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RESEARCH ARTICLE

Preparation and evaluation of zolmitriptan submicron emulsion for rapid and effective nasal absorption in beagle dogs

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Abstract

Submicron emulsion was prepared for rapid and effective nasal absorption of zolmitriptan (ZT). The different charge inducers and pH values of the formulations were evaluated to optimize the formulations. Submicron emulsion prepared by using stearylamine as positive charge inducer with pH of 5.0 was stable and most of ZT was freely dispersed in the aqueous phase of the preparation. *In vitro* release study demonstrated that ZT from the submicron emulsion preparation could be released as fast as that from the solution preparation. The pharmacokinetics was studied after intranasal administration of the submicron emulsion and solution preparation of ZT to beagle dogs. ZT from the submicron emulsion was absorbed much more rapidly and the absolute availability of the submicron emulsion preparation was significantly higher compared with the solution preparation. The nasal ciliotoxicity of the preparations was evaluated by using *in situ* toad palate model, which indicated that the submicron emulsion of ZT did not exhibit any obvious nasal ciliotoxicity. These results demonstrated that the submicron emulsion preparation of ZT was a relatively safe dosage form for rapid and effective intranasal delivery of ZT.

Keywords: Zolmitriptan, submicron emulsion, stearylamine, nasal absorption, beagle dogs

Introduction

Zolmitriptan (ZT) is a 5-HT1B/1D receptor partial agonist for use in the acute treatment of migraine and related vascular headaches[1]. At present, ZT is commercially available on the market in the form of oral tablets (conventional and orodispersible) and nasal spray[2,3]. However, the current oral therapies have some drawbacks. A literature reveals that ZT undergoes first-pass metabolism and has poor bioavailability (40%)[4]. In addition, an oral formulation may not be suitable for the treatment of some kinds of migraine attacks, particularly those associated with nausea and vomiting, which make it difficult for migraine patients to take tablets. Furthermore, gastric stasis is a common feature of migraine attacks, and this can limit the absorption of orally administered medications[5,6]. Consequently, a nasal spray formulation of ZT has been developed, which demonstrates good efficacy, high tolerability, and a very fast onset of action[7]. The main goals in the treatment of migraine are to provide patients with highly effective and rapid relief from migraine symptoms during an attack[8,9]. However, although absorption of ZT is initially more rapid after intranasal administration than oral administration, pharmacokinetic parameters for ZT such as plasma concentration–time curve (AUC) or C_{max} values were similar for nasal spray and oral formulations[10]. It has been reported that the absolute biological availability of ZT from the nasal spray was only 40% after administration to healthy volunteers[2]. In healthy volunteers, after administration, some of the ZT given in the form of nasal spray is swallowed and enters the gastrointestinal tract[11,12]. As a result, a new dosage form that can make ZT be absorbed much more rapidly and effectively is needed to offer benefits of highly effective and rapid relief from migraine for patients.

Emulsion formulations have been used as vehicles for drugs with a poor aqueous solubility to improve the nasal absorption of drugs[13,14]. The mechanism for the enhancement of nasal absorption of emulsions is not clear.

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Incorporation of drug to the oily phase of submicron emulsion formulation presented a higher bioavailability than the solution preparation after intranasal administration[15]. However, a report revealed that increase in amount of drug in the oil droplets of emulsion delayed intranasal absorption of drug by the perfusion method[16]. But the pharmacokinetics was not evaluated for the emulsion formulations with drug dispersed in the aqueous phase[16]. It seems reasonable that dispersing drug into the aqueous phase of emulsions can make drug absorbed more rapidly than incorporating drug to the oily phase.

The current nasal spray of ZT is a simple solution preparation, which presents a relatively low bioavailability. In this study, a submicron emulsion formulation of ZT with drug dispersing into the aqueous phase was prepared and pharmacokinetics of the preparation was evaluated in beagle dogs. The prepared submicron emulsion formulation of ZT has an advantage of faster and more effective nasal absorption than the current nasal spray.

Materials and methods

Materials

ZT was obtained from Beijing Gaobo Pharm-Chemicals Tech. Co. (China); egg lecithin (Lipoid E170) and medium-chain triglyceride (MCT) were both supplied by Lipoid KG (Ludwigshafen, Germany); glycerol was supplied by Zhejiang Suichang Glycerol Plant (Zhejiang, China); Poloxamer 188 (Pluronic F68®) was obtained from BASF AG (Ludwigshafen, Germany). All other reagents were of analytical grade or of the highest grade commercially available. The beagle dogs used for pharmacokinetic study were supplied by the Animal Center of Shenyang Pharmaceutical University (Shenyang, China).

Preparation of ZT solution and ZT submicron emulsions

The ZT solution (ZTS) was prepared by dissolving citric acid (1.3%, w/w), disodium hydrogen phosphate (2.8%, w/w), and ZT (0.5%, w/w) in water. After stirring to obtain a clear solution, the pH was adjusted to about 5.0 with disodium hydrogen phosphate to give 5 mg/mL ZTS for intravenous and intranasal administration.

In order to choose the most suitable charge inducer, submicron emulsion of ZT prepared using oleic acid as negative charge inducer (ZTSE-1) and submicron emulsion of ZT using stearylamine as positive charge inducer (ZTSE-2) were prepared. Oleic acid (0.05%, w/w) was added to the water phase and stearylamine (0.5%, w/w) was added to the oil phase, respectively, to prepare the two submicron emulsion preparations. In brief, egg lecithin (3%, w/w) and ZT (0.5%, w/w) were dissolved in alcohol (30%, v/w) and the solution was stirred at 75°C until the alcohol completely evaporated. The lecithin–ZT mixture was obtained after evaporation of alcohol and was dissolved in MCT (20%, w/w) to produce the oil phase by stirring. More ZT could be dissolved in MCT by dissolving the lecithin–ZT mixture rather than just dissolving physical mixture of ZT and lecithin because ZT–phospholipid complex was probably formed by this method[17]. In addition, egg lecithin (1%, w/w), Poloxamer 188 (0.4% w/w), glycerol (2.5%, w/w), EDTA-2Na (0.05%, w/w), and benzalkonium bromide (0.01%, w/w) were dispersed in water at 75°C to produce the water phase. The oil phase was added to the aqueous phase with continuous stirring using a high shear mixer (ULTRA TURRAX T18 basic, IKA WORKS Guangzhou, Germany) at 14,000 rpm for 5 min and the primary emulsion was obtained. After passing the primary emulsion through the high-pressure homogenizer (Niro Soavi NS10012k, Niro Soavi S.p.A., Via M. Da Erba, Italy), a 5 mg/mL submicron emulsion of ZT for intranasal administration was obtained. The pH value of the final submicron emulsion using oleic acid as charge inducer (ZTSE-1) and stearylamine as charge inducer (ZTSE-2) was 9.5 and 10.0, respectively. To optimize to dosage forms, the final ZTSE-1 was adjusted to pH 8.0, 7.0, and 6.0, respectively. The final ZTSE-2 was adjusted to pH 9.2, 7.0, and 5.0, respectively. The index of physical property of the preparations was evaluated to optimize the formulation.

Particle size and zeta potential

The particle size and zeta potential of ZTSE were measured using a NICOMP™ 380 Zeta Potential/Particle Sizer (Particle Sizing Systems, Santa Barbara, CA). The mean particle size and distribution were measured using photon correlation spectroscopy (PCS; dynamic light scattering, DLS), which is a powerful and versatile tool for estimating the particle size distribution of fine particle materials ranging from a few nanometers to several micrometers[18]. The zeta potential was determined based on an electrophoretic light scattering (ELS) technique. Measurement of particle size and zeta potential were performed immediately after dilution of the emulsion sample with double-distilled water. The temperature was maintained at 25°C for the determination.

Drug content and entrapment efficiency of ZTSE

The content of ZT was determined using a high-performance liquid chromatographic method at a wavelength of 229 nm. A C18 column (250 mm × 4.6 mm, particle size 5 μm) was used for the separation of ZT and a mixture of methanol, 0.025 mol/L potassium dihydrogen phosphate, and triethanolamine (25:75:0.5, v/v/v, pH was adjusted to 3.5 with phosphoric acid) was used as the mobile phase. ZT could be completely separated from the other components by this HPLC method. The linear range of ZT was 2.0–100.0 μg/mL. The repeatability variation was 0.72% and recovery was 99.9 ± 1.2%. The method had an acceptable linear range, good precision, and accuracy.

The microdialysis (MD) method was chosen to determine the entrapment efficiency of ZTSE[19]. The MD probes for evaluation of the entrapment efficiency were the same as described in an earlier study[19]. The membrane

of MD probes had a molecular weight cutoff of 5000 Da, so nano-sized oil droplets incorporating drug in submicron emulsion could not pass through the semipermeable membrane. As a result, the drug concentration of free drug in the aqueous phase was determined by MD. Then, drug concentrations in the aqueous phase and in the whole submicron emulsion were compared with calculate the drug entrapment[19].

Transmission electron microscopy

The morphology of the submicron emulsion was examined by transmission electron microscopy (TEM) (H-600, Hitachi, Japan). Before visualization, diluted dispersions of submicron emulsion were negatively stained with 1% phosphotungstic acid and finally examined through TEM.

Apparent partition coefficient determinations of ZT

Equal volumes of water-saturated octanol and different pH buffers containing about 1 mg/mL ZT were mixed and shaken at 37°C for 3 days until equilibrium was reached. The concentrations of drug in the oil phase and aqueous phases were determined by HPLC as described above. The logarithms of the apparent partition coefficients of ZT were calculated.

Measurement of viscosity

The dynamic viscosity of ZTSE-2 (pH 5.0) and ZTS was evaluated using an AR2000 Rheometer (TA Instruments, Leatherhead, UK) in the flow mode with a plate diameter of 60 mm diameter. The shear rate was kept constant at 50 s^{-1} for 60 min at the controlled temperature of 37°C.

In vitro drug release

The *in vitro* drug release behavior of ZTSE-2 (pH 5.0) was studied compared with ZTS. A dialysis tube containing 1 mL ZTSE-2 (pH 5.0) or ZTS was immersed in 50 mL of phosphate buffer (pH 6.4) maintained at 37°C, under shaking at 100 rpm. Then 0.1 mL samples of medium were withdrawn at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, and replaced with 0.1 mL blank medium. The drug content in the release medium was determined by HPLC as described above.

Animal experiments

Pharmacokinetic study was carried out after the ZTSE-2 (pH 5.0) and ZTS were intranasally administrated to beagle dogs. In addition, surfactants acting as penetration enhancers can improve transcellular transport of drugs across nasal mucosa[20]. To study the effect of surfactants in ZTSE-2 (pH 5.0) on nasal absorption of ZT alone, a control preparation was prepared by dissolving ZT in water with the same amount of surfactants as that in ZTSE-2 (pH 5.0). The control system was prepared by simply dissolving ZT (0.5%, w/w), egg lecithin (4%, w/w), Poloxamer 188 (0.4% w/w), glycerol (2.5%, w/w), EDTA-2Na (0.05%, w/w), and benzalkonium bromide (0.01%, w/w) in water and the pH was adjusted to 5.0 with 0.1 M HCl solution.

Twenty-four female and male beagle dogs, weighing 9.5 to 13.5 kg, were randomly divided into four groups (n = 6/group). One group received ZTS intravenously at a dose of 1.3 mg per dog. The other three groups, respectively, received ZTS, ZTSE-2 (pH 5.0), and ZT in surfactants control preparation by the intranasal route. Intranasal administration was carried out by a manual pump spray unit that delivers 70 μL of the formulation per spray. A total volume of 280 μL of the formulation was sprayed into two nostrils of the dogs at a dose of 1.4 mg for each dog (each nostril received two sprays). The experimental procedures complied with the University Animal Ethics Committee Guidelines.

Blood samples (3 mL) were withdrawn from the tube in the femoral vein and transferred to heparin stabilized test tubes at different times after administration: 0.083, 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 h. Plasma was separated by centrifugation at 3000 rpm for 15 min and kept frozen at –20°C for subsequent analysis.

Then, 0.4 mL plasma was spiked with 20 μL internal standard (sumatriptan succinate, 0.5 ng/mL) methanol solution and 0.1 M NaOH 160 μL. The sample was then vortexed with methyl *tert*-butyl ether (3 mL) for 2 min and, after 10 min mechanical shaking it was centrifuged for 5 min at 4000 rpm. The supernatant was transferred to a new tube and then evaporated to dryness in a water bath at 40°C under N_2. The residue was dissolved in 100 μL methanol, and 5 μL was injected into the UPLC-MS/MS system for analysis.

Sample determination by UPLC-ESI-MS/MS

Liquid chromatography was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA) with a conditioned autosampler maintained at 4°C. The separation was performed at a column temperature of 35°C using an ACQUITY UPLC™ BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp.). The analysis was carried out with gradient elution and the mobile phase consisted of acetonitrile (A) and water (B, containing 0.1% formic acid). The gradient conditions of the mobile phase were as follows: A was increased linearly from initially 10% to 60% during the first 0.8 min and then held for 0.8 min. Then the composition was reset to the initial composition (10% A) within 0.05 min and equilibrated at the initial composition for 0.35 min. The flow rate was kept constant at 0.2 mL/min and the sample injection volume was 5 μL using the partial loop mode.

The Waters ACQUITY™ TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system via the electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode for both ZT and IS. The ESI source parameters were as follows: capillary 0.4 kV, cone voltage 34 V, extractor 3.0 V, and RF 0.0 V. The temperature of the source and desolvation were set at 100°C and 400°C, respectively. Nitrogen was used

as the desolvation gas (550 L/h) and cone gas (50 L/h) for nebulization. For collision-induced dissociation (CID), argon was used as the collision gas at a pressure of approximately 2.91×10^{-3} mbar. The collision energy was 20 eV for both ZT and IS. The multiple reaction monitoring (MRM) mode was used for quantification. The fragmentation transitions for MRM were m/z 288.13 → 58.13 amu for ZT and m/z 296.12 → 57.90 amu for IS. All data collected in centroid mode were acquired using MassLynx™ NT4.1 software (Waters Corp., Milford, MA). Post-acquisition quantitative analyses were carried out using a QuanLynx™ program (Waters Corp., Milford, MA). In plasma, ZT can be detected sensitively with an acceptable linear range from 0.25 to 125 ng/mL. The ZT relative recovery from the plasma at three concentrations (0.5, 5, 100 ng/mL) was higher than 50%. The analytical method showed acceptable precision and accuracy, confirming that the method used was suitable for the studies planned.

Data analysis

Pharmacokinetic calculations were performed on each individual set of animal data using the pharmacokinetic calculation software DAS (drug and statistics) version 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China) by the statistical moment method. The absolute bioavailability was calculated using the equation below:

$$F = \frac{AUC_{0\to t(i.n.)}/X_{i.n.}}{AUC_{0\to t(i.n.)}/X_{i.v.}} \times 100\%$$

where $X_{i.n.}$ is the dose administered intranasally and $X_{i.v.}$ is the dose administered intravenously.

Statistical differences between groups after intravenous and intranasal administration were examined using the unpaired Student's t-test (two-tailed) and a value of $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered highly significant. Results were presented as mean values ± standard deviation of the mean pharmacokinetic parameters of ZT.

Nasal ciliotoxicity

Nasal ciliotoxicity studies were carried out using the *in situ* toad palate model[15,21]. In brief, the upper palate of toad (30–40 g, ♂♀ animal house; Shenyang Pharmaceutical University) was exposed and treated with 0.5 mL test formulations for 30 min and then rinsed with saline. The palate was isolated and the mucocilia was examined using a 400-fold Motic DMBA 450 microscope (MoticChina Group Co. Ltd., Beijing, China). Beating of the cilia was clearly observed and the duration of the ciliary movement was recorded. Physiological saline and sodium deoxycholate (a serious nasal mucociliary toxicity agent) were used as a negative and positive control, respectively.

Results

Physicochemical properties of submicron emulsions

The entrapment efficiency, particle size, and zeta potential of ZTSE-1 with pH 9.5 was 77.8%, 152.0 ± 42.6 nm and –42.0 mv, respectively. The physicochemical properties of ZTSE-1 with pH 9.5 were stable. However, ZTSE-1 exhibited creaming within 24 h after being adjusted to pH 8.0, 7.0 and 6.0, respectively. To avoid nasal irritation, the pH of the nasal formulation should be adjusted to 4.5–6.5[22]. As a result, high pH value of ZTSE-1 made it unsuitable for intranasal administration.

The effect of the pH on the index of physical properties of ZTSE-2 was shown in Table 1. ZTSE-2 with pH 9.2, 7.0, and 5.0 exhibited good appearance without oil droplets. The zeta potential of ZTSE-2 was positive and at lower pH the zeta potential was much higher. The entrapment efficiency of ZTSE-2 was much higher at the higher pH. When the pH value of ZTSE-2 was adjusted to 5.0, the entrapment efficiency was extremely low and the drug was almost completely dispersed into the water phase of the submicron emulsion. As a result, the optimized preparation of submicron emulsion was to choose stearylamine as positive charge inducer and to adjust the pH to about 5.0. Another batch of ZTSE-2 with pH 5.0 was prepared to evaluate to stability. The pH of the primary emulsion was adjusted to 5.0 before passing the primary emulsion through the high-pressure homogenizer. The results of stability investigation were showed in Table 2. The physicochemical properties of ZTSE-2 with pH 5.0 were stable at 4°C for at least 6 months. TEM image of ZTSE-2 (pH 5.0) was presented in Figure 1. This result conformed that the emulsions were circular in shape within diameters of about 100–200 nm.

Apparent partition coefficients and viscosities

The logarithms of the apparent partition coefficients of ZT for pH 4.0, 5.0, 7.0, 7.8, and 9.0 buffer were –2.8, –2.2, –0.87, –0.20, and 0.67, respectively. The mean values of viscosities of ZTS and ZTSE-2 (pH 5.0) were 0.74 and 3.94 mPa·s, respectively.

Table 1. The effect of the pH on the indexes of physical properties of ZTSE-2.

pH after homogenization	Particle size (nm)	ξ-potential (mv)	Physical appearance 24 h after adjusting pH	EE (%)
10.0	289.6 ± 119.9	+9.2	Creaming	—
9.2	165.6 ± 51.3	+14.9	Good appearance	31.0
7.0	153.1 ± 54.2	+28.1	Good appearance	11.4
5.0	160.0 ± 56.8	+31.5	Good appearance	5.2

EE indicate the entrapment efficiency of ZTSE-2.

Table 2. Stability of ZTSE-2 (pH 5.0) at 4°C for 6 months.

	Particle size (nm)	ξ-potential (mv)	Drug content (%)	Physical appearance
0 time	167.3 ± 35.9	+40.0	105.35	Good appearance
6 months	168.0 ± 47.7	+40.8	102.51	Good appearance

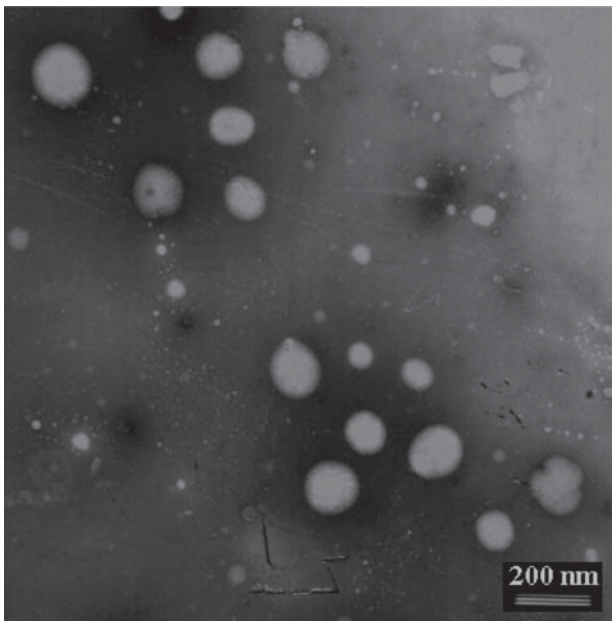

Figure 1. Transmission electron microscopic (TEM) image of ZTSE-2 (pH 5.0).

In vitro drug release

The *in vitro* release profiles of ZT from ZTSE-2 (pH 5.0) and ZTS were showed in Figure 2. ZT from the two preparations was released completely within 1.5 h and the dialysis tube method slowed drug release through the dialysis membrane into the medium. However, ZT from ZTSE-2 (pH 5.0) and ZTS exhibited similar release profiles. The results demonstrated that ZT from ZTSE-2 (pH 5.0) could be released as fast as from ZTS.

Pharmacokinetic studies

The mean plasma concentration–time curves of ZT after intranasal and intravenous administration are shown in Figure 3. For intravenous delivery, the peak plasma concentration (C_{max} 100.7 ± 14.3 ng/mL) was reached at the first beginning and the drug concentration–time curve exhibited exponential decline. The pharmacokinetic parameters were calculated from the observed plasma concentration–time profiles. The values of AUC, $t_{1/2}$, T_{max}, C_{max}, and absolute bioavailability are shown in Table 3. The absolute bioavailability of ZTSE-2 (pH 5.0) group (80.68 ± 16.84%) was significantly higher than that of ZTS group (53.23 ± 19.86%) and the ZT in surfactants control preparation group (62.70 ± 5.06%) ($P < 0.05$). However, no significant difference was found in the absolute bioavailability values between the ZTS group and the ZT in surfactants control preparation group ($P > 0.05$). T_{max} value of the ZTSE-2 (pH 5.0) group was significantly shorter than that of ZTS group ($P < 0.05$). C_{max} values for the three groups after intranasal administration were in the order

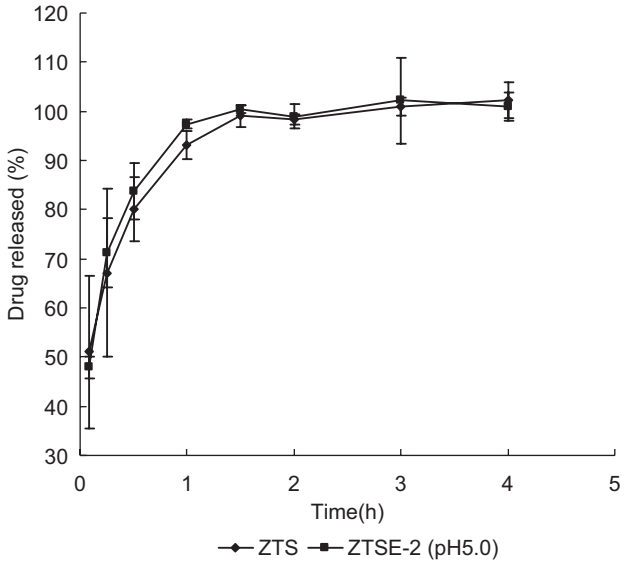

Figure 2. *In vitro* release profiles of zolmitriptan (ZT) from ZTSE-2 (pH 5.0) and solution preparations ($n = 3$).

of ZTSE-2 (pH 5.0) > ZT in surfactants control preparation > ZTS. The C_{max} value of ZTSE (pH 5.0) group was statistically significantly higher than that of ZTS group and ZT in surfactants control preparation group ($P < 0.01$). A significant difference was also found for C_{max} values between ZT in surfactants control preparation group and ZTS group ($P < 0.05$).

Nasal ciliotoxicity

The optical microscopy results showed that all cilia fell off from the edge of the mucosa and no cilia on the mucosa was observed after the upper palate of toad was treated with 1% sodium deoxycholate solution. However, there were a great number of cilia with a fast beating rate on the edge of mucosa after being treated with physiological saline, ZTS, ZTSE-2 (pH 5.0), and ZT in surfactants control preparation, respectively. The optical microscope photographs for the edge of mucosa after being treated with 1% sodium deoxycholate solution and ZTSE-2 (pH 5.0) were shown in Figure 4. The duration of the ciliary movement of all the test formulations was about 11–13 h and no significant difference were found for the duration of ciliary beating between the test formulations. The results indicated that ZTS, ZTSE-2 (pH 5.0), and ZT in surfactants control preparation did not show obvious nasal ciliotoxicity.

Discussion

Since ZT is a faintly alkaline drug, after ZTSE-1 was adjusted to a lower pH, a high number of cations were formed due to the ionization of ZT. The cations formed

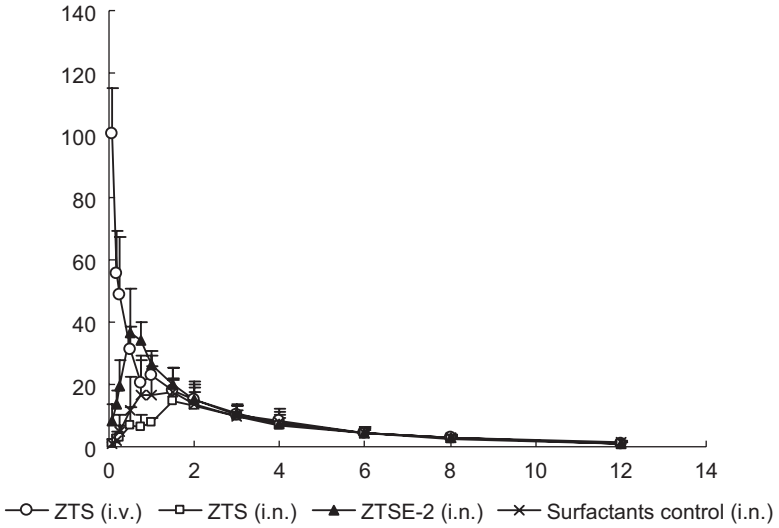

Figure 3. The mean plasma concentration–time curves of zolmitriptan (ZT) after administration of ZTS (i.v.), ZTS (i.n.), ZTSE-2 (pH 5.0), and ZT in surfactants control preparation (i.n.) to beagle dogs.

Table 3. Pharmacokinetic parameters of zolmitriptan (ZT) after administration of ZTS (i.v.), ZTS (i.n.), ZTSE-2 with pH 5.0 (i.n.), and ZT in surfactants control preparation (i.n.) to beagle dogs ($n=6$, mean ± SD).

Parameters	$AUC_{0\to t}$ (ng/mL h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	F (%)
ZTS (i.v.)	110.3 ± 27.7	2.4 ± 0.5	0.083 ± 0	100.7 ± 14.3	—
ZTS (i.n.)	63.2 ± 23.6	2.9 ± 0.7	1.3 ± 0.6	16.3 ± 6.2	53.23 ± 19.86
ZTSE-2 (i.n.)	95.8 ± 20.0	3.1 ± 0.7	0.58 ± 0.13	39.7 ± 8.8	80.68 ± 16.84
Surfactants control (i.n.)	74.4 ± 6.0	2.7 ± 0.7	1.4 ± 1.3	24.8 ± 6.2	62.70 ± 5.06

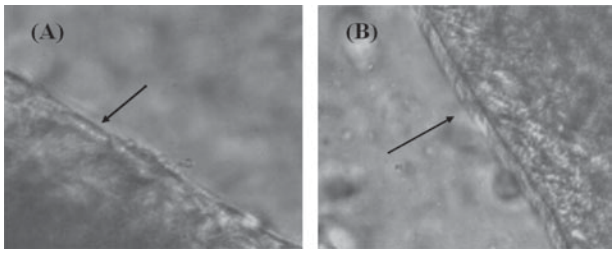

Figure 4. Optical microscopic images of (A) positive control (1% sodium deoxycholate solution, no cilia on the mucosa was observed. (B) ZTSE-2 with pH 5.0 (cilia was beating fast on the edge of mucosa) ($n=3$).

made the submicron emulsions unstable because the zeta potential of the ZTSE-1 was negative. Adding stearylamine to the submicron emulsion of ZT made the zeta potential of the submicron emulsion positive. The zeta potential of ZTSE-2 with pH 5.0 was higher than 20 mv and was responsible for the stability by preventing coalescence of droplets following random collision[23]. ZTSE-2 at pH 10.0 was unstable and the particle size increased. The reason was that stearylamine was alkaline and at a relatively high pH, the zeta potential made by ionization of stearylamine was not high enough to keep the submicron emulsion stable.

With the increase of the pH value of buffers, logarithms of the apparent partition coefficients of ZT increased. ZT has a pK_a of 9.6 and is a faintly alkaline drug. At low pH, more drugs are in the ionized form, which has a higher hydrophilicity than the unionized form. As a result, dissolving ZT in the oily phase of the emulsion did not result in a high entrapment efficiency of the final submicron emulsion with low pH. The entrapment efficiency of ZTSE-2 (pH 5.0) calculated by MD method was just 5.2%, which meant that most of ZT was freely dispersed in the aqueous phase of submicron emulsion. As ZT was not incorporated in the oil phase of the submicron emulsion, the release of ZT from ZTSE-2 (pH 5.0) cannot be slowed down. The *in vitro* drug release study demonstrated that ZT released from ZTSE-2 (pH 5.0) as fast as from ZTS. The fast release of drug from ZTSE-2 (pH 5.0) was beneficial for rapid absorption of ZT after intranasal administration.

In this study, the prepared ZTS was the same as the commercial nasal spray of ZT, which was also a buffered solution (pH 5.0) containing citric acid and disodium phosphate. Pharmacokinetic studies demonstrated that the ZTSE-2 (pH 5.0) group exhibited the significantly shorter T_{max}, higher C_{max}, and higher absolute bioavailability than the ZTS. ZT from ZTSE-2 (pH 5.0) was absorbed much more rapidly and effectively than the ZTS. As a result, ZTSE-2 (pH 5.0) will result in a faster and more effective relief of migraine symptoms for patients than the current nasal spray of ZT.

Some factors affecting the nasal absorption of ZT from ZTSE-2 (pH 5.0) should be considered. First, a higher viscosity of the formulation could increase the contact time between the drug and the nasal mucosa and thus the time for drug permeation could be increased[22,24]. However, the enhanced penetration effect cannot be attributed to

the slight increase in viscosity of the ZTSE-2 with pH 5.0 (3.2 mPa·s) compared with ZTS.

In addition, penetration enhancers such as surfactants, β-cyclodextrins, bile salts, phospholipids, and lysophospholipids can increase the permeability across the nasal mucosa, thereby promoting the observed transcellular transport of drugs[20]. The C_{max} value of ZT in the surfactants control preparation group was significantly higher than that of the ZTS group although no statistically significant differences were found for the absolute availability values between these two groups. Hence, the egg lecithin and poloxamer acting as surfactants probably acted on the nasal mucosa and enhanced the nasal absorption of ZT to some extent. However, the ZTSE-2 (pH 5.0) group exhibited significantly higher level of drug absorption than that of ZT in surfactants control preparation group. ZT in surfactants control group contains the same amount of egg lecithin and poloxamer as that in ZTSE-2 (pH 5.0). The results demonstrated that the enhancement of nasal absorption of ZT in ZTSE-2 (pH 5.0) was not mainly caused by the egg lecithin and poloxamer. It was presumed that the nano-sized oil droplets in the ZTSE-2 (pH 5.0) might enhance the action of the surfactants on the nasal mucosa.

Conclusions

In this study, a stable ZTSE-2 (pH 5.0) was prepared by using stearylamine as positive charge inducer and ZT was mainly dispersed in the aqueous phase of the submicron emulsion. The pharmacokinetic studies demonstrated that ZT from ZTSE-2 (pH 5.0) was absorbed much more rapidly and effectively compared with ZTS and ZT in surfactants control preparation after intranasal administration. The enhanced nasal absorption of ZT in ZTSE-2 (pH 5.0) was not mainly caused by the egg lecithin and poloxamer. It was presumed that the nano-sized oil droplets in the ZTSE-2 (pH 5.0) might enhance the action of the surfactants on the nasal mucosa. The nasal ciliotoxicity was evaluated by using *in situ* toad palate model, which indicated that ZTSE-2 (pH 5.0) did not show any obvious nasal ciliotoxicity. The results demonstrated that ZTSE-2 (pH 5.0) was a relatively safe dosage form for rapid and effective intranasal delivery of ZT.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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