Utility of a Rectal Suppository Containing the Antiepileptic Drug Zonisamide

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A suppository of zonisamide (ZNS) was investigated from the viewpoint of pharmaceutical evaluation, pharmacokinetics and pharmacological effect. Two types of ZNS suppositories were prepared. One used Witexsol (H-15:S-55 = 3:1) as a lipophilic base and the other polyethylene glycol (PEG, 4000:1500 = 4:1) as a hydrophilic base. The in vitro release rate of ZNS from the PEG suppository was significantly higher compared with that of ZNS from Witexsol. Male Wistar rats were administered ZNS (20 mg/kg) using an intravenous, oral or rectal (PEG or Witexsol) route. The absorption of ZNS from the PEG suppository was more rapid than that of ZNS from the Witexsol suppository or from the oral preparation. The peak plasma concentration (C_max) after a rectal administration of ZNS with Witexsol or PEG suppository was significantly higher than that after the oral administration of ZNS. However, the bioavailability of the three preparations was approximately 100%. Male ICR mice were administered ZNS (80 mg/kg) using the oral or rectal (PEG or Witexsol) route. A positive correlation was observed between the electroshock seizure (ES) threshold and ZNS concentration in plasma or brain. Further, there was no significant difference in the ES threshold or the ZNS concentration in plasma or brain among the three preparations. These results indicate that a ZNS suppository is a very useful preparation from the viewpoint of both pharmacokinetics and pharmacological action.

Key words zonisamide; rectal suppository; seizure threshold; pharmacokinetics; drug evaluation

Antiepileptic drugs are used for the treatment of various seizures. In terms of pharmacotherapy, the most suitable drug is selected by means of clinical symptoms and brain waves in the epileptic patient. The drugs are generally used as an internal preparation such as tablets or powders. However, it is often difficult to administer the drug by the oral route for pre- and post-surgical patients operated on the brain neurosurgery region. Therefore, the rectal administration of a suppository is considered as a superior route to oral administration. Up to the present, only diazepam (Diapp, Wakodo Co., Ltd., Tokyo, Japan) among antiepileptic drugs has been available in the formulation of a rectal suppository.

Zonisamide (1,2-benzisoxazole-3-methansulphonamide, ZNS), which has benzisoxazole groups, has been reported to have an anticonvulsant effect in experimental animals.1,2) ZNS shows clinical efficacy upon various types of epilepsy, particularly in refractory seizures. Recently, ZNS has been available in order to control convulsions during or after surgery on patients within the brain neurosurgery region. ZNS is available in two types of formulations, tablets and powders, and the bioavailability is approximately 100%. However, neither tablet nor powder is a suitable preparation for patients who are difficult for oral administration. Therefore, the rectal route is considered as a preferable route for these patients. However, no animal study or pharmacokinetic analysis on the clinical application of ZNS suppositories has yet been examined.

In this study, two types of ZNS suppositories were prepared in order to examine the possibility of clinical application, and they were investigated in terms of pharmaceutical evaluation, pharmacokinetics and pharmacological efficacy of rectal routes in an animal.

MATERIALS AND METHODS

Drugs ZNS and ZNS sodium salt (ZNS-Na) were kindly supplied by Dainippon Pharmaceutical Co., Ltd. (Tokyo, Japan). Witexsol H-15 and S-55 were obtained from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Polyethylene glycol (PEG) 1500 and 4000 were obtained from Nakalai Tesque Co., Ltd. (Kyoto, Japan). Allobarbital and tert-butyl methyl ether were obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of Drug A ZNS suppository was prepared by the fusion method. ZNS was mixed with a molten suppository base, either Witexsol (H-15:S-55 = 3:1) or PEG (4000:1500 = 4:1). The mixture was then poured into a 2.25 ml plastic mold. In the case of the animal study, the mixture was solidified in a 1 ml syringe for rats (5 mm i.d.) or polyethylene tubing for mice (3 mm i.d.). The administered volume of the suppository was 100 mg/100 g body weight of rat or 20 mg/10 g body weight of mouse. Both suppositories were stored at 4°C in a refrigerator. For intravenous administration, ZNS-Na was dissolved in isotonic sodium chloride solution. For oral administration, ZNS was suspended in 5% arabic gum solution. The administered volume of drug solutions was 0.1 ml/100 g body weight of rat or 0.1 ml/10 g body weight of mouse.

In Vitro Release Study The release of ZNS (20 mg) from a suppository (2 g) was determined using a suppository release tester (model TMS-103, Toyama Sango Co., Ltd.).
Co., Ltd., Osaka, Japan) and MF-millipore membrane filters (filter type: ss, pore size: 3.0 μm, diameter: 47 mm, Nihon Millipore, Ltd., Tokyo, Japan) according to the procedure reported by Muranishi et al.31 Five hundred ml of phosphate buffer (pH 7.4) was used as a release phase. The buffer was rotated at 100 rpm with a magnetic stirrer and maintained at 37°C. The release cell was placed into 5 ml of phosphate buffer and rotated at 25 rpm. Samples (1 ml) were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7 h. The amount of ZNS released was determined by a spectrophotometer (DU-64, Beckman Instruments, Inc., Fullerton, U.S.A.) at a wavelength of 239 nm.

**In Vivo Absorption Study** Male Wistar rats weighing 190 to 250 g (Kyudo Co., Ltd., Saga, Japan) were used in the experiments. They were fasted for 24 h before the administration of ZNS. Before the experiment, the jugular vein was cannulated with silicon tubing. For intravenous administration, ZNS-Na solution (10 mg/kg: 100 mg/10 ml, 20 mg/kg: 200 mg/10 ml, 40 mg/kg: 400 mg/10 ml) was injected into the tail vein. For oral administration, a ZNS suspension (20 mg/kg: 200 mg/10 ml) was directly administered into the stomach using a sonde. For rectal administration, a ZNS suppository (20 mg/kg: 4 mg/200 mg base) was inserted to a depth of 1 cm from the anus. Then, the anus was closed with an adhesive, Alone Alpha A (Sankyo Co., Ltd., Tokyo, Japan). Blood samples were drawn at 0.5, 1, 2, 4, 8, 10, 16 and 24 h by the cannula implanted into the jugular vein. Plasma samples were immediately obtained by centrifugation and frozen at −30°C until being analyzed.

**Determination of Electroshock Seizure (ES) threshold** Male ICR mice (7 weeks old, Clea Japan, Inc., Osaka, Japan) were used in the experiments and fasted for 24 h before the administration of ZNS. ZNS was administered at a dose of 80 mg/kg for oral and rectal routes (oral: 80 mg/10 ml, rectal: 2.4 mg/60 mg base). Each preparation without ZNS was administered as the control. The ES threshold was determined by a stimulator (E.C. stimulator, model MK-800, Muromachi Kikai Co., Ltd., Tokyo, Japan), which increased the direct current (0.1 mA) stepwise every prefixed period of time (0.2 s).4,5 The stimulus was delivered through copper corneal electrodes placed on the eyes. The ES threshold was defined as the amount of current in milliamperes (mA) delivered through corneal electrodes, which resulted in a detectable tonic forelimb flexion and immediate forelimb extension. Plasma and whole brain samples were obtained under deep ether anesthesia immediately after ES threshold determination and were frozen at −30°C until being analyzed.

**Determination of ZNS Concentration** The ZNS concentration in plasma and whole brain was determined by HPLC (Waters 510 HPLC Pump-Waters 484 Tenable Absorbance, Waters Division of Millipore, Milford, U.S.A.). For the determination of ZNS concentration in plasma, 50 μl alobarbital as an internal standard (I.S. solution, 48 μg/ml in distilled water) and 900 μl phosphate buffer (pH 6.8) were added to a 100 μl plasma sample and mixed with a vortex mixer. For the determination of ZNS concentration in whole brain, the brain was homogenized with phosphate buffer (pH 6.8) using a glass homogenizer (Ikemoto Rika Kogyo Co., Ltd., Tokyo, Japan). Three hundred μl brain homogenate was deproteinated with 100μl 10% (w/v) trichloroacetic acid. The homogenate was centrifuged at 3000 rpm for 10 min. Fifty μl I.S. solution, 20 μl 2.5% (w/v) NaOH and 930 μl phosphate buffer were added to 100 μl of brain supernatant. The mixture was passed through an extrelute column (Extrelute 1, Merck Co., Ltd., Darmstadt, Germany) and eluted by tert-butyl methyl ether after 10 min. The eluate was evaporated for 70 min at room temperature. The residue was dissolved in 100 μl 50% (v/v) methanol in distilled water and then injected onto the HPLC column (LiChroCART 75-4, Merck Co., Ltd., Darmstadt, Germany). The mobile phase was 20% (v/v) methanol in distilled water. The flow rate was 1.5 ml/min, and the detection wavelength was 210 nm.

**Pharmacokinetic Analysis** The peak plasma concentration (Cmax) and time required to reach the peak concentration (tmax) were obtained from the observed values. The area under the plasma concentration–time curve (AUC0−∞) was calculated using the trapezoidal rule, while the extrapolation to infinity was carried out by dividing the last measured plasma concentration value by the value of the slope. The absolute bioavailability was calculated using the AUC value.

**Data Analysis** The means of all data were presented with their standard deviation (mean ± S.D.). Statistical evaluation was performed by analysis of variance (ANOVA). The difference between two groups was determined by Student’s t-test. Significance was defined as p < 0.05.

**RESULTS**

**Release of ZNS from Suppository Base in Vitro** Two types of ZNS suppository bases were evaluated from pharmaceutical viewpoints by a ZNS release test in vitro (Fig. 1). The release rate of ZNS from the PEG suppository base was significantly higher than that from the Witepsol suppository base (after 2.5 h: 90.80 ± 7.16%, 73.51 ± 5.63%, after 3 h: 94.38 ± 5.79%, 79.78 ± 4.91%, p < 0.05, respectively). However, there was no significant difference.

**Fig. 1. Release Profiles of ZNS from Suppository**

- ○, Witepsol suppository; ■, PEG suppository. Each point represents the mean ± S.D. of 3 experiments. *significantly different from Witepsol (p < 0.05).
at any other sampling time between the two suppository bases. After 6 h, the release amount of ZNS from both suppository bases reached nearly 100%.

**Pharmacokinetics of ZNS after an Intravenous, Oral or Rectal Administration of ZNS** Figure 2 showed the time course of plasma concentrations after an intravenous administration of ZNS (10, 20 or 40 mg/kg). ZNS after intravenous administration was eliminated from the plasma in a monoeponential manner, and the elimination half-life was 6.12—6.53 h at each dose examined. The $AUC_{\text{0-\infty}}$ after the intravenous administration of ZNS increased depending on the dose (Table 1). In the dose range of 10—40 mg/kg, a positive linear relationship was observed between the $AUC$ and the dose of ZNS ($y = 36.27x - 211.14$, $r = 1.000$, $p < 0.01$). The plasma ZNS concentration-time profiles and ZNS pharmacokinetic parameters are shown in Figs. 3A, B and Table 1. $C_{\text{max}}$ was significantly higher after the rectal administration of ZNS with Witepsol or PEG than after the oral administration of ZNS ($p < 0.05$, $p < 0.01$ respectively). $t_{\text{max}}$ was shorter after the rectal administration of ZNS with PEG than after the rectal administration of ZNS with Witepsol or the oral administration of ZNS alone. The bioavailability of the three preparations was approximately 100%.

**Relationship between ES Threshold and ZNS Concentration in Plasma or Brain** Table 2 shows the ZNS concentrations in the plasma or brain at 1 h after the oral or rectal administration of ZNS (80 mg/kg) in mice. There was no significant difference of ZNS concentration in the plasma or brain among the three preparations. Figure 4 showed the ES threshold at 1 h after the oral or rectal administration of ZNS (80 mg/kg) in mice. The ES threshold values of three preparations with ZNS were significantly higher than those of the control prepara-

**Table 1. Pharmacokinetic Parameters of ZNS after Intravenous, Oral or Rectal Administration of ZNS in Rats**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$AUC_{\text{0-\infty}}$ (µg h/ml)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>151.82 ± 6.64</td>
<td>2.47</td>
<td>270.43 ± 34.53</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>332.59 ± 24.76</td>
<td>2.60</td>
<td>695.76 ± 72.04</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>695.76 ± 72.04</td>
<td>2.60</td>
<td>1391.52 ± 148.12</td>
<td>100</td>
</tr>
<tr>
<td>Oral</td>
<td>20</td>
<td>18.97 ± 2.47</td>
<td>4</td>
<td>270.43 ± 34.53</td>
</tr>
<tr>
<td>Rectal</td>
<td>40</td>
<td>31.82 ± 2.46</td>
<td>2</td>
<td>358.87 ± 60.68</td>
</tr>
<tr>
<td>Witepsol sup.</td>
<td>20</td>
<td>26.11 ± 4.09</td>
<td>4</td>
<td>319.08 ± 49.31</td>
</tr>
<tr>
<td>PEG sup.</td>
<td>20</td>
<td>31.82 ± 2.46</td>
<td>2</td>
<td>358.87 ± 60.68</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3—5 rats. a) Comparisons of $C_{\text{max}}$ between oral and rectal administration were done by Student’s t-test. b) Statistical evaluation for $AUC_{\text{0-\infty}}$ was performed by ANOVA. c) Bioavailability = $[AUC_{\text{0-\infty}}]$ oral or rectal/[AUC$_{\text{0-\infty}}$] intravenous $\times 100$. d) Significantly different from oral ($p < 0.05$). e) Significantly different from oral ($p < 0.01$).

**Table 2. ZNS Concentrations in Plasma and Brain at 1 h after Oral or Rectal Administration of ZNS (80 mg/kg) in Mice**

<table>
<thead>
<tr>
<th>ZNS concentration in plasma (µg/ml)</th>
<th>ZNS concentration in brain (µg/g)</th>
</tr>
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<tbody>
<tr>
<td>Oral</td>
<td>96.33 ± 18.89</td>
</tr>
<tr>
<td>Rectal</td>
<td>86.52 ± 20.65</td>
</tr>
<tr>
<td>Witepsol sup.</td>
<td>103.32 ± 21.29</td>
</tr>
<tr>
<td>PEG sup.</td>
<td></td>
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</table>

Each value represents the mean ± S.D. of 9—12 mice.

**Fig. 2. Time Courses of ZNS Concentrations in Plasma after Intravenous Administration of ZNS (■ 10 mg/kg; ● 20 mg/kg; ▲ 40 mg/kg) in Rats**

Each point represents the mean ± S.D. of 3 rats.

**Fig. 3. Time Courses of ZNS Concentrations in Plasma after Oral (A) or Rectal (B) Administration of ZNS (20 mg/kg) in Rats**

▲ oral; ● Witepsol suppository; ■ PEG suppository. Each point represents the mean ± S.D. of 3—5 rats.
tions without ZNS (oral: 7.73 ± 0.88 mA (with ZNS) and 5.29 ± 0.54 mA (without ZNS), Witepsol: 7.24 ± 0.48 mA and 5.40 ± 0.38 mA, PEG: 7.48 ± 0.60 mA and 5.22 ± 0.50 mA, p < 0.01, respectively). However, no significant difference was observed among the three preparations. Figures 5A and B showed a positive correlation between the ES threshold and ZNS concentrations in the plasma or brain. The inclination and intercept in the regression line did not differ among the three preparations. The ES threshold showed a good positive correlation with ZNS concentrations in plasma (r = 0.742, p < 0.01) or brain (r = 0.656, p < 0.05) after the oral administration of ZNS or ZNS concentrations in plasma (r = 0.686, p < 0.05) after the rectal administration of ZNS with Witepsol.

DISCUSSION

The release profile of a drug from a suppository in vitro is important for estimating its in vivo action. First, a drug must be released into the rectal fluid in order to reach systemic circulation after rectal administration of the drug.

Therefore, the selection of a physicochemically suitable base for the drug is essential for the preparation of the suppository. ZNS (pK_a = 9.66) is very slightly soluble in water, ether and chloroform, but is sparingly soluble in ethanol. Consequently, two types of suppository bases were examined. One was Witepsol as a lipophilic base and the other polyethylene glycol as a hydrophilic base. Witepsol H-15 and S-55 were mixed at a ratio of 3 to 1, while polyethylene glycol 4000 and 1500 were mixed at a ratio of 4 to 1 in order to make up the favorable conditions for ZNS dispersion. The in vitro release rate of ZNS from the suppository prepared with a hydrophilic base (PEG) was significantly rapid compared with that from the suppository prepared with a lipophilic base (Witepsol). PEG shows a solubilizing effect, increased wettability, and a decrease in aggregation and agglomeration of lipophilic drugs. The release rate of aspirin and chloramphenicol from a hydrophilic base such as PEG is more rapid than that of the drugs from a lipophilic base such as cocoa butter and castor oil. The rate is associated with the water solubility of the hydrophilic base. Also, the release rate of a poorly soluble drug, such as sulfaguanidine, from PEG is more rapid than that of the drug from Witepsol, because the solubility of the drug in an aqueous media is increased by hydrophilic bases. Such characteristics of bases seem to cause the differences in the release profiles of ZNS from the suppositories made up of Witepsol or PEG.

ZNS shows linear pharmacokinetics after an oral dose of ZNS (200 or 400 mg) in healthy male volunteers, whereas in epileptic patients ZNS has nonlinear pharmacokinetics under steady-state plasma levels. In the present intravenous study with rats, ZNS showed linear pharmacokinetics in the range of 10—40 mg/kg. Therefore, the pharmacokinetic parameters of ZNS after the oral or rectal administration of ZNS (20 mg/kg) were analyzed by the simple linear compartment model.

The absorption of ZNS from a suppository prepared with PEG was more rapid than that from the other two preparations. The result corresponded nicely to the release characteristics of ZNS in vitro. It is known that PEG has a cosolvency property. The rectal absorption of phenytoin
and carbamazepine, which are poorly water soluble drugs, is increased by PEG.\textsuperscript{13}) The drugs in suppositories prepared with PEG are dissolved in the base which is dissolved in the rectal fluid, then absorbed from the rectum, whereas the drugs in suppositories prepared with Wittepsol are released from the melted base into the rectal fluid, and then absorbed, or are absorbed directly from the melted base through the mucous membrane of the rectum.\textsuperscript{1,3}) The $C_{\text{max}}$ was significantly higher after the rectal administration of ZNS prepared with Wittepsol or PEG suppository base than after the oral administration of ZNS. However, the bioavailability of the three preparations was approximately 100%.

Although the ES threshold values of the three preparations with ZNS were significantly higher than those of the control preparations without ZNS, no significant difference was observed among the three preparations. The ES threshold method can exactly evaluate the concentration-dependent change in the drug-induced anticonvulsant effect.\textsuperscript{4,5,14}) Certainly, the ES threshold showed good positive correlation with the ZNS concentration in the plasma and brain after either oral or rectal administration in mice, as shown in Fig. 5. There was no significant difference in ZNS concentration in the plasma or brain among the three preparations. This seems to coincide with there being no significant difference of ES threshold among the three preparations.

The present study indicates that a ZNS suppository is a very useful preparation from the viewpoint of pharmacokinetic and pharmacological action in rats and mice. Although a clinical study is necessary, a ZNS suppository may be a very effective preparation in place of an oral preparation.

REFERENCES