Abstract

The aim of this investigation was to prepare microemulsions containing zolmitriptan (ZT) for rapid drug delivery to the brain to treat acute attacks of migraine and to characterize microemulsions and evaluate biodistribution in rats. Zolmitriptan microemulsions (ZME) were prepared using the titration method and were characterized for globule size distribution and zeta potential. ZT was radiolabeled using $^{99m}$Tc (technetium) and radiolabeled-drug formulations of ZT were used to carry out biodistribution of drug in the brain of Swiss albino rats after intranasal and intravenous administration. The pharmacokinetic parameters, drug targeting efficiency (%DTE) and direct nose-to-brain drug transport (%DTP) were calculated. Brain scintigraphy imaging in rats were also performed to ascertain the uptake of drug into the brain. ZME were transparent and stable with mean globule size of 35 nm ± 25 nm and zeta potential of -38 mV to -52 mV. $^{99m}$Tc-labeled-drug formulations of ZT were found to be stable and suitable to perform in vivo studies. Following intranasal administrations of zolmitriptan mucoadhesive microemulsion (ZMME), ZME, Zolmitriptan solution (ZS) and intravenous administration of ZS, brain/blood uptake ratios at 0.50 h were found to be 0.70, 0.56, 0.27 and 0.13 respectively, indicating effective brain-targeting following intranasal administration of ZMME. Comparing intranasal administration of ZMME with intravenous administration of ZME, the %DTE and %DTP were found higher indicating effective drug transport following intranasal administration and highest brain-targeting following ZMME administration. Rat brain scintigrams showed substantial uptake of drug into the brain after intranasal administration of ZMME. Studies of this investigation conclusively demonstrated rapid and larger extent of transport into the rat brain following intranasal administration of ZMME and can play promising role in the treatment of acute attacks of migraine.
Keywords: Intranasal, Microemulsion, Zolmitriptan, Radiolabel, Brain-targeting

Introduction

Migraine attack is a troublesome physiological condition associated with throbbing, intense headache in one half of the head. During an attack, the blood vessels in the brain dilate and then draw together with stimulation of nerve endings near the affected blood vessels. These changes in the blood vasculature may be the responsible for the pain. However, the exact cause of migraine - whether it is a vascular or a neurological dysfunction still remains unclear [1, 2]. Therapeutic approaches for management of migraine has a strong rationale however, it is still a poorly understood phenomenon [3]. Zolmitriptan (ZT), a triptan derivative is a serotonin (5-hydroxy tryptamine) agonist, used widely in the effective treatment of migraine associated with menses, migraine with aura and cluster headache. ZT reduces incidence of persistent migraine and recurrent migraine headache [4]. Data on comparative clinical studies reveal that ZT has a similar or superior efficacy to sumatriptan in the treatment of migraine. Substantial proportion of the migraine patients not only suffer from gastric stasis but have also been associated with severe nausea and vomiting, at large [5]. These circumstances may lead to erratic absorption of the drug from the gastrointestinal tract and may result into ineffective treatment. Reports in the literature reveal that zolmitriptan undergoes first-pass metabolism and has poor bioavailability (40 percent) [4, 5]. In addition, zolmitriptan gets cleared rapidly from the circulation by hepatic metabolism and renal clearance and the half-life is 1-2 h [6]. Presently, ZT is available on the market in form of tablet, nasal spray and orodispersible tablet dosage forms. Later two dosage forms release ZT at a fast rate compare to conventional tablet dosage form and are preferred in the treatment of acute attacks of migraine [4, 5]. Delivery system which can preferentially target the drug to the site of action such as brain and vasculature [7] may be advantageous in the
effective treatment of acute attacks of migraine [8, 9]. Therefore, an alternative drug delivery system which can enhance the rate and extent of release of ZT is needed.

Direct transport of drugs to the brain circumventing the brain-barriers following intranasal administration provides a unique feature and better option to target drugs to the brain [10, 11]. However, to enhance effectiveness of the drug, a few issues should be considered by the formulator when designing intranasal drug delivery. Intranasal drug delivery system must be meticulously designed to provide rapid transport of drug across nasal mucosa and longer residence time in nasal cavity [12]. Microemulsions, by virtue of being lipophilic in nature and having low globule size, are explored widely as a delivery system to enhance uptake across nasal mucosa [13]. Addition of a mucoadhesive agent such as a polyelectrolyte polymer helps in retention of formulation in nasal cavity [14, 15].

The objective of this investigation was to prepare and to characterize microemulsion/mucoadhesive microemulsion of ZT, and to study biodistribution of the microemulsions/drug solution in rats using radiolabeled-drug formulations, and to assess direct nose-to-brain transport. It was hypothesized that microemulsion/mucoadhesive microemulsion based drug delivery system will result in effective nose-to-brain transport, enhanced rate and extent of drug transport, and greater distribution of ZT into and within the brain. This can help to maximize the therapeutic index of the drug, reduce its side effects, and reduce the dose and frequency of dosing, and perhaps result into cost effectiveness.
Materials and Methods

Chemicals

ZT was a gift from Zydus Cadila Healthcare, Ahmedabad, India (manufactured by Archerchem, Mumbai, India). Fatty acid ester of polyglycerol, caprylocaproyl macrogolglyceride and purified diethylene glycol monoethyl ether were received as gifts from Gattefosse (Saint-Priest, France). Polycarbophil (AA-1, pharmagrade, mol wt. approx. 3.50 billion) was purchased from Noveon (Mumbai, India). Citric acid (monohydrate) and disodium phosphate (dihydrate) were purchased from Merck Chemicals (Mumbai, India). Diethylene triamine penta acetic acid (DTPA) and stannous chloride dihydrate (SnCl2.2H2O) were purchased from Sigma Chemical Co (St. Louis, MO). Sodium pertechnetate, separated from molybdenum-99 (99m) by solvent extraction method, was provided by Regional Center for Radiopharmaceutical Division (Northern Region), Board of Radiation and Isotope Technology (BRIT, Delhi, India). All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Preparation and Characterization of Microemulsions

Zolmitriptan solution (ZS, 50 mg/mL ZT) was prepared by addition of ZT (500 mg) to 8 mL distilled water with continuous stirring. The pH was adjusted to 5.0 ± 0.25 using citric acid (approx. 0.08 mg/mL) and disodium phosphate (approx. 0.12 mg/mL). The mixture was stirred for 10 minutes and clear solution was obtained. Final volume was made up to 10 mL using distilled water.

Zolmitriptan microemulsion (ZME, 50 mg/mL zolmitriptan) was prepared using medium chain triglyceride (MCT) as an oil (20% w/w), caprylocaproyl macrogol glyceride as a surfactant (S, 27.50% w/w). Mixture of purified diethylene glycol monoethyl ether and fatty acid ester of polyglycerol (1:1 w/w) was used as a co-surfactant (CoS, 12.50% w/w)
and distilled water (40% w/w) as an aqueous phase. Formulations were prepared by
dissolving ZT at 60°C ± 5°C in S, CoS and oil mixture. The solution was cooled to 30°C ± 5°C temperature. Distilled water was added gradually with continuous stirring, which resulted in transparent and homogenous ZME (% transmittance at 630 nm > 99%). Zolmitriptan mucoadhesive microemulsion (ZMME) was prepared by addition of polycarbophil (0.50% w/w) to ZME and the dispersion was stirred for 1 h.

ZT content in the formulations was determined using a high performance liquid chromatographic method [16] at 229 nm wavelength ($\lambda_{\text{max}}$). C$_8$ column (150 mm x 6 mm, 10 μm) was used for separation of ZT; mixture of acetonitrile and phosphate buffer-10 mmol/L (25:75, pH 7.5) was used as mobile phase. The mobile phase was degassed and isocratically run at a flow rate of 1 mL/minute.

The globule size determination [17] was performed using photon correlation spectroscopy with in-built Zetasizer (model: Nano ZS, Malvern Instruments, UK) at 633 nm. Helium-neon gas laser having intensity of 4 mW was the light source. The equipment was programmed to provide 18 mm laser width. Electrophoretic mobility (μm/s) was measured using small volume disposable zeta cell and converted to zeta potential [17] by in-built software using Helmholtz-Smoluchowski equation.

**Preparation of radiolabeled-drug formulations of ZMME, ZME and ZS**

Zolmitriptan was labeled using $^{99m}$Tc by direct labeling method [18-22] and formulations were prepared using radiolabeled-drug. ZT (62.5 mg) was added in the mixture of oil (2000 mg) and surfactant (150 mg) with constant stirring. The temperature of the dispersion was adjusted to 55° C ± 5° C and was constantly stirred for 30 minutes. The clear drug solution was cooled to 30° C ± 5° C. One hundred microgram of stannous chloride dihydrate in 100 μL of 0.10 N HCl was added and pH was adjusted to 6.60 ± 0.20 using 50 mM sodium bicarbonate solution. To the mixture, 1 mL of sterile $^{99m}$Tc-
Pertechnetate (75 to 400 MBq, filtered through 0.22 μm nylon 66 membrane filters) was added gradually over a period of 60 seconds with constant stirring. The solution was incubated at room temperature for 30 minutes with continuous nitrogen purging. To this solution, surfactant (2600 mg) and co-surfactant were added and stirred for 5 minutes. Distilled water (approx. 4 mL) was added over a period of 2 minutes with constant stirring. The final volume was made up to 10 mL using distilled water. Similarly, for preparation of radiolabeled-drug formulation of ZS, the drug was dissolved in distilled water (5 mL) and the pH was adjusted to 5.0 ± 0.25 using citric acid (approx. 0.08 mg/mL) and disodium phosphate (approx. 0.12 mg/mL). The drug is radiolabeled using 99mTc and final volume was made up to 10 mL using distilled water.

The radiochemical purities [20, 21] of 99mTc-ZME (99m-Technetium-labeled-drug formulation ZME), 99mTc-ZMME (99m-Technetium-labeled-drug formulation ZMME) and 99mTc-ZS (99m-Technetium-labeled-drug formulation ZS) were estimated using ascending instant thin layer chromatography. Silica gel coated fiber glass sheets (Gelman Sciences Inc., Ann Arbor, MI, USA) and dual solvent systems consisting of acetone and pyridine: acetic acid: water (3: 5: 1.50 v/v) were used as mobile phases. The effect of incubation time, pH and stannous chloride concentration on radiolabeling efficiency were studied to achieve optimum reaction conditions. The radiolabeled-drug formulations were challenged to assess bonding strength at different molar concentrations (25 mM to 100 mM) of diethylene triamine penta acetic acid (DTPA) [21]. The optimized radiolabeled-drug formulations were assessed for in vitro stability in 0.90% (w/v) sodium chloride (normal saline) and in rat plasma [20]. The composition, mean globule size distribution, zeta potential and radiolabeling efficiency of zolmitriptan formulations are recorded in Table 1. Consequently, optimized, stable radiolabeled-drug formulations of ZT were used to study biodistribution in rats.
Bio-distribution Studies

All experiments conducted on animals were approved by the Social Justice and Empowerment Committee, Ministry of Government of India. Swiss albino rats (male, aged 4 to 5 months), weighing between 200 to 250 g were selected for the study.

Four rats for each formulation per time point were used to carry out biodistribution study. Radiolabeled-drug formulation $^{99m}$Tc-ZME (100 µCi/50 µL) containing 0.10 mg to 0.15 mg zolmitriptan (equivalent to 0.083 mg/kg body weight) was injected through tail vein of Swiss albino rats [18]. Similarly, radiolabeled-drug formulations $^{99m}$Tc-ZS/ZME/ZMME (100 µCi /20 µL) containing 0.10 mg to 0.15 mg ZT (equivalent to 0.083 mg/kg body weight) were administered (10 µL) in each nostril. Formulations were administered in the nostrils using micropipette (100 µL) fixed with low density polyethylene tube having 0.10 mm internal diameter at the delivery site. The rats were held from the back in slanted position during nasal administration. The rats were sacrificed with mercy at different time intervals and blood was collected using cardiac puncture. Subsequently, tissues/organs such as heart, lung, liver, stomach, intestine, kidney, spleen, brain and spinal cord were dissected, washed twice using normal saline and made free from adhering tissue/fluid and weighed. The radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter. The radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of the administered dose [21] and the results are recorded in Table 2. The pharmacokinetic parameters for ZT formulations were calculated from the Figure 1 and Figure 2 using Kinetica software (version 4.10, Innaphase, USA) and are recorded in Table 3. Brain-targeting efficiency for ZT formulations were calculated using two indexes (%DTE and %DTP) as mentioned below [23, 24].
Drug targeting efficiency (DTE) [11]: DTE represents time average partitioning ratio of intranasal to intravenous route.

\[
DTE(\%) = \left\{ \frac{(\text{AUC}_{\text{brain}} / \text{AUC}_{\text{blood}})_{\text{i.n.}}}{(\text{AUC}_{\text{brain}} / \text{AUC}_{\text{blood}})_{\text{i.v.}}} \right\} \times 100 \quad \text{(1)}
\]

Nose-brain direct transport was calculated using “brain-drug-direct-transport percentage (DTP)”; which has been calculated from equations (i) and (ii) as mentioned below.

\[
\text{DTP}(\%) = \left\{ \frac{(B_{\text{i.n.}} - B_{\text{x}})}{B_{\text{i.n.}}} \right\} \times 100 \quad \text{(i)}
\]

Where,

\[
B_{\text{x}} = (B_{\text{i.v.}} / P_{\text{i.v.}}) \times (P_{\text{i.n.}}) \quad \text{(ii)}
\]

\(B_{\text{x}}\) = Brain AUC fraction contributed by systemic circulation through the blood-brain-barrier (BBB) following intranasal administration.

\(B_{\text{i.v.}}\) = AUC\(_{0\rightarrow480}\) (brain) following intravenous administration.

\(P_{\text{i.v.}}\) = AUC\(_{0\rightarrow480}\) (blood) following intravenous administration.

\(B_{\text{i.n.}}\) = AUC\(_{0\rightarrow480}\) (brain) following intranasal administration.

\(P_{\text{i.n.}}\) = AUC\(_{0\rightarrow480}\) (blood) following intranasal administration.

\(\text{AUC} = \text{Area under the curve.}\)

Reports in the literature reveal that the drug uptake into the brain from the nasal mucosa mainly occurs via two different pathways. One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. The other is the olfactory pathway by which partly the drug travels from the nasal cavity to CSF and/or brain tissue\(^{10}\). We can conclude that the amount of drug in the brain tissue after nasal administration is attributed to these two pathways. Since, zolmitriptan displays linear pharmacokinetics, the amount of drug is proportional to AUC. Thus, we can assume that the brain AUC fraction contributed by systemic circulation through BBB (represented by \(B_{\text{x}}\)), divided by plasma AUC from nasal route is equal to that of i.v. route (see Equation (1)). Therefore, DTP (%) represents the
percentage of drug directly transported to the brain via the olfactory pathway. DTP (%) and DTE (%) were calculated using tissue/organ distribution data following intranasal and intravenous administrations and are recorded in Table 4.

**Gamma Scintigraphy Imaging**

The albino rats (200 g to 250 g) were selected for the study. Radiolabeled-drug formulation $^{99m}$Tc-ZME (100 μCi/50 μL) containing 0.10 mg to 0.15 mg ZT (equivalent to 0.083 mg/kg body weight) was injected through tail vein of Swiss albino rats. Similarly, radiolabeled-drug formulation $^{99m}$Tc-ZS/ZME/ZMME (100 μCi /20 μL) containing 0.10 mg to 0.15 mg ZT (equivalent to 0.083 mg/kg body weight) were administered (10 μL) in each nostril. The rats were anaesthetized using 0.25 mL ketamine hydrochloride intramuscular injection (50 mg/mL) and placed on the imaging board. Imaging was performed using Single Photon Emission Computerized Tomography (SPECT, LC 75-005, Diacam, Siemens AG, Erlanger, Germany) gamma camera [22, 25, 26]. The scintigraphy images following intravenous administration of ZME and intranasal administrations of ZME and ZMME are shown in Figure 3.

**Statistical Analysis**

All data are reported as mean ± SEM and the difference between the groups were tested using Student’s t test at the level of $P< 0.05$. More than two groups were compared using ANOVA and differences greater at $P< 0.05$ were considered significant.
Results and Discussion

ZS, ZME and ZMME were prepared and characterized for assay, globule size and zeta potential (Table 1). The ZT content was found to be 98.78%, 97.22% and 98.95% for ZS, ZME and ZMME respectively. The mean globule size and zeta potential of ZME was found to be 31.56 nm ± 17.12 nm and -38.90 ± 2.05 mV whereas for ZMME, the globule size was found to be 34.79 nm ± 21.80 nm and zeta potential -51.94 mV ± 0.89 mV. ZME showed net negative charge and addition of mucoadhesive agent further contributed negatively to the system. This may be attributed to the fact that increase in surfactant level resulted in a decrease in surface tension and surface free energy of the formed micelles. Therefore, net negative charge (anionic) of the microemulsion increased [27]. The microemulsions were expected to have good physical stability (phase separation) as zeta potential is less than -30 mV [28, 29]. Moreover, addition of mucoadhesive polymer (Polycarbophil P) may further stabilize the system since it increased negative charge of the system.

ZT was effectively labeled using 99mTc and radiolabeled-drug formulations of ZS, ZME and ZMME were optimized for maximum labeling efficiency and stability and radiochemical purity achieved was 98.77%, 96.30% and 96.99% for ZS, ZME and ZMME respectively when evaluated for reduced/hydrolyzed (R/H) 99mTc and free 99mTc. Optimum SnCl2.2H2O concentration was found to be 100 µg/mL at pH 6.60 ± 0.20 with an incubation time of 15 minutes. The 99mTc-ZS/ZME/ZMME were found to be stable in 0.90% (w/v) sodium chloride solution (saline) and in rat serum up to 24 hours (degradation<3% w/w). Bonding strength of the 99mTc- ZS/ZME/ZMME were also investigated using DTPA challenging test and the percent transechelation of the labeled complex was 1.35% w/w at 25 mM DTPA concentration, while at 100 mM it increased...
to 2.95% w/w. The results suggested high bonding strength and stability $^{99m}$Tc-ZS/ZME/ZMME and hence, were used to study biodistribution of the drug in rats.

Biodistribution studies of $^{99m}$Tc-ZT formulations following i.v. administration (ZME) and intranasal (ZS, ZME and ZMME) administration on Swiss albino rats were performed and the radioactivity was estimated at different intervals up to 8 h (Table 2). The brain/blood ratio of the drug at all sampling time points for different formulations were also calculated and recorded in Table 2. The pharmacokinetic parameters were calculated from Figure 1 and Figure 2 and are recorded in Table 3.

Lower $T_{max}$ values (brain) for nasally administered formulations (0.50 h) compared to ZME (i.v.) (1 h) is indicative of direct nose-to-brain transport (Figure 2). The brain/blood ratios of the drug were found to be higher for formulations administered intranasally (Table 2). The results suggest direct nose-to-brain transport. The concentrations of drug in the brain following intranasal administration of ZME and ZMME were found to be significantly higher at all sampling time points compared to ZME (intravenous) up to 8 h. The substantial higher uptake in the brain is suggestive of a larger extent of preferential nose-to-brain transport of ZT. Many scientists have reported unique connection between the nose and the brain, and intranasal delivery of drug circumvents the blood-brain-barrier resulting into enhanced rate and extent of transport of drugs to the brain [10, 11, 30]. The $T_{1/2}$ was found between 1.50 and 3.60 h (blood) and 1.25-5.50 h (brain), and $K_{el}$ of 0.10 to 0.26 (blood) and 0.01 to 0.31 (brain) and found to be insignificant irrespective of the routes of administration and the type of the formulations.

Significantly higher $C_{max}$ (brain) and AUC (brain) were observed when ZME nasal and ZMME nasal were compared to ZME i.v. This may be attributed to preferential nose-to-brain transport of the drug following intranasal administration [32, 33].
Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance. However, when mucoadhesive agent was incorporated in the formulation (ZMME), significantly higher $C_{\text{max}}$ and AUC were observed compared to ZS and ZME (nasal). The results demonstrated the importance of mucoadhesive agent in prolonging the contact time of the formulation with the nasal mucosa and thereby enhancing rate and extent of absorption of the drug [14, 15].

When ZME nasal was compared to ZS nasal, significantly higher AUC and $C_{\text{max}}$ were observed. This may be attributed to the fact that microemulsion enhances transport of drug across nasal mucosa. These findings are in congruence with the observations reported by Qizhi et al. that microemulsion enhances transport of drug across nasal mucosa resulting in direct nose-to-brain transport of the drugs [23, 31]. Drug targeting efficiency (DTE (%)) and brain drug-direct-transport percentage (DTP (%)) were also calculated (Table 4) from the pharmacokinetics data (Table 3). Amongst all nasally administered formulations, ZMME showed highest DTE (%) and DTP (%) values followed by ZME and then ZS. Three-fold higher DTE (%) and two-fold higher DTP (%) for ZMME compared to ZS demonstrated the significance of the mucoadhesive microemulsion formulation. The higher DTE (%) and DTP (%) demonstrated that ZMME (nasal) has greater brain-targeting efficiency compared to ZME and ZS, may be because of preferential nose-to-brain transport.

In order to ascertain the brain uptake following intranasal and intravenous administrations of $^{99m}$Tc-zolmitriptan microemulsion, we performed gamma scintigraphy and scintigrams of rats at 0.50 h post-intravenous injection and intranasal administrations are shown in Figure 3. The major radioactivity accumulation was seen in the abdominal region, which is in conformity with the results of biodistribution studies. In case of formulations administered intranasally, a part of radioactivity was noticed in the
esophagus. The scintigrams clearly demonstrate the accumulation of formulations in brain administered via both the routes. However, the accumulation of radioactivity was higher following intranasal administration of ZMME compared to intravenous administration CME.

Conclusions

To conclude, mucoadhesive microemulsion of zolmitriptan was successfully prepared and demonstrated in rats to deliver zolmitriptan in larger quantity, quickly and effectively to the brain following intranasal administration. This study aptly demonstrated the effectiveness of intranasal delivery of zolmitriptan. However, the ratio of benefits vs. the risks must be evaluated and clinical intricacies must be scientifically established for its efficacy in clinical practice, in the treatment of acute attacks of migraine.

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References


### Table 1: Composition and characterization* of zolmitriptan formulations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Formulation</th>
<th>O (%)</th>
<th>S (%)</th>
<th>CoS (%)</th>
<th>AQ (%)</th>
<th>Drug content (%)</th>
<th>Globule size (nm)</th>
<th>Zeta potential (mV)</th>
<th>Radiolabeled complex (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZS</td>
<td>Zolmitriptan solution</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.0</td>
<td>98.78 ± 0.18</td>
<td>-</td>
<td>98.77 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>ZME</td>
<td>Zolmitriptan microemulsion</td>
<td>20.0</td>
<td>37.5</td>
<td>12.5</td>
<td>30.0</td>
<td>97.22 ± 0.34</td>
<td>31.56 ± 17.12</td>
<td>-38.90 ± 2.05</td>
<td>96.30 ± 0.57</td>
</tr>
<tr>
<td>ZMME</td>
<td>Zolmitriptan mucoadhesive microemulsion</td>
<td>20.0</td>
<td>37.5</td>
<td>12.5</td>
<td>30.0</td>
<td>98.95 ± 0.39</td>
<td>34.79 ± 21.80</td>
<td>-51.94 ± 0.89</td>
<td>96.99 ± 0.24</td>
</tr>
</tbody>
</table>

*The results are mean values ± SEM derived from six different experimental batches. O is denoted for Oil Phase (Medium chain triglyceride), S for surfactant (Mixture (1:1) of caprylocaproyl macrogol glyceride and purified diethylene glycol), CoS for co-surfactant (fatty acid ester of polyglycerol) and AQ is denoted for aqueous phase (purified water). The formulations (ZS, ZME and ZMME) mentioned in Table 1 contain zolmitriptan 50 mg/mL.
Table 2 Compartmental distribution of $^{99m}$Tc-ZME (i.v.), $^{99m}$Tc-ZS/ZME/ZMME (intranasal) at predetermined time intervals in normal Swiss albino rats *

<table>
<thead>
<tr>
<th>Formulation and route of administration</th>
<th>0.50 h</th>
<th>1.00 h</th>
<th>2.00 h</th>
<th>4.00 h</th>
<th>8.00 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZME (i.v.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2.36 ± 0.21</td>
<td>1.63 ± 0.13</td>
<td>1.15 ± 0.07</td>
<td>0.75 ± 0.11</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Brain</td>
<td>0.30 ± 0.06</td>
<td>0.38 ± 0.09</td>
<td>0.18 ± 0.02</td>
<td>0.13 ± 0.05</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>ZS (i.n.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.09 ± 0.11</td>
<td>0.61 ± 0.05</td>
<td>0.38 ± 0.04</td>
<td>0.09 ± 0.04</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Brain</td>
<td>0.29 ± 0.10</td>
<td>0.24 ± 0.07</td>
<td>0.21 ± 0.06</td>
<td>0.13 ± 0.04</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>ZME (i.n.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.47 ± 0.09</td>
<td>1.33 ± 0.08</td>
<td>0.83 ± 0.05</td>
<td>0.25 ± 0.03</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>Brain</td>
<td>0.82 ± 0.07</td>
<td>0.53 ± 0.11</td>
<td>0.44 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>ZMME (i.n.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.54 ± 0.12</td>
<td>1.09 ± 0.12</td>
<td>0.85 ± 0.06</td>
<td>0.54 ± 0.02</td>
<td>0.26 ± 0.03</td>
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<tr>
<td>Brain</td>
<td>1.07 ± 0.05</td>
<td>0.88 ± 0.06</td>
<td>0.67 ± 0.08</td>
<td>0.53 ± 0.08</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>ZME (i.v.) Brain/Blood</td>
<td>0.13 ± 0.08</td>
<td>0.23 ± 0.11</td>
<td>0.16 ± 0.05</td>
<td>0.17 ± 0.04</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>ZS (i.n.) Brain/Blood</td>
<td>0.27 ± 0.07</td>
<td>0.39 ± 0.09</td>
<td>0.55 ± 0.05</td>
<td>0.14 ± 0.04</td>
<td>3.00 ± 0.08</td>
</tr>
<tr>
<td>ZME (i.n.) Brain/Blood</td>
<td>0.56 ± 0.12</td>
<td>0.40 ± 0.07</td>
<td>0.53 ± 0.11</td>
<td>0.32 ± 0.06</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>ZMME (i.n.) Brain/Blood</td>
<td>0.70 ± 0.16</td>
<td>0.81 ± 0.14</td>
<td>0.79 ± 0.16</td>
<td>0.98 ± 0.15</td>
<td>1.19 ± 0.12</td>
</tr>
</tbody>
</table>

* The rats were administered with 100 µCi $^{99m}$Tc-zolmitriptan formulations and the radioactivity was measured in percent per g of tissue of the administered dose. Each value is the mean ± SEM of 4 estimations.
Table 3 Pharmacokinetics of $^{99m}$Tc-ZME (i.v.), $^{99m}$Tc-ZS/ZME/ZMME (intranasal) at predetermined time intervals in normal Swiss albino rats

<table>
<thead>
<tr>
<th>Formulation and route of administration</th>
<th>Organ/tissue</th>
<th>$C_{\text{max}}$ (%/g)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC$_{0\rightarrow480\text{min}}$ (h* %/g)</th>
<th>AUC$_{0\rightarrow\infty}$ (h* %/g)</th>
<th>$K_{dL}$ (L/h)</th>
<th>$T_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZME (i.v.)</td>
<td>Blood</td>
<td>2.36 ± 0.21</td>
<td>0.50 ± 0.10</td>
<td>6.79 ± 0.51</td>
<td>7.52 ± 0.61</td>
<td>0.10 ± 0.02</td>
<td>1.67 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.38 ± 0.09</td>
<td>1.00 ± 0.15</td>
<td>1.13 ± 0.17</td>
<td>1.27 ± 0.14</td>
<td>0.01 ± 0.01</td>
<td>1.38 ± 0.30</td>
</tr>
<tr>
<td>ZS (i.n.)</td>
<td>Blood</td>
<td>1.09 ± 0.11</td>
<td>0.50 ± 0.10</td>
<td>1.79 ± 0.23</td>
<td>1.95 ± 0.16</td>
<td>0.08 ± 0.01</td>
<td>1.45 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.29 ± 0.10</td>
<td>0.50 ± 0.10</td>
<td>0.57 ± 0.09</td>
<td>0.73 ± 0.06</td>
<td>0.11 ± 0.01</td>
<td>1.38 ± 0.15</td>
</tr>
<tr>
<td>ZME (i.n.)</td>
<td>Blood</td>
<td>1.47 ± 0.09</td>
<td>0.50 ± 0.10</td>
<td>3.80 ± 0.31</td>
<td>4.25 ± 0.39</td>
<td>0.09 ± 0.02</td>
<td>1.98 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.82 ± 0.07</td>
<td>0.50 ± 0.10</td>
<td>1.62 ± 0.18</td>
<td>1.78 ± 0.13</td>
<td>0.02 ± 0.01</td>
<td>1.38 ± 0.10</td>
</tr>
<tr>
<td>ZMME (i.n.)</td>
<td>Blood</td>
<td>1.54 ± 0.12</td>
<td>0.50 ± 0.15</td>
<td>4.90 ± 0.17</td>
<td>6.32 ± 0.28</td>
<td>0.26 ± 0.02</td>
<td>3.55 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>1.07 ± 0.05</td>
<td>0.50 ± 0.10</td>
<td>4.36 ± 0.48</td>
<td>6.82 ± 0.44</td>
<td>0.31 ± 0.02</td>
<td>5.36 ± 0.45</td>
</tr>
</tbody>
</table>

* The rats were administered with 100 μCi $^{99m}$Tc-zolmitriptan formulations and the radioactivity was measured in percent per g of tissue of the administered dose. The pharmacokinetic parameters are derived using mean ± SEM of four estimations.
Table 4 Drug targeting efficiency and direct nose-to-brain transport* following intranasal administration of $^{99m}$Tc-ZS/ZME/ ZMME

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug targeting efficiency (%DTE)*</th>
<th>Direct drug nose to brain transport (%DTP)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZS (i.n.)</td>
<td>189 ± 9</td>
<td>47 ± 1.5</td>
</tr>
<tr>
<td>ZME (i.n.)</td>
<td>255 ± 11</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>ZMME (i.n.)</td>
<td>533 ± 18</td>
<td>81 ± 4</td>
</tr>
</tbody>
</table>

* Parameters are derived using mean ± SEM values of four different estimations.
Figure Legends

**Figure 1** Blood concentrations vs. time (h) plot following intranasal and intravenous administrations of $^{99m}$Tc-zolmitriptan formulations.

**Figure 2** Brain concentrations vs. time (h) plot following intranasal and intravenous administrations of $^{99m}$Tc-zolmitriptan formulations.

**Figure 3** Gamma scintigraphy images of rat (A/P view) showing the presence of radioactivity into the brain (arrows). (A) intravenous and (B) intranasal administration of $^{99m}$Tc-ZME (100 μCi); and (C) intranasal administration of $^{99m}$Tc-ZMME (100 μCi).
Figure 1

Concentration in Blood vs. Time (h) for different routes of administration:
- ZME i.v.
- ZMME i.n.
- ZS i.n.
- ZME i.n.
Figure 2

Concentration in Brain vs. Time (h)

- ZMME i.n.
- ZMME i.v.
- ZS i.n.

[Graph showing concentration over time for different treatments]