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Gastrointestinal Absorption of a New Reversible Proton Pump Inhibitor, YJA-20379-8, and its Pharmacokinetics after Oral Administration in Acetic Acid-induced Gastric Ulcer in Rats

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Abstract

The absorption of YJA-20379-8 (3-butyryl-4-[5-(*R*)-(+)-methylbenzylamino]-8-ethoxy-1,7-naphthyridine) from various rat gastrointestinal segments was evaluated using in-situ closed-loops. The pharmacokinetics of the drug were also evaluated after oral administration to rats with acetic acid-induced gastric ulcer (AIURs). The concentrations of YJA-20379-8 in the biological samples were analyzed by HPLC.

The absorption of YJA-20379-8 from stomach and jejunum was fast, but approximately 50% of the drug was recovered from each segment at 24 h. The total areas under the plasma concentration–time curves from time zero to 24 h ($AUC_{0-24\,h}$) were 161, 392, 233, 365, and 226 $\mu g\,min\,mL^{-1}$ for stomach, duodenum, jejunum, ileum, and colon, respectively. After oral administration of the drug, the plasma concentrations and the resultant $AUC_{0-12\,h}$ were not significantly different between control and AIURs. The detection limits of YJA-20379-8 in human plasma and urine were 50 and 100 $ng\,mL^{-1}$, respectively.

The results suggest that modification of the oral dose of YJA-20379-8 may not be required in gastric ulcer patients if the present rat pharmacokinetic data could be extrapolated to man.

YJA-20379-8 (3-butyryl-4-[5-(R)-(+)-methylbenzylamino]-8-ethoxy-1,7-naphthyridine, Figure 1) is a new reversible proton pump inhibitor. The stability, blood partition between plasma and blood cells, and pharmacokinetics of YJA-20379-8 have been previously reported (Chung et al 1998). The pharmacokinetic parameters of YJA-20379-8 were dose-independent after intravenous administration of the drug, 10, 20, and 50 $mg\,kg^{-1}$, to rats (Chung et al 1998). After oral administration of YJA-20379-8, the extent of absolute oral bioavailability was 9·00, 16·7, and 11·5% for 20, 50, and 100 $mg\,kg^{-1}$, respectively (Chung et al 1998). YJA-20379-8 is now being evaluated in a preclinical study. YJA-20379-8 was developed for oral administration, therefore the gastrointestinal absorption of the drug was investigated using in-situ gastrointestinal closed-loops (Hsu et al 1987; Lee et al 1994; Lee & Lee 1996; Han et al 1998).

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YJA-20379-8 is a new anti-ulcer drug and the pharmacokinetics were investigated after oral administration to rats with acetic acid-induced gastric ulcer (AIURs). Gastric ulcers can be induced by a number of chemicals including acetic acid (Szelenyi et al 1983; Fukuda et al 1989; Ito et al 1989; Ogihara & Okabe 1993).

The aim of this study was to determine the gastrointestinal absorption of YJA-20379-8, and the pharmacokinetics after oral administration of the drug, 50 $mg\,kg^{-1}$, to AIURs using HPLC analysis.

Materials and Methods

Chemicals

YJA-20379-8 was supplied by the Pharmacology and Toxicology Laboratory of Yung Jin Pharmaceutical Company (Hwasung, Korea). Cremophor (a derivative of castor oil and ethylene oxide) was a product of Sigma Chemical (St Louis, MO). Ketamine and cefazolin were obtained from Research Centre of Yuhan Corporation (Kunpo,

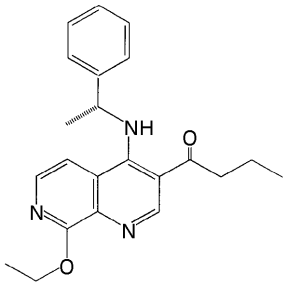

Figure 1. Chemical structure of YJA-20379-8.

Korea). Xylazine was purchased from Bayer Korea (Seoul, Korea). Other chemicals were of reagent or HPLC grade.

HPLC analysis

Preparation of stock and standard solutions. A stock solution of YJA-20379-8 was prepared in methanol (1 mg mL^{-1}). Appropriate dilutions of the stock solution were made with methanol. Standard solutions of YJA-20379-8 in human plasma and urine were prepared by mixing with an appropriate volume (less than 10 μg mL^{-1} human plasma or urine) of the diluted stock solutions giving final concentrations of 0·05, 0·1, 0·2, 0·5, 1, 2, and 5 μg mL^{-1}.

Sample preparation

A 2·5-volume of acetonitrile was added to deproteinize the sample (Chiou et al 1978; Lee & Lee 1994). After vortex-mixing for 1 min and centrifugation at 9000 *g* for 10 min, a 50-μL sample of supernatant was injected directly onto the HPLC column.

Apparatus

The HPLC system consisted of a model 7125 injector (Rheodyne, Cotati, CA), a model 400 pump (Xper-Chrom, St Louis, MO), a reversed-phase column (C_{18}; 25 cm × 4·6 mm, i.d.; particle size, 4 μm; YMC, Tokyo, Japan), a model 118 UV/Vis detector (Gilson, Middleton, WI), and a model 1200 recorder (Linear, Reno, NV). The mobile phase, acetonitrile–H_2O (5 : 1, v/v), was run at a flow rate of 1·3 mL min^{-1} and the column effluent was monitored by a UV detector set at 255 nm.

Animals

Male Sprague–Dawley rats, 260–305 g, were purchased from Charles River Company (Atsugi, Japan). They were housed in a clean room (Animal Centre for Pharmaceutical Research, College of Pharmacy, Seoul National University, Korea) and had free access to food (Samyang Company, Korea) and water.

Gastrointestinal absorption of YJA-20379-8

The procedures are similar to those reported previously (Hsu et al 1987; Lee et al 1994; Lee & Lee 1996; Han et al 1998) except that the whole gastrointestinal tract was not flushed with a buffer. The carotid artery was cannulated with a polyethylene tube (Clay Adams, Parsipanny, NJ) under light ether anaesthesia (Lee & Lee 1996). The cannula was exteriorized to the dorsal side of the neck where it terminated with a long Silastic tube (Dow Corning, Midland, MI). The Silastic tube was covered with wire to allow free movement of the rat. YJA-20379-8 powder was dissolved in polyethylene glycol 400–Cremophor (5 : 1, v/v). After abdominal incision under light ether anaesthesia, 50 mg kg^{-1}, YJA-20379-8 was injected directly into the stomach (n = 5), a 5-cm loop of the duodenum (n = 5), jejunum (n = 5), ileum (n = 6), and colon (n = 6) using a 23-gauge needle in a total volume of approximately 0·5 mL. The exposed areas were surgically sutured. Rats were individually housed in metabolic cages (Daejong Scientific Company, Seoul, Korea). Blood (0·12 mL approx.) was collected via the carotid artery at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, 600, 720, 960 and 1440 min after YJA-20379-8 administration. After centrifugation, a 50-μL plasma sample was stored in the freezer pending HPLC analysis. After 24 h, the rats were killed by cervical dislocation. Each closed loop was removed, transferred to a beaker containing 50 mL methanol (to facilitate drug extraction), and cut into small pieces using scissors. After stirring with a glass rod, two 100-μL samples of the supernatant were collected from each beaker and stored in the freezer pending HPLC analysis.

To determine whether bile juice enhances the absorption of YJA-20379-8 (a poorly water-soluble drug), the bile duct was cannulated with a polyethylene tube (Clay Adams). YJA-20379-8 was injected into the closed-loop of the duodenum with (n = 3) or without (n = 4) 1·5 mL bile juice (collected from other rats) injected just before drug administration. Other procedures were similar to those in the closed-loop study.

Acetic acid-induction of gastric ulceration

The rats were randomly divided into two groups, control and AIURs. After overnight fasting with

free access to water, each rat was anaesthetized with ketamine (0·3 mL; 50 mg mL^{-1}) and xylazine (0·4 mL; 20 mg mL^{-1}), and then the abdomen was opened. For AIURs, 30% acetic acid (Szelenyi et al 1983; Fukuda et al 1989; Ito et al 1989; Ogihara & Okabe 1993) was injected (total injection volume 0·25 mL) directly into the epithelium of the fundus part of the stomach using a microsyringe. The open areas were surgically sutured. To prevent microbial infection, 300 mg cefazolin (dissolved in 0·9% NaCl injectable solution), was injected (total injection volume 1·5 mL) intraperitoneally. Control rats were sham-operated.

Oral administration of YJA-20379-8 to control and AIURs

Ten days after surgery, the carotid artery was cannulated with a polyethylene tube (Clay Adams) under light ether anaesthesia. Other procedures were the same as those used in the closed-loop study. YJA-20379-8 (YJA-20379-8 powder was suspended in 0·2% carboxymethylcellulose), 50 mg kg^{-1} , was administered orally (total oral volume approximately 1·5 mL) using a feeding tube to control rats (n = 9) and AIURs (n = 10). An approximate 0·12-mL sample of blood was collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600, and 720 min after oral administration. After centrifugation, a 50-μL plasma sample was stored in the freezer until HPLC analysis. Urine was collected for 24 h, and the metabolic cage was rinsed with 10 mL of distilled water, these rinsings were then combined with the urine sample. After measuring the exact volume of the combined 24-h urine sample, two 100-μL samples of the combined urine sample were stored in the freezer until HPLC analysis. Each rat was killed by cervical dislocation at the end (24 h) of the experiment, and the whole gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 200 mL methanol (to facilitate the extraction of the drug), and then cut into small pieces using scissors. After stirring with a glass rod, two 100-μL samples of the supernatant were collected from each beaker and stored in the freezer until HPLC analysis. At the same time, the stomach was examined to determine whether gastric ulcers had been induced.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to the last measured time (12 or 24 h) in plasma ($AUC_{0-12\,h}$ or $AUC_{0-24\,h}$) was calculated by the trapezoidal rule (Kim et al 1993), employing the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase (Chiou 1978) and the linear trapezoidal rule for the calculation of the area during the rising plasma-level phase.

Statistical analysis

Levels of statistical significance were assessed using Duncan's multiple range test of a posteriori analysis of variance using unpaired data of the mean, or the *t*-test between two means for unpaired data. Significant differences were judged for $P < 0.05$. Data are expressed as mean ± s.d.

Results and Discussion

HPLC analysis

The peak of YJA-20379-8 was symmetrical and eluted at approximately 5·2 min. The detection limits for YJA-20379-8 were 50 ng mL^{-1} in human plasma and 100 ng mL^{-1} in urine, based on a signal-to-noise ratio of 3·0 using the deproteinization method. The mean within-day response factors (the peak height of YJA-20379-8 in mm divided by the concentration of YJA-20379-8 in μg mL^{-1}) were 41·7 and 19·6 for human plasma and urine, respectively. The lower response factor seen in human urine compared with that in human plasma could be the result of binding and/or adsorption of the drug to endogenous compounds in urine. Similar results were also found with azosemide (Lee & Lee 1994), methotrexate (Chen & Chiou 1981), and YH 1885, a new proton pump inhibitor

Table 1. Pharmacokinetics of 50 mg kg^{-1} YJA-20379-8 after direct injection into various gastrointestinal segments.

Gastrointestinal closed loops	AUC (μg min mL^{-1})[a]	Dose recovered as unchanged drug (%)
Stomach (n = 5)	161 ± 80·1*	52·4 ± 19·0
Duodenum		
without bile duct cannulation (n = 5)	392 ± 115	41·9 ± 11·4
with bile duct cannulation (n = 4)	198 ± 43·5	51·2 ± 15·9
with bile duct cannulation and bile juice injection (n = 3)	304 ± 86·4	34·7 ± 29·9
Jejunum (n = 5)	233 ± 69·9	47·4 ± 19·0
Ileum (n = 6)	365 ± 147	45·7 ± 2·17
Colon (n = 6)	226 ± 71·0	52·4 ± 11·5

Data are mean ± s.d. [a]$AUC_{0-24\,h}$ except duodenum with bile-duct cannulation and with bile juice injection ($AUC_{0-12\,h}$). *$P < 0.05$ compared with duodenum without bile-duct cannulation and ileum.

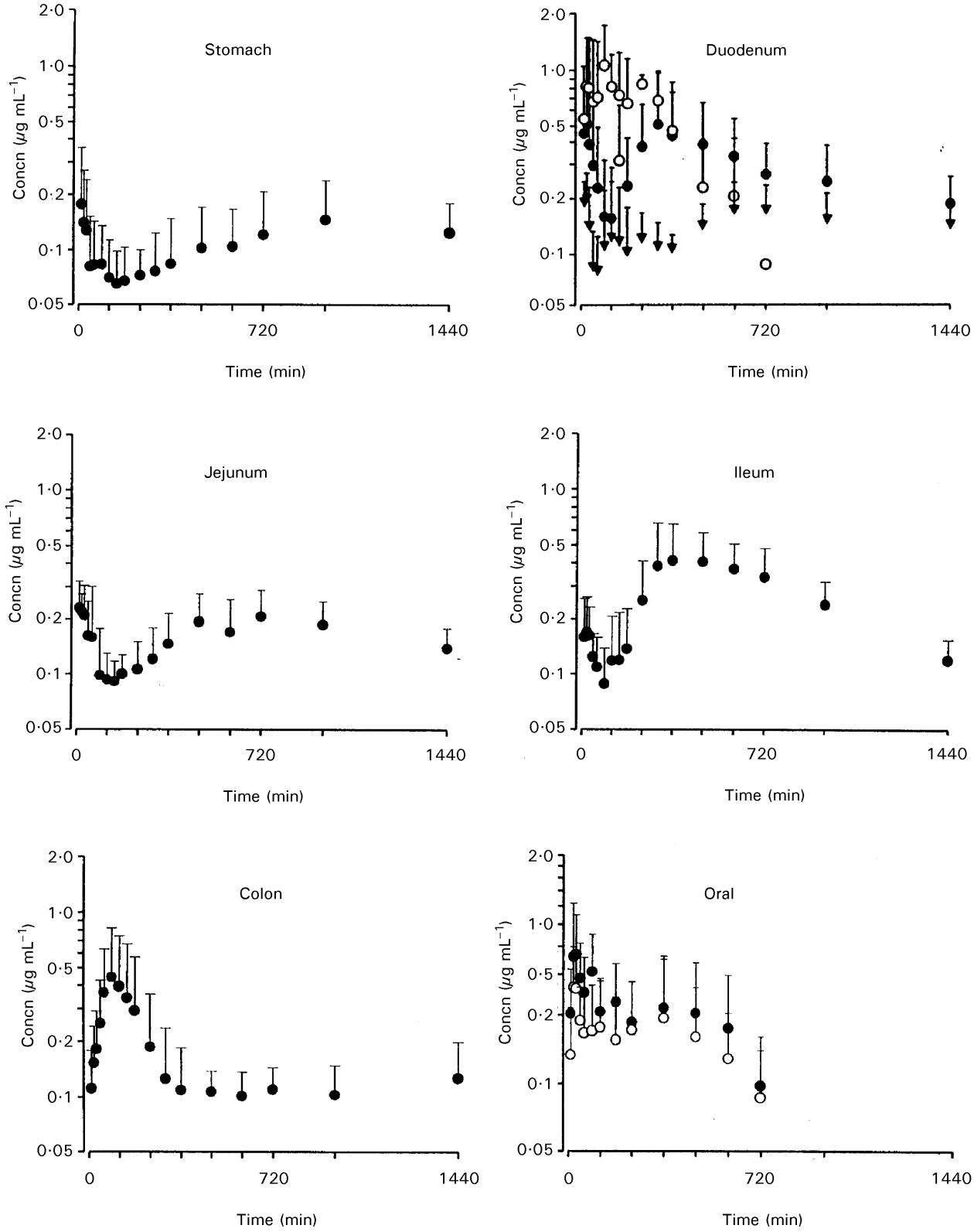

Figure 2. Plasma concentration–time profiles of 50 mg kg^{-1} YJA-20379-8 in the stomach; duodenum without bile duct cannulation (●), with bile duct cannulation with (○) or without (▼) bile juice; jejunum; ileum; colon; and oral administration in control rats (●) and rats with acetic acid-induced gastric ulceration (○). Data are mean ± s.d., $*P < 0{\cdot}05$.

(Han et al 1997). The mean within- and between-day coefficients of variation of the analysis of the same samples on three consecutive days for YJA-20379-8 in human plasma and urine were lower than 9·49% using the deproteinization method.

Closed-loop study

The mean arterial plasma concentration–time profiles of YJA-20379-8, 50 mg kg^{-1}, after administration into the stomach, duodenum, jejunum, ileum, and colon are shown in Figure 2; selected pharmacokinetic parameters are listed in Table 1. The absorption of YJA-20379-8 from the stomach and jejunum was fast; the drug was detected at the first sampling time (10 min) and reached its peak at 10, 300 (and 90), 10, 360, and 90 min for stomach, duodenum, jejunum, ileum, and colon, respectively (Figure 2). The absorption of YJA-20379-8 from the gastrointestinal tract was not complete; 52·4, 41·9, 47·4, 45·7, and 52·4% of the injected dose was recovered at 24 h for stomach, duodenum, jejunum, ileum, and colon, respectively (Table 1); these values were not significantly different. The contribution of biliary excretion of unchanged YJA-20379-8 to the amount of injected drug recovered from each closed-loop at 24 h was negligible (Table 1). This was because less than 0·5% of the intravenous dose of YJA-20379-8 was excreted as unchanged drug in 24-h bile after intravenous administration of the drug, 20 mg kg^{-1}, to three rats (Chung et al 1998). Therefore, the values listed in Table 1 could represent the unabsorbed fraction of YJA-20379-8 assuming that the drug was stable for up to 24 h in gastrointestinal fluids.

Approximately 50% of the injected YJA-20379-8 was absorbed from the gastrointestinal segments studied. The $AUC_{0-24\,h}$ in the stomach was significantly smaller than those in duodenum and ileum (161, 392, 233, 365, and 226 μg min mL^{-1} for stomach, duodenum, jejunum, ileum, and colon, respectively; Table 1). The percentages of the injected YJA-20379-8 recovered from each segment at 24 h as unchanged drug were not significantly different among stomach, duodenum, jejunum, ileum, and colon (Table 1); the differences in $AUC_{0-24\,h}$ in each closed-loop could represent the degree of first-pass effect of YJA-20379-8. The first-pass effects of YJA-20379-8 by the duodenum and ileum were not considerable.

The effects of bile juice on the absorption of YJA-20379-8 from the duodenum were inconclusive. Although in these experiments, the plasma concentrations of YJA-20379-8 were higher (Figure 2), $AUC_{0-12\,h}$ was ($P < 0{\cdot}154$) greater (54%), and the percentages of injected dose recovered at 24 h as unchanged drug was less ($P < 0{\cdot}452$, 32%) than in rats without bile juice injection after bile duct cannulation, the lack of significance between these values in the limited number of rats studied precluded any interpretation. However, enhanced absorption of poorly water-soluble drugs, phenytoin (Shinkuma et al 1985) and vitamin E (Traber et al 1986) by bile juice has been reported.

Pharmacokinetics of YJA-20379-8 in rats with acetic acid-induced gastric ulcer

The mean arterial plasma concentration–time curves of YJA-20379-8 after oral administration of the drug, 50 mg kg^{-1}, to control (n = 9) and AIURs (n = 10) are shown in Figure 2. The plasma concentrations of YJA-20379-8 were similar for the two groups (Figure 2). The $AUC_{0-12\,h}$ values were also not significantly different between the two groups (137 ± 55·2 (control rats) and 119 ± 63·4 μg min mL^{-1} (AIURs)). The absorption of YJA-20379-8 from the gastrointestinal tract was not complete; the percentages of oral dose recovered from the gastrointestinal tract at 24 h as unchanged drug were 20·2 and 22·8% for control and AIURs, respectively. YJA-20379-8 was below the detection limit in rat urine.

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