Effect of Simultaneous Insertion of Oleaginous Base on the Absorption and on the Anticonvulsant Effect of Diazepam Suppository

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To clarify the drug-base interaction between diazepam (DZP) suppository and oleaginous base, we investigated the effect of simultaneous combination of oleaginous base on the absorption and on the anticonvulsant effect of DZP suppository in rats. Simultaneous insertion of DZP suppository and oleaginous base significantly decreased maximum concentration (C max) and area under the blood concentration–time curve (AUC) of plasma DZP concentrations. Administration of DZP suppositories (2.5, 5 mg/kg) dose-dependently suppressed the pentylentetrazol (PTZ)-induced seizures, and the anticonvulsant effect of DZP suppository (5 mg/kg) was reduced by simultaneous insertion of oleaginous base. In an in vitro study using a suppository release apparatus, simultaneous combination of DZP suppository and oleaginous base (1.5—98 mg) significantly decreased the accumulative release of DZP in a dose-dependent manner. These results suggested that when DZP suppository and oleaginous base are inserted simultaneously, the released DZP distributes partially to the oleaginous base, and this phenomenon is related to the decreases in the plasma concentration and the anticonvulsant effect of DZP.

Key words diazepam suppository; oleaginous base; drug–base interaction; anticonvulsant effect

Diazepam (DZP) suppositories are widely used in infants suffering from febrile convulsions. Previous studies of clinical applications of rectal DZP have shown that intermittent administration of DZP suppositories during the febrile period prevents recurrent febrile convulsions. In addition to DZP suppository, acetaminophen (AAP) suppositories are occasionally used to ease suffering by high fever. However, the simultaneous combination of DZP and AAP suppositories has been reported to reduce the plasma concentration of DZP in infants with recurrent febrile convulsions. It is speculated that when an AAP suppository prepared with an oleaginous base is administered simultaneously with a DZP suppository, portions of the once-dissolved lipid-soluble DZP are reincorporated into the oleaginous base of the AAP suppository, which has fused in the rectal cavity. However, the reason for these drug-base interactions of DZP and AAP suppositories is not clear. Thus, in the present study, we investigated the effect of simultaneous administration of DZP suppository and oleaginous base on the pharmacokinetics and pharmacodynamic action of DZP.

MATERIALS AND METHODS

Materials Polyethylene glycol (PEG) 1540 and 4000 were purchased as the water-soluble base of DZP suppository (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Witepsol H-15 (Vasco H-15, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), which is widely used for oleaginous bases of suppository, was purchased as the oleaginous base. DZP and pentylentetrazol (PTZ) were purchased from Wako Pure Chemical Industries, Ltd. Fludiazepam was obtained from Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan).

Animals Male Wistar rats weighing 240 to 300 g were obtained from Charles River Japan, Inc. (Yokohama, Japan). Rats were fasted for the nights before the experiments, but water was given freely. They were housed at 24 ± 2 °C with a 12-h light period (7:00 am to 7:00 pm). To whom correspondence should be addressed.

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each extraction column for drug elution. Five hundred microliters of acetonitrile was added to each column, and then eluted in a vacuum. To complete the elution, 500 µl of acetonitrile were added to each column. The eluent was evaporated to dryness at 40 °C under a gentle stream of nitrogen for 15 min. The residue was reconstituted in 100 µl of methanol, and 50 µl was injected into the chromatograph. The calibration curve for determining plasma DZP was linear at the concentration of 10—500 ng/ml ($R^2=0.99$). A high-performance liquid chromatograph (LC-10AD) equipped with variable-wavelength UV detector (SPD-10AVVP), column oven (CTO-10A), automatic injector (SIL-10A VP) and data processor (C-R7A plus) was used (all apparatus was obtained from Shimadzu Co., Ltd., Kyoto, Japan). A reverse phase column (YMC-Pack Pro C 18, 150×4.5 mm I.D. S-5 µm, 120 Å, YMC Co., Ltd., Tokyo, Japan) was maintained at 40 °C. The mobile phase was a mixture of acetonitrile/water (40:60, v/v), and the flow rate was 1.5 ml/min. The detection was performed at 245 nm (0.002 AUFS).

**Pentylentetrazol-Induced Seizures** Four groups of animals were established for the experiment of PTZ-induced seizures: PEG suppository (group 1), DZP suppository 2.5 mg/kg (group 2), DZP suppository 5 mg/kg (group 3), simultaneous insertion of DZP suppository (5 mg/kg) and WTS suppository (group 4). PEG and WTS suppositories were administered using the same volume/body weight as for DZP suppository. PTZ (100 mg/kg) was administered intraperitoneally (i.p.) 30 min after insertion of suppositories and behavioral changes were observed for 30 min. The incidence of clonic convolution (CL) and the latency for the first CL appearance (B) induced by PTZ (100 mg/kg, i.p.) in rats, respectively. PTZ-induced CL of all rats treated with PEG suppository. DZP suppositories (2.5, 5 mg/kg) decreased the incidence of CL dose-dependently, and there was a significant difference ($p<0.01$) between 5 mg/kg DZP and PEG suppositories. The inhibitory effect of DZP suppository (5 mg/kg) was significantly suppressed by simultaneous insertion of WTS suppository and the incidence of CL was equivalent to insertion of 2.5 mg/kg DZP suppository.

The latency of the first CL was prolonged by insertion of

![Fig. 1. Effect of Simultaneous Insertion of DZP Suppository and WTS Suppository on Plasma DZP Concentration in Rats](image)

<table>
<thead>
<tr>
<th>Drug Combination</th>
<th>DZP suppository alone</th>
<th>Combination of DZP and WTS suppository</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>106.32±12.89</td>
<td>58.23±7.82**</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.575±0.075</td>
<td>0.45±0.062</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng·h/ml)</td>
<td>199.78±18.36</td>
<td>133.97±16.50*</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng·h/ml)</td>
<td>87.53±9.75</td>
<td>48.86±6.10**</td>
</tr>
<tr>
<td>$MRT_{0-\infty}$ (h)</td>
<td>1.91±0.33</td>
<td>2.30±0.47</td>
</tr>
</tbody>
</table>

Each result is the mean of 10 rats with S.E. The words in parentheses are units. Dose of DZP suppository is 5 mg/kg. The significance of differences is indicated as follows: *$p<0.05$, **$p<0.01$ vs. DZP suppository alone.
DZP suppositories (2.5, 5 mg/kg) dose-dependently, and there was a significant difference ($p < 0.01$) between 5 mg/kg DZP and PEG suppositories. The increase in latency at a dose of 5 mg/kg was suppressed by simultaneous insertion of WTS suppository to the same degree as 2.5 mg/kg DZP suppository.

Figure 3 shows the percentage of accumulative release of DZP from suppository using a suppository release apparatus. The percentage of accumulative release increased until 8 h, and DZP suppository alone was 56% at 8 h. When WTS suppository was administered simultaneously at doses of 1.5, 98, 245 and 490 mg, the percentages of accumulative release of DZP were 34, 17.9, 17.7 and 16% at 8 h, respectively. To evaluate the effect of simultaneous administration of WTS suppository, a repeated two-way ANOVA was applied to four doses of WTS suppository compared with the DZP suppository alone. There were significant differences in the percentage of accumulative release of DZP between four doses and DZP suppository alone. The $post hoc$ comparisons using Tukey's test showed significant differences between four doses at all time examined in this study and DZP suppository alone. Simultaneous administration of DZP suppository with WTS suppositories (1.5—98 mg) decreased the rate of release dependent on the dose of oleaginous base.

DISCUSSION

Benzodiazepines (BZPs) are widely used because of their multiple actions: sedative, anxiolytic, hypnotic, muscle relaxant and anticonvulsant. Previous studies have shown the drug–drug interaction between BZPs and CYP3A4 inhibitors. For example, azole antifungals and human immunodeficiency virus (HIV) protease inhibitors increase the blood concentration of BZPs, for example midazolam and triazolam, which are mainly metabolized by CYP3A4. On the other hand, drugs in a suppository must be released into the rectal fluid in order to reach systemic circulation. The release rates of drugs from suppositories are influenced by bases and the water solubility of the drugs. Therefore, the selection of a physicochemically suitable base for the drug is essential for the preparation of the suppositories, and it is proposed that simultaneous insertion of two kinds of suppositories may cause drug-base interaction.

Previous study has shown that the combination of morphine suppository and indomethacin suppository prepared with a water-soluble base reduce the plasma concentration of morphine. It is speculated that the rectal fluid is used for a water-soluble base dissolution of indomethacin suppository, and result in insufficient morphine dissolution. Thus, in this study, we investigated effect of simultaneous combination of DZP suppository and oleaginous base, and compared with the action of DZP suppository alone.

The simultaneous insertion of a DZP suppository prepared with a water-soluble base and an AAP suppository prepared with an oleaginous base has been reported to reduce the plasma concentration of DZP in infants with febrile convulsion. However, it was not clear whether AAP or the oleaginous base caused this phenomenon. In the present pharmacokinetic study using rats, simultaneous insertion of DZP suppository and oleaginous base decreased the $C_{\text{max}}$ and $AUC$, but not $T_{\text{max}}$, consistent with the previous clinical reports. In addition, the simultaneous insertion of DZP suppository and oleaginous base attenuated the anticonvulsant effect of DZP suppository in rats. Therefore, the results of current study suggested that the decrease of serum DZP concentration in combination with DZP and AAP suppositories is due to the AAP suppository containing WTS of oleaginous base.

Generally, there are several possible explanations for the decrease in the blood concentrations after rectal administration of suppositories. First, this reducing phenomenon may result from changes in absorption of drugs via the membrane permeability of the rectum. Nonsteroidal anti-inflammatory drugs (NSAIDs), indomethacin, diclofenac sodium and as-
pirin, have conversely been reported to enhance the rectal membrane permeability to drugs in rats.\textsuperscript{19,20} Similarly, there is an example in which the rectal membrane permeability was changed by the medicine, but in this case the plasma DZP concentration was decreased by simultaneous insertion of DZP and WTS suppositories. Another possible explanation for the decrease of plasma DZP concentration may be due to the increased drug clearance by the induction of metabolizing enzyme. However, WTS suppository of oleaginous base has not been reported to affect the membrane permeability of the rectum or the metabolism of drugs. The final possible explanation is a decrease in absorption of drugs via suppression of the release rate from the base. In the present \textit{in vitro} study using a suppository release apparatus, simultaneous combination of DZP suppository and WTS suppository of oleaginous base significantly decreased the accumulative release of DZP dependent on the dose of oleaginous base (1.5—98 mg). The suppository prepared with water-soluble base is liquefied by the rectal fluid, and DZP, which is a high lipophilic drug (distribution coefficient; 1-octanol/pH 7.2 phosphate buffer=382), is released into the rectal fluid. These results suggested that a part of the released DZP was distributed to the oleaginous base.

In conclusion, the results of the current study indicate when DZP suppository and oleaginous base are inserted simultaneously, a part of the DZP released from the suppository is distributed to the oleaginous base, which had fused in the rectal cavity. This process leads to decreases in the plasma concentration and the anticonvulsant effect of DZP. Thus, it is necessary to avoid inserting DZP suppository and oleaginous base simultaneously and to leave a sufficient interval after administration of DZP suppository to avoid the interaction between drugs and bases. However, further studies are needed to determine the insertion interval.

\textbf{REFERENCES}