C_{max} (ng/ml)	2.15	0.427	1.42	0.445	1.52
AUC_{0-t} (ng·min/ml)	231	54.8	122	41.4	1.89
$AUC_{0-\infty}$ (ng·min/ml)	237	53.4	131	38.3	1.82

Table 5.9 Pharmacokinetic parameters of males and females after intravenous administration

Parameter	Pharmacokinetic parameters after IV administration (1.0 mg/dog)				
	Male (n=4)		Female (n=4)		Male/Female
	Mean	SD	Mean	SD	
$t_{1/2}$ (min)	68.8	1.74	62.9	15.5	1.09
t_{max} (min)	6.25	2.50	5.00	0	1.25
C_{max} (ng/ml)	25.2	3.77	22.3	1.79	1.13
AUC_{0-t} (ng·min/ml)	1924	422	1482	198	1.30
$AUC_{0-\infty}$ (ng·min/ml)	1936	427	1492	197	1.30



(a)



(b)

Figure 5.4 The linear relationship of pharmacokinetic parameters (a: C_{max}, b: $AUC_{0-\infty}$) and doses after intranasal administration of granisetron

5.4.4 The pharmacokinetics of the major metabolite (7-OH granisetron)

The plasma concentration versus time profiles of the major metabolite (7-OH granisetron) after intranasal, oral and intravenous administration at a single dose of 1.0 mg/dog are presented in Figure 5.5. The pharmacokinetic parameters of 7-OH granisetron are summarized in Table 5.10. The plasma concentration of 7-OH granisetron increased rapidly in the first hour after doing, with the maximum plasma concentration (C_{max}) ranging from 2.18 ng/ml to 5.73 ng/ml. The C_{max} obtained from different administration routes can be ranked in the increasing order of intranasal < intravenous < oral administration ($P < 0.05$). After the maximum plasma concentration, all the pharmacokinetic profiles declined, followed by comparable elimination rates with the half-lives ($t_{1/2}$) ranging from 262 minutes to 276 minutes. The systemic exposure of 7-OH granisetron was evaluated by the $AUC_{0-\infty}$ values shown in Table 5.10, indicating the lowest exposure of the major metabolite after intranasal administration, in comparison to the oral administration ($P < 0.05$). The $AUC_{0-\infty}$ of 7-OH granisetron was almost doubled after oral administration ($AUC_{0-\infty}$: 1246 ng·min/ml) as compared to intranasal administration ($AUC_{0-\infty}$: 691 ng·min/ml).

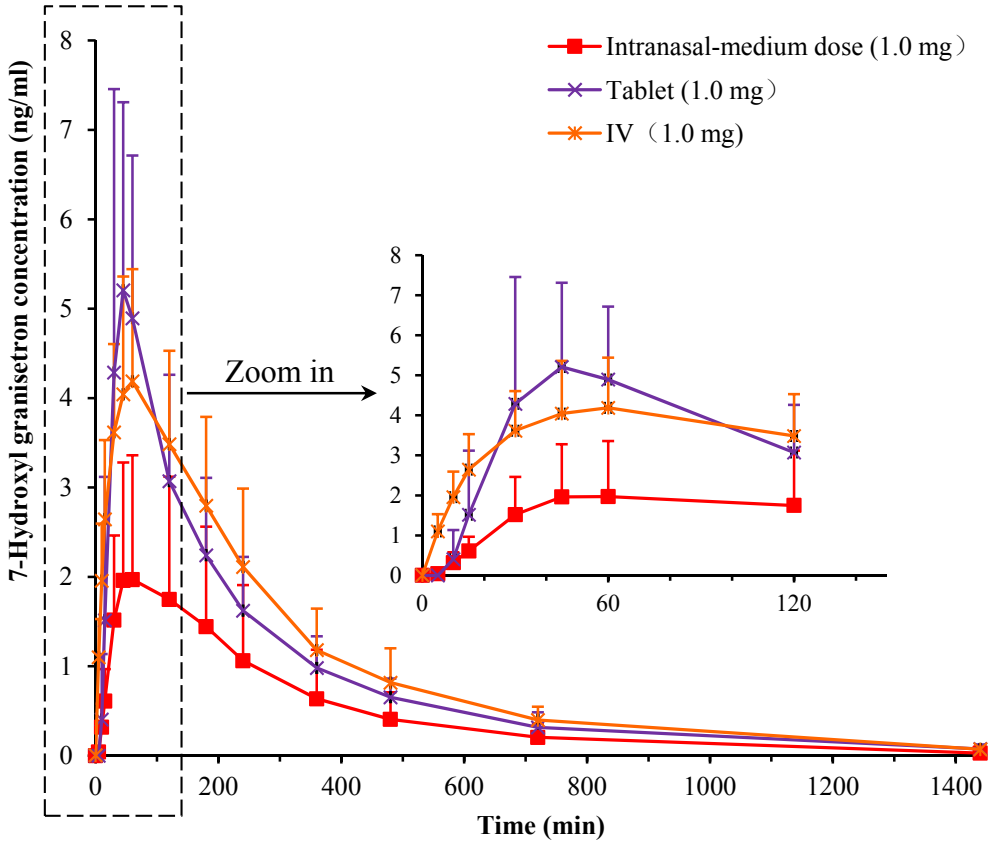


Figure 5.5 The plasma concentration versus time profiles of 7-OH granisetron after intranasal, oral and intravenous administration of granisetron at the dose of 1.0 mg/dog

In the dose escalation studies after intranasal administration, the major metabolite of granisetron (7-OH granisetron) was also found to be a dose-proportional increase over the dose range of 0.5 mg/dog and 2.0 mg/dog (Figure 5.6). After intranasal administration of granisetron, 7-OH granisetron increased rapidly in all dosing groups, followed by a gradual decline till 24 hours. The half-lives of 7-OH granisetron increased from 233 minutes to 306 minutes over the dose range of 0.5 mg/dog and 2.0 mg/dog after intranasal administration, indicating the elimination rate decreased slightly with the increase of the dose. The decrease in the clearance rate (CL/F) of 7-OH granisetron was also observed with the dose escalation, which declined from 2.98 L/min to 1.50 L/min when the dose increased from 0.5 mg/dog to 2.0 mg/dog.

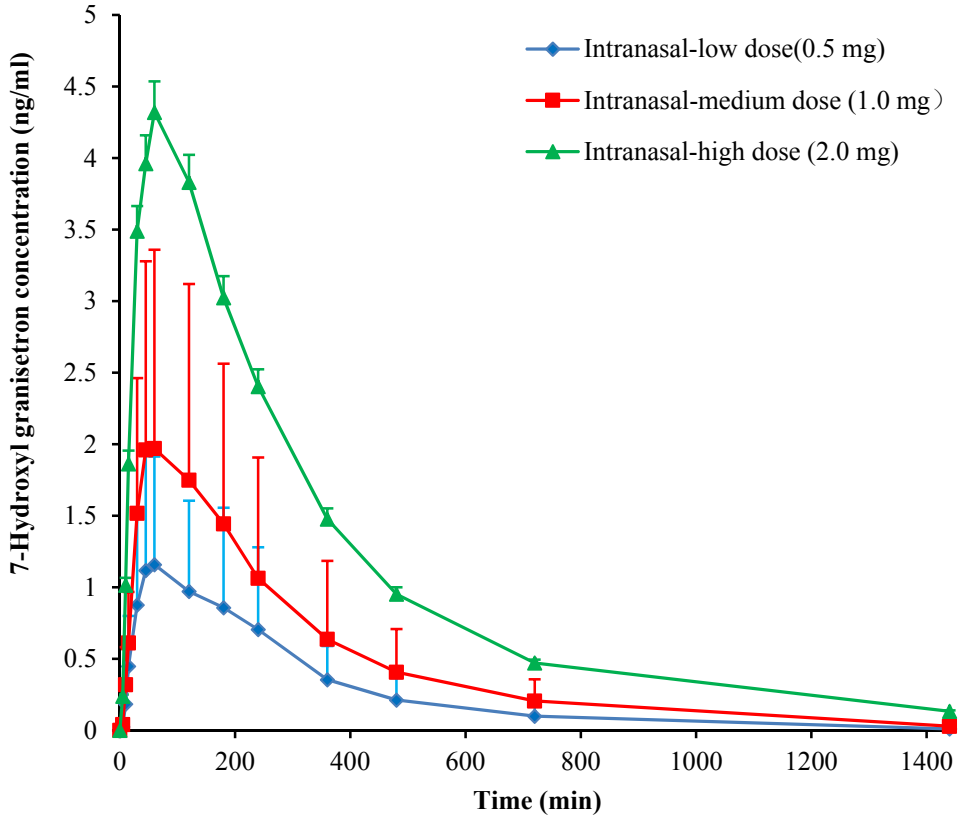
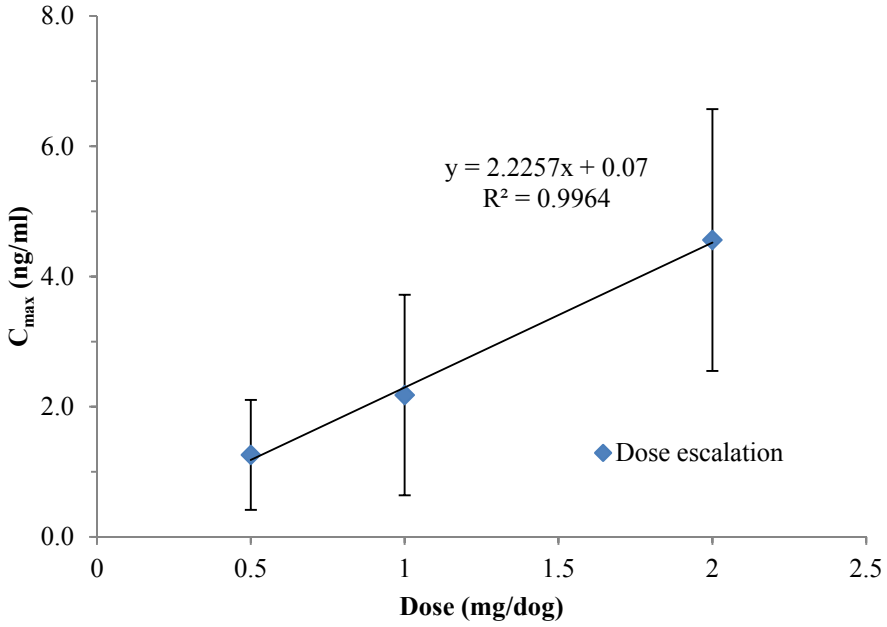
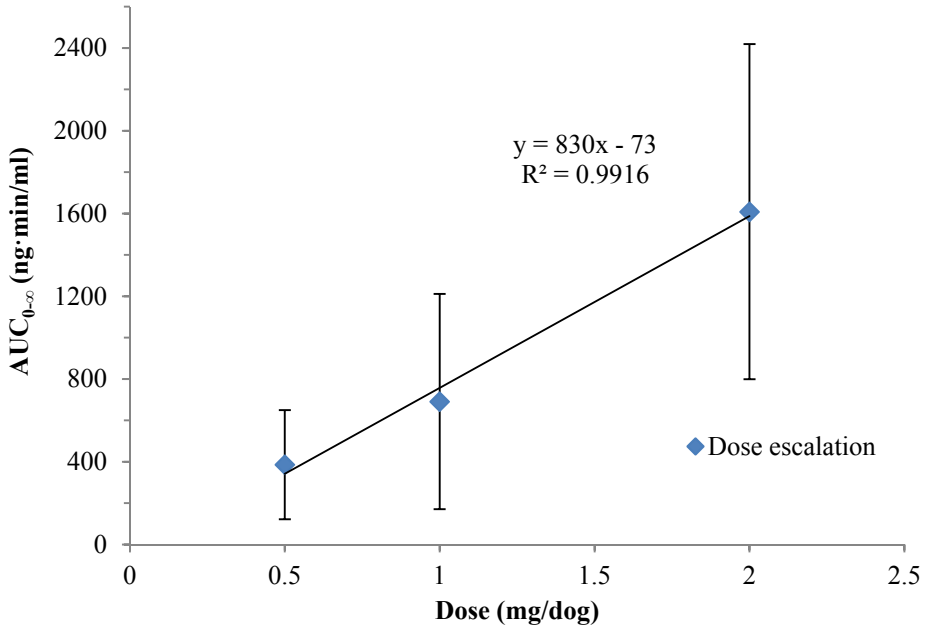


Figure 5.6 The plasma concentration of 7-OH granisetron versus time profiles after intranasal administration of granisetron at different doses



(a)



(b)

Figure 5.7 The linearity of pharmacokinetic parameters (a: C_{max}, b: $AUC_{0-\infty}$) for 7-OH granisetron versus intranasal doses

Table 5.10 Pharmacokinetic parameters of metabolite 7 OH-Granisetron in Beagle dogs

Analyte	7-OH Granisetron				
Route	Nasal Administration			Oral	IV
Dosage (mg)	0.5	1.0	2.0	1.0	1.0
PK Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C_{max} (ng/ml)	1.26 ± 0.845	2.18 ± 1.54	4.56 ± 2.01	5.73 ± 2.17	4.28 ± 1.20
t_{max} (min)	60.0 ± 25.4	63.8 ± 23.7	65.6 ± 35.8	37.5 ± 16.0	48.8 ± 13.3
$t_{1/2}$ (min)	233 ± 71.5	262 ± 47.2	306 ± 33.3	275 ± 60.6	276 ± 0.211
MRT (min)	235± 48.9	265 ± 47.9	325 ± 21.1	270 ± 40.6	297 ± 18.8
CL/F (L/min)	2.98 ± 4.01	2.70 ± 2.36	1.50 ± 0.608	0.899 ± 0.321	0.774± 0.248
Vz/F (L)	1055 ± 1518	996 ± 871	672 ± 322	342 ±102	317 ± 142
AUC_{0-t} (ng/ml·min)	364 ± 263	664 ± 520	1549 ± 771	1197 ± 446	1389 ± 465
$AUC_{0-\infty}$ (ng/ml·min)	386 ± 264	691 ± 520	1609 ± 810	1246 ± 473	1419 ± 470

5.5 Discussion

Besides the desirable aqueous solubility to concentrate the target dose in a limited volume of vehicle (< 300 μL), a desirable molecular weight of the drug is also a prerequisite for intranasal delivery, which should be less than 1000 g/mol [58]. Granisetron has a high aqueous solubility (> 300 mg/ml in water at room temperature) with a suitable molecular weight (312.409 g/mol) for intranasal drug delivery, indicating satisfactory physicochemical characteristics of granisetron to be administered via nasal route. Nasal cavity is covered by a thin, highly permeable and vascularized mucosa where the drug can easily penetrate and enter into the systemic circulation quickly. The mucosal thickness of dog's nasal cavity varies from 0.07 mm to 6.0 mm, which gradually increases from the caudal portion of the non-olfactory area to rostral part in the vestibular area of the nasal cavity. The ethmoid conchae and caudal area of the nasal septum are covered by the olfactory mucosa with the thickness between 0.15 mm and 0.69 mm. Approximately, 35% of the nasal mucosa of Beagle dogs is occupied by dense blood vessels, varying with the different locations in the rostrocaudal area [194], which makes the nasally administered drugs quickly enter into the systemic circulation. All these properties contribute to quick absorption and high bioavailability of granisetron intranasal dosage form, which were observed in the pharmacokinetic profiles of Beagle dogs dosed by granisetron intranasal spray solutions. After administration of granisetron via intranasal, oral and intravenous routes, the Beagle dogs were exposed to the parent drug. The absorption of granisetron by intranasal administration was faster than that of oral administration. However, the elimination half-lives of granisetron for all the administration routes were around 60 minutes.

In our studies, the absolute bioavailability of granisetron followed by oral

administration was only 10.7%, which was much lower than that of intranasal administration due to the hepatic first-pass metabolism. However, the absolute bioavailability of oral granisetron tablets in Beagle dogs is much higher than that in rats, which may be attributed to the differences of cytochrome P450 between Beagle dogs and rats. The total CYP P450 content in rats is more than two fold higher than the value in Beagle dogs [195] [196]. After oral administration of granisetron, the drug is absorbed in gastrointestinal tract and delivered to the liver through the hepatic portal vein. A portion of granisetron is metabolized in the liver before entering into the blood stream. Drug metabolism in liver was responsible for the poor oral bioavailability of granisetron. As to the intranasal administration of granisetron, improved absolute bioavailability was achieved due to the bypass of hepatic first-pass metabolism. The abundant vascular plexus in the nasal cavity and the highly permeable epithelium in the nasal cavity enable the rapid and complete drug absorption into the systemic circulation. In comparison with oral administration, the direct absorption of granisetron through nasal route could reduce the dose and minimize the dose-related side effects. Furthermore, the bioavailability of nasally administered granisetron is more predictable than the orally administered drug, due to the low inter-subject variability. In our dose-escalation study after intranasal administration of granisetron, a well fitted linear regression was obtained between the $AUC_{0-\infty}$ and doses. In the pharmacokinetic study in Beagle dogs, the nasal bioavailability of granisetron was around 30%, which was lower than that in rats (refer to Chapter 4). The relatively higher ratio of the nasal epithelium area to body weight in rats may account for the complete absorption of granisetron through intranasal route.

Granisetron is metabolized extensively by the cytochrome P450 (CYP) monooxygenase system. N-demethylation and aromatic ring oxidation are involved in

the metabolism of granisetron. In humans, granisetron is primarily metabolized into 7-hydroxygranisetron (7-OH granisetron) with small amount into 9'-desmethylgranisetron. The CYP 3A4 has been reported to be the major enzyme for 9'-desmethylgranisetron, although CYP 1A1 is an alternative enzyme responsible for 9'-demethylation [197] [198]. 7-OH granisetron is mainly metabolized by CYP 1A1, which is a major catalyst responsible for the metabolism of granisetron through a 7-hydroxylation route [199]. In dogs, the 7-OH granisetron is also one of the major metabolites. Therefore, 7-OH granisetron was monitored as the main metabolite of Beagle dogs in our study. 7-OH granisetron was detected after administration of granisetron through intranasal, oral and intravenous routes, indicating the metabolic patterns of granisetron via different administrations were similar. Therefore, the metabolic pathway of granisetron was generally irrelevant to the gender, administration routes and doses. Meanwhile, the AUC of the major metabolite 7-OH granisetron after intranasal administration was lower than that after oral administration, due to bypass of the hepatic first-pass metabolism from intranasal route.

In summary, rapid absorption with high bioavailability of granisetron, as well as lower level of main metabolite were achieved by intranasal administration in Beagle dogs. The bioadhesive formulations for intranasal spray can be further developed into an alternative to conventional intravenous and oral products, with the advantages of non-invasive route, self-dose, rapid onset of antiemetic effects and minimal side effects.

5.6 Conclusion

After administration of granisetron via different routes, all the Beagle dogs were exposed to the parent drug. The absorption of granisetron by intranasal administration was faster than that of oral administration. The elimination half-lives for all dose levels were around 60 minutes for both intranasal and oral administrations. The drug exposure after intranasal administration of granisetron was much larger than that of oral administration at the similar dose level. Specifically, the exposure of granisetron after intranasal administration was evaluated with C_{max} and $AUC_{0-\infty}$, which was twice as large as that of oral administration. Following intranasal administration of granisetron, C_{max} and $AUC_{0-\infty}$ increased with the dose escalation in a dose-proportional manner over the dose range of 0.5 - 2.0 mg/dog, indicating a predictable drug exposure of granisetron could be obtained by nasally dosing. After oral administration of granisetron, the exposure of metabolite 7-OH granisetron was at least twice as much as that after intranasal administration due to the hepatic first-pass metabolism. Granisetron absorbed via nasal route could avoid the destruction by hepatic first-pass metabolism, and thus achieve a higher drug exposure with limited main metabolite 7-OH granisetron. Overall, nasal delivery is a promising noninvasive alternative route for administration of granisetron to achieve a desirable bioavailability and minimize the exposure of metabolites.

Chapter 6

A Phase I pharmacokinetic study of granisetron hydrochloride nasal spray in healthy volunteers

6.1 Introduction

Granisetron is an effective and well-tolerated 5-HT3 receptor antagonist with little or no affinity for other serotonin receptors for the management of CINV. It was first approved as injection (Kytril®) by US FDA in 1993. Since then, granisetron was successively developed into several other commercial products, such as oral tablets (Kytril®), oral solution (Kytril®), transdermal patch (Sancuso®) and extended-release subcutaneous injection (Sustol®). Furthermore, other innovative drug delivery routes have been explored for granisetron recently, e.g., nasal drug delivery.

Nasal drug delivery is an alternative administration route which could provide an access to vascularized mucosa for drugs acting locally or systemically. The nasal drug delivery provides a practicable way for absorbing drugs under the conditions that is unfeasible to apply oral administration, e.g., patients with compromised swallowing ability and nausea and vomiting. Other advantages of nasal drug delivery include non-invasive administration, rapid onset of action, good compliance of patients, without sterile preparation, etc. [200] One of the foremost limitations on the absorption of drugs through the intranasal route is the nasal mucociliary clearance, which is an innate protection mechanism of nose to defend the respiratory system by capturing inhaled matter. The trapped foreign matter is subsequently transported to the oropharynx with the mucus covering on the nasal epithelium, impelled by the coordinated mobility of nasal cilia [201]. Therefore, the mucoadhesion of dosage forms is considered as a

prerequisite for nasal drug delivery. In decades, the application of bioadhesive technology has gained mounting interest to prolong the residence time of drugs in nasal cavity for facilitating the drug absorption, such as in situ gelling matrix and polymeric vehicles [202] [87] [203].

In our previous studies, granisetron was developed into a bioadhesive spray formulation based on hypromellose. Compared to normal saline solution, the formulations containing HPMC could effectively prolong the nasal mucociliary transport time (MTT) in rats. Faster absorption and higher bioavailability were also observed after intranasal administration in Beagle dogs, as compared to oral tablets. Moreover, the systemic exposure of granisetron major metabolite (7-OH gransietron) was much lower following intranasal administration than that of oral administration of granisetron. In acute toxicology and toxicology studies, no abnormalities and drug accumulation were observed in rats.

The efficacy and safety profiles of oral and injection granisetron hydrochloride formulations were well established. However, no clinical studies have been performed with Granisetron Nasal Spray. In this chapter, a Phase I clinical study (open-label and parallel-group) is designed to investigate the pharmacokinetics, safety and tolerability of granisetron in healthy volunteers following intranasal administration of granisetron nasal spray at a single dose, as compared to granisetron intravenous injection and granisetron oral tablets.

6.2 Materials

6.2.1 Chemicals and reagents

Granisetron hydrochloride (Lot#: G0K401, Purity: 99.7%) was purchased from USP. Granisetron-d_3 (Lot#: 1034-146A2, Purity: 99.5%) was obtained from the TLC Pharmachem. Acetonitrile (HPLC grade) was purchased from Spectrum® Chemical MFG Corp. Methanol and methyl tert-butyl ether were acquired from Macron™, Avantor Performance Materials, LLC.

6.2.2 Test articles

The test articles for the clinical trial are listed as Table 6.1.

Table 6.1 Information of test articles for clinical trials

Treatment	GNS 0.5 mg	GNS 1.0 mg	GNS 2.0 mg	Kytril® IV injection 1.0 mg	Kytril® Tablet 1.0 mg
Product Name	Granisetron hydrochloride Nasal Spray 0.5 mg/spray (GNS-B01-05)	Granisetron hydrochloride Nasal Spray 0.5 mg/spray (GNS-B01-05)	Granisetron hydrochloride Nasal Spray 1.0 mg/spray (GNS-B01-10)	Kytril® 3 mg in 3 ml	Kytril® 1 mg F.C. (Film-coated) Tablets
Manufacturer	Loyal Advance Ltd.	Loyal Advance Ltd.	Loyal Advance Ltd.	Cenexi SAS	F. Hoffmann-La Roche Ltd.
Active Ingredient	Granisetron hydrochloride	Granisetron hydrochloride	Granisetron hydrochloride	Granisetron hydrochloride	Granisetron hydrochloride
Dosage Form	Nasal Spray	Nasal Spray	Nasal Spray	Injection	Tablet
Strength	Granisetron base 0.5% w/v, 3 ml/bottle	Granisetron base 0.5% w/v, 3 ml/bottle	Granisetron base 1.0% w/v, 3 ml/bottle	Granisetron base 3 mg/3 ml/vial	Granisetron base 1.0 mg/tablet
Dose	Granisetron 0.5 mg (1 spray into right nostril)	Granisetron 1.0 mg (2 sprays, 1 spray per nostril)	Granisetron 2.0 mg (2 sprays, 1 spray per nostril)	Granisetron 1.0 mg (1 ml)	Granisetron 1.0 mg (1 tablet)
Mode of Administration	IN, single dose	IN, single dose	IN, single dose	IV, single dose	PO, single dose

6.3 Methods

6.3.1 Starting dose justification

The starting dose in the clinical trial is proposed to be 0.5 mg/day. Such a dose should be safe in humans, based on our non-clinical intranasal PK study results in the dogs and previous human experience at similar or higher doses of granisetron hydrochloride administered via oral or IV routes.

In an acute (7-day) toxicity study in SD rats, the no observed adverse effect level (NOAEL) was established to be 3.2 mg/kg for IN administration, equivalent to a human IN dose of 31.1 mg/subject (body weight: 60 kg) based on body weight divided by body surface area, which was calculated by the Equation 6.1 [204]. The first dose in human was obtained by further dividing 31.1 mg/subject by 10 (safety factor), which was established to be 3.1 mg/subject.

$$\text{Human equivalent dose (mg/kg)} = \text{Rat doses (mg/kg)} \times (K_{m\,(rat)} / K_{m\,(human)}) \qquad \text{(Eq. 6.1)}$$

Where, $K_{m\,(rat)}$ - the correction factor for rats, which equals 6 mg/m^2 (see Table 6.2).

$K_{m\,(human)}$ - the correction factor for human, which equals 37 mg/m^2.

On grounds of the above estimations on pharmacokinetics and toxicology, as well as human clinical trial experience, together with careful clinical monitoring, we conclude that the human health risk associated with an intranasal starting dose of 0.5 mg/day and the proposed dosing range (0.5-2.0 mg), if any, is minimal.

Table 6.2 The correction factors (K_m) for different species [204]

Species	K_m (mg/m^2)
Human	37
Mouse	3
Hamster	5
Rat	6
Ferret	7
Guinea pig	8
Rabbit	12
Dog	20

6.3.2 Study design

An open-label, single-dose, parallel-group and dose increasing clinical trial was conducted to investigate the pharmacokinetics, safety and tolerability of granisetron in healthy male and female volunteers.

At least 50 volunteers (25 males and 25 females) were recruited and assigned into the intranasal (IN, granisetron dose: 0.5 mg, 1.0 mg and 2.0 mg), intravenous (IV, dose: 1.0 mg), and oral (PO, dose: 1.0 mg) groups. In the 3 intranasal (IN) cohorts, each 10 volunteers (5 males and 5 females) received an IN dose of Granisetron hydrochloride Nasal Spray (GNS), starting with the 0.5 mg cohort, followed by the 1.0 mg cohort and 2.0 mg cohort. In the intravenous (IV) group, 10 volunteers (5 males and 5 females) received a single intravenous dose of Kytril® IV Injection (1.0 mg). In the Oral (PO) group, 10 volunteers (5 males and 5 females) received a single oral dose of Kytril® Tablet (1.0 mg). The dose schedule is provided in Table 6.3.

Table 6.3 Study scheme of clinical trials

Group No.	Cohorts	Assigned Intervals	PK	Safety and Tolerability
1 (IN Group)	1st *	Granisetron dose 0.5 mg, n=10	√	√
	2nd *	Granisetron dose 1.0 mg, n=10	√	√
	3rd	Granisetron dose 2.0 mg, n=10	√	√
2 (IV Group)	1	Kytril® IV injection (dose 1.0 mg), n=10	√	√
3 (PO Group)	1	Kytril® Tablet (dose 1.0 mg), n=10	√	√

*Data Review Committee (DRC) reviewed the preliminary PK, safety and tolerability results from earlier IN cohort(s) and determined if the higher IN dose could be administered.

All subjects were admitted at least 10 hours before dosing to ensure an overnight fast of at least 10 hours. After administration, all subjects fasted for 4 hours. All subjects were discharged after 12 hours after dose and returned at 24 hours and 36 hours (only for the oral group) post dose for PK blood sample collection. All IN treatments were administered by study site personnel.

6.3.3 Patient selection

The inclusion criteria (Table 6.4) had to be met by all subjects to be eligible to participate in the study.

Table 6.4 Inclusion criteria of patient selection

No.	Inclusion criteria
1	Healthy males or females between the ages of 20-64 years.
2	Body Mass Index (BMI) of 18.5 (inclusive) to 24 kg/m^2; and a total body weight >45 kg.
3	Accessible vein for blood sampling.
4	No significant abnormalities in electrocardiogram (ECG) recording as per sites' local practice.

5	No significant abnormalities in general physical examination as per sites' local practice.
6	No significantly abnormal findings in laboratory assessments including hematology, biochemistry and urinalysis as per site's local practice.
7	A signed and dated written informed consent must be obtained from the subject prior to study participation.
8	Capable of understanding and willing to comply with study procedures.
9	A negative serum pregnancy test before the first dose of study drug must be available for women of childbearing potential.

Subjects were excluded if they meet any of the following criteria shown as Table 6.5.

Table 6.5 Exclusion criteria of subjects

No.	Exclusion criteria
1	Females who are pregnant, breast-feeding or have positive pregnancy test.
2	History of hypersensitivity to granisetron or its analogs.
3	Subjects with nasal ulcer, septal perforation, or other nasal conditions that may interfere with nasal administration and determined by the investigator to be ineligible.
4	Subjects with a QT interval greater than 500 ms or with acute ischemic changes or cardiac abnormality predisposing to arrhythmia on screening electrocardiogram (ECG) or by history.
5	Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or other significant disease or clinical findings at screening and determined by the investigator to be ineligible.
6	Subjects with a history of drug and/or alcohol abuse within 12 months prior to dosing.
7	Inability to read and/or sign the consent form.
8	Treatment with any other investigational drug during the 4 weeks prior to the initial dosing for this study.
9	Subjects who have donated or lost more than 250 ml blood within 2 months prior to the initial dosing for this study.
10	Male and female subjects with reproductive potential who are not willing to use effective method of contraception. Use of hormonal contraceptive is not allowed during the study period.

11	Clinical significant rhinitis or rhinorrhea at screening determined by the investigator to be ineligible.
12	Use of prescription or nonprescription drugs and dietary supplements within 7 days or 5 half-lives (whichever is longer) prior to dosing of study medication.
13	For subjects who smoke or use tobacco products or are currently using nicotine products (patches, gums, etc.), 2 weeks abstinence is required.
14	Conditions upon screening which might contraindicate or require that caution be used in the administration of granisetron.

Protocol deviations or other deviations including issues such as the timing of administration, dosage, or the timing of blood sampling may lead to removal of subjects from assessment. However, removal of any subject from assessment should be discussed with the medical advisor.

6.3.4 Experimental procedure

For the granisetron 0.5 mg cohort, Granisetron hydrochloride Nasal Spray 0.5 mg/spray (GNS-B01-05) was sprayed once into the right nostril. For the granisetron 1.0 mg cohort, Granisetron hydrochloride Nasal Spray 0.5 mg/spray (GNS-B01-05) was sprayed once into both left and right nostrils (totally 2 sprays, 1 per nostril). For the granisetron 2.0 mg cohort, Granisetron hydrochloride Nasal Spray 1.0 mg/spray (GNS-B01-10) was sprayed once into both left and right nostrils (totally 2 sprays, 1 per nostril). All granisetron spray solutions were administered by site staff and under investigator supervision.

The procedure and instructions of IN drug administration are described below:

1. Remove the cap from the nasal spray container. In a separate room with good ventilation, prime the spray device 6-8 times for the first time use. Spray it into the air, away from faces.

2. With the head upright and tilted forward slightly, press fingers against left side of the nose to close the nostril.

3. Gently insert the tip of the applicator into the right nostril.

4. With the thumb supporting the bottom of the bottle, press down evenly on the white applicator with two fingers. Spray once in the right nostril. Breathe in through the nose and out through the mouth. Avoid blowing the nose for several minutes after using spray.

5. If the spray is used in both nostrils, repeat Step 4 for the left nostril (for the GNS 1.0 mg and 2.0 mg cohorts).

6. Replace the clear plastic cover and place the bottle in a cool, dry location out of direct sunlight.

For the IV treatment group, a dose of 1.0 mg of granisetron IV injection (Kytril® 1 ml, 3 mg/3 ml/vial) was administered over approximately 30 seconds. All IV administrations were conducted by site staff and under investigator supervision.

For the Oral treatment group, subject took a single dose (Kytril® 1.0 mg, one tablet) orally with 240 ml of water under investigator's supervision. A mouth check was performed by the investigator immediately after drug administration to ensure that the study drug had been swallowed.

6.3.5 Blood sample and vital sign collection

Venous blood samples were collected at 0 (pre-dose), 5, 10, 15, 20, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post-dose in the IN and IV treatment groups; or 0 (pre-dose), 15, 30, 45 minutes and 1, 1.33, 1.66, 2, 2.5, 3, 4, 6, 8, 10, 12, 24 and 36 hours post dose in the oral treatment group. Vital signs including blood pressure (BP),

heart rate, respiration rate, and body temperature were also collected approximately 30 minutes pre-dose, every hour during the first 3 hours after dosing, and then collected every 3 hours until discharging from the hospital as well as at each return visit for blood sample collection at 24 and 36 hours post dose. All the blood samples were kept at -70^{o}C until analysis. For safety, the frequency and type of clinical adverse effects (AEs), as well as results of clinical laboratory tests were recorded during the study period.

6.3.6 Blood sample processing

The plasma samples were obtained from the whole blood by a refrigerated (4^{o}C) centrifuge at 3000 rpm for 10 minutes. 100 μL of plasma was spiked with 100 μL of IS solution (1 ng/ml in acetonitrile) and mixed for 1 minute with a vortex mixer. Then the plasma sample was basified by adding 50 μL of 0.1 N NaOH solution and mixed for 1 minute. 2 ml of methyl tert-butyl ether was added into the plasma sample and mixed for 2 minutes with a vortex mixer, followed by a refrigerated centrifuge (4^{o}C) at 3000 rpm for 10 minutes. After the centrifuge, approximate 1.5 ml of the supernatant was transferred into a tube and evaporated to dryness in a 40 ^{o}C bath under nitrogen stream for about 15 minutes. The remainder was reconstituted with 250 μL of reconstitution solvent (Acetonitrile:H_2O:Formic acid = 20:80:0.1, V/V) and mixed for 1 minute. The mixed solution was centrifuged at 3000 rpm for 5 minutes. A 10 μL of supernatant aliquot was injected and analyzed on the LC-MS/MS system.

6.3.7 Analytical method

The plasma samples were analyzed for the assay of granisetron by a validated method described below (Table 6.6 and Table 6.7).

Table 6.6 HPLC method information

Chromatography Settings							
Column type	Synergi Polar-RP, 50 × 2.00 mm, 4 µm, Phenomenex						
Column switching	0.9-1.7 min to mass spec						
Column oven temperature	40ºC						
Mobile phase composition	A: Water: 1M Ammonium Acetate: Formic Acid /1000:5:1 (V:V:V) B: Acetonitrile: Formic Acid / 1000:1 (V:V)						
Autoinjector temperature	Ambient temperature						
Autoinjector wash solvent 1	Water: Formic Acid / 100:2 (V:V)						
Autoinjector wash solvent 2	Acetonitrile: Formic Acid / 100:2 (V:V)						
Flow rate	0.5 ml/min						
Analysis time	~4.5 min						
Injection volume	10 µL						
Retention time	Granisetron = ~1.19 min Granisetron-d_3 (IS) = ~1.18 min						
Program							
Time (min)	0.5	1.0	1.5	1.6	3.1	3.2	3.8
%B	20	60	60	95	95	20	20
Flow Rate (ml/min)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 6.7 Mass spectrometer parameters

Mass Spectrometer Settings	
Source Temperature (TEM)	550ºC
Collision Gas (CAD):	7 psig N_2
Curtain Gas (CUR):	20 psig N_2
Ion Source Gas 1 (GS1):	60 psig N_2

Ion Source Gas 2 (GS2):		70 psig N_2				
Ion Spray Voltage (IS):		5500 V				
Entrance Potential (EP):		8 V				
Scan duration:		2.5 min				
Compound	Ionization Mode	Dwell Time (msec)	Declustering Potential (V)	Collision Energy (eV)	Collision Exit Potential (V)	Transition (m/z)
Granisetron	TIS+	200	80	35	12	313.2 → 138.1
Granisetron-d_3 (IS)	TIS+	200	80	35	12	316.2 → 138.1

6.3.8 Data analysis

The PK parameters were estimated by using Phoenix WinNonlin®, version 6.3. Statistical analysis of PK data was performed by using SAS®, version 9.3. The estimated PK parameters of granisetron were maximum observed concentration (C_{max}), time of the maximum concentration (t_{max}), terminal elimination rate constant (K_{el}), area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), terminal elimination half-life ($t_{1/2}$), area under the concentration-time curve from time zero to time of the last quantifiable concentration (AUC_{0-t}), apparent total body clearance (CL/F) or total body clearance (CL), and absolute bioavailability (F_{abs}).

6.4 Results

6.4.1 Pharmacokinetic profiles and parameters of granisetron

The plasma concentration versus time curves of granisetron after intranasal, intravenous and oral administrations are shown as Figure 6.1. The pharmacokinetic parameters are listed in Table 6.8.

After intranasal administration of granisetron at the dose of 0.5 mg/subject, 1.0 mg/subject and 2.0 mg/subject, the plasma concentration versus time profiles showed that granisetron concentration increased to 3.47 ng/ml, 7.43 ng/ml and 8.50 ng/ml respectively by the first 15 minutes, followed by a gradual decrease to 0.108 ng/ml, 0.497 ng/ml and 0.782 ng/ml after 24 hours. The granisetron concentration rapidly reached the maximum value of 17.8 ng/ml within 5 minutes after intravenous administration of Kytril® IV Injection at the dose of 1.0 mg/subject. Nonetheless, the plasma concentration immediately dropped to 8.20 ng/ml by 15 minutes, implying a fast distribution of granisetron in body. Then the granisetron concentration decreased gradually to 0.556 ng/ml by 24 hours after dosing. Following oral administration of Kytril® Tablet (1.0 mg), the granisetron levels reached a concentration of 3.72 ng/ml by 2 hours after dosing, indicating a slower absorption of granisetron comparing to the intranasal administration. The plasma concentration dropped to a concentration of 0.154 ng/ml by 36 hours after dosing.

Table 6.8 provides the mean and standard deviation (SD) of pharmacokinetic parameters of granisetron administered via intranasal, intravenous and oral routes. Granisetron was rapidly absorbed after intranasal administration at the doses of 0.5 mg, 1.0, or 2.0 mg/subject. The maximum plasma concentrations of granisetron were attained at the mean t_{max} of approximate 15 minutes, regardless of different granisetron

doses. The mean t_{max} of granisetron after intranasal administration at the dose of 1.0 mg was 0.14 hour longer as compared to Kytril® IV Injection 1.0 mg, but was 1.68 hours shorter as compared to Kytril® Tablet 1.0 mg.

The mean $t_{1/2}$ of granisetron after intranasal dosing at 0.5, 1.0, and 2.0 mg/subject were 5.12, 5.99, and 6.95 hours, respectively. The mean $t_{1/2}$ of granisetron appears to be comparable among the intranasal cohorts with different granisetron doses. The mean $t_{1/2}$ of granisetron after Kytril® IV Injection 1.0 mg and Kytril® Tablet 1.0 mg were 6.11 hours and 7.03 hours, respectively. The mean $t_{1/2}$ of granisetron appears to be similar regardless of the administration routes.

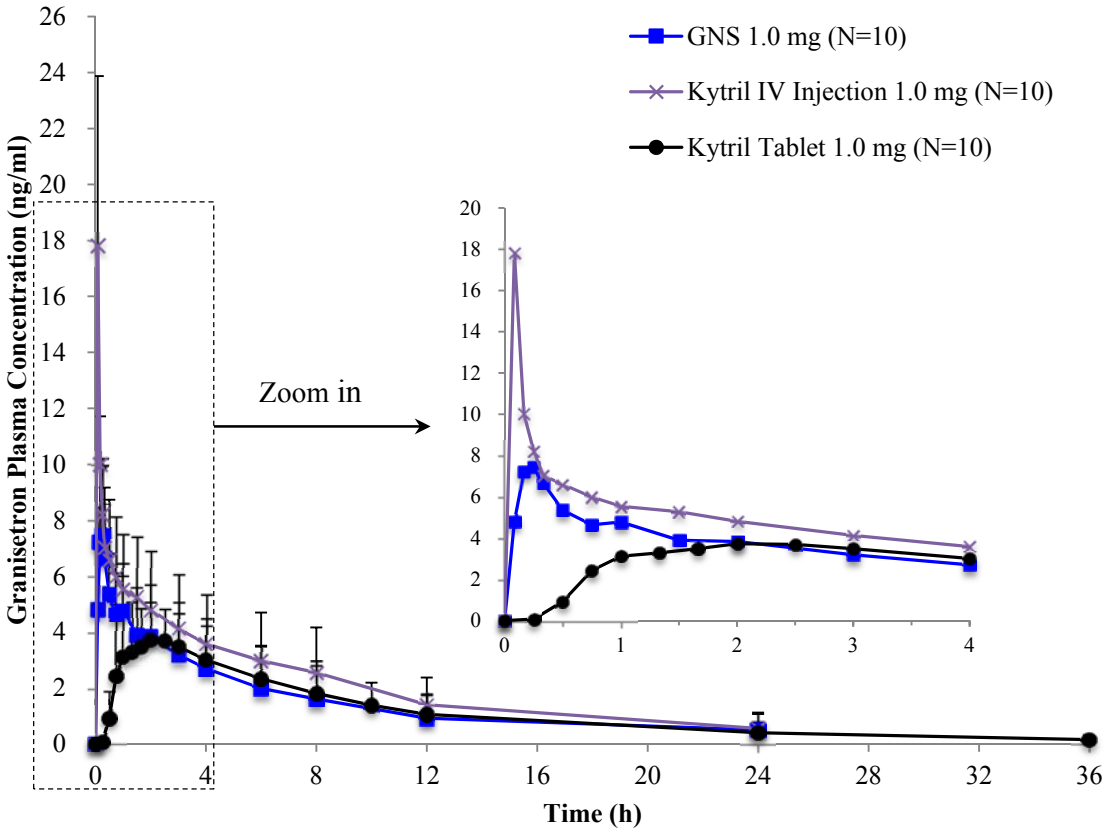
Following nasal administration of granisetron at 0.5, 1.0, and 2.0 mg/subject, the mean C_{max} of granisetron increased with the escalation of doses. The mean C_{max} of granisetron after dosing 1.0 mg of granisetron via nasal route was lower comparing to Kytril® IV Injection 1.0 mg (8.03 vs. 17.9 ng/ml), but was higher than that of Kytril® Tablet 1.0 mg (8.03 vs. 4.55 ng/ml).

The AUC_{0-t} and $AUC_{0-\infty}$ of nasally administered granisetron increased when the doses of intranasal administration increased from 0.5 to 2.0 mg/subject. Although the mean AUCs of nasally dosed granisetron at 1.0 mg/subject were less than those of Kytril® IV Injection 1.0 mg (AUC_{0-t}: 37.1 vs. 52.8 h·ng/ml; $AUC_{0-\infty}$: 44.7 vs. 59.2 h·ng/ml), they were comparable to those after Kytril® Tablet 1.0 mg (AUC_{0-t}: 37.1 vs. 37.6 h·ng/ml; $AUC_{0-\infty}$: 44.7 vs. 40.0 h·ng/ml).

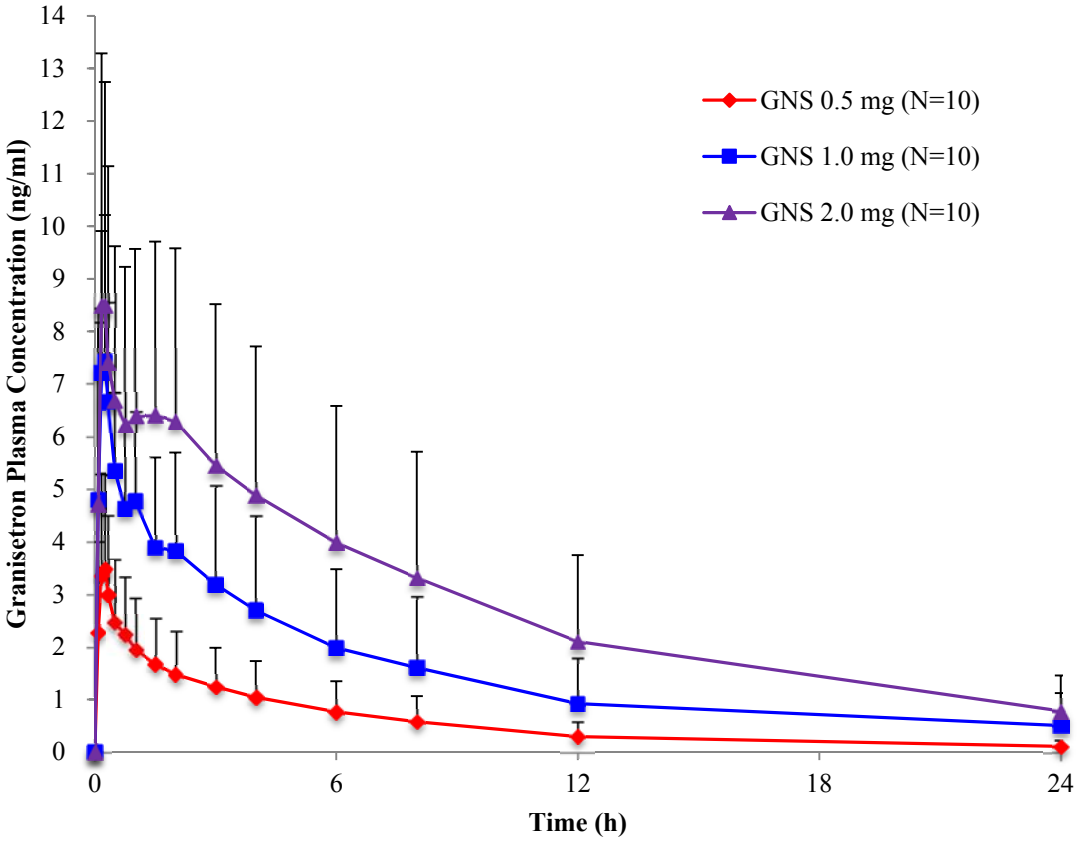
The mean CL/F of granisetron after intranasal administration at 0.5, 1.0, and 2.0 mg/subject were 65.5, 47.1, and 56.8 L/h, respectively. The mean CL/F of granisetron did not change significantly when the dose increased, which implied CL/F of granisetron was not dose-dependent after intranasal administration. The mean CL/F of

nasally administered granisetron was higher as compared to that of Kytril® Tablet at the dose of 1.0 mg/subject (47.1 vs. 35.9 L/h).

The absolute bioavailability (F_{abs}) of granisetron after intranasal administration at 0.5, 1.0, and 2.0 mg/subject was 50.4%, 75.5%, and 64.0%, respectively. The F_{abs} of granisetron was not changed by the increase of the doses, suggesting the intranasal absolute bioavailability of granisetron was not dose-dependent. The absolute bioavailability of nasally dosed granisetron was higher than that of Kytril® Tablet at the dose of 1.0 mg/subject (75.5% vs. 67.5%).



(a)



(b)

Figure 6.1 (a) Plasma concentration of granisetron in healthy volunteers after intranasal, oral and intravenous administrations. (b) Plasma concentration of granisetron through nasal route at the doses of 0.5, 1.0 and 2.0 mg/subject

Table 6.8 Summary (Mean and SD) of pharmacokinetic parameters of granisetron in healthy volunteers

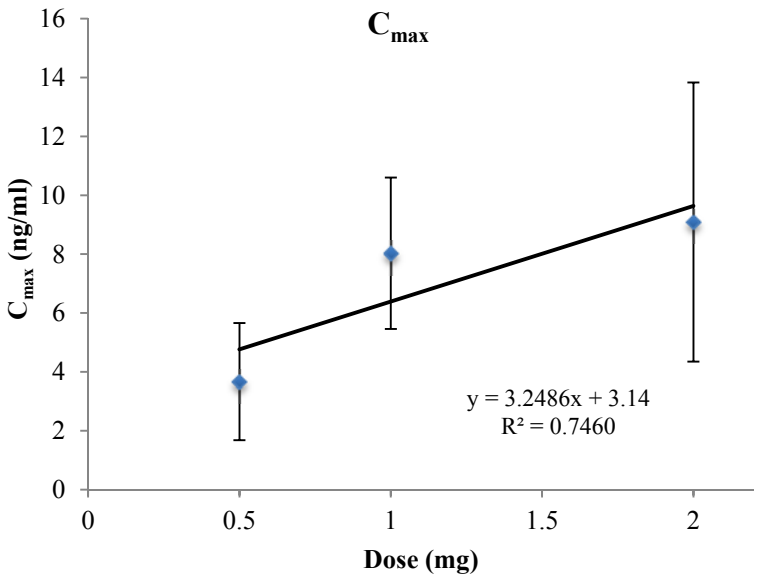
PK Parameter	Statistics	Intranasal			Intravenous	Oral
		GNS 0.5 mg (n=10)	GNS 1.0 mg (n=10)	GNS 2.0 mg (n=10)	Kytril® IV Injection 1.0 mg (n=10)	Kytril® Tablet 1.0 mg (n=10)
C_{max}, ng/ml	Mean (SD)	3.67 (1.99)	8.03 (2.57)	9.09 (4.74)	17.9 (5.97)	4.55 (2.07)
t_{max}, h		0.25 (0.07)	0.23 (0.05)	0.26 (0.10)	0.09 (0.03)	1.91 (1.05)
AUC_{0-t}, h·ng/ml		13.6 (8.48)	37.1 (25.1)	66.7 (42.9)	52.8 (28.3)	37.6 (19.9)
$AUC_{0-\infty}$, h·ng/ml		14.9 (9.33)	44.7 (35.2)	75.7 (50.8)	59.2 (34.7)	40.0 (22.4)
K_{el}, 1/h		0.210 (0.149)	0.203 (0.172)	0.120 (0.0829)	0.146 (0.107)	0.115 (0.0512)
$t_{1/2}$, h		5.12 (3.44)	5.99 (4.16)	6.95 (2.06)	6.11 (2.40)	7.03 (2.86)
CL/F, L/h		65.5 (85.7)	47.1 (42.9)	56.8 (81.1)	29.7 (32.7)	35.9 (26.0)
F_{abs}, % [a]		50.4	75.5	64.0	-	67.5

a: The ratio of mean $AUC_{0-\infty, treatment}$/Dose $_{treatment}$ to mean $AUC_{0-\infty, IV}$/Dose $_{IV}$.

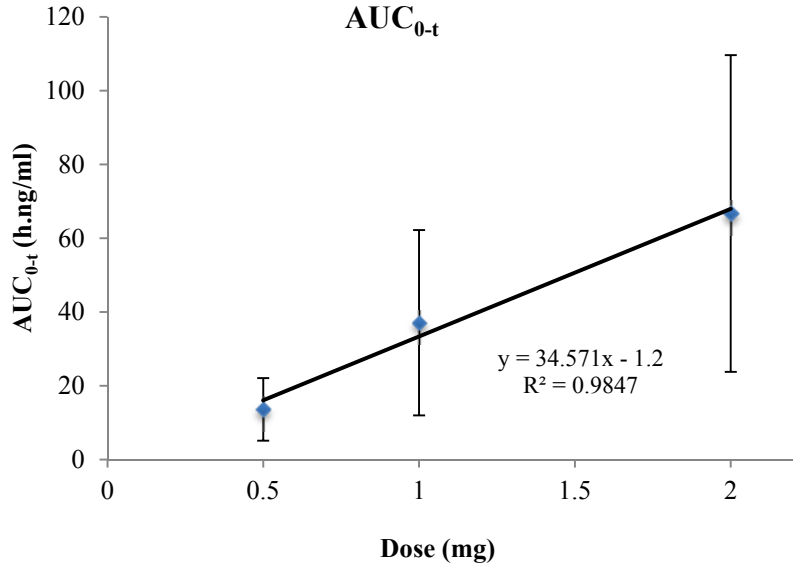
6.4.2 Dose Proportionality of granisetron after intranasal administration

After intranasal administration of granisetron at 0.5, 1.0 and 2.0 mg/subject, the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ increased proportionally with the dose escalation, which is shown as Figure 6.2. A linear fitting was conducted between the PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) and the doses, with the R-square values of 0.7460, 0.9847 and 0.9684 respectively.

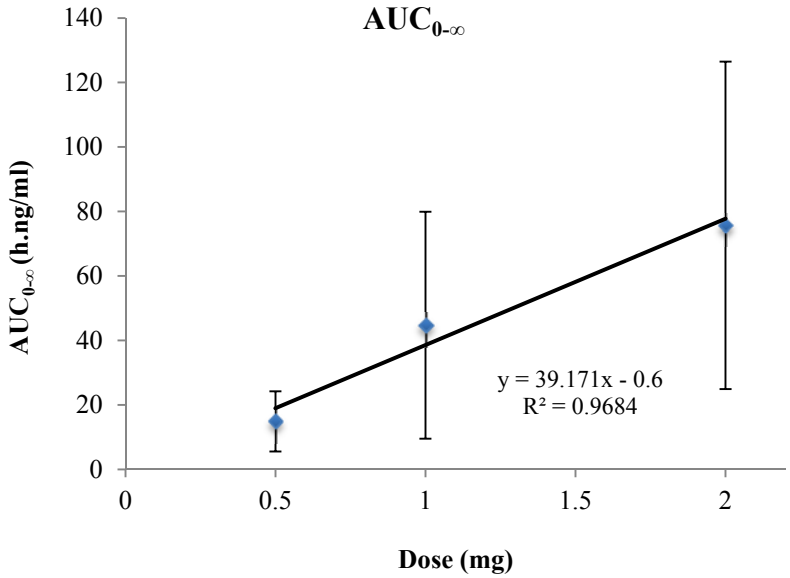
The logarithmic transformation was performed on C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Following GNS 0.5, 1.0, and 2.0 mg, the slope (β) for the logarithmic values of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 0.693 (95% confidence interval, 0.297-1.09), 1.13 (95% confidence interval, 0.579, 1.68), and 1.15 (95% confidence interval, 0.558-1.74), respectively (Table 6.9). The analysis revealed that the 95% confidence interval of the slope (β) included 1, indicating that the C_{max} and AUCs of granisetron were dose proportional over the dose range of 0.5 to 2.0 mg/subject after intranasal administration.



(a)



(b)



(c)

Figure 6.2 Regression analysis of mean (a) maximum plasma concentration (C_{max}) and (b, c) area under the curve on the ascending dose of intranasal granisetron (Mean ± SD, n=10, half male and half female)

Table 6.9 Statistical evaluation dose proportionality of pharmacokinetic parameters of granisetron after three intranasal doses of granisetron nasal spray at 0.5, 1.0 and 2.0 mg/subject in healthy volunteers

Parameter	N	β	
		Point Estimate	95% Confidence Interval
C_{max}, ng/ml	30	0.693	(0.297, 1.09)
AUC_{0-t}, h·ng/ml	30	1.13	(0.579, 1.68)
$AUC_{0-\infty}$, h·ng/ml	30	1.15	(0.558, 1.74)

6.4.3 Tolerability and Safety

A total of 55 treatment-emergent adverse events (TEAEs) were reported by 25 subjects in the study (Table 6.10). All events were mild, unrelated to test drugs, and resolved without change in test drugs and treatment required. All TEAEs were resolved by the end of the study or without any complaint at follow-up telephone contact.

Table 6.10 Treatment-Emergent Adverse Events

Variable	1st IN Group GNS 0.5 mg (N=10)			2nd IN Group GNS 1.0 mg (N=10)			3rd IN Group GNS 2.0 mg (N=10)			IV Group (N=10)			PO Group (N=10)		
	Event	Subject		Event	Subject		Event	Subject		Event	Subject		Event	Subject	
	E	n	(%)	E	n	(%)	E	n	(%)	E	n	(%)	E	n	(%)
Subject with any AE*	19	7	70.00	14	7	70.00	6	3	30.00	8	5	50.00	8	3	30.00
Subjects with SAE**	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00

*AE: Adverse Event. **SAE: Severe Adverse Event.
The AE incidence rate: 100%×The number of subjects with event (n) / The number of subjects in the treatment group (N), where N=10.

The most common TEAE in this study was asymptomatic hypotension (defined as systolic BP less than 90 mmHg or diastolic BP less than 60 mmHg), which was reported in 7/10 (70.00%), 6/10 (60.00%), 3/10 (30.00%), 4/10 (40.00%), and 3/10 (30.00%) subjects in the intranasal (GNS 0.5, 1.0, and 2.0 mg), intravenous and oral groups, respectively. Asymptomatic heart rate decreased (defined as heart rate less than 60

beats/min) was the next common TEAE reported in this study, with 4/10 (40.00%), 2/10 (20.00%), 2/10 (20.00%), 2/10 (20.00%), and 2/10 (20.00%) subjects reported in the intranasal (GNS 0.5, 1.0, and 2.0 mg), intravenous, and oral groups, respectively (Table 6.11). All these AEs are unrelated to the study medication. There was no correlation between dose and AEs, and no difference in the control groups (PO and IV) as compared with the treatment group (IN). None of the AEs required intervention.

Table 6.11 Adverse events by treatments

Preferred Term n (%)	GNS 0.5 mg (N=10)	GNS 1.0 mg (N=10)	GNS 2.0 mg (N=10)	IV Group (N=10)	PO Group (N=10)
Hypotension	7 (70.00%)	6 (60.00%)	3 (30.00%)	4 (40.00%)	3 (30.00%)
Heart rate decreased	4 (40.00%)	2 (20.00%)	2 (20.00%)	2 (20.00%)	2 (20.00%)
Dizziness	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (10.00%)	0 (0.00%)
Haematuria	1 (10.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Hypertension	0 (0.00%)	1 (10.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Statistical analysis was performed to compare the incidence of asymptomatic hypotension (defined as systolic BP less than 90 mmHg or diastolic BP less than 60 mmHg) and asymptomatic heart rate decreased (defined as heart rate less than 60 beats/min) among groups in this study (Table 6.12). The Statistical results showed that there were no statistically significant differences among groups for the incidence of both heart rate decreased and hypotension (p-value > 0.05, Table 6.12), either with or without subjects who had systolic BP less than 90 mmHg, diastolic BP less than 60 mmHg or heart rate less than 60 beats/min at screening visit or pre-dose. There appeared to be no consistent relationship between the dose or route of drug administration and these vital sign parameters.

Table 6.12 Statistical analysis for Treatment-Emergent Adverse Events

System Organ Class / Preferred Term	1st IN Group GNS 0.5 mg (N=10)			2nd IN Group GNS 1.0 mg (N=10)			3rd IN Group GNS 2.0 mg (N=10)			IV Group (N=10)			PO Group (N=10)			
	Event	Subject		Event	Subject		Event	Subject		Event	Subject		Event	Subject		
	E	n	(%)	E	n	(%)	E	n	(%)	E	n	(%)	E	n	(%)	P-value*
Heart rate disorders																
------Total	6	4	40.00	3	2	20.00	3	2	20.00	2	2	20.00	4	2	20.00	NA
Heart rate decreased - with subjects who had heart rate < 60 beats/min at screening visit or pre-dose	6	4	40.00	3	2	20.00	3	2	20.00	2	2	20.00	4	2	20.00	1.0000
Heart rate decreased - without subjects who had heart rate < 60 beats/min at screening visit or pre-dose	4	3	30.00	3	2	20.00	3	2	20.00	2	2	20.00	3	1	10.00	0.8829
Vascular disorders																
------Total	12	7	70.00	11	7	70.00	3	3	30.00	5	4	40.00	4	3	30.00	NA
Hypotension - with subjects who had DBP < 60 mmHg or SBP < 90 mmHg at screening visit or pre-dose	12	7	70.00	10	6	60.00	3	3	30.00	5	4	40.00	4	3	30.00	0.4545
Hypotension - without subjects who had DBP < 60 mmHg or SBP < 90 mmHg at screening visit or pre-dose	12	7	70.00	7	5	50.00	3	3	30.00	4	3	30.00	4	3	30.00	0.4461

The AE incidence rate: 100%×The number of subjects with event (n) / The number of subjects in the treatment group (N)
*Fisher's exact test was performed to examine among groups. NA: not applicable.

6.5 Discussion

The Phase I clinical trial provides preliminary information on how the human bodies cope with investigational new drugs. The primary aims of Phase I clinical trial are two-pronged: (1) to evaluate the pharmacologic activities of the investigational drug in vivo, involving the side effects associated with the increase of doses, and the initial efficacy information of the drug; (2) to collect enough pharmacokinetic and pharmacological information of the investigational drug in human for the Phase II clinical trial. In this study, the pharmacokinetics, safety and tolerability of 3 intranasal doses (0.5, 1.0 and 2.0 mg/subject) of granisetron hydrochloride nasal sprays were determined and compared to those of granisetron intravenous injection and granisetron oral tablets in healthy volunteers.

Granisetron is a 5-HT3 receptor antagonist which is available as intravenous injection, oral tablet, oral solution, transdermal patch and subcutaneous injection on the market. This Phase I clinical trial of granisetron nasal spray is the first study to administer the drug in human through intranasal route. The first dose in human was determined from no observed adverse effect level (NOAEL) in rat toxicology study, after dose conversion and safety factor [205]. The NOAEL is derived from appropriate animal studies and regarded as a vital benchmark for determining a relatively safe starting dose of new drugs in healthy volunteers. In an acute (7-day) toxicity study in SD rats, the SD rats were administered by intranasal instillation of granisetron solution at increasing doses of 0.8, 1.6 and 3.2 mg/kg for 7 consecutive days. No accumulation of granisetron or any other adverse effect was observed in the rats after once daily repeated administration for 7 consecutive days. Therefore, the NOAEL is 3.2 mg/kg for intranasal granisetron in SD rats. After the determination of NOAEL in rats, the

parameter was converted to human equivalent dose (HED) according to body surface area. There are several rules for dose conversion: (1) larger animals usually have slower physiological process and lower metabolic rates; (2) the dose on weight basis for large animals is lower than the small animals; (3) the interspecies variation in pharmacokinetics is believed to be the consequence of the body size (allometry); (4) the allometric scaling is not applicable to the dose conversion between adults and kids. Although normalization based on body surface area (mg/m^2) is prevalently used in dose conversion between different species, it is not recommended to extrapolate the doses using the body surface area normalization method when the drug is administered via alternative routes (e.g., intranasal, topical, subcutaneous, intramuscular). In this case, the K_m factors (see Table 6.2), which is obtained by dividing the average body weight (kg) by the body surface area (m^2), were used to convert the rat dose to human equivalent dose (HED) according to the Equation 6.1. For safety's purpose, the HED was further divided by the safety factor of 10 as the first intranasal dose in human. Granisetron is considered to be safe with minor side effects in patients. It has been reported that a case of granisetron overdose up to 38.5 mg by IV injection, resulting only slight headache in patient. Granisetron at ascending intravenous doses up to 300 μg/kg (~15 mg/subject) was reported to be well tolerated without serious adverse events in healthy male volunteers [206]. Our clinical results showed that the initial dose of 0.5 mg/subject should be safe and well tolerated.

In this clinical study in 50 healthy volunteers (25 males and 25 females), the pharmacokinetics of granisetron after intranasal administration was evaluated and compared with those after IV and PO administration. The PK parameters of granisetron following oral administration of Kytril® Tablet (1.0 mg) in this study was comparable to the data in previous studies [207] (Table 6.13).

Table 6.13 The comparison of pharmacokinetic parameters of granisetron

PK Parameter Mean (Range)	Current Study (single oral dose)	Published Data (single oral dose)
N	10	25
Dose, mg	1.0	1.0
C_{max}, ng/ml	4.55 (2.01-9.76)	4.10 (0.58-7.37)
$AUC_{0-\infty}$, h·ng/ml	40.0 (10.6-80.8)	43.7 (2.85-142)
$t_{1/2}$, h	7.03 (3.09-13.0)	8.74 (2.40-19.9)

Granisetron is predominantly metabolized in the liver through oxidation and subsequent conjugation. The major metabolite of granisetron in human is 7-hydroxy granisetron after oral and intravenous administration, and is independent of the administration route, gender and dose level [95]. It is generally believed that 7-hydroxy granisetron has low affinity to the 5HT3 receptors and negligible contribution to the antiemetic pharmacological effects in clinical use, therefore 7-hydroxy granisetron was not monitored and quantified in the current study.

It was reported the adverse events after Kytril® IV injection included headache (14%), asthenia (5%), somnolence (4%), diarrhea (4%) and constipation (3%) in a clinical trial with 1268 patients. However, the study could not determine the ratio of the adverse events caused by the administration of Kytril® due to the absence of a control group. In our study, no clinically significant abnormal nasal cavity examination results, no abnormal physical examination findings, and no clinically significant abnormal electrocardiogram (ECG) results were observed. Several subjects in the study had blood pressure recordings that were outside of the "normal" range. However, these blood pressure or pulse rates are not unusual for young healthy subjects. And in this study, there were no symptoms associated with these vital sign measurements. Abnormal vital

signs were recorded in all dose groups without a dose related increase in incidence. There was no difference in the frequency of abnormal vital sign measurements between the routes of administration. In summary, granisetron hydrochloride nasal sprays (0.5, 1.0, and 2.0 mg) are generally safe and well tolerated in healthy subjects as compared to Kytril® IV Injection (1.0 mg) and Kytril® Tablet (1.0 mg).

The granisetron nasal spray is designed to be given in two dosing regimens. One is to use 30 minutes before chemotherapy, which is similar with the dosing regimen of Kytril® IV Injection. Another regimen is designed as a rescue treatment. The patient can use it by themselves when they experience vomiting or feel nausea. If the patients feel nausea at home, there's no time to go to hospital or clinic for treatment. Furthermore, taking Kytril® Tablets is not ideal due to ongoing nausea. In that case, our product is an excellent alternative treatment with rapid onset and non-invasive administration. Further clinical trials are required for determining the dosing regimens.

6.6 Conclusion

According to the results of the current clinical trial involving 50 healthy volunteers (25 males and 25 females), granisetron could be absorbed rapidly through intranasal route and attain comparable bioavailability as oral administration. The absolute bioavailability of granisetron after intranasal and oral administrations at the dose of 1.0 mg was 75.5% and 67.5%, respectively. However, the absorption of granisetron after oral administration is much slower than that of intranasal administration, with the t_{max} of 0.23 hour (IN) and 1.91 hours (PO) respectively.

In the dose escalation study, granisetron hydrochloride nasal spray exhibited dose-proportional pharmacokinetics (C_{max} and AUCs) over the dose range of 0.5 to 2.0 mg. As to the safety and tolerability studies, no clinically significant abnormal nasal cavity examination results, no abnormal physical examination findings, and no clinically significant abnormal electrocardiogram (ECG) results were observed. For the adverse events (AEs) during the current clinical trial, no correlation between the AEs and the doses of granisetron nasal spray was found. There was no difference in the frequency of abnormal vital sign measurements in the treatment group (IN) as compared with the control groups (PO and IV). Granisetron hydrochloride nasal sprays (dose: 0.5, 1.0, and 2.0 mg) are generally safe and well tolerated in comparison with intravenous and oral administrations of Kytril® (1.0 mg).

Chapter 7

Overall Conclusion

In this study, granisetron was successfully formulated into bioadhesive nasal spray. HPMC was used as mucoadhesive vehicle to improve drug intranasal absorption by prolonging the residence time of drug solution in nasal cavity. The rats were exposed to the parent drug after intranasal administration of granisetron formulations. Compared to the slow and incomplete absorption of oral granisetron, rapid and complete absorption of granisetron was achieved in rats, with significant earlier and higher drug plasma concentration which was comparable to intravenous administration. In pharmacokinetic study in rats, the granisetron nasal spray solution containing 0.25% of HPMC exhibited the highest bioavailability and rapid absorption. A dose-proportional manner was observed over the dose ranges of 0.4 to 1.6 mg/kg in rats after intranasal administration of granisetron formulations. In the brain pharmacokinetic study, no obvious nose-to-brain transport was observed in rats after intranasal administration of granisetron.

In the dog pharmacokinetic study, the granisetron nasal spray solution can achieve faster absorption with higher bioavailability comparing to oral administration. The C_{max} and $AUC_{0-\infty}$ also increased with the dose in a dose-proportional manner over the dose range of 0.5 - 2.0 mg/dog after intranasal administration of granisetron formulations. The intranasally administered granisetron could bypass the hepatic first-pass metabolism, resulting in 50% reduction of the major metabolite (7-OH granisetron) as compared to that after oral administration.

In the clinical trial involving 50 healthy volunteers (25 males and 25 females), the

bioavailability of intranasal granisetron was comparable to that of oral granisetron, but with faster absorption. The dose escalation study exhibited dose proportional pharmacokinetics after intranasal administration over the dose range of 0.5 to 2.0 mg. There was no correlation between the adverse events and the intranasal doses of granisetron, indicating the granisetron nasal sprays were generally safe and well tolerated comparing to intravenous and oral administration of Kytril®.

References

1. de Boer-Dennert, M., et al., *Patient perceptions of the side-effects of chemotherapy: the influence of 5HT3 antagonists.* Br J Cancer, 1997. **76**(8): p. 1055-61.

2. Hickok, J.T., et al., *Nausea and emesis remain significant problems of chemotherapy despite prophylaxis with 5-hydroxytryptamine-3 antiemetics: a University of Rochester James P. Wilmot Cancer Center Community Clinical Oncology Program Study of 360 cancer patients treated in the community.* Cancer, 2003. **97**(11): p. 2880-6.

3. Schnell, F.M., *Chemotherapy-induced nausea and vomiting: the importance of acute antiemetic control.* Oncologist, 2003. **8**(2): p. 187-98.

4. Lindley, C.M., et al., *Quality of life consequences of chemotherapy-induced emesis.* Qual Life Res, 1992. **1**(5): p. 331-40.

5. Rhodes, V.A. and R.W. McDaniel, *Nausea, vomiting, and retching: complex problems in palliative care.* CA Cancer J Clin, 2001. **51**(4): p. 232-48; quiz 249-52.

6. Sanger, G.J. and P.L. Andrews, *Treatment of nausea and vomiting: gaps in our knowledge.* Auton Neurosci, 2006. **129**(1-2): p. 3-16.

7. Wilhelm, S.M., M.L. Dehoorne-Smith, and P.B. Kale-Pradhan, *Prevention of postoperative nausea and vomiting.* Ann Pharmacother, 2007. **41**(1): p. 68-78.

8. Andrews, P.L., W.G. Rapeport, and G.J. Sanger, *Neuropharmacology of emesis induced by anti-cancer therapy.* Trends Pharmacol Sci, 1988. **9**(9): p. 334-41.

9. Osoba, D., et al., *Effect of postchemotherapy nausea and vomiting on health-related quality of life. The Quality of Life and Symptom Control Committees of the National Cancer Institute of Canada Clinical Trials Group.* Support Care Cancer, 1997. **5**(4): p. 307-13.

10. Sussman, N., *Reactions of patients to the diagnosis and treatment of cancer.* Anticancer Drugs, 1995. **6 Suppl 1**: p. 4-8.

11. Naeim, A., et al., *Evidence-based recommendations for cancer nausea and vomiting.* J Clin Oncol, 2008. **26**(23): p. 3903-10.

12. Herrstedt, J., *Nausea and emesis: still an unsolved problem in cancer patients?* Support Care Cancer, 2002. **10**(2): p. 85-7.

13. Network, N.C.C., *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): antiemesis.* Version 2.2015. September 22, 2015. .

14. Durand, J.P., I. Madelaine, and F. Scotte, *[Guidelines for prophylaxis and treatment of chemotherapy-induced nausea and vomiting]*. Bull Cancer, 2009.

96(10): p. 951-60.

15. Basch, E., et al., *Antiemetics: american society of clinical oncology clinical practice guideline update.* J Oncol Pract, 2011. **7**(6): p. 395-8.

16. Jordan, K., H.J. Schmoll, and M.S. Aapro, *Comparative activity of antiemetic drugs.* Crit Rev Oncol Hematol, 2007. **61**(2): p. 162-75.

17. Roila, F., et al., *Delayed emesis: incidence, pattern, prognostic factors and optimal treatment.* Support Care Cancer, 2002. **10**(2): p. 88-95.

18. Roscoe, J.A., et al., *Anticipatory nausea and vomiting.* Support Care Cancer, 2011. **19**(10): p. 1533-8.

19. Morrow, G.R., et al., *Nausea and emesis: evidence for a biobehavioral perspective.* Support Care Cancer, 2002. **10**(2): p. 96-105.

20. Darmani, N.A. and A.P. Ray, *Evidence for a re-evaluation of the neurochemical and anatomical bases of chemotherapy-induced vomiting.* Chem Rev, 2009. **109**(7): p. 3158-99.

21. *ASHP Therapeutic Guidelines on the Pharmacologic Management of Nausea and Vomiting in Adult and Pediatric Patients Receiving Chemotherapy or Radiation Therapy or Undergoing Surgery.* Am J Health Syst Pharm, 1999. **56**(8): p. 729-64.

22. Hornby, P.J., *Central neurocircuitry associated with emesis.* Am J Med, 2001. **111 Suppl 8A**: p. 106s-112s.

23. Rubenstein, E.B., et al., *New approaches to chemotherapy-induced nausea and vomiting: from neuropharmacology to clinical investigations.* Cancer J, 2006. **12**(5): p. 341-7.

24. Feyer, P. and K. Jordan, *Update and new trends in antiemetic therapy: the continuing need for novel therapies.* Ann Oncol, 2011. **22**(1): p. 30-8.

25. Kobrinsky, N.L., *Regulation of nausea and vomiting in cancer chemotherapy. A review with emphasis on opiate mediators.* Am J Pediatr Hematol Oncol, 1988. **10**(3): p. 209-13.

26. Borison, H.L., *Area postrema: chemoreceptor circumventricular organ of the medulla oblongata.* Prog Neurobiol, 1989. **32**(5): p. 351-90.

27. Maolood, N. and B. Meister, *Protein components of the blood-brain barrier (BBB) in the brainstem area postrema-nucleus tractus solitarius region.* J Chem Neuroanat, 2009. **37**(3): p. 182-95.

28. Willis, C.L., C.J. Garwood, and D.E. Ray, *A size selective vascular barrier in the rat area postrema formed by perivascular macrophages and the extracellular matrix.* Neuroscience, 2007. **150**(2): p. 498-509.

29. Navari, R.M., *Olanzapine for the prevention and treatment of chronic nausea*

and chemotherapy-induced nausea and vomiting. European Journal of Pharmacology, 2014. **722**: p. 180-186.

30. Johnston, K.D., *The potential for mu-opioid receptor agonists to be anti-emetic in humans: a review of clinical data.* Acta Anaesthesiol Scand, 2010. **54**(2): p. 132-40.

31. Apfel, C.C. and L. Jalota, *Can central antiemetic effects of opioids counter-balance opioid-induced nausea and vomiting?* Acta Anaesthesiol Scand, 2010. **54**(2): p. 129-31.

32. Ho, K.Y. and T.J. Gan, *Pharmacology, pharmacogenetics, and clinical efficacy of 5-hydroxytryptamine type 3 receptor antagonists for postoperative nausea and vomiting.* Curr Opin Anaesthesiol, 2006. **19**(6): p. 606-11.

33. Perez, E.A., *Use of dexamethasone with 5-HT3-receptor antagonists for chemotherapy-induced nausea and vomiting.* Cancer J Sci Am, 1998. **4**(2): p. 72-7.

34. Jordan, K., et al., *Granisetron versus tropisetron for prophylaxis of acute chemotherapy-induced emesis: a pooled analysis.* Support Care Cancer, 2005. **13**(1): p. 26-31.

35. Warr, D., et al., *Use of 5-HT3 receptor antagonists in patients receiving moderately or highly emetogenic chemotherapy*. Vol. 8. 2001. 69-82.

36. del Giglio, A., et al., *Granisetron is equivalent to ondansetron for prophylaxis of chemotherapy-induced nausea and vomiting: results of a meta-analysis of randomized controlled trials.* Cancer, 2000. **89**(11): p. 2301-8.

37. Lopez-Rodriguez, M.L., et al., *Benzimidazole derivatives. 2. Synthesis and structure-activity relationships of new azabicyclic benzimidazole-4-carboxylic acid derivatives with affinity for serotoninergic 5-HT(3) receptors.* J Med Chem, 1999. **42**(24): p. 5020-8.

38. Perez, E.A., et al., *Comparison of single-dose oral granisetron versus intravenous ondansetron in the prevention of nausea and vomiting induced by moderately emetogenic chemotherapy: a multicenter, double-blind, randomized parallel study.* J Clin Oncol, 1998. **16**(2): p. 754-60.

39. Rockville, *Guidance for industry: food-effect bioavailability and fed bioequivalence studies*, F.D.A. Center for Drug Evaluation and Research, Editor. 2002 Dec.

40. Hesketh, P.J., et al., *Methodology of antiemetic trials: response assessment, evaluation of new agents and definition of chemotherapy emetogenicity.* Support Care Cancer, 1998. **6**(3): p. 221-7.

41. Kamanabrou, D., *Intravenous granisetron--establishing the optimal dose. The Granisetron Study Group.* Eur J Cancer, 1992. **28A Suppl 1**: p. S6-11.

42. Ettinger, D.S., et al., *A double-blind comparison of the efficacy of two dose regimens of oral granisetron in preventing acute emesis in patients receiving moderately emetogenic chemotherapy.* Cancer, 1996. **78**(1): p. 144-51.

43. Uchida, M., et al., *Antiemetic efficacy and safety of granisetron or palonosetron alone and in combination with a corticosteroid for ABVD therapy-induced nausea and vomiting.* J Pharm Health Care Sci, 2018. **4**: p. 1.

44. Roila, F., et al., *2016 MASCC and ESMO guideline update for the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting and of nausea and vomiting in advanced cancer patients.* Ann Oncol, 2016. **27**(suppl 5): p. v119-v133.

45. Oge, A., et al., *Comparison of granisetron, ondansetron and tropisetron for control of vomiting and nausea induced by cisplatin.* J Chemother, 2000. **12**(1): p. 105-8.

46. Yalcin, S., et al., *Serotonin receptor antagonists in prophylaxis of acute and delayed emesis induced by moderately emetogenic, single-day chemotherapy: a randomized study.* Am J Clin Oncol, 1999. **22**(1): p. 94-6.

47. Ahmed, S., et al., *Provesicular granisetron hydrochloride buccal formulations: in vitro evaluation and preliminary investigation of in vivo performance.* Eur J Pharm Sci, 2014. **60**: p. 10-23.

48. Shankar, A., et al., *Current Trends in Management of Oral Mucositis in Cancer Treatment.* Asian Pacific journal of cancer prevention : APJCP. **18**(8): p. 2019-2026.

49. Mitra, A.K. and R. Krishnamoorthy, *Prodrugs for nasal drug delivery.* Adv Drug Deliv Rev, 1998. **29**(1-2): p. 135-146.

50. AlBalawi, Z.H., S.S. Othman, and K. Alfaleh, *Intranasal ipratropium bromide for the common cold.* Cochrane Database Syst Rev, 2013(6): p. Cd008231.

51. Berkhout, M.C., et al., *Systemic absorption of nasally administered tobramycin and colistin in patients with cystic fibrosis.* J Antimicrob Chemother, 2014. **69**(11): p. 3112-5.

52. Haque, S., et al., *Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression.* J Psychiatr Res, 2014. **48**(1): p. 1-12.

53. Fortuna, A., et al., *Intranasal delivery of systemic-acting drugs: small-molecules and biomacromolecules.* Eur J Pharm Biopharm, 2014. **88**(1): p. 8-27.

54. Kapoor, M., J.C. Cloyd, and R.A. Siegel, *A review of intranasal formulations for the treatment of seizure emergencies.* Journal of Controlled Release, 2016. **237**: p. 147-159.

55. Chaturvedi, M., M. Kumar, and K. Pathak, *A review on mucoadhesive polymer*

used in nasal drug delivery system. J Adv Pharm Technol Res, 2011. **2**(4): p. 215-22.

56. Jones, N., *The nose and paranasal sinuses physiology and anatomy.* Adv Drug Deliv Rev, 2001. **51**(1-3): p. 5-19.

57. Huang, C.H., et al., *Mechanism of nasal absorption of drugs I: Physicochemical parameters influencing the rate of in situ nasal absorption of drugs in rats.* J Pharm Sci, 1985. **74**(6): p. 608-11.

58. Mathias, N.R. and M.A. Hussain, *Non-invasive systemic drug delivery: developability considerations for alternate routes of administration.* J Pharm Sci, 2010. **99**(1): p. 1-20.

59. Duvvuri, S., S. Majumdar, and A.K. Mitra, *Drug delivery to the retina: challenges and opportunities.* Expert Opin Biol Ther, 2003. **3**(1): p. 45-56.

60. Dhuria, S.V., L.R. Hanson, and W.H. Frey, 2nd, *Intranasal delivery to the central nervous system: mechanisms and experimental considerations.* J Pharm Sci, 2010. **99**(4): p. 1654-73.

61. Alam, M.I., et al., *Strategy for effective brain drug delivery.* Eur J Pharm Sci, 2010. **40**(5): p. 385-403.

62. Bojsen-Moller, F., *Demonstration of terminalis, olfactory, trigeminal and perivascular nerves in the rat nasal septum.* J Comp Neurol, 1975. **159**(2): p. 245-56.

63. Gänger, S. and K. Schindowski, *Tailoring Formulations for Intranasal Nose-to-Brain Delivery: A Review on Architecture, Physico-Chemical Characteristics and Mucociliary Clearance of the Nasal Olfactory Mucosa.* Pharmaceutics, 2018. **10**(3): p. 116.

64. Tayebati, S.K., I.E. Nwankwo, and F. Amenta, *Intranasal drug delivery to the central nervous system: present status and future outlook.* Curr Pharm Des, 2013. **19**(3): p. 510-26.

65. Lochhead, J.J. and R.G. Thorne, *Intranasal delivery of biologics to the central nervous system.* Adv Drug Deliv Rev, 2012. **64**(7): p. 614-28.

66. Jansson, B. and E. Bjork, *Visualization of in vivo olfactory uptake and transfer using fluorescein dextran.* J Drug Target, 2002. **10**(5): p. 379-86.

67. Thorne, R.G. and W.H. Frey, 2nd, *Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations.* Clin Pharmacokinet, 2001. **40**(12): p. 907-46.

68. Hussain, A., et al., *Does increasing the lipophilicity of peptides enhance their nasal absorption?* J Pharm Sci, 1991. **80**(12): p. 1180-1.

69. Corbo, D.C., J.C. Liu, and Y.W. Chien, *Characterization of the barrier*

properties of mucosal membranes. J Pharm Sci, 1990. **79**(3): p. 202-6.

70. Inagaki, M., et al., *Macromolecular permeability of the tight junction of the human nasal mucosa.* Rhinology, 1985. **23**(3): p. 213-21.

71. Lee, V.H., *Enzymatic barriers to peptide and protein absorption.* Crit Rev Ther Drug Carrier Syst, 1988. **5**(2): p. 69-97.

72. Westin, U., et al., *Transfer of morphine along the olfactory pathway to the central nervous system after nasal administration to rodents.* Eur J Pharm Sci, 2005. **24**(5): p. 565-73.

73. Merkus, F.W., et al., *Nasal mucociliary clearance as a factor in nasal drug delivery.* Adv Drug Deliv Rev, 1998. **29**(1-2): p. 13-38.

74. Washington, N., et al., *Determination of baseline human nasal pH and the effect of intranasally administered buffers.* Int J Pharm, 2000. **198**(2): p. 139-46.

75. Ohwaki, T., et al., *Effects of dose, pH, and osmolarity on nasal absorption of secretin in rats.* J Pharm Sci, 1985. **74**(5): p. 550-2.

76. Zaki, N.M., et al., *Rapid-onset intranasal delivery of metoclopramide hydrochloride. Part I. Influence of formulation variables on drug absorption in anesthetized rats.* Int J Pharm, 2006. **327**(1-2): p. 89-96.

77. Haschke, M., et al., *Pharmacokinetics and pharmacodynamics of nasally delivered midazolam.* Br J Clin Pharmacol, 2010. **69**(6): p. 607-16.

78. Tachibana, T., et al., *Predicting drug-drug interactions involving the inhibition of intestinal CYP3A4 and P-glycoprotein.* Curr Drug Metab, 2010. **11**(9): p. 762-77.

79. Donnelly, A., et al., *Absorption enhancers as tools to determine the route of nasal absorption of peptides.* J Drug Target, 1998. **5**(2): p. 121-7.

80. Karasulu, E., et al., *Permeation studies and histological examination of sheep nasal mucosa following administration of different nasal formulations with or without absorption enhancers.* Drug Deliv, 2008. **15**(4): p. 219-25.

81. R Putheti, R., M. C Patil, and O. Omokaro, *Nasal Drug delivery in Pharmaceutical and biotechnology: Present and future*. Vol. 4. 2009.

82. Alagusundaram, M., et al., *Nasal drug delivery system - an overview*. Vol. 1. 2010.

83. Mahdi, M.H., B.R. Conway, and A.M. Smith, *Development of mucoadhesive sprayable gellan gum fluid gels.* Int J Pharm, 2015. **488**(1-2): p. 12-9.

84. Hansen, K., et al., *Feasibility Investigation of Cellulose Polymers for Mucoadhesive Nasal Drug Delivery Applications.* Mol Pharm, 2015. **12**(8): p. 2732-41.

85. Rassu, G., et al., *Solid microparticles based on chitosan or methyl-beta-cyclodextrin: a first formulative approach to increase the nose-to-brain transport of deferoxamine mesylate.* J Control Release, 2015. **201**: p. 68-77.

86. Vidgren, M.T. and H. Kublik, *Nasal delivery systems and their effect on deposition and absorption.* Adv Drug Deliv Rev, 1998. **29**(1-2): p. 157-177.

87. Galgatte, U.C., A.B. Kumbhar, and P.D. Chaudhari, *Development of in situ gel for nasal delivery: design, optimization, in vitro and in vivo evaluation.* Drug Deliv, 2014. **21**(1): p. 62-73.

88. Madan, M., et al., *In situ forming polymeric drug delivery systems.* Indian J Pharm Sci, 2009. **71**(3): p. 242-51.

89. Sherafudeen, S.P. and P.V. Vasantha, *Development and evaluation of in situ nasal gel formulations of loratadine.* Research in Pharmaceutical Sciences, 2015. **10**(6): p. 466-476.

90. Cao, S.L., et al., *In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate.* Int J Pharm, 2009. **365**(1-2): p. 109-15.

91. Shelke, S., et al., *Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies.* J Liposome Res, 2016. **26**(4): p. 313-23.

92. Yadav, S., et al., *Comparative Biodistribution and Pharmacokinetic Analysis of Cyclosporine-A in the Brain upon Intranasal or Intravenous Administration in an Oil-in-Water Nanoemulsion Formulation.* Mol Pharm, 2015. **12**(5): p. 1523-33.

93. Miyamoto, M., et al., *Improved nasal absorption of drugs using poly-L-arginine: effects of concentration and molecular weight of poly-L-arginine on the nasal absorption of fluorescein isothiocyanate-dextran in rats.* Eur J Pharm Biopharm, 2001. **52**(1): p. 21-30.

94. Sherr, J.L., et al., *Glucagon Nasal Powder: A Promising Alternative to Intramuscular Glucagon in Youth With Type 1 Diabetes.* Diabetes Care, 2016. **39**(4): p. 555-62.

95. Clarke, S.E., et al., *Metabolism and disposition of 14C-granisetron in rat, dog and man after intravenous and oral dosing.* Xenobiotica, 1994. **24**(11): p. 1119-31.

96. Ibrahim, H.K., N.S. Abdel Malak, and S.A. Abdel Halim, *Formulation of Convenient, Easily Scalable, and Efficient Granisetron HCl Intranasal Droppable Gels.* Mol Pharm, 2015. **12**(6): p. 2019-25.

97. López-Rodríguez, M.L., et al., *Benzimidazole Derivatives. 2. Synthesis and Structure−Activity Relationships of New Azabicyclic Benzimidazole-4-carboxylic Acid Derivatives with Affinity for Serotoninergic*

5-HT3 Receptors. Journal of Medicinal Chemistry, 1999. **42**(24): p. 5020-5028.

98. Arora, P., S. Sharma, and S. Garg, *Permeability issues in nasal drug delivery.* Drug Discov Today, 2002. **7**(18): p. 967-75.

99. Illum, L., *Nasal drug delivery—possibilities, problems and solutions.* Journal of Controlled Release, 2003. **87**(1): p. 187-198.

100. Lorenzi, G., et al., *Correlation between rheologic properties and in vitro ciliary transport of rat nasal mucus.* Biorheology, 1992. **29**(4): p. 433-40.

101. King, M., *Relationship between mucus viscoelasticity and ciliary transport in guaran gel/frog palate model system.* Biorheology, 1980. **17**(3): p. 249-54.

102. Macchione, M., et al., *Rheological determinants of mucociliary transport in the nose of the rat.* Respir Physiol, 1995. **99**(1): p. 165-72.

103. Popov, T.A., et al., *Methyl-cellulose powder for prevention and management of nasal symptoms.* Expert Rev Respir Med, 2017. **11**(11): p. 885-892.

104. Mahmoodi, N.S., et al., *The Comparison of Nasaleze and Mometasone Nasal Spray to Control the Symptoms of Allergic Rhinitis.* Advanced Biomedical Research, 2018. **7**: p. 27.

105. Tanaka, A., et al., *Nasal drug absorption from powder formulations: The effect of three types of hydroxypropyl cellulose (HPC).* Eur J Pharm Sci, 2017. **96**: p. 284-289.

106. Cassandro, E., et al., *Hyaluronan in the Treatment of Chronic Rhinosinusitis with Nasal Polyposis.* Indian J Otolaryngol Head Neck Surg, 2015. **67**(3): p. 299-307.

107. Fisher, A., et al., *Pharmacokinetic comparisons of three nasal fentanyl formulations; pectin, chitosan and chitosan-poloxamer 188.* Int J Clin Pharmacol Ther, 2010. **48**(2): p. 138-45.

108. Cao, S.-l., et al., *In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate.* International Journal of Pharmaceutics, 2009. **365**(1): p. 109-115.

109. Cai, Z., et al., *Formulation and Evaluation of In Situ Gelling Systems for Intranasal Administration of Gastrodin.* AAPS PharmSciTech, 2011. **12**(4): p. 1102-1109.

110. Paul, A., K.M. Fathima, and S.C. Nair, *Intra Nasal In situ Gelling System of Lamotrigine Using Ion Activated Mucoadhesive Polymer.* The Open Medicinal Chemistry Journal, 2017. **11**: p. 222-244.

111. Dahl, T.C., et al., *Influence of physico-chemical properties of hydroxypropyl methylcellulose on naproxen release from sustained release matrix tablets.* Journal of Controlled Release, 1990. **14**(1): p. 1-10.

112. Chonkar, A., U. Nayak, and N. Udupa, *Smart Polymers in Nasal Drug Delivery.* Indian Journal of Pharmaceutical Sciences, 2015. **77**(4): p. 367-375.

113. Everaert, A., et al., *Optimisation of HPMC ophthalmic inserts with sustained release properties as a carrier for thermolabile therapeutics.* International Journal of Pharmaceutics, 2017. **528**(1): p. 395-405.

114. Chandak, A.R. and P.R.P. Verma, *Development and Evaluation of HPMC Based Matrices for Transdermal Patches of Tramadol.* Clinical Research and Regulatory Affairs, 2008. **25**(1): p. 13-30.

115. Research., F.a.D.A.-C.f.D.E.a., *Inactive Ingredient Search for Approved Drug Products*. Accessed on October 12, 2018.

116. Pennington, A.K., et al., *The influence of solution viscosity on nasal spray deposition and clearance.* International Journal of Pharmaceutics, 1988. **43**(3): p. 221-224.

117. Funami, T., et al., *Molecular structures of gellan gum imaged with atomic force microscopy in relation to the rheological behavior in aqueous systems in the presence or absence of various cations.* J Agric Food Chem, 2008. **56**(18): p. 8609-18.

118. Cao, S.-l., Q.-z. Zhang, and X.-g. Jiang, *Preparation of ion-activated in situ gel systems of scopolamine hydrobromide and evaluation of its antimotion sickness efficacy.* Acta Pharmacologica Sinica, 2007. **28**: p. 584.

119. Jansson, B., et al., *The influence of gellan gum on the transfer of fluorescein dextran across rat nasal epithelium in vivo.* European Journal of Pharmaceutics and Biopharmaceutics, 2005. **59**(3): p. 557-564.

120. Mahajan, H.S. and S.G. Gattani, *Gellan Gum Based Microparticles of Metoclopromide Hydrochloride for Intranasal Delivery: Development and Evaluation.* Chemical and Pharmaceutical Bulletin, 2009. **57**(4): p. 388-392.

121. Cabana, A., A. Aït-Kadi, and J. Juhász, *Study of the Gelation Process of Polyethylene Oxidea–Polypropylene Oxideb–Polyethylene OxideaCopolymer (Poloxamer 407) Aqueous Solutions.* Journal of Colloid and Interface Science, 1997. **190**(2): p. 307-312.

122. Pund, S., G. Rasve, and G. Borade, *Ex vivo permeation characteristics of venlafaxine through sheep nasal mucosa.* Eur J Pharm Sci, 2013. **48**(1-2): p. 195-201.

123. Basu, S. and A.K. Bandyopadhyay, *Development and characterization of mucoadhesive in situ nasal gel of midazolam prepared with Ficus carica mucilage.* AAPS PharmSciTech, 2010. **11**(3): p. 1223-31.

124. Schmolka, I.R., *Artificial skin. I. Preparation and properties of pluronic F-127 gels for treatment of burns.* J Biomed Mater Res, 1972. **6**(6): p. 571-82.

125. Gilbert, J.C., et al., *The effect of solutes and polymers on the gelation properties of pluronic F-127 solutions for controlled drug delivery.* Journal of Controlled Release, 1987. **5**(2): p. 113-118.

126. Tao, T., et al., *Preparation of huperzine A nasal in situ gel and evaluation of its brain targeting following intranasal administration*. Vol. 41. 2006. 1104-10.

127. Zaki, N.M., et al., *Enhanced bioavailability of metoclopramide HCl by intranasal administration of a mucoadhesive in situ gel with modulated rheological and mucociliary transport properties.* Eur J Pharm Sci, 2007. **32**(4-5): p. 296-307.

128. Phaechamud, T., J. Mahadlek, and T. Chuenbarn, *In situ forming gel comprising bleached shellac loaded with antimicrobial drugs for periodontitis treatment.* Materials & Design, 2016. **89**: p. 294-303.

129. Erfani Jabarian, L., et al., *In vitro and in vivo evaluation of an in situ gel forming system for the delivery of PEGylated octreotide.* European Journal of Pharmaceutical Sciences, 2013. **48**(1): p. 87-96.

130. Lale, A.M., J.D. Mason, and N.S. Jones, *Mucociliary transport and its assessment: a review.* Clin Otolaryngol Allied Sci, 1998. **23**(5): p. 388-96.

131. Gurny, R., E. Doelker, and N.A. Peppas, *Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers.* Biomaterials, 1982. **3**(1): p. 27-32.

132. Polli, J.E., et al., *Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets.* J Pharm Sci, 1997. **86**(6): p. 690-700.

133. Higuchi, T., *Rate of release of medicaments from ointment bases containing drugs in suspension.* J Pharm Sci, 1961. **50**: p. 874-5.

134. Peppas, N.A., *Analysis of Fickian and non-Fickian drug release from polymers.* Pharm Acta Helv, 1985. **60**(4): p. 110-1.

135. Hyman, W.A., *Rheology of Power Law Fluids.* Industrial & Engineering Chemistry Fundamentals, 1976. **15**(3): p. 215-218.

136. Panzade, P., et al., *Enhanced transdermal delivery of granisetron by using iontophoresis.* Iran J Pharm Res, 2012. **11**(2): p. 503-12.

137. Li, C., et al., *Enhancement in bioavailability of ketorolac tromethamine via intranasal in situ hydrogel based on poloxamer 407 and carrageenan.* Int J Pharm, 2014. **474**(1-2): p. 123-33.

138. Keck, T., et al., *Temperature profile in the nasal cavity.* Laryngoscope, 2000. **110**(4): p. 651-4.

139. Edsman, K., J. Carlfors, and R. Petersson, *Rheological evaluation of poloxamer*

as an in situ gel for ophthalmic use. Eur J Pharm Sci, 1998. **6**(2): p. 105-12.

140. Gilbert, J.C., et al., *Drug release from Pluronic F-127 gels.* International Journal of Pharmaceutics, 1986. **32**(2): p. 223-228.

141. Escobar-Chavez, J.J., et al., *Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations.* J Pharm Pharm Sci, 2006. **9**(3): p. 339-58.

142. Bhandwalkar, M.J. and A.M. Avachat, *Thermoreversible nasal in situ gel of venlafaxine hydrochloride: formulation, characterization, and pharmacodynamic evaluation.* AAPS PharmSciTech, 2013. **14**(1): p. 101-10.

143. Wang, Y., et al., *A mucoadhesive, thermoreversible in situ nasal gel of geniposide for neurodegenerative diseases.* PloS one, 2017. **12**(12): p. e0189478-e0189478.

144. Anderson, B.C., N.K. Pandit, and S.K. Mallapragada, *Understanding drug release from poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) gels.* Journal of Controlled Release, 2001. **70**(1): p. 157-167.

145. Moore, T., et al., *Experimental investigation and mathematical modeling of Pluronic F127 gel dissolution: drug release in stirred systems.* J Control Release, 2000. **67**(2-3): p. 191-202.

146. Wei, G., et al., *Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies.* J Control Release, 2002. **83**(1): p. 65-74.

147. Juhasz, J., et al., *Effect of sodium chloride on physical characteristics of poloxamer 407 solutions.* Journal of Colloid and Interface Science, 1990. **136**(1): p. 168-174.

148. Choi, H.-G., et al., *Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository.* International Journal of Pharmaceutics, 1998. **165**(1): p. 33-44.

149. Corbo, G.M., et al., *Measurement of nasal mucociliary clearance.* Archives of disease in childhood, 1989. **64**(4): p. 546-550.

150. Ojima, F., et al., *Development of a new method for simultaneously evaluating mucociliary clearance and pulmonary epithelial permeability in rabbit experiments by means of 18FDG, three-dimensional positron emission tomography and rectilinear scan.* Ann Nucl Med, 1998. **12**(5): p. 231-5.

151. Rutland, J. and P.J. Cole, *Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis.* Thorax, 1981. **36**(9): p. 654-658.

152. Blower, P.R., *Granisetron: relating pharmacology to clinical efficacy.* Support Care Cancer, 2003. **11**(2): p. 93-100.

153. Proctor DF, A.I., eds., *The Nose: Upper Airway Physiology and the Atmospheric Spheric Environment.* 1982: Amsterdam: Elsevier Science Ltd.

154. Chien YW, S.K., Chang SF, eds., *Nasal Systemic Drug delivery.* 2nd ed. ed. 1989, New York: NY: Marcel Dekker, Inc.

155. Kadian, N., et al., *Comparative assessment of bioanalytical method validation guidelines for pharmaceutical industry.* J Pharm Biomed Anal, 2016. **126**: p. 83-97.

156. Cupissol, D., et al., *Evaluation of the bioequivalence of tablet and capsule formulations of granisetron in patients undergoing cytotoxic chemotherapy for malignant disease.* J Pharm Sci, 1993. **82**(12): p. 1281-4.

157. Marks, D.R., et al., *Awake intranasal insulin delivery modifies protein complexes and alters memory, anxiety, and olfactory behaviors.* J Neurosci, 2009. **29**(20): p. 6734-51.

158. Jogani, V.V., et al., *Nose-to-brain delivery of tacrine.* J Pharm Pharmacol, 2007. **59**(9): p. 1199-205.

159. Jiang, Y., et al., *Rapid determination of granisetron in human plasma by liquid chromatography coupled to tandem mass spectrometry and its application to bioequivalence study.* J Pharm Biomed Anal, 2006. **42**(4): p. 464-73.

160. McElvain, J.S., V.J. Vandiver, and L.S. Eichemeier, *Validation of a reversed-phase HPLC method for directly quantifying the enantiomers of MDL 74,156, the primary metabolite of dolasetron mesylate, in human plasma.* J Pharm Biomed Anal, 1997. **15**(4): p. 513-21.

161. Nirogi, R.V., et al., *Quantification of granisetron in human plasma by liquid chromatography coupled to electrospray tandem mass spectrometry.* Biomed Chromatogr, 2006. **20**(9): p. 888-97.

162. Mayor, S.H. and L. Illum, *Investigation of the effect of anaesthesia on nasal absorption of insulin in rats.* International Journal of Pharmaceutics, 1997. **149**(1): p. 123-129.

163. Liu, P.T., et al., *Effects of ether anaesthesia and fasting on various cytochromes P450 of rat liver and kidney.* Biochem Pharmacol, 1993. **45**(4): p. 871-7.

164. Loch, J.M., J. Potter, and K.A. Bachmann, *The influence of anesthetic agents on rat hepatic cytochromes P450 in vivo.* Pharmacology, 1995. **50**(3): p. 146-53.

165. Plate, A.Y., D.L. Crankshaw, and D.D. Gallaher, *The effect of anesthesia by diethyl ether or isoflurane on activity of cytochrome P450 2E1 and P450 reductases in rat liver.* Anesth Analg, 2005. **101**(4): p. 1063-4, table of contents.

166. Fritz, H.G., et al., *The effect of mild hypothermia on plasma fentanyl concentration and biotransformation in juvenile pigs.* Anesth Analg, 2005. **100**(4): p. 996-1002.

167. Dede, S., Y. Deger, and I. Meral, *Effect of short-term hypothermia on lipid peroxidation and antioxidant enzyme activity in rats.* J Vet Med A Physiol Pathol Clin Med, 2002. **49**(6): p. 286-8.

168. van den Broek, M.P., et al., *Effects of hypothermia on pharmacokinetics and pharmacodynamics: a systematic review of preclinical and clinical studies.* Clin Pharmacokinet, 2010. **49**(5): p. 277-94.

169. Siegel, R., et al., *Cancer statistics, 2014.* CA Cancer J Clin, 2014. **64**(1): p. 9-29.

170. Hassan, B.A. and Z.B. Yusoff, *Negative impact of chemotherapy on breast cancer patients QOL - utility of antiemetic treatment guidelines and the role of race.* Asian Pac J Cancer Prev, 2010. **11**(6): p. 1523-7.

171. Janelsins, M.C., et al., *Current pharmacotherapy for chemotherapy-induced nausea and vomiting in cancer patients.* Expert Opin Pharmacother, 2013. **14**(6): p. 757-66.

172. Li, C.L., et al., *The use of hypromellose in oral drug delivery.* J Pharm Pharmacol, 2005. **57**(5): p. 533-46.

173. Jagdale, S., N. Shewale, and B.S. Kuchekar, *Optimization of Thermoreversible In Situ Nasal Gel of Timolol Maleate.* Scientifica, 2016. **2016**: p. 6401267-6401267.

174. Everaert, A., et al., *Optimisation of HPMC ophthalmic inserts with sustained release properties as a carrier for thermolabile therapeutics.* Int J Pharm, 2017. **528**(1-2): p. 395-405.

175. Sarkar, G., et al., *Taro corms mucilage/HPMC based transdermal patch: an efficient device for delivery of diltiazem hydrochloride.* Int J Biol Macromol, 2014. **66**: p. 158-65.

176. Kaur, R., et al., *Preparation and characterization of spray-dried inhalable powders containing nanoaggregates for pulmonary delivery of anti-tubercular drugs.* Artif Cells Nanomed Biotechnol, 2016. **44**(1): p. 182-7.

177. Dodov, M.G., et al., *Formulation and evaluation of diazepam hydrogel for rectal administration.* Acta Pharm, 2005. **55**(3): p. 251-61.

178. Jaipal, A., et al., *Effect of HPMC and mannitol on drug release and bioadhesion behavior of buccal discs of buspirone hydrochloride: In-vitro and in-vivo pharmacokinetic studies.* Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society, 2015. **23**(3): p. 315-326.

179. Costantino, H.R., et al., *Intranasal delivery: physicochemical and therapeutic aspects.* Int J Pharm, 2007. **337**(1-2): p. 1-24.

180. Zhang, Q., et al., *Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain.* Int J Pharm, 2004. **275**(1-2): p. 85-96.

181. Kim, J.S., M.S. Kim, and I.H. Baek, *Enhanced Bioavailability of Tadalafil after Intranasal Administration in Beagle Dogs.* Pharmaceutics, 2018. **10**(4).

182. Abou-Taleb, H.A., R.A. Khallaf, and J.A. Abdel-Aleem, *Intranasal niosomes of nefopam with improved bioavailability: preparation, optimization, and in-vivo evaluation.* Drug Des Devel Ther, 2018. **12**: p. 3501-3516.

183. Zhou, J. and S.M. Poloyac, *The effect of therapeutic hypothermia on drug metabolism and response: cellular mechanisms to organ function.* Expert Opin Drug Metab Toxicol, 2011. **7**(7): p. 803-16.

184. Merkus, P., et al., *Nasal drug delivery to the cerebrospinal fluid: transport of a lipophilic compound.* British Journal of Clinical Pharmacology, 2002. **54**(5): p. 560-560.

185. P.A.M., V.D., *Brain Size in Vertebrates. In: The Central Nervous System of Vertebrates.* 1998: Springer, Berlin, Heidelberg.

186. Herrstedt, J., *Nausea and emesis: still an unsolved problem in cancer patients?* Supportive Care in Cancer, 2002. **10**(2): p. 85-87.

187. Shumay, D.M., et al., *Why some cancer patients choose complementary and alternative medicine instead of conventional treatment.* J Fam Pract, 2001. **50**(12): p. 1067.

188. Bayo, J., et al., *Chemotherapy-induced nausea and vomiting: pathophysiology and therapeutic principles.* Clin Transl Oncol, 2012. **14**(6): p. 413-22.

189. Higgins, G.A., et al., *5-HT3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret.* Br J Pharmacol, 1989. **97**(1): p. 247-55.

190. Illum, L., *Nasal drug delivery - recent developments and future prospects.* J Control Release, 2012. **161**(2): p. 254-63.

191. Inoue, D., et al., *Quantitative Estimation of the Effect of Nasal Mucociliary Function on In Vivo Absorption of Norfloxacin after Intranasal Administration to Rats.* Mol Pharm, 2018.

192. Perry, M.R., J. Rhee, and W.L. Smith, *Plasma levels of peptide YY correlate with cisplatin-induced emesis in dogs.* J Pharm Pharmacol, 1994. **46**(7): p. 553-7.

193. Sharma, S.S., et al., *Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs.* J Ethnopharmacol, 1997. **57**(2): p. 93-6.

194. Adams, D.R. and D.K. Hotchkiss, *The canine nasal mucosa.* Anat Histol Embryol, 1983. **12**(2): p. 109-25.

195. Shimada, T., et al., *Cytochrome P450-dependent drug oxidation activities in liver microsomes of various animal species including rats, guinea pigs, dogs, monkeys, and humans.* Archives of Toxicology, 1997. **71**(6): p. 401-408.

196. Tanaka, E., A. Ishikawa, and T. Horie, *In vivo and in vitro trimethadione oxidation activity of the liver from various animal species including mouse, hamster, rat, rabbit, dog, monkey and human.* Human & Experimental Toxicology, 1999. **18**(1): p. 12-16.

197. Bloomer, J.C., et al., *Characterisation of the cytochrome P450 enzymes involved in the in vitro metabolism of granisetron.* British journal of clinical pharmacology, 1994. **38**(6): p. 557-566.

198. Clarke, S.E., et al., *Metabolism and disposition of 14C-granisetron in rat, dog and man after intravenous and oral dosing.* Xenobiotica, 1994. **24**(11): p. 1119-1131.

199. Nakamura, H., et al., *CYP1A1 is a major enzyme responsible for the metabolism of granisetron in human liver microsomes.* Curr Drug Metab, 2005. **6**(5): p. 469-80.

200. Ugwoke, M.I., et al., *Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives.* Adv Drug Deliv Rev, 2005. **57**(11): p. 1640-65.

201. Mall, M.A., *Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models.* J Aerosol Med Pulm Drug Deliv, 2008. **21**(1): p. 13-24.

202. Ahmed, O.A. and S.M. Badr-Eldin, *In situ misemgel as a multifunctional dual-absorption platform for nasal delivery of raloxifene hydrochloride: formulation, characterization, and in vivo performance.* Int J Nanomedicine, 2018. **13**: p. 6325-6335.

203. Walsh, S., et al., *Extended nasal residence time of lysostaphin and an anti-staphylococcal monoclonal antibody by delivery in semisolid or polymeric carriers.* Pharm Res, 2004. **21**(10): p. 1770-5.

204. Nair, A.B. and S. Jacob, *A simple practice guide for dose conversion between animals and human.* Journal of basic and clinical pharmacy, 2016. **7**(2): p. 27-31.

205. Dorato, M.A. and J.A. Engelhardt, *The no-observed-adverse-effect-level in drug safety evaluations: Use, issues, and definition(s).* Regulatory Toxicology and Pharmacology, 2005. **42**(3): p. 265-274.

206. Allen, A., et al., *Pharmacokinetics and tolerability of ascending intravenous doses of granisetron, a novel 5-HT3 antagonist, in healthy human subjects.* Eur J Clin Pharmacol, 1994. **46**(2): p. 159-62.

207. *Kytril® (granisetron hydrochloride tablets) [Package insert]*. June 6, 2014, Hoffmann-La Roche Limited: Mississauga, Ontario.