Pharmaceutical Evaluation of Carbamazepine Suppositories in Rats

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The absorption of carbamazepine (CBZ) after rectal administration in the form of a suppository was studied in rats. CBZ suppositories were prepared with Witepsol H-15 (H-15), Witepsol S-55 (S-55) or polyethylene glycol 6000 (PEG) bases by moulding procedure. An *in vitro* investigation was undertaken using a dissolution apparatus. The *in vitro* dissolution rate of CBZ showed marked differences among the bases in the order PEG > H-15 > S-55. An *in vivo* study demonstrated marked differences in the time required to reach peak plasma concentration (T_{max}) of CBZ after rectal and oral administration: the absorption of CBZ from PEG base was the most prolonged among these bases (in the order PEG > H-15 > S-55 > oral). Comparison of the area under the plasma concentration–time curve (AUC) of CBZ after rectal administration of the three different suppositories with those after intravenous and oral administration showed no significant differences in the AUC among the five preparations. These results suggest the possibility that CBZ suppositories can replace oral treatment for epilepsy.

Key words carbamazepine; suppository; bioavailability; rectal administration; rat

Carbamazepine (CBZ) is widely used for the treatment of partial and secondarily generalized seizures. However, neither tablets nor powders are suitable formulations for children who dislike oral dosing or for some of the epileptic patients who have difficulty in oral ingestion or poor absorption from the gastrointestinal tract (70–80% of human patients).^{1,2)} Furthermore, the absorption of CBZ from the gastrointestinal tract is slow and extremely variable, due to the low water-solubility of CBZ.¹⁾ Therefore, rectal administration is considered a preferable route for these pediatric patients with epilepsy.³⁾ There is little information available regarding the clinical application of CBZ suppositories. The aim of the present study was to evaluate the possible clinical use of CBZ suppositories prepared with Witepsol H-15 and Witepsol S-55 bases consisting of triglycerides of saturated fatty acids, and polyethylene glycol. The bioavailability of CBZ from the three types of suppositories was compared with that observed after intravenous and oral administration of CBZ in rats. The release characteristics of CBZ from the suppositories was also examined *in vitro*.

MATERIALS AND METHODS

Preparation of CBZ Suppositories CBZ (Ciba-Geigy Japan, Ltd., Takarazuka, Japan) was dissolved in the lipid soluble bases, Witepsol H-15 (H-15), Witepsol S-55 (S-55); (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) or a water-soluble base, polyethylene glycol 6000 (PEG); (Wako Pure Chemical Industries, Ltd., Osaka, Japan). CBZ (6 mg) was dissolved in each suppository base at 60 °C, followed by a moulding procedure.⁴⁾ Suppositories weighing 0.94 g for H-15, 0.95 g for S-55 and 1.16 g for PEG were used in this study; the length of each one was 2.65 cm. As PEG solidifies faster than polyethylene glycol 4000, it was chosen as a water-soluble base. A preliminary experiment showed that CBZ in each suppository was stable for 3 months at 4 °C.

Content of CBZ in Various Suppositories CBZ in each

suppository was extracted with methanol in a water bath kept at 60 °C. The amount of CBZ was measured by HPLC.^{5–8)}

In Vitro Study The *in vitro* test was carried out using a dissolution apparatus (Toyama Sangyo Co., Ltd., Osaka, Japan) according to the procedure reported by Muranishi *et al.*⁹⁾ The release phase used was phosphate buffer (pH 7.4), stirred with a magnetic stirrer at 100 rpm at 37 °C.^{7–11)} The rotation rate of the steel rod in the suppository chamber was 25 rpm. Samples (1 ml) were collected at designated intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 h), and the amount of CBZ in each sample was determined by HPLC.

Administration of CBZ to Rats Nine-week-old male Wistar rats (Nippon SLC, Hamamatsu, Japan) weighing 270 to 310 g were used in these experiments. One day before the experiment, rats were anesthetized with sodium pentobarbital (25 mg/kg) and cannulated with polyethylene tubing into the right jugular vein, then allowed to recover. After fasting overnight but with free access to water, rats were divided into five treatment groups: intravenous, oral and three rectal administration groups of H-15, S-55 and PEG bases. Each group consisted of five rats. CBZ was dissolved in a solution of propylene glycol: distilled water:ethanol=2:2:1¹¹⁾ and administered intravenously or orally at a dose of 20 mg/kg. Oral administration of CBZ was performed by the stomach intubation method at a dose of 20 mg/kg. CBZ suppositories were administered by the common method.^{3,10)} Blood samples were collected at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 6 h after administration, and plasma samples were obtained by centrifugation at 6000 rpm for 5 min and stored at –35 °C.

Drug Analysis The HPLC apparatus was a LC-4A system (Shimadzu Co., Kyoto, Japan) with a Cosmosil 5C₈ column (5 µm, 16 mm i.d. × 15 cm, Shimadzu Co.). The mobile phase was acetonitrile–5 mM KH₂PO₄ buffer (31:69; v/v), and the flow rate was 1 ml/min. Elution was carried out at room temperature and the eluates

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were monitored at 210 nm.

In a 1.5 ml plastic centrifuge tube, 50 μ l of each sample and 250 μ l of the internal standard solution (methyl-*p*-hydroxybenzoate 3 μ g/ml in acetonitrile) were mixed and centrifuged at 10000 rpm for 5 min. The resulting supernatant (10 μ l) was injected directly into the column. Standard curves for CBZ were measured over a range of 1–25 μ g/ml and shown to be linear. The coefficients of variation for the desired concentrations (5, 10 and 15 μ g/ml) ranged from 2.4 to 4.0%. The detection limit was 0.5 μ g/ml. The recovery from plasma ranged from 99 to 105%. Blank plasma samples showed no interference with the peak corresponding to CBZ and its metabolite, carbamazepine-10,11-epoxide.^{12,13)}

Solubility of CBZ in PEG Solution We also determined the solubility of CBZ in the phosphate buffer (pH 7.4) solution containing various concentrations of PEG according to the Pharmacopoeia of Japan.

Pharmacokinetic Analysis The plasma concentration–time data of CBZ for each rat in each study was analyzed by the model-independent method. The bioavailability parameters, time to maximum concentration (T_{max}) and maximum plasma concentration (C_{max}) were obtained from the observed data. The area under the plasma concentration–time curve (AUC) was determined by the linear trapezoidal rule up to the last measured plasma concentration and was extrapolated to infinity by adding the value of the last measured plasma concentration divided by the mean value of the terminal–elimination rate constant (K_e) obtained in the intravenous administration study. K_e was calculated from the slope of the terminal portion of the log concentration–time data by linear least-squares regression.

Statistical Analysis Results were expressed as mean $\pm$ S.E. Statistical analysis was performed by one-way analysis of variance using Tukey's test. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Our investigation was on the bioavailability of CBZ from suppositories and we assessed the pharmacokinetic characteristics of CBZ from these preparations both *in vitro* and *in vivo*.

The content of CBZ in each suppository ranged from 5.81 to 5.97 mg, and the percentage of recovery was more than 96% as shown in Table 1. The coefficients of variation were below 2.4%, indicating that there was pharmaceutical equivalence among the three types of suppositories used.

The results of the *in vitro* dissolution test are shown in Fig. 1. Marked differences were observed in the release profile of CBZ among the three suppositories. The percentage of CBZ released at 3 h ranged from 56 to 98% in the order PEG > H-15 > S-55. The percentage of CBZ released at 3 h was approximately 80% and 60% for H-15 and S-55 base, respectively. The percentage of CBZ from H-15 released at 3 h was greater than that from S-55, probably due to the difference in ability to release CBZ from bases between H-15 and S-55.¹⁴⁾ The dissolution of CBZ from the suppositories prepared with lipid-soluble bases was slow or incomplete compared with that from

Table 1. Content of Carbamazepine in Various Suppositories

	S-55	H-15	PEG
Content (mg)	5.81 $\pm$ 0.09	5.88 $\pm$ 0.14	5.97 $\pm$ 0.09

S-55, Witepsol S-55; H-15, Witepsol H-15; PEG, polyethylene glycol 6000. Each value represents the mean $\pm$ S.E. ($n = 5$).

Fig. 1. Release Profiles of Carbamazepine from Suppositories

○, H-15; △, S-55; □, PEG. Each vertical bar represents the mean $\pm$ S.E. ($n = 5$).

Fig. 2. Mean Plasma Concentration–Time Curves of Carbamazepine (CBZ) after Intravenous (i.v.), Oral and Rectal Administrations to Rats

×, i.v.; ○, H-15; △, S-55; □, PEG; ●, oral. Each vertical bar represents the mean $\pm$ S.E. ($n = 5$).

Table 2. Bioavailability Parameters of Carbamazepine after Intravenous, Oral and Rectal Administration

	AUC (μ g $\cdot$ h/ml)	C_{max} (μ g/ml)	T_{max} (h)	Bioavailability (%)
i.v.	24.57 $\pm$ 1.39			100
Oral	16.81 $\pm$ 2.39	5.82 $\pm$ 0.34	0.74 $\pm$ 0.15	69
S-55	19.34 $\pm$ 3.54	5.76 $\pm$ 0.75	1.25 $\pm$ 0.16	79
H-15	25.15 $\pm$ 5.04	5.47 $\pm$ 0.61	1.45 $\pm$ 0.08 ^{a)}	102
PEG	24.80 $\pm$ 3.38	5.89 $\pm$ 1.21	1.86 $\pm$ 0.25 ^{b)}	101

Each value represents mean $\pm$ S.E. of 5 rats. a) $p < 0.05$, b) $p < 0.01$ vs. oral administration.

suppositories prepared with water-soluble bases.

Mean plasma CBZ concentration–time data for five rats after intravenous, oral and rectal administration of CBZ are shown in Fig. 2. The corresponding pharmacokinetic parameters of CBZ administered rectally and orally in rats are compared with those administered intravenously in Table 2. Plasma concentration of CBZ increased rapidly after oral administration and C_{max} reached 5.8 μ g/ml after 0.75 h. The suppositories prepared with PEG produced a

slow increase in plasma concentration of CBZ, and $C_{\max}$ of 5.9 $\mu\text{g/ml}$ was attained 2 h after administration. The $T_{\max}$ values of CBZ on the two suppositories prepared with H-15 and PEG bases were significantly larger than that after oral administration. The shorter $T_{\max}$ of the suppository form realized more rapid efficacy of CBZ for seizures. The findings demonstrated that the $T_{\max}$ of CBZ from suppositories prepared with S-55 and H-15 bases was shorter than that prepared with PEG base. Although there was no significant difference in the $T_{\max}$ among these bases, it was indicated that CBZ suppositories prepared with lipid-soluble bases were absorbed more rapidly than those prepared with water-soluble bases. The bioavailability of CBZ in oral and rectal administration relative to intravenously administered CBZ is also summarized in Table 2. The estimated pharmacokinetic parameters of CBZ following intravenous administration (K_e , 0.93 h^{-1} ; volume of distribution, 0.93 l/kg; systemic clearance, 0.83 l/kg/h) were consistent with a previous report.¹⁵⁾ The rapid absorption of CBZ from the gastrointestinal tract observed in this study may be due to its being in solution form. The AUC of CBZ in suppositories prepared with S-55 and in oral administration had a tendency to be less than those in suppositories prepared with H-15 and PEG, although there were no significant differences in the AUC of CBZ among the four preparations. This finding in the *in vivo* study may be due to interindividual variations. Data obtained from this study showed that these suppositories have adequate bioavailability, which is comparable to data on the administration using suspension or gel of CBZ in human or dog¹⁶⁻²⁰⁾ and CBZ suppository containing Polysorbate 80 in rabbits.²¹⁾ CBZ suppositories are advantageous, since they are easier to administer rectally when the patient has a convulsion, compared with suspension or gel form.

The *in vitro* release characteristics of CBZ in this study could not explain the plasma concentration-time profiles of CBZ after rectal administration with suppositories, as reported by other investigators.²²⁾ We believe that PEG concentration in rectal fluid was not sufficient to enhance the dissolution of CBZ in the first hour, and CBZ particles might have been in a state of suspension. Further research will be needed to clarify this. Muranishi has reported that drugs in suppositories prepared with lipid-soluble bases including H-15 and S-55 are released from the melted base into the rectal fluid and then absorbed, or absorbed directly from the melted base through the mucous membrane of the rectum.²²⁾ He also showed that drugs in suppositories prepared with a PEG base were dissolved in PEG solution in the rectal fluid and absorbed from the rectum.²²⁾ Stavchansky and Gowan²³⁾ demonstrated that the absorption of phenytoin, which has the same poor water-soluble characteristics as CBZ, was increased by using a PEG base in dogs. Muranishi also reported that the drugs which had poor water-solubility were markedly dissolved by PEG at high concentrations in the rectal fluid, but not at low concentrations.²²⁾ In this study, the solubility of CBZ in the solution at various concentrations

Table 3. Solubility of Carbamazepine in Various Concentrations of PEG

Concentration of PEG (%)	0	0.1	0.5	1
Solubility of CBZ (mg/l)	249	263	334	408

of PEG was 249 mg/l for 0%, 263 mg/l for 0.1%, 334 mg/l for 0.5% and 408 mg/l for 1.0% as shown in Table 3. The solubility of CBZ was increased by PEG in a concentration dependent fashion. The discrepancy between *in vivo* and *in vitro* results observed here may be explained by these findings.

In conclusion, the bioavailability of rectal administration of CBZ was equivalent to that of oral administration in rats. Further studies, however, will be necessary to evaluate the clinical applicability of rectal administration of CBZ in humans.

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