Absorption Characteristics of Azasetron from Rectal and Oral Routes in Rabbits

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The absorption characteristics of azasetron, a serotonin type 3 (5-HT₃) receptor antagonist which is used for the treatment of chemotherapy-induced emesis and nausea, were investigated in rabbits. The serum concentrations of azasetron following rectal administration as a suppository increased rapidly and showed the mean $t_{\max}$ value of 0.18 h. The concentrations were greater after rectal administration than those after oral administration. The absolute bioavailability was significantly different between the rectal, 52.9% and oral doses, 21.6%. The mean $C_{\max}$ and $t_{\max}$ values after the rectal dose were 904.8 ng/ml and 0.18 h, respectively, whereas those after the oral dose averaged 124.7 ng/ml and 0.85 h, respectively.

These results indicate that azasetron is absorbed to a greater extent and more rapidly into the systemic circulation *via* the rectum than *via* the intestine in rabbits. Consequently, the suppository form of azasetron hydrochloride may be feasible for the treatment of chemotherapy-induced acute emesis and nausea.

Key words azasetron; suppository; serotonin type 3 inhibitor; bioavailability

Azasetron, 3,4-dihydro-3-oxo-1,4-benzoxazine-8-carboxamide derivative, is a novel serotonin type 3 (5-HT₃) receptor antagonist efficacious in the treatment of the severe nausea and emesis induced by chemotherapy in cancer patients.^{1,2)} It has been reported that azasetron has a potent and selective affinity for 5-HT₃ receptors.^{3,4)}

There is great interest in the development of alternative dosage forms of 5-HT₃ receptor antagonists such as oral and rectal dosage forms in addition to the injection. Granisetron and ondansetron, which have similarly selective antagonistic characteristics at 5-HT₃ receptors to azasetron, have already been marketed in tablet and powder forms in Japan.^{5–7)} However, it is difficult to give oral preparations to patients who show symptoms of nausea and vomiting. The rectal administration of 5-HT₃ receptor antagonist is one of the most reasonable routes for such patients who can not take the drug by mouth. Azasetron is currently used only as an intravenous injection in spite of its excellent antiemetic activity.^{8,9)} There are no reports on its rectal absorption compared with other dosage routes.

This study was therefore designed to investigate the absorption characteristics of azasetron from the rectum and to evaluate the utility of the drug as a suppository.

MATERIALS AND METHODS

Materials Azasetron hydrochloride was generously supplied by Yoshitomi Pharmaceutical Co. (Osaka, Japan). Vosco H-15 (equivalent to Witepsol H-15) was purchased from Maruishi Pharmaceutical Co. (Osaka). Normal saline for injection was of JP XIII grade and all other reagents and solvents were commercial products of reagent grade.

Preparation of Suppository and Solutions A suppository was prepared by suspending azasetron hydrochloride (10 mg) in a suppository base (Vosco H-15) after the base had been melted at 50 °C in a hot water bath. The molten mass was cooled down to 35–37 °C, and poured into disposable plastic molds (Maruishi Pharmaceutical Co.). The suppositories were kept at 4 °C until the start of

experiments. The drug content in the suppositories was determined by spectrophotometry at 220 nm to be 98.7% ($n=5$) of the calculated amount. An oral solution was prepared by dissolving the drug in distilled water at the concentration of 0.5 mg/ml, and a solution for intravenous injection was prepared by dissolving the drug in normal saline at the concentration of 10 mg/ml.

Animal Studies Male white rabbits, 2.3 to 2.8 kg were fasted for 16 h prior to the experiments but allowed free access to water. Intravenous administration: after fixing a rabbit in the crouching position, 1 ml of azasetron hydrochloride solution was injected into the ear vein. Rectal administration: After fixing a rabbit in a supine position, a suppository containing 10 mg of azasetron hydrochloride was inserted into the rectum, and the anus was closed with a plastic clamp to prevent leakage of the rectal contents during the experiment. Oral administration: after fixing a rabbit in a supine position, the mouth was forcedly opened with a wooden rod having a hole at the center. A rubber stomach tube was inserted into the stomach and 20 ml of azasetron hydrochloride solution was poured into the stomach. After the administration, blood samples were taken periodically from the marginal ear vein of the animal. Serum was separated by centrifugation at 3000 rpm for 15 min and frozen at –20 °C until analysis.

Analytical Methods A high-performance liquid chromatograph (HPLC; Shimadzu LC-6A, Kyoto, Japan) equipped with a fluorescence detector was used to determine the serum levels of azasetron. To 0.2 ml of serum were added 20 μ l of 2 N NaOH and 2 ml of dichloroethane. The mixture was shaken for 10 min, and then centrifuged for 5 min at 3000 rpm. A 1.6 ml portion of the upper organic layer was transferred to a conical tube and 1.5 ml of hexane and 80 μ l of 0.1 N HCl were added. After centrifugation for 5 min at 3000 rpm, the upper organic layer was removed and 50 μ l of the lower layer was injected into the HPLC column. Separation was performed with a reversed phase-type column (Senshu Pak, ODS-0151-N, 4 mm i.d. $\times$ 150 mm). The mobile phase consisted of 0.1 M

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ammonium acetate (adjusted by acetate to pH 5), acetonitrile and tetrahydrofuran (84:9.8:6.2). The flow rate was 1.0 ml/min and the column temperature was maintained at 50 °C. The eluate was monitored at an excitation wavelength of 318 nm and emission wavelength of 382 nm. The between-day coefficients of variation for azasetron determination were less than 10% in serum over a concentration range of 50–5000 ng/ml.

Pharmacokinetic Analysis The maximal serum azasetron concentration ($C_{\max}$) and the time to reach this concentration ($t_{\max}$) were determined from the individual serum concentration–time profiles after rectal and oral administration. The terminal elimination rate constant (β) was determined by a nonlinear regression analysis of the serum levels. The area under the serum concentration–time curve (AUC) was calculated according to the trapezoidal integration to the last sampling point with a measurable concentration (C_n) to which C_n/β was added. The mean residence time (MRT) was calculated using the equation reported by Yamaoka *et al.*¹⁰⁾

Comparisons of pharmacokinetic parameters between two groups and among three groups were performed by Student's *t*-test and analysis of variance (ANOVA), followed by Tukey's studentized range test, respectively. A probability value smaller than 0.05 was considered statistically significant. Data are represented by mean $\pm$ S.E.M.

RESULTS AND DISCUSSION

Choice of the 10-mg dose was based on the amount being adequate to determine the quantity remaining in serum even after oral administration, *i.e.* to give serum concentrations above an assay limit of azasetron (50 ng/ml).

Figure 1 shows the serum concentration time profiles of azasetron following intravenous, rectal and oral administrations of 10 mg azasetron hydrochloride to rabbits. There was a considerable difference in the absorption pattern of the drug between the rectal and oral administrations. Azasetron was very rapidly absorbed from the rectum and the serum concentrations reached the maximal value by the first sampling time after the administration in 3 of 5 rabbits. Thereafter, the concentrations decayed exponentially and the mean $C_{\max}$ of 904.8 ng/ml was reached at 0.18 h on average. Furthermore, the mean serum azasetron concentrations were greater at all time points with the rectal dose than those with the oral dose. The serum concentrations of azasetron after oral administration did not increase as sharply as those after rectal administration, and it took 0.85 h on average to reach the $C_{\max}$. The statistical difference in $C_{\max}$ and $t_{\max}$ was detected between the values of rectal and oral administrations. The pharmacokinetic parameters are summarized in Table 1.

The mean AUC value after the intravenous injection of 10 mg azasetron hydrochloride averaged 1889 ng·h/ml, the mean after rectal administration being about 2.5 times greater than that after oral administration, although no statistical difference was detected between them. The rectal and oral bioavailabilities of azasetron, calculated using the AUC values of the corresponding rabbits adminis-

Fig. 1. Serum Concentration–Time Profiles of Azasetron after Intravenous, Rectal and Oral Administrations at a Dose of 10 mg in 5 Rabbits, Mean $\pm$ S.E.M.

Table 1. Mean Pharmacokinetic Parameters of Azasetron in Five Rabbits

Parameter	Route and dosage forms		
	Intravenous injection	Rectal suppository	Oral solution
AUC (ng·h/ml)	1889 $\pm$ 301.6 ^{a)}	1084.7 $\pm$ 339.5	420.4 $\pm$ 104.8 ^{a)}
$C_{\max}$ (ng/ml)	—	904.8 $\pm$ 214.5 ^{b)}	124.7 $\pm$ 13.4 ^{b)}
$t_{\max}$ (h)	—	0.18 $\pm$ 0.03 ^{b)}	0.85 $\pm$ 0.06 ^{b)}
MRT (h)	1.81 $\pm$ 0.58	1.75 $\pm$ 0.48	2.67 $\pm$ 0.35
F (%)	(100)	52.9 $\pm$ 9.3 ^{b)}	21.6 $\pm$ 2.6 ^{b)}

a) Mean value of oral solution was significantly different from that of intravenous injection ($p < 0.05$). b) Mean values were significantly different from each other ($p < 0.05$).

tered intravenously, were 52.9 and 21.6%, respectively; there was a statistical difference between the absolute bioavailabilities (F) of rectal and oral administration. No statistical difference in the MRT values was detected among the dosage forms. It has been reported that $t_{\max}$ of ondansetron after rectal administration was 1.2 h and its bioavailability was 87% in rabbits.¹¹⁾ In addition, $t_{\max}$ of the drug after rectal administration was 6.8 h and its bioavailability was 58% in human volunteers.¹²⁾ Comparing with earlier papers,^{11,12)} the present results suggest that the rectal administration of azasetron hydrochloride may be useful for patients with nausea and vomiting or those with difficulty in swallowing a drug.

As shown in Table 1, the pharmacokinetic parameters of azasetron on AUC , $C_{\max}$ and $t_{\max}$ differed between the rectal and oral administrations. Especially, $t_{\max}$ after rectal administration was about one fourth of that after oral administration, which may be due to the well-matched combination of the drug with its extremely hydrophilic nature (coefficient of octanol/water: 0.017 at 25 °C)¹³⁾ and the suppository base with the fat excipient. The oleaginous base releases azasetron hydrochloride rapidly and the drug subsequently dissolves into the rectal fluid, resulting in immediately high concentration levels. It is well known that lipophilic drugs dissolved in a fat suppository base diffuse much less rapidly out of the base than hydrophilic drugs.^{14,15)} The larger value of $t_{\max}$ after oral administra-

tion than that of rectal administration may also be due to gastric emptying time. The larger values of AUC and C_{max} after rectal administration may be due to avoidance of the first pass effect in the liver.

In conclusion, we found that azasetron was rapidly and efficiently absorbed through the rectal mucosa in the absence of any promoters for rectal absorption. The rectal dosage form of azasetron hydrochloride thus may be a useful alternative for patients suffering from nausea and vomiting induced by chemotherapy. Possible species difference between humans and rabbits, however, has to be examined.

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