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Gastrointestinal First-pass Effect of YJA-20379-8, a New Reversible Proton Pump Inhibitor, in Rats

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Abstract

Since low bioavailability of YJA-20379-8 (3-butyryl-4-[5-*R*-(+)-methylbenzylamino]-8-ethoxy-1,7-naphthyridine), a new reversible proton pump inhibitor, has been reported after oral administration of the drug to rats, the first-pass organ of the drug was investigated in rats. YJA-20379-8, 50 mg kg^{-1}, was infused over 1 min via the jugular vein (n = 5) or the portal vein (n = 5), or was instilled directly into the stomach (n = 5) or the duodenum (n = 5).

After intravenous or intraportal infusion of the drug, the total body clearance of YJA-20379-8 (18·1 and 19·7 mL min^{-1} kg^{-1} based on plasma data) was considerably lower than the reported cardiac output (296 mL min^{-1} kg^{-1} based on blood data) in rats. This data indicated that the first-pass effect of YJA-20379-8 by the lung and heart was negligible. The areas under the plasma concentration–time curve from time zero to time infinity (AUC) after intravenous or intraportal administration of YJA-20379-8 (2760 and 2540 μg min mL^{-1}) were not significantly different, indicating that the hepatic first-pass effect of the drug was also negligible in rats.

After intragastric or intraduodenal instillation of YJA-20379-8, the extent of absolute oral bioavailability was 18·2 and 33·8%, respectively. Based on gastrointestinal recovery studies, approximately 86·5 and 91·2% of YJA-20379-8 was absorbed from rat gastrointestinal tract after intragastric or intraduodenal instillation, respectively. The data indicated that gastrointestinal and intestinal first-pass effects of YJA-20379-8 were approximately 68% (86·5–18·2) and 57% (91·2–33·8), respectively. The $AUC_{0-24\,h}$ values of YJA-20379-8 were significantly different between intragastric and intraduodenal instillation, indicating that the gastric first-pass effect of the drug was approximately 10% in rats. Therefore, it could be concluded that the low F value of YJA-20379-8 after oral administration of the drug could be due to a considerable (approx. 60%) intestinal first-pass effect in rats.

Although the liver is the most studied organ for the first-pass effect of many drugs, the gastrointestinal tract is an additional first-pass organ of drugs such as azosemide (Kim et al 1997), furosemide (Lee & Chiou 1983), bumetanide (unpublished data), and 2-(allylthio)pyrazine, a new chemoprotective agent (Han & Lee 1999).

YJA-20379-8 (3-butyryl-4-[5-*R*-(+)-methylbenzylamino]-8-ethoxy-1,7-naphthyridine, Figure 1) is a new reversible proton pump inhibitor. YJA-20379-8 is a basic drug with a pK_a of 5·3 and is lipophilic with log(n-octanol/water) of 1·4 in pH 1·2 buffer, with values of infinity in both water and pH 6·8 buffer. The stability, blood partition, and pharmacokinetics of YJA-20379-8 have been reported (Chung et al 1998). Chung et al (1998) reported that after the oral administration of YJA-20379-8 (20, 50, or 100 mg kg^{-1}) to rats, the percentages of unchanged drug recovered from the gastrointestinal tract were 2·85, 20·2, and 23·9%, respectively. The estimated extent of absolute oral bioavailability (F) of YJA-20379-8 was 9·0, 16·7, and 11·3% respectively after oral administration of 20, 50, or 100 mg kg^{-1} drug to rats (Chung et al 1998). The low values of F were surprising, considering the considerable absorption of YJA-20379-8 from rat gastrointestinal tract; after oral administration of

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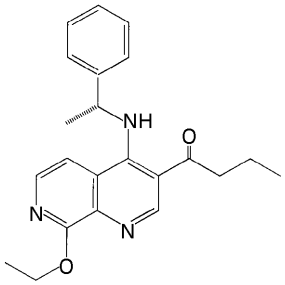

Figure 1. Chemical structure of YJA-20379-8.

the drug (20, 50, or 100 mg kg^{-1}), approximately 97, 80, and 76% of the dose was absorbed, respectively. This could be due to considerable first-pass hepatic, gastric, or intestinal effects of the drug in rats. The pharmacokinetic parameters of YJA-20379-8 were dose-independent after intravenous administration of 10, 20, or 50 mg kg^{-1} drug to rats (Chung et al 1998). YJA-20379-8 is now being evaluated in preclinical study.

The purpose of this study was to report the hepatic, gastric, and intestinal first-pass effects of YJA-20379-8 after intravenous, intraportal, intragastric, or intraduodenal administration of 50 mg kg^{-1} drug to rats.

Materials and Methods

Chemicals

YJA-20379-8 was donated by the Pharmacology & Toxicology Laboratory of Yung Jin Pharmaceutical Company (Hwasung, Korea). Cremophor (a derivative of castor oil and ethylene oxide) was purchased from Sigma Chemical (St Louis, MO). Other chemicals were of reagent grade or HPLC grade and used without further purification.

Animals

Male Sprague–Dawley rats, 260–280 g, were purchased from Charles River Company (Atsugi, Japan). The animals were housed in a clean room (Animal Centre for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea) and had free access to food (Samyang Company, Seoul, Korea) and water.

Measurement of hepatic first-pass effect of YJA-20379-8

The jugular vein and the carotid artery of each rat were catheterized with polyethylene tube (Clay Adams, Parsippany, NJ) under light ether anaesthesia (Yoon et al 1998). Both cannulae were exteriorized to the dorsal side of the neck and terminated with a long Silastic tube (Dow Corning, Midland, MI). At the same time, the portal vein was cannulated (Kim et al 1997) by the modified Suzuki method (Xu et al 1992). After a midline abdominal incision, the middle portion of the portal vein was isolated. The tapered end of the 23-gauge needle, bent at a 60° angle, was inserted into the pyloric vein, the tributary flow directly into the hepatic portal vein (to minimize the impairment of blood flow in the portal vein). Bleeding was prevented by an application of epoxy glue (Krazy Glue, Krazy Glue Inc., Itasca, IL). A 5-cm piece of the Silastic tube was attached to the other end of the needle which linked the dorsal-side cannula of the neck. All of the three Silastic tubes were covered with wire to allow free movement of the rat. The exposed areas, the neck and abdomen, were closed using surgical suture. Each animal was kept individually in a metabolic cage (Daejong Scientific Company, Seoul, Korea) for 2–3 h to recover from the anaesthesia. The rats were not restrained during the experimental period.

YJA-20379-8 powder was dissolved in a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v), and 50 mg kg^{-1} was infused over 1 min via the jugular vein (n = 5) or the portal vein (n = 5). The total injection volume was approximately 0·5 mL. At the same time, 0·5 mL of the mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v) was infused over 1 min via the portal vein for intravenous study and via the jugular vein for intraportal study. Blood samples (0·12 mL) were collected via the carotid artery at 0 (to serve as a control), 1 (at the end of infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 960, and 1440 min. After centrifugation, 50 μL of each plasma sample was stored in the freezer until HPLC analysis of YJA-20379-8. Heparinized 0·9% NaCl injectable solution (0·3 mL; 20 units mL^{-1}), was used to flush the cannula immediately after each blood sampling to prevent blood clotting. At the end of 24 h, each rat was killed by cervical dislocation. The entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 50 mL methanol (to facilitate the extraction of YJA-20379-8), and cut into small pieces with scissors. After shaking manually and stirring with a magnetic stirrer for 2 min, 100 μL supernatant was collected from each beaker and stored in the freezer until HPLC analysis of YJA-20379-8.

Measurement of gastric and intestinal first-pass effects

Rats were fasted overnight with free access to water. The carotid artery was catheterized with a

polyethylene tube (Clay Adams) under light ether anaesthesia. The cannula was exteriorized to the dorsal side of the neck and terminated with a long Silastic tube (Dow Corning). At the same time, the portal vein was similarly cannulated (Kim et al 1997) by the modified Suzuki method (Xu et al 1992). For intraportal administration, a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v) was instilled (0·5 mL) into the stomach and the duodenum, respectively, using a 23-gauge needle. Thereafter, YJA-20379-8 (the same solution as described for the measurement of hepatic first-pass effect), 50 mg kg^{-1}, was infused (0·5 mL) over 1 min via the portal vein (n = 6). For intraduodenal instillation, 50 mg kg^{-1} YJA-20379-8 was instilled (0·5 mL) into the duodenum (n = 5), followed by intragastric instillation (0·5 mL) of a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v, 0·5 mL) using a 23-gauge needle. At the same time, a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v) was similarly infused (0·5 mL) over 1 min via the portal vein. For intragastric instillation, a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v) was instilled (0·5 mL) into the duodenum and 50 mg kg^{-1} YJA-20379-8 was instilled (0·5 mL) intragastrically (n = 5). At the same time, 0·5 mL of a mixed solution of polyethylene glycol 400 and Cremophor (5 : 1, v/v) was infused over 1 min via the portal vein. Blood samples (0·12 mL) were collected at 0 (to serve as a control), 1 (at the end of infusion for intraportal infusion only), 5 (for intraportal infusion only), 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 960, and 1440 min. Other procedures were similar to those described for the measurement of hepatic first-pass effect.

HPLC analysis of YJA-20379-8

The concentrations of YJA-20379-8 in the samples were analysed by a reported HPLC method (Chung 1998). A 2·5-volume of acetonitrile was added to deproteinize the sample. After vortexing for 1 min and centrifugation for 10 min, 50 μL supernatant was injected directly onto the HPLC column. The mobile phase, acetonitile–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 UV detection at 255 nm. The retention time for YJA-20379-8 was approximately 5·2 min. The detection limits of YJA-20379-8 were 50 and 100 ng mL^{-1} for human plasma and urine, respectively. The intraday and interday coefficients of variation of YJA-20379-8 in human plasma and urine, and rat tissue homogenates were lower than 9·49%.

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).

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) or to the last measured time, up to 24 h, in plasma ($AUC_{0-24\,h}$) was calculated by the trapezoidal rule-extrapolation method (Kim et al 1993) using the logarithmic trapezoidal rule (Chiou 1978) for the calculation of the area during the declining plasma-level phase, and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity (for the calculation of AUC) was estimated by dividing the last measured concentration by the terminal rate constant.

A standard method (Gibaldi & Perrier 1982) was used to calculate the time-averaged total body clearance (CL), the area under the first moment of plasma concentration–time curve (AUMC), the mean residence time (MRT), and the apparent volume of distribution at steady state (Vd_{SS}).

The extent of absolute oral (intragastric and intraduodenal instillation) bioavailability (F) was estimated by comparing the AUC after intravenous administration and $AUC_{0-24\,h}$ after oral administration, since the pharmacokinetic parameters of YJA-20379-8 were dose-independent after intravenous administration of the drug (10–50 mg kg^{-1}) to rats (Chung et al 1998).

The mean value of each terminal half-life (Eatman et al 1977), Vd_{SS} (Chiou 1979), and CL (Chiou 1980) was determined by the harmonic-mean method.

Statistical analysis

$P < 0{\cdot}05$ was considered to be statistically significant using an unpaired *t*-test. All results are expressed as mean ± s.d.

Results and Discussion

Measurement of hepatic first-pass effect of YJA-20379-8

The mean arterial plasma concentration–time curves of YJA-20379-8 after intravenous (n = 5) or intraportal (n = 5) administration of 50 mg kg^{-1}

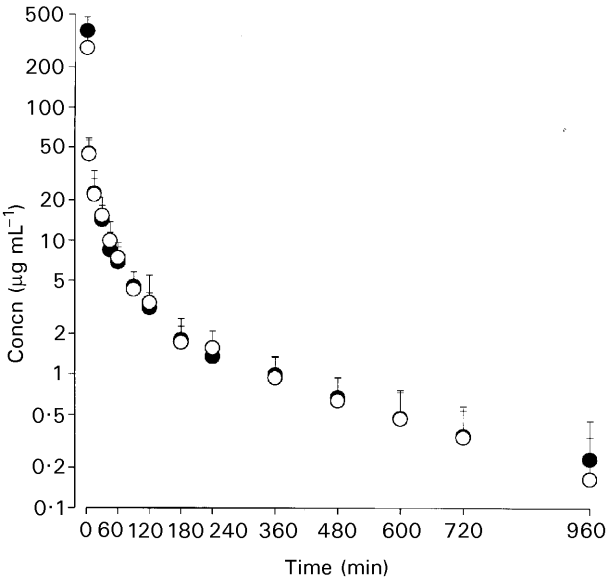

Figure 2. Mean arterial plasma concentration–time curves of YJA-20379-8 after 1-min intravenous (●, n = 5) and intraportal (○, n = 5) infusion of 50 mg kg^{-1} drug to rats. Vertical bars represent standard deviation.

drug to rats are shown in Figure 2; selected relevant pharmacokinetic parameters are listed in Table 1. After 1-min intravenous infusion, the plasma concentrations of YJA-20379-8 declined in a parallel fashion for both groups of rats (Figure 2). The pharmacokinetic parameters of YJA-20379-8 were not significantly different between both groups of rats (Table 1). The CL values of YJA-20379-8 based on plasma data after intravenous (18·1 mL min^{-1} kg^{-1}) or intraportal (19·7 mL min^{-1} kg^{-1}) administration of the drug (Table 1) were considerably lower than the reported (Davies & Morris 1993) cardiac output in rats based on blood data (296 mL min^{-1} kg^{-1}), indicating that first-pass effects of YJA-20379-8 by the lung and heart could be negligible in rats. Although the AUC after intraportal administration was 92·0% of that after intravenous administration (Table 1), they were not significantly different, indicating that hepatic first-pass effect of YJA-20379-8 was also almost negligible in rats.

Table 1. Pharmacokinetic parameters of YJA-20379-8 after intravenous and intraportal infusion of 50 mg kg^{-1} drug to rats.

Parameters	Intravenous	Intraportal
Body weight (g)	283 ± 12·2	290 ± 19·6
Terminal half-life (min)	314 ± 154	180 ± 104
Area under the plasma concentration–time curve from time zero to time infinity (μg min mL^{-1})	2760 ± 343	2540 ± 710
Mean residence time (min)	180 ± 83·9	135 ± 51·9
Time-averaged total body clearance (mL min^{-1} kg^{-1})	18·1 ± 6·06	19·7 ± 7·56
Volume of distribution at steady state (mL kg^{-1})	2940 ± 1360	2520 ± 687
Amount of YJA-20379-8 recovered from gastrointestinal tract at 24 h as unchanged drug (% of dose)	0·0571 ± 0·00737	0·137 ± 0·120

Values are mean ± s.d., n = 5.

Measurement of gastric and intestinal first-pass effects of YJA-20379-8

The mean arterial plasma concentration–time curves of YJA-20379-8 after intraportal infusion, and intraduodenal or intragastric instillation of 50 mg kg^{-1} drug to rats are shown in Figure 3; relevant pharmacokinetic parameters are listed in Table 2. After intraportal infusion, the plasma concentrations of YJA-20379-8 declined in a polyexponential fashion (Figure 3) with a mean terminal half-life of 215 min (Table 2). After intraduodenal and intragastric instillation, however, the plasma concentrations of YJA-20379-8 were almost constant from 2 to 24 h (Figure 3), and this could be due to continuous absorption of the drug from the various rat gastrointestinal segments. Approximately 50% of the instilled YJA-20379-8 was absorbed from each gastrointestinal segment (stomach, duodenum, jejunum, ileum, and colon) in an in-situ closed-loop study in rats (Chung et al 1999). YJA-20379-8 was below the detection limit in all rat urine studied, indicating that the drug was completely metabolized. Chung et al (1998) reported that in three rats administered 20 mg kg^{-1} YJA-20379-8 intravenously, less than 0·5% of the dose was excreted in 24-h bile as unchanged drug. Therefore, the CL values listed in Tables 1 and 2 could represent non-renal clearance (metabolic clearance) of YJA-20379-8.

The extent of absolute oral bioavailability of YJA-20379-8 was 18·2 and 33·8% for intragastric and intraduodenal instillation, respectively. After intragastric and intraduodenal instillation of YJA-20379-8, the percentages of dose recovered from the entire gastrointestinal tract at 24 h as unchanged drug were 13·5 and 8·9%, respectively (Table 2). It is possible that this unchanged YJA-20379-8 might be partly attributed to the gastrointestinal excretion (including biliary excretion) of the absorbed drug. Based on the linear pharmacokinetics, the mean true fraction of dose unabsorbed (F_{unabs}) in this study may be estimated by the following equations (Lee & Chiou 1983);

Table 2. Mean (± s.d.) pharmacokinetic parameters of YJA-20379-8 after intraportal infusion, and intraduodenal and intragastric instillation of the drug (50 mg kg^{-1}), to rats.

Parameters	Intraportal	Intraduodenal	Intragastric
Body weight (g)	260 ± 8·37	272 ± 6·71	269 ± 13·4
Terminal half-life (min)	215 ± 119		
Area under the plasma concentration–time curve from time zero to the last measured time, 24 h, in plasma (μg min mL^{-1})	2700 ± 1190	934 ± 133	501 ± 296
Mean residence time (min)	165 ± 110		
Time-averaged total body clearance (mL min^{-1} kg^{-1})	17·9 ± 8·28		
Volume of distribution at steady state (mL kg^{-1})	2580 ± 1600		
Amount of YJA-20379-8 recovered from gastrointestinal tract at 24 h as unchanged drug (% of dose)	0·113 ± 0·0332	8·90 ± 13·1	13·5 ± 12·7

For intraportal studies n = 6, for intraduodenal and intragastric studies n = 5.

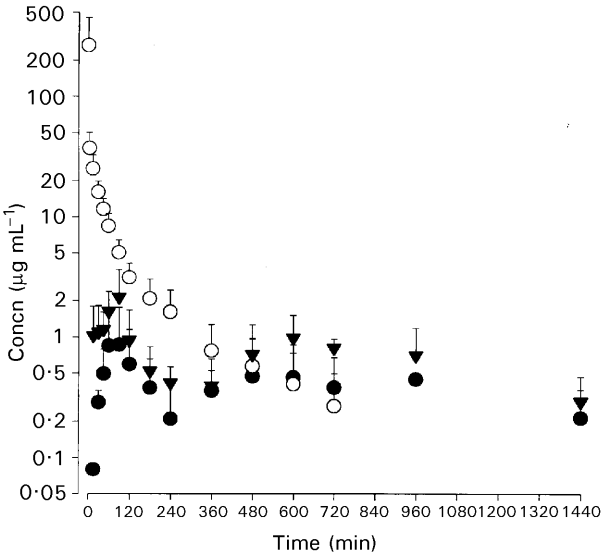

Figure 3. Mean arterial plasma concentration–time curves of YJA-20379-8 after intraportal infusion (○, n = 6), and intraduodenal (▼, n = 5) and intragastric (●, n = 5) instillation of 50 mg kg^{-1} drug to rats. Vertical bars represent standard deviation.

$$0{\cdot}135 = F_{unabs} + (0{\cdot}1815 \times 0{\cdot}000571)$$

for intragastric instillation

and

$$0{\cdot}089 = F_{unabs} + (0{\cdot}3384 \times 0{\cdot}000571)$$

for intraduodenal instillation

where 0·1815 and 0·3384 are the F values for intragastric and intraduodenal instillation, respectively, and 0·000571 is the mean fraction of the intravenous dose of YJA-20379-8 recovered from the entire gastrointestinal tract at 24 h as unchanged drug (Table 1). The calculated F_{unabs} values were 13·5 and 8·88% for intragastric and intraduodenal instillation, respectively, indicating that the contribution of gastrointestinal excretion (including biliary excretion) of the drug to the total drug recovered from the gastrointestinal tract following intragastric and intraduodenal instillation was negligible.

Therefore, it could be concluded that approximately 86·5% (100−13·5) and 91·2% (100−8·88) of YJA-20379-8 was absorbed or metabolized after intragastric and intraduodenal instillation, respectively. Since the hepatic first-pass effect of YJA-20379-8 was almost negligible in rats as discussed earlier, the major site for first-pass metabolism of YJA-20379-8 in rats could be the gastrointestinal tract.

In this study, the F values of YJA-20379-8 were 18·2 and 33·8% after intragastric and intraduodenal instillation, respectively, and based on the gastrointestinal recovery study, 86·5 and 91·2% were found to be absorbed or metabolized after intragastric and intraduodenal instillation, respectively. Therefore, 68·3% (86·5−18·2) and 57·4% (91·2−33·8) of instilled YJA-20379-8 were lost through metabolism by gastrointestinal and intestinal first-pass effects, respectively. This was proved by significantly smaller $AUC_{0-24\,h}$ values of YJA-20379-8 after intragastric and intraduodenal instillation than that after intraportal infusion of the drug; the $AUC_{0-24\,h}$ values after intragastric and intraduodenal instillation were 34·6 and 18·6%, respectively, of that after intraportal infusion (Table 1). Approximately 10·9% (68·3−57·4) of YJA-20379-8 was lost by gastric first-pass effect after intragastric instillation. This was shown by significantly different $AUC_{0-24\,h}$ values after intragastric and intraduodenal instillation; the value after intragastric instillation was 54% of that after intraduodenal instillation (Table 2). Therefore, it could be concluded that approximately 57·4% of YJA-20379-8 was lost by an intestinal first-pass

effect and the intestinal first-pass effect mainly contributed to the low F values in rats.

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