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Effect of Food on the Pharmacokinetics of YH439 and Its Metabolites in Rats

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The pharmacokinetics of YH439 and its metabolites were investigated after oral administration of YH439 to rats to investigate the food effect. After oral administration of YH439, its metabolites, M4 and M5 were detected in plasma. YH439 was not detected in the plasma for both fasted and fed rats for all doses studied. The pharmacokinetic parameters of the metabolites were not affected by food at all doses studied. The results of this study indicated that there are no significant food effects on the pharmacokinetics of YH439 and its metabolites in rats.

Key words : YH439, Pharmacokinetics, Effect of food

INTRODUCTION

Recently, Yuhan Research Center of Yuhan Corporation (Kunpo, Korea) has developed YH439 (Fig. 1), a malotilate analog, as a hepatoprotective agent which is currently evaluated in Phase II clinical trials. YH439 is expected to improve the symptoms of various hepatic disorders including viral hepatitis. The metabolites of YH439, M3, M4, M5, and M7 (Fig. 1) were identified by GC/MS spectroscopy in blood, urine, or bile samples after intravenous or oral administration of YH439 to rats (Park, 1994). The metabolites seemed to have negligible hepatoprotective activity compared with YH439. Simultaneous HPLC analysis of YH439 and its metabolites in human plasma and urine (Yoon *et al.*, submitted), stability and blood partitioning of YH439 (Yoon *et al.*, 1996), factors influencing the protein binding of YH439 using an equilibrium dialysis technique (Lee *et al.*, 1995), and pharmacokinetics of YH439 and its metabolites in rats, rabbits and dogs (Yoon *et al.*, submitted) have been reported.

Some pharmacokinetic studies have demonstrated that food can increase, decrease, or no effect on oral absorption of drugs. The reported mechanisms of the drug-food interaction include formation of chelation (Disler *et al.*, 1975; Schuna *et al.*, 1983; Siegel, 1978), and changes in dissolution rate of drugs (Greenblatt *et al.*, 1978), solubility of poorly water-soluble drugs (Palma *et al.*, 1986), gastric emptying pattern (Walter-Sack, 1987),) and/or first-pass metabolism of drugs (Leidholm *et al.*, 1990; Melander *et al.*, 1977). Since YH439 is poorly water-soluble and undergoes GI and/or liver extensive first-pass metabolism, it is important to investigate the effect of food on the absorption of the drug. Therefore, we administered YH439 orally at three doses to fasted and fed rats and attempted to

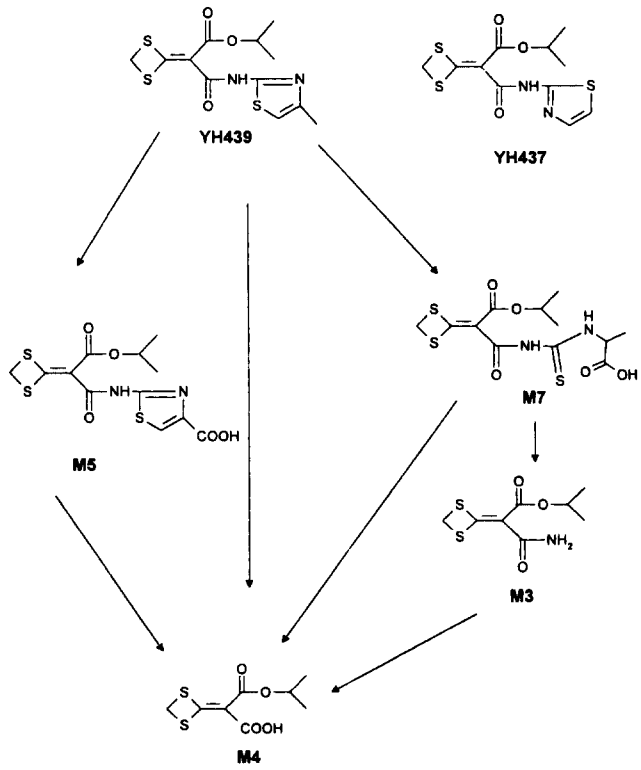

Fig. 1. Structures of YH439, M3, M4, M5, M7, and YH437 (internal standard) and presumed metabolic pathways of YH 439 in rats.

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evaluate the effect of food on the pharmacokinetics of YH439 and its metabolites.

MATERIALS AND METHODS

Materials

YH439, M3, M4, M5, M7, and YH437(as an internal standard for the HPLC analysis, Fig. 1) were synthesized in Yuhan Research Center of Yuhan Corporation. Carboxymethylcellulose sodium was purchased from Aldrich Chemical Company (Milwaukee, WI, USA). All other chemicals and solvents were of analytical or HPLC grade, and therefore used without further purification. The chemical names of YH439, M3, M5, M7 and YH437 are as follows; YH439, isopropyl 2-(1,3-dithietan-2-ylidene-2-[N-(4-methyl-2-thiazolyl)carbamoyl]acetate; M3, isopropyl 2-(1,3-dithietan-2-ylidene)-2-carbamoylacetate; M4, isopropyl 2-(1,3-dithietan-2-ylidene)-2-carboxyacetate; M5, isopropyl 2-(1, 3-dithietan-2-ylidene)-2-[N-(4-carboxy-2-thiazolyl)carbamoyl]acetate; M7, isopropyl 2-(1,3-dithietan-2-ylidene)-2-[N-[N'-(±)-carboxyethylthioureido]carbonyl]acetate; and YH437, isopropyl 2-(1,3-dithietan-2-ylidene)-2-[N-(2-thiazolyl) carbamoyl]acetate.

Animals

Seven weeks old, male Sprague-Dawley rats (Yuhan Research Center of Yuhan Corporation), weighing between 260 and 280 g, were kept in a room maintained on a 12 h light/12 h dark cycle at 23±2°C and 30~70% relative humidity. Animals were fasted with free access to water overnight (about 24 h) prior to the study for the fasted group. For the fed group, the rats were allowed to take food for about 18 h prior to the study. Each rat consumed about 36 g of food per day.

Oral studies

Under light ether anesthesia, the carotid artery was cannulated with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) for blood sampling. The cannula was exteriorized to the dorsal side of the neck, and covered with a Silastic tubing to allow free movement of the rats. The exposed area was closed using a surgical clip (Autoclip, Clay Adams). Each rat was allowed to recover for about 4 h from anesthesia. An aliquot of heparinized normal saline injectable solution (300 μl, 20 units/ml) was used for flushing each cannula to prevent blood clotting. YH439 (suspended in 0.5% carboxymethylcellulose sodium) was administered orally using feeding sonde (total oral volume was 5 ml/kg) at the doses of 50, 100, and 200 mg/kg (n=6 for each group). Blood samples (300 μl) were collected through cannula at 0 (to serve as a control), 15, 30, 45 60, 90, 120, 180, 240, 360, 480, 600, and 1440 min after the drug administration. Equal volume of the heparinized normal saline injectable solution was replaced after each blood sampling. Blood samples were immediately centrifuged at 10,000 rpm for 1 min with MicroMax (International Equipment Company, Needham Heights, MA), and an aliquot of plasma (100 μl) was separated and kept frozen at -20°C until HPLC analysis for YH439 and its metabolites.

HPLC analysis

The plasma concentrations of YH439 and its metabolites were determined by high-performance liquid chromatography (HPLC). An aliquot of acetonitrile (270 μl) and acetonitrile (30 μl) containing internal standard (YH437, 10 μg/ml) were added to the plasma sample (100 μl). After centrifugation, an aliquot (30 μl) of the supernatant was injected directly onto the HPLC column. The HPLC system consisted of an autosampler (851-AS, Jasco, Tokyo, Japan), a pump (PU-980, Jasco), a reversed-phase column (C_{18}; 4.6 mm, I.D.×250 mm, L; 5 μm particle size; Shiseido, Tokyo, Japan), an UV-detector (UV-975, Jasco) and an integrator (D-7500, Hitachi, Tokyo, Japan). The mobile phase, acetonitrile-H_2O (48:52, v/v) containing trifluoroacetic acid (0.05 %) was run at a flow rate of 1 ml/min. The effluent was monitored using UV detector set at 317 nm and column temperature was maintained at an ambient temperature (about 20°C). Stock solutions of each compound were made using acetonitrile (100 μg/ml). An appropriate volume (less than 10 μl per ml plasma) of the stock solution was spiked into plasma to make standard solution. Standard curves for YH439, M3, M4, M5, and M7 in plasma were constructed from 0.05 to 1.00 μg/ml of each compound. The retention times of M3, M4, M5, YH437 (internal standard), M7, and YH439 were approximately 5.2, 7.6, 6.8, 13.2, 13.9, and 14.8 min, respectively. The detection limits of YH439, M3, M4, M5 and M7 were all 0.05 μg/ml.

Pharmacokinetic analysis

Pharmacokinetic parameters were obtained from the plasma concentration-time data for YH439 and its metabolites in each animal. The total area under the plasma concentration-time curve from time 0 to the last measured time (AUC_{0-t}) was calculated using a trapezoidal rule method. Maximum plasma concentration (C_{max}) and time to reach a C_{max} (t_{max}) was read directly from the experimental data.

Statistical analysis

Data were analyzed using the unpaired Student's t test, and a p value of less than 0.05 was considered

to be statistically significant. All the data were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

The plasma concentration-time profiles of M4 and M5 after oral administration of YH439 to rats are shown in Figs. 2-4, and their pharmacokinetic parameters are listed in Table I. After oral administration, YH439 was not detected in the plasma in all the rats studied. This could be due to 1) poor absorption of poorly water-soluble YH439 from rat GI tract (Yoon *et al.*, submitted), 2) considerable first-pass metabolism of the absorbed YH439 (to form M4 and M5), and 3) our HPLC assay sensitivity. YH439 was found to be metabolized to M7 by esterase, and M7 is further metabolized to M4 by amidase (Fig. 1). YH439 is also oxidized to M5 and M5 is further metabolized to M4 (Fig. 1). After oral administration of YH439, M7 and M3 were also not detected in plasma in all the rats studied. This was due to fact (Yoon *et al.*, submitted) that M4 was formed directly from YH439. However, M7 was found from plasma in dogs (Yoon *et al.*, submitted).

However, M4 and M5 were detected in plasma in all rats studied after oral administration of YH439. After oral administration of YH439 to rats, the formation of M4 was fast; the plasma concentration of M4 reached its peak rapidly and declined fast (Fig. 2-4). However, the formation of M5 seemed to be slow; the plasma concentrations of M5 increased continually up to approximately 600 min (Fig. 2-4). Similar results were also obtained from other rat studies (Yoon *et al.*, submitted). The plasma concentrations of both M4 and M5 were not significantly different at all three doses for both groups of rats (although they were significantly different up to 45 min at 200 mg/kg, inset of Fig. 4), and similar results were also obtained from pharmacokinetic parameters listed in Table I. Above data indicated that the rate and extent of oral absorption of YH439 and the formation of its metabolites, M4 and M5 were not affected by food in rats.

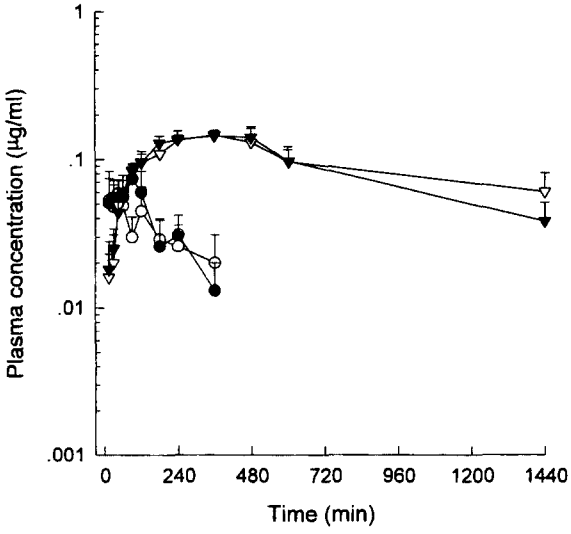

Fig. 2. Mean plasma concentration-time profiles of M4 in fasted (○) and fed rats (●), and M5 in fasted (▽) and fed rats (▼) after oral administration of YH439, 50 mg/kg to rats. Bars represent SEM (n=6).

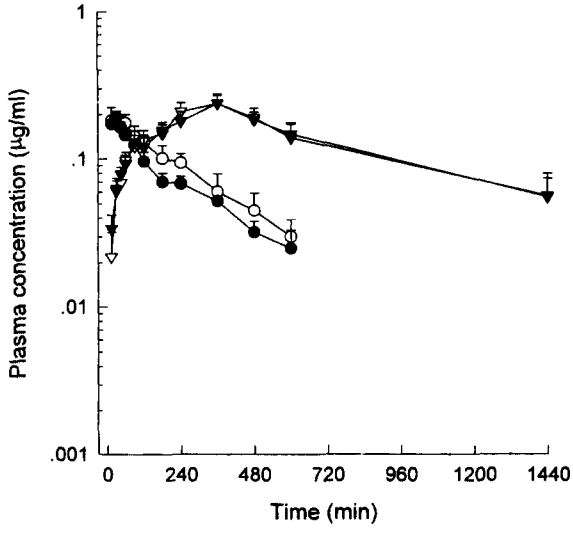

Fig. 3. Mean plasma concentration-time profiles of M4 in fasted (○) and fed rats (●), and M5 in fasted (▽) and fed rats (▼) after oral administration of YH439, 100 mg/kg. Bars represent SEM (n=6).

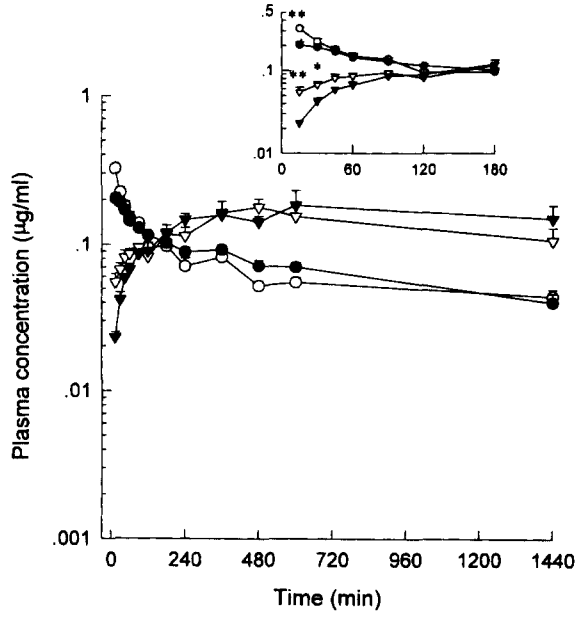

Fig. 4. Mean plasma concentration-time profiles of M4 in fasted (○) and fed rats (●), and M5 in fasted (▽) and fed rats (▼) after oral administration of YH439, 200 mg/kg. Bars represent SEM (n=6). Inset shows the profiles up to 180 min. *p<0.05, **p<0.01.

Table I. Pharmacokinetic parameters of M4 and M5 after oral administration of YH439 in fasted and fed rats (n=6)

Dose	Group	Pharmacokinetic parameters					
		C_{max} (μg/ml)		t_{max} (min)		AUC (μg · min/ml)	
		M4	M5	M4	M5	M4	M5
50 mg/kg	Fasted	0.107±0.0268	0.158±0.00831	48.0±10.7	312±26.3	15.6±4.75	126±23.2
	Fed	0.0930±0.0150	0.167±0.0136	42.5±15.6	420±46.9	19.3±5.85	133±15.2
100 mg/kg	Fasted	0.210±0.0248	0.238±0.0328	22.5±4.68	340±18.3	63.5±13.4	175±33.9
	Fed	0.201±0.0129	0.242±0.0328	22.5±3.06	380±18.3	51.8±5.80	183±29.4
200 mg/kg	Fasted	0.322±0.00905	0.192±0.0313	15.0±0.00	560±169	92.0±5.80	184±28.4
	Fed	0.212±0.0132	0.232±0.0323	20.0±2.89	620±153	105±2.41	217±28.8

Each value represents the mean±SEM.

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