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Bioavailabilities of Omeprazole Administered to Rats through Various Routes

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Omeprazole, a proton pump inhibitor, was given intravenously (iv), orally (po), intraperitoneally (ip), hepatoportalvenously (pv), and intrarectally (ir) to rats at a dose of 72mg/kg in order to investigate the bioavailability of the drug. The extent of bioavailabilities of omeprazole administered through pv, ip, po, and ir routes were 88.5, 79.4, 40.8, and 38.7%, respectively. Pharmacokinetic analysis in this study and literatures (Regardh *et al.*, 1985 : Watanabe *et al.*, 1994) implied significant dose-dependency in hepatic first-pass metabolism, clearance and distribution, and acidic degradation in gastric fluid. The high bioavailability from the pv administration (88.5%) means that only 11.5% of dose was extracted by the first-pass metabolism through the liver at this dose (72 mg/kg). The low bioavailability from the oral administration (40.8%) in spite of minor hepatic first-pass extraction indicates low transport of the drug from GI lumen to portal vein. From the literature (Pilbrant and Cederberg, 1985), acidic degradation in gastric fluid was considered to be the major cause of the low transport. Thus, enteric coating of oral preparations would enhance the oral bioavailability substantially. The bioavailability of the drug from the rectal route, in which acidic degradation and hepatic first-pass metabolism may not occur, was low (38.7%) but comparable to that from the oral route (40.8 %) indicating poor transport across the rectal membrane. In this case, addition of an appropriate absorption enhancer would improve the bioavailability. Rectal route seems to be an possible alternative to the conventional oral route for omeprazole administration.

Key words : Omeprazole, Dose-dependent pharmacokinetics, Bioavailability

INTRODUCTION

Omeprazole, 5-methyl-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl}-1H-benzimidazole, is a substituted benzimidazole which selectively inhibits the proton pump in the gastric mucosa (Larsson *et al.*, 1985; Wallmark *et al.*, 1985). Omeprazole is very slightly soluble in water and degrades very rapidly in aqueous solutions at low pH (Pilbrant and Cederberg, 1985). Various oral formulations of the drug have been tried to limit preabsorptive degradation and consequently improve systemic bioavailability. Coadministration of basic buffers had been tried to avoid acidic degradation of the drug (Pilbrant and Cederberg, 1985). More recently, an enteric-coated formulation of omeprazole has been introduced which seems to overcome many problems associated with oral administration.

Omeprazole is rapidly absorbed in mouse, rat, dog and man with systemic availability of >40% provided the drug is protected from acidic degradation in the stomach (Regardh *et al.*, 1985). Omeprazole is eliminated by hepatic metabolism. The major fraction (~80%) of its metabolites is excreted by the kidney, and the rest in feces, primarily originated from bile secretion (Lind *et al.*, 1987; Regardh *et al.*,1990). Previous studies indicate that impaired renal function has no essential influence on the absorption and disposition of omeprazole, while impaired hepatic function significantly decreases the metabolism of this drug (And-ersson *et al.*, 1993; Naesdal *et al.*, 1986).

The pharmacokinetics of omeprazole has been known well. But further informations are needed to clarify quantitatively the contribution of each process during absorption to the bioavailability of omeprazole orally administered. The process includes degradation in the GI fluid, transport through the GI membrane, metabolism in the GI mucosa, and presystemic hepatic elimination.

On the other hand, whether and how much omeprazole is absorbed from the rectal route are also of

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great interest. The purpose of this study was to investigate the pharmacokinetic behaviors of omeprazole administered via various routes. For this purpose, plasma concentration profiles of omeprazole in rats after oral (po), hepatoportal (pv), intraperitoneal (ip), intrarectal (ir), and intravenous (iv) administration were examined, and their absolute bioavailabilities were compared. Based on these informations, each transport steps involved in the process of oral absorption was discussed for its contribution to the overall bioavailability, and rectal route was examined for its feasibility as an alternative to the oral route of administration of omeprazole.

MATERIALS AND METHODS

Materials

Omeprazole was obtained from Han-Mi Pharmaceutical Inc., (Seoul, Korea). HPLC grade methanol and acetonitrile were from Fisher Scientific International (Springfield, NJ). All other reagents were analytical grade and used as purchased. Male Wistar rats weighing 230-280 g (Experimental Animal Center, Seoul National University) were used in all experiments.

Administration of omeprazole via various routes

Rats were starved for 36 hrs before experiment. The rats were fixed at supine during experiment. Under light ether anesthesia, the femoral artery of each rat was cannulated with polyethylene tubings (PE-50, Intramedic, Clay Adams, USA) for blood sampling. Femoral vein was also cannulated with PE-50 for iv administration. For pv administration, the pyloric vein was cannulated as follows. The abdomen was opened through a midline incision and the tip of an injection needle (25 gauge) attached to a PE-50 was inserted into hepatic portal vein and was fixed with surgical glue (Aron Alpha, Sankyo Co, Tokyo, Japan). The needle was bent 120° for convenience of insertion. After surgical suture of the incision, this catheter was connected to a 1-ml syringe and then omeprazole was given through the syringe into the portal vein. Oral administration was performed by insertion of a round-tip needle connected to a 1-ml syringe. Intraperitoneal administration was performed with a 26-gauge needle.

After complete recovery (1 hr) from the anesthesia, a 1.8% (w/v) solution of omeprazole in the mixture of PEG 400 and physiological saline (7:3 v/v ratio) was administered at a dose of 4 ml/kg (72 mg/kg) through intravenous (iv), oral (po), portal venous (pv), and intraperitoneal (ip) routes, respectively. For intrarectal (ir) administration, omeprazole suppository was inserted into rectum at a dose of 1.88 g/kg (72 mg of omeprazole per kg rat). The suppository was made by suspending omeprazole in the melted mixture of Witepsol H containing 1% (w/w) arginine.

After drug administration, blood samples (250 μl) were collected at the designated time interval into heparinized tubes from the femoral artery via PE-50 catheter. The heparinizton was conducted by treating the tubes with 10μl of heparinized 0.9% NaCl solution (150 IU/ml) and successive drying the solution. Blood samples were withdrawn at 0, 2, 5, 10, 20, 30, 40, 60, 120, 180, 300 and 360 min postadministration afer iv and pv administration, and at 0, 5, 10, 15, 30, 45, 60, 120, 160, 200, 240, 300 and 360 min after po administration, and at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after ip administration and at 0, 10, 20, 30, 40, 60, 80, 120, 180, 240, 300, and 360 min after ir administration. Plasma samples were separated by centrifuging the blood samples at 6,000 g for 1 min and were stored at -20°C until HPLC analysis.

HPLC assay of omeprazole

A HPLC assay for omeprazole in plasma was developed by modifying the reported HPLC methods (Amantea and Narang, 1988; Nakashima *et al.*, 1988). To 100 μl of plasma sample in polypropylene tube, 200 μl of 1 M carbonate buffer (pH 9.3) was added and vortexed for 20 sec. The sample was then extracted with 4 ml of acetonitrile : methylenechloride (1:1 in volume ratio) by vortexing for 5 min. After centrifugation at 4000g for 10 min, 2.0 ml aliquots of the aqueous phase (lower layer) were transferred to another tube and evaporated to dryness using a Speed Vac Concentrator (Savant Instruments Inc., Famingdale, NY). The residue was dissolved in 200 μl of the mobile phase described below by vortexing for 30 sec, and then aliquots of 100 μl were in jected directly onto the guard column (μ-Bondapak, 10-μm) connected to a C18 reversed-phase column (Shim-pack, 5-μm silica, 1500x4.6-mm id). The pump (Model SCL-6B) and variable UV spectrophotometric detector (Model SPD-6A) were from Shimadzu Ltd. (Tokyo, Japan). The mobile phase was a mixture of 0.025 M phosphate buffer (pH 7.4), methanol, and acetonitrile (52:40:8, v/v). The flow rate of the mobile phase was 1.1 ml/min and the wavelength of the detector was set at 302 nm.

Omeprazole was appropriately separated from the other substances in the plasma with a retention time of 10 min. The total run time of 20 min per injection was necessary to eliminate any possible interfering peaks in plasma. Recovery of omeprazole from the plasma sample was more than 80% in the concentration ranges of 20-1000 μg/ml, and the calibration curve was linear in these concentration range. Intra-

day and interday coefficients of variation were all below 5.0%, and the detection limit was 5 ng/ml.

Pharmacokinetic analysis

Total-body clearance (CL_t), apparent volume of distribution at steady-state (Vd_{ss}), and half-life ($t_{1/2\beta}$) of omeprazole were calculated using iv data by Eqs. (1)-(3). Mean residence time (MRT) of omeprazole following each administration was calculated by Eq. (4) using respective AUC and AUMC data.

$$CL_t = D/AUC \quad (1)$$

$$Vd_{ss} = D.\ AUMC/AUC^2 \quad (2)$$

$$t_{1/2\beta} = 0.693/\beta \quad (3)$$

$$MRT = AUMC/AUC \quad (4)$$

where D, AUC, AUMC, and β, respectively, denote dose, area under the plasma omeprazole concentration-time curve from time 0 to time infinity, area under the first moment of the plasma omeprazole concentration-time curve from time 0 to infinity, and elimination rate constant at postdistributive phase (β-phase). The AUC and AUMC were calculated by the trapezoidal method from time 0 to the last measured time and extrapolated from the time to infinity using β. β was obtained after fitting the plasma concentration data to a conventional two-compartment model using program MULTI (Yamaoka *et al.*, 1981).

Fraction of omeprazole transported (availability=1-extraction ratio) across the liver (F1) was calculated from Eq. (5). In this model, omeprazole was presumed not to be cleared in the lung and not to be subject to enterohepatic recirculation, although these assumptions were not tested.

$$F_1 = AUC_{pv}/AUC_{iv} \quad (5)$$

Absolute bioavailability (bioavailability %) was calculated from Eq. (6) using AUC value of each administration route (AUC_{AR}).

$$\text{Bioavailability (\%)} = 100 \times AUC_{AR}/AUC_{iv} \quad (6)$$

Statistical analysis

The statistical significance of the differences in the pharmacokinetic parameters between the administration routes was determined using the one-way analysis of variance (ANOVA) for unpaired data. A P value of <0.05 was chosen as the level of statistical significance. All results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Plasma levels of omeprazole following administration via various routes at the dose of 72 mg/kg are plotted as a function of time and are shown in Fig. 1. The profiles following iv and pv administrations showed biexponential decay. The terminal slopes of the curves following po, pv, ip, and ir administration were almost comparable to that of iv administration.

Pharmacokinetic parameters obtained from the iv data are summarized in Table I together with those of other routes. The volume of distribution at steady state (Vd_{ss}) of omeprazole in this study (0.18 l/kg) was comparable to the post pseudodistributive volume of distribution (V_β) of the drug in man (0.19 to 0.45 l/kg, Regardh *et al.*, 1985), which is compatible to the volume of the extracellular water in the body. Plasma protein binding was reported to be about 95, 90 and 87% in plasma of man, dog and rat, respectively (Regardh *et al.*, 1985). And the penetration into the red blood cells was also known to be rather low, the concentration ratio in whole blood to plasma being about 0.6 (Regardh *et al.*, 1985). Therefore, the poor body distribution of omeprazole might be mainly attributed to its high plasma protein binding.

The Vd_{ss} in this study is much smaller than that reported for 2.5-10 mg/kg dose in rats (0.64-0.75 l/kg, Watanabe *et al.*, 1994). The difference in Vd_{ss} between Watanabe *et al.* (1994) and this study, needs further explanation. At present, there is no way to explain other than introducing dose-dependent pharmacokinetic characteristics of omeprazole (Regardh *et al.* did (2.5-10 mg/kg). However, the extrapolation of the dose-dependency in hepatic metabolism to body distribution needs experimental verification.

Omeprazole was eliminated rapidly with a half-life ($t_{1/2\beta}$) of about 1 hr in this study, which is consistent with the report of Regardh *et al.* in man (1985), but much longer than the reported value in rats (15-18 min, Watanabe *et al.*, 1994).

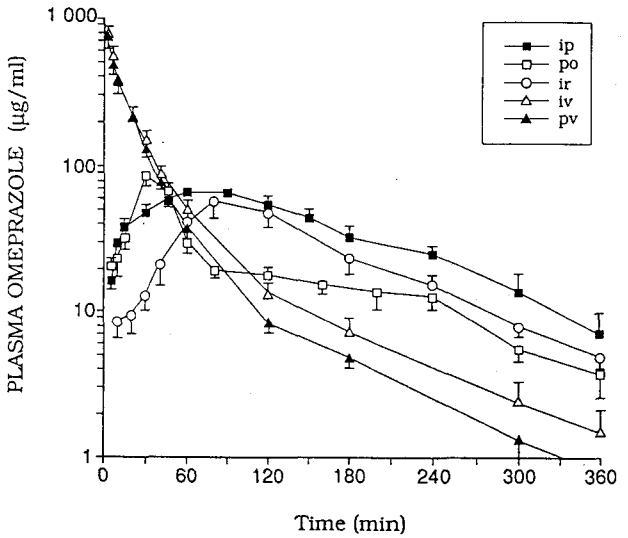

Fig. 1. Plasma concentration-time profiles of omeprazole following administraion via various routes to rats at a dose of 72 mg/kg (Mean ± SE, n=3)

Table I. Pharmacokinetic and bioavailability parameters of omeprazole in rats[a]

Administration routes	Vd_{ss} (ml/kg)	CL_t (ml/min/kg)	MRT (min)	C_{max} (min)	T_{max} (ng/ml)	AUC (μg.min/ml)	Bioavailability (%)
iv	181.4 (18.2)	4.9 (1.5)	37.8 (11.7)			15,916.7 (4,435.5)	100
po			198.2 (87.7)	868.3 (101.6)	35.0 (8.7)	6,488.4 (2,536.3)	40.8
pv			40.0 (5.5)			14,092.4 (2,321.3)	88.5
ip			151.6 (16.5)	701.9 (85.9)	80.0 (17.3)	12,641.6 (2,348.3)	79.4
ir			195.1 (32.8)	675.2 (31.7)	93.3 (23.1)	6,159.3 (1,155.2)	38.7

[a]Each value represents the mean (±SD) of three experiments. Omeprazole was administered at a dose of 72 mg/kg in all the routes.

This, together with Vd_{ss}, suggests again the nonlinear pharmacokinetics of omeprazole in rats. The elimination of omeprazole may be due to hepatic metabolism since insignificant amounts of unchanged drug were excreted via kidney and stools in man (Regardh *et al.*, 1985). The total plasma clearance (CL_t) of omeprazole in this study (4.9 ml/min/kg) is comparable with the reported value (8.8 ml/min/kg) in man (Regardh *et al.*, 1985), but much smaller than that reported in rats (38-39 ml/min/kg) for 2.5-10 mg/kg dose (Watanabe *et al.*, 1994). The difference between this study and Watanabe *et al.* (1994) supports the dose-dependent hepatic metabolism in the liver.

MRT following each administration will increase as absorption through the route occurs slowly. As expected, it increased in the order of iv, pv, ip, ir and po administration reflecting slowest absorption through po and ir routes followed by ip and pv routes.

The peak plasma concentrations (C_{max}) of omeprazole following po, ip, and ir administration were not significantly different each other. The absorption of omeprazole following po administration proceeded rapidly reaching its peak plasma concentration at 35 min, which was significantly faster than those following ip (80 min) and ir (93 min) administration. The time to reach its peak (T_{max}) in this study was 35 min, which is somewhat slower than that of suspensions in rats (12-16 min, Watanabe *et al.*, 1994) and human subjects (13 min), but much earlier than that of enteric-coated granules in human subjects (3 hrs) (Regardh *et al.*, 1985). The different T_{max} between Watanabe *et al.* (1994) and this study may be explained by the dose-dependency. The dose in this study (72 mg/kg) was much higher than that of Watanabe *et al.* (<40 mg/kg).

Bioavailability of omeprazole calculated on AUC according to Eq. (6) was in the order of pv (88.5%), ip (79.4%), po (40.8%), and ir (38.7%) administration (Table I). The oral bioavailability is comparable to that in man (40.3-58.2%, Regardh *et al.*, 1985), but much larger than that in rats (6.4-12.6%, Watanabe *et al.*, 1994). The difference between this study and Watanabe *et al.* can be explained by the saturation of first-pass metabolism in the liver at higher doses (Regardh *et al.*, 1985; Watanabe *et al.*, 1994). The oral bioavailability of omeprazole administered in alkaline buffer increased from 40 to 58% in man when the dose was raised from 10 to 40 mg (Regardh *et al.*, 1985). It also increased from 6.4 to 12.6% in rats at doses from 10 to 40 mg/kg (Watanabe *et al.*, 1994). Considering the dose-dependent first-pass metabolism in man, much higher oral bioavailability than 40% is expected in this study since much larger dose (72 mg/kg) than in human study (10-40 mg/body) was administered to rats. However, the oral bioavailability in this study was comparable to that in human study. One possible explanation of the inconsistency may be the species-difference between rat and man especially in the metabolic activity of omeprazole in the liver. But, we should note that omeprazole degrades with a half-life of less than 10 min in aqueous solution of pH <4 (Pilbrant and Cederberg, 1985). Therefore, acid-instability of the drug may be mostly responsible for the lower oral bioavailability of omeprazole than expected. The oral bioavailability of omeprazole may also be influenced by some physiological factors like gastric emptying rate and pH of the small intestinal fluid etc..

The mean availability of omeprazole across the liver (F_1) can be calculated according to Eq. (5). It was 0.89, which means 11.5% of dose administered through the pv route was extracted by the liver (the first-pass ef-

fect). Then, 88.5% of dose should reach systemic circulation after oral administration if all the dose transport across the gastrointestinal (GI) tract to reach portal vein. However, the oral bioavailability was only 40% in this study which was lower than expected 88.5%. In this case, the well-known acidic degradation in the stomach, low permeability across the GI membrane and/or enzymatic degradation in the GI mucosa can be suspected as causes of the low bioavailbility. Omeprazole undergoes slight GI mucosal metabolism (Watanabe *et al.,* 1994), and permeates across the GI mucosal membrane almost completely (Regardh *et al.,* 1985). Therefore, the lower oral bioavailability (40%) than expected 88.5% could be attributed mainly to acidic degradation in the stomach fluid and partly to hepatic first-pass metabolism. The acid-instability may be overcome by enteric coating of the oral preparations, for example, resulting in improved bioavailability of the drug. However, it should be noted that above discussion may lack strict meaning since F_1 in this study was calculated using AUC_{iv} and AUC_{pv} obtained from the different experimental conditions.

AUC from ip administration was comparable to that from pv administration. It means that omeprazole is transported in a good efficiency (90%) through GI mucosa and/or mesenteric veins to the portal vein. Assuming the transport through mesenteric veins is negligible, the transport efficiency across the GI mucosa will be fairly high resulting in the minimal first-pass metabolism in the GI mucosa as suggested previously (Watanabe *et al.,* 1994). This conclusion is consistent with Regardh *et al.* (1985), who showed almost complete GI absorption of omeprazole.

The bioavailability of omeprazole administered intrarectally (38.7%) was the smallest among administration routes examined but comparable to that of oral administration (41%). The low bioavailability of omeprazole from the rectum may be attributed to poor absorption since either acidic degradation or the first-pass metabolism will not be significant, if any in the rectal administration. Rectal administration may have advantage over oral administration in spite of low bioavailability because dose adjustment in the rectal administration is easy since dose-dependent first-pass metabolism can be avoided. In this sense, rectal route is worth developing as an alternative route of oral administration. Addition of appropriate absorption enhancers to the rectal preparations is expected to improve the poor bioavailability of omeprazole.

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