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Abstract

The effects of immobilization stress on the pharmacokinetics of omeprazole were studied in rats. The immobilization stress for 30 or 60 min immediately after oral administration of the drug caused an increase in the time to reach the maximum concentration. However, such stress did not alter the area under the plasma concentration-time curve (AUC). When administered intravenously, the half-life during the elimination phase was significantly prolonged by 30 min of immobilization stress, but the AUC value remained unchanged. The intestinal propulsive activity was significantly decreased by immobilization stress. These findings suggest that immobilization stress reduces gastrointestinal motility. A resulting delay during the absorption phase of omeprazole occurs, although the degree of influence on overall pharmacokinetics is relatively insignificant.

KEYWORDS: omeprazole, pharmacokinetics, stress, immobilization

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Original Article

The Effect of Immobilization Stress on the Pharmacokinetics of Omeprazole in Rats

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The effects of immobilization stress on the pharmacokinetics of omeprazole were studied in rats. The immobilization stress for 30 or 60 min immediately after oral administration of the drug caused an increase in the time to reach the maximum concentration. However, such stress did not alter the area under the plasma concentration-time curve (AUC). When administered intravenously, the half-life during the elimination phase was significantly prolonged by 30 min of immobilization stress, but the AUC value remained unchanged. The intestinal propulsive activity was significantly decreased by immobilization stress. These findings suggest that immobilization stress reduces gastrointestinal motility. A resulting delay during the absorption phase of omeprazole occurs, although the degree of influence on overall pharmacokinetics is relatively insignificant.

Key words: omeprazole, pharmacokinetics, stress, immobilization

The pharmacokinetics of drugs are influenced by various factors, such as age, food intake, body weight, and drug interactions. Emotional stress is one such factor as well. We have previously reported that the pharmacokinetics of nicorandil and theophylline were influenced by emotional stress [1, 2]. However, emotional stress is closely related to the pathogenesis of peptic ulcers [3, 4]. No studies have thus far been conducted on the effects of emotional stress on the pharmacokinetics of antiulcer drugs. In the present study, we examined the effect of immobilization stress in rats on the pharmacokinetics of omeprazole, a gastric proton pump inhibitor.

Materials and Methods

Materials. The omeprazole was donated by Fujisawa-Astra Ltd. (Osaka, Japan). Since omeprazole is unstable under acidic conditions, the compound was suspended in 0.5% methylcellulose containing 2.5% $NaHCO_3$ (pH 9) for oral (p.o.) administration or dissolved in 40% polyethylene glycol 400 containing 0.1% $NaHCO_3$ (pH 8) for intravenous (i.v.) injection. The volume of administration was adjusted to 1 (p.o.) or 0.5 (i.v.) ml/kg body weight. All other reagents used were of analytical grade.

Animals and Drug Administration. Male Wistar rats (Charles River Lab., Atsugi, Japan), each weighing 230-400 g, were kept in groups of 3-4 in a room maintained on a 12-h light/12-h dark cycle (lights on at 07:00) at 22 ± 1 °C and approximately 60% relative humidity. The animals were allowed free access to food and water, except for a 12-h fast before the experiments.

When omeprazole was administered p.o. (20 mg/kg), the animals received an additional intake of 2.5% $NaHCO_3$ (3 ml/kg body weight) immediately after dosing. For i.v. bolus administration (5 mg/kg), the drug was injected into the femoral vein under light ether anesthesia.

Immobilization stress was administered by restraining

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the rats in a wire net. In the first experiment, the animals were exposed to immobilization stress for 30 or 60 min immediately after the oral administration of omeprazole. In the second experiment, the rats were exposed to immobilization stress for 30 min immediately after the intravenous administration of the compound. At various times after the drug administration, 60 μl blood samples were collected from the tail vein under light local anesthesia with ethyl aminobenzoate ointment, and the plasma concentrations of omeprazole were determined by high performance liquid chromatography, as described previously [5].

The principles of laboratory animal care and all experimental procedures were in strict accordance with the Guidelines for Animal Experiments of Okayama University Medical School.

Pharmacokinetic Analysis. Pharmacokinetic parameters were obtained from the plasma concentration-time measurements of omeprazole for each animal, using a personal computer program for nonlinear least squares regression analysis (MULTI, Nankodo, Tokyo, Japan) [6]. The maximum plasma concentration (C_{max}), the time to reach the maximum concentration (T_{max}), the half-life during the elimination phase ($T_{1/2}$), and the steady state volume of distribution (Vd_{ss}) were estimated from these pharmacokinetic parameters. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal method during a 0–3 h period for p.o. administration, and a 0–2 h period for i.v. administration.

Measurement of intestinal propulsive activity. Immediately after oral administration of 0.5% suspension of carbon powder at a volume of 0.2 ml/100 g body weight, the rats were exposed to immobilization stress for 30 min. One hour after the administration, the animals were decapitated and laparotomized, and the destination of carbon powder in the intestine was macroscopically measured. The intestinal propulsive activity was obtained as a percentage of the destination of carbon powder/ the total length of the small intestine.

Statistics. The findings of p.o. administration were statistically evaluated by analysis of variance followed by Dunnett's test, and the other findings were analyzed by two-tailed Student's t test or the Aspin-Welch method.

Results

Effect of immobilization stress on the oral pharmacokinetics of omeprazole. Fig. 1 shows the time-course of the plasma omeprazole concentration after oral administration in each group. The plasma concentration of omeprazole in the control group increased rapidly, reached a maximum at about 15 min, and decreased to a level of < 0.1 μg/ml by 2 h after the administration. The plasma concentration 10 min after the administration was significantly decreased by 30 min of immobilization stress ($P < 0.05$), and tended to be decreased by 60 min of immobilization stress ($P = 0.074$). The plasma concentration 20 min after the administration was significantly decreased by 60 min of immobilization stress ($P < 0.05$). Table 1 summarizes the pharmacokinetic parameters after oral administration of omeprazole. The T_{max} value was significantly increased by 30 min of

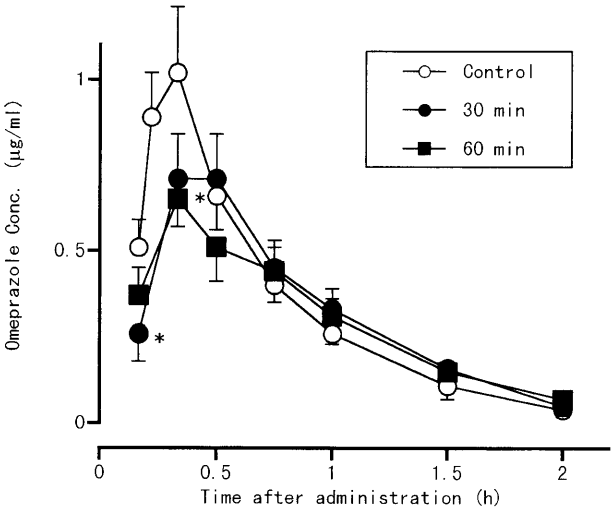

Fig. 1 Time course of plasma omeprazole concentration in rats. Animals were exposed to immobilization stress for 30 or 60 min immediately after the oral administration of omeprazole at a dose of 20 mg/kg. Each point indicates the mean ± SEM. *$P < 0.05$ *vs.* control group.

Table 1 Pharmacokinetic parameters of omeprazole after oral administration in rats

Parameter	Control (n = 8)	Stressed	
		30 min (n = 7)	60 min (n = 7)
C_{max} (μg/ml)	1.01 ± 0.17	0.76 ± 0.12	0.69 ± 0.09
T_{max} (h)	0.25 ± 0.02	0.41 ± 0.05*	0.36 ± 0.04
AUC (μg · h/ml)	0.73 ± 0.06	0.69 ± 0.09	0.66 ± 0.07

Each value represents the mean ± SEM.
*$P < 0.05$ *vs.* control group.

immobilization stress ($P < 0.05$), and the T_{max} value tended to be increased by 60 min of immobilization stress ($P = 0.092$). There was no significant difference in the C_{max} and AUC values among the 3 groups.

Effect of immobilization stress on the intravenous pharmacokinetics of omeprazole. The decline in the plasma concentration of omeprazole after i.v. administration in the control group showed a biexponential curve (Fig. 2). The plasma concentrations 20 and 30 min after administration were significantly increased by 30 min of immobilization stress ($P < 0.05$). Table 2 shows the pharmacokinetic parameters after i.v. administration of omeprazole. The $T_{1/2}$ value in the stressed group was significantly longer than that of the control group ($P < 0.01$). There was no significant difference in the Vd_{ss} and AUC values between the groups.

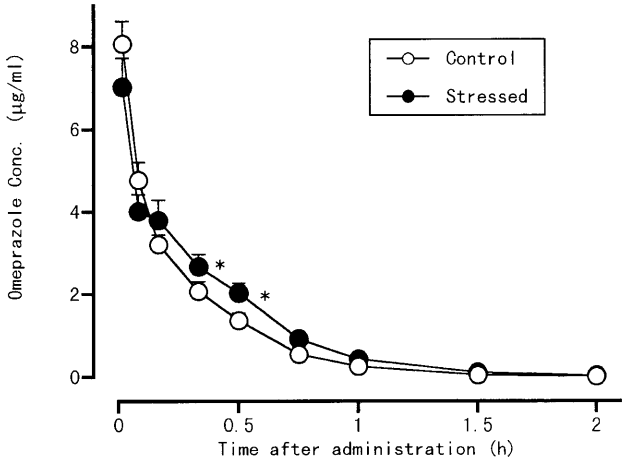

Fig. 2 Time course of plasma omeprazole concentration in rats. Animals were exposed to immobilization stress for 30 min immediately after the intravenous administration of omeprazole at a dose of 5 mg/kg. Each point indicates the mean ± SEM. *$P < 0.05$ *vs.* control group.

Table 2 Pharmacokinetic parameters of omeprazole after intravenous administration in rats

Parameter	Control (n = 8)	Stressed (n = 9)
$T_{1/2}$ (h)	0.23 ± 0.01	0.35 ± 0.02**
Vd_{ss} (l/kg)	0.79 ± 0.06	0.99 ± 0.09
AUC (μg · h/ml)	2.02 ± 0.20	2.37 ± 0.24

Each value represents the mean ± SEM.
**$P < 0.01$ *vs.* control group.

Effect of immobilization stress on intestinal propulsion. The intestinal propulsive activity in the stressed group (50.4 ± 2.4%) was significantly lower than that of the control group (73.7 ± 2.1%, $P < 0.01$).

Discussion

Omeprazole is a potent inhibitor of gastric acid secretion [7], and it is effective for intractable peptic ulcers that fail to respond to histamine H_2 receptor antagonists [8]. Recently, proton pump inhibitors including omeprazole have been used in combination with antibiotics to eradicate *Helicobacter pylori* [9, 10], which is involved in the pathogenesis of gastritis and peptic ulcers [11]. Omeprazole undergoes extensive presystemic metabolism (first-pass metabolism) after oral administration. Previously, we reported that omeprazole is metabolized in the intestinal tract and in the liver and that only 6–13% of the oral dose reaches systemic circulation [5]. Accordingly, factors that affect first-pass metabolism are thought to markedly alter the oral pharmacokinetics of omeprazole.

We intended to examine the effect of emotional stress on the pharmacokinetics of omeprazole. In studies on the influence of emotional stress on animal responses, stimuli such as foot shock, immobilization, heat, or cold have often been used. Since immobilization stress is a simple method and is still being used in a number of studies [12–14], we chose to apply immobilization stress in the present study.

In the experiment involving oral administration of the compound, a decrease in plasma omeprazole concentration and a prolongation of the T_{max} value were induced by immobilization stress. These phenomena appeared to be caused by the impairment of drug absorption in the gastrointestinal tract. This hypothesis was supported by the present findings that immobilization stress inhibited intestinal propulsion activity. It was reported that acoustic stress, cold-restrain stress, and wrap-restrain stress delay gastric emptying and decrease gastrointestinal motility [15–17].

In the experiment involving the intravenous administration of omeprazole, immobilization stress caused an increase in plasma omeprazole concentrations and an increase in the $T_{1/2}$ value. It has been reported that omeprazole is completely metabolized in the liver after i.v. administration, and that unchanged drug is negligible in feces and urine [18]. Therefore, the metabolism in the liver plays a principal role in the elimination of omepr-

azole. Hepatic blood flow has been reported to be the most influential factor in the elimination of highly extracted drugs (*i.e.*, drugs which are largely extracted from the blood via hepatic metabolism or biliary excretion) [19]. Since it has been reported that the hepatic extraction ratio of omeprazole is relatively high (0.59–0.80) [5], changes in hepatic blood flow appear to markedly influence the hepatic clearance of omeprazole. It is possible that immobilization stress decreases hepatic blood flow, followed by a delay in the elimination of the drug. In the present study, we did not measure hepatic blood flow in the rats, but it has previously been reported that restraint and water immersion stress caused a marked decrease in hepatic blood flow in mice [20].

When omeprazole was administered orally, there was no change in the AUC value among the groups. This finding suggests that the total amount of the drug absorbed from the gastrointestinal tract was not altered by immobilization stress, although the absorption of the drug was delayed. The inhibition in the elimination of omeprazole may contribute to the unchanged AUC value. Thus, an inhibition of gastric acid secretion may be attenuated during the early phase after omeprazole administration. However, the overall effect of immobilization stress on acid secretion appears to be weak.

In the present study, we were interested in the effects of emotional stress on the first-pass metabolism of omeprazole. However, the present findings indicated that immobilization stress does not greatly affect the first-pass metabolism of omeprazole, although stress does inhibit the elimination of the compound. It should be noted that since the first-pass metabolism of omeprazole might have occurred before the immobilization stress had a chance to influence hepatic clearance of the drug, the first-pass metabolism might not have been altered.

In conclusion, the present study showed that immobilization stress reduced gastrointestinal motility with a resulting delay during the absorption phase of omeprazole. A decrease in the rate of elimination of the drug was observed. Consequently, the AUC value was not affected by immobilization stress. Hence, the effects of such stress on the anti-secretory properties of omeprazole might be insignificant.

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