Thermal Reversible Microemulsion System for Poorly Water-Soluble YH439 for Oral Delivery

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To improve bioavailability of poorly water-soluble YH439, a thermal reversible microemulsion system was prepared using modified fatty acids such as capric acid and palmitic acid with PEG 400. A combination of Capric-PEG 400 and Palmitic-PEG 400 with a ratio of 1 : 3 used as a lipid matrix and Cremophor RH40 and Neobee M-5® were selected as an oil and a surfactant, respectively. The microemulsion with melting point of 36.5 °C was produced by mixing the lipid matrices, Cremophor RH40® and Neobee M-5® with a volume ratio of 5 : 4 : 1. After the microemulsion was dispersed in the aqueous medium, the average particle size of 28 nm was obtained. At the release measurements of YH439 after 45 min suspension in pH 1.2 aqueous medium, about 80%, 65%, 10% and less than 5% of drug were released from the thermal reversible microemulson, Gelucire® formulation, 5% Ca-carboxymethylcellulose (CMC) suspension and YH439 powder, respectively. The apparent permeability of YH439 in microemulsion either from apical to basolateral or basolateral to apical after measuring YH439 across a Caco-2 cell monolayer in a Transwell® larger than Gelucire® formulation or 5% Na-CMC suspension. The area under the drug concentration–time curves (*AUC*) and maximal blood concentration (C_{max}) after oral administration of YH439 loaded on thermal reversible microemulsion were significantly increased than drug loaded on either Gelucire formulation or 5% Na-CMC suspension. Thus, the present work demonstrates that the thermal reversible microemulsion system of YH439 greatly enhances the bioavailability of YH439 after oral administration due to the improvement of solubility and dispersion of the drug in the artificial gastrointestinal tract without pepsin.

Key words YH439; thermal reversible microemulsion; Caco-2 cell line; the apparent permeability; bioavailability

There have been many attempts to improve absorption and bioavailability of poorly water-soluble drugs.[1—3] One of attractive systems is microemulsion which is composed of fine oil-in-water droplets in aqueous medium.[4—8] When such a formulation is released into the lumen of gut, it disperses to form a fine emulsion, so that the drug remains as a liquid state in the gut avoiding dissolution step that frequently limits the rate of absorption of liphophilic drug.[9] The smaller sizes of droplets will have a larger interfacial surface areas per unit volume and correspondingly large free energy contribution from the liquid–liquid interfacial tension.[10]

To avoid the precipitation of poorly water soluble drugs in the pre-microemulsion within the period of expiration, we hypothesize if the dosage form in the solid or semi-solid state at the room temperature during the storage and turn into the liquid state at body temperature, It will be overcome hydrophobic drugs rapid precipitate in the liquid formulation. Under this concept, a poorly water-soluble drug has been designed as a thermal reversible microemulsion system which can disperse rapidly in the aqueous contents of stomach and form fine oil in water droplets, and thus leads to improve absorption of the poorly water soluble drugs.

YH439, {isopropyl 2-(1,3-dithioetane-2-ylidene) 2-(*N*-4-methylthiazol-2-yl)carbamoylacetate}, a malotilate analog, has been developed as a hepatoprotective drug for the treatment of chronic hepatitis and liver cirrhosis by Yuhan Research Center of Yuhan Co.[11] YH439 has been shown to rapidly and potently inhibit the transcription of the *CYP2E1* gene, whose protein product is believed to be responsible for the generation of reactive metabolites or oxygen species and free radicals.[12] Due to its poorly water solubility (<1 μg/ml in water at 25 °C), the bioavailabilty of YH439 is very low by oral administration.[13,14]

Therefore, in the present study, the poorly water-soluble YH439 has been developed by a thermal reversible microemulsion system for oral delivery using a combination of modified capric acid and palmitic acid with polyethylene glycol (PEG) 400 as lipid matrices and Cremophor RH40® and Neobee M-5® as an oil and a surfactant, respectively. The system was designed to have a melting point to close body temperature around 36.5 °C that could rapidly disperse in stomach as fine droplets.

Experimental

Materials YH439 and [^{14}C]YH439 were kindly supplied by Yuhan Research Center of Yuhan Co. (Seoul, Korea). [^{14}C]Manitol purchased from New England Nuclear, Boston MA, U.S.A. PEG 400, capric acid and palmitic acid were purchased from Junsei Chemical Co. (Tokyo, Japan). Gelucrie® and Cremophor RH40® were obtained from Gatteffosse (Saint-Priest Cedex, France). Neobee M-5® was provided by Stepan Co. (Maywood, NJ, U.S.A.). Capsizapine was kindly provided by Dong-A Pharm. Co. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

Synthesis of PEG Derivatives Fatty acids modified with PEG 400 were prepared using capric acid and palmitic acid. The capric acid and palmitic acid were individually dissolved in ethanol and PEG 400 was added to each solution with a molar ratio of 1 : 1. The mixture was enclosed in a vessel and heated at 80 °C for 4 h, and then the ethanol was evaporated under reduced pressure. After purification of the residue using a silica gel column chromatography, Capric-PEG 400 (Ca-PEG) and Palmitic-PEG 400 (Pa-PEG) were obtained. Chemical structure of each compound was confirmed by ^{1}H-NMR (Bruker ARX300 spectrophotometer, 300 MHz, Madison, WI, U.S.A.). The synthesized compounds were caricaturized by GPC (Agilent 1100 Series with RI detector, Santa Clara, CA, U.S.A.).

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Solubility of YH439 in Various Composition The solubility of YH439 was determined in Neobee M-5®, Cremorphor RH40®, Ca-PEG, Pa-PEG and Gelucire. An excess amount of YH439 was introduced to 2 ml of each dissolution medium and the solutions were stirred at 60 °C for 48 h. Triplicate samples were centrifuged at 3000 ***g*** for 5 min to remove the insoluble drug. Then, aliquots of supernatant were taken and the content of YH439 was quantified by HPLC after dilution with 50% acetonitrile.

HPLC Method The amount of YH439 was quantified using HPLC system (Hitachi, model L-6000, Tokyo, Japan) equipped with a reverse phase C18 column (3.9×300 mm, Bondapak™, Milford, MA, U.S.A.) and UV detector. A mixture of distilled water and acetonitrile (3 : 7, v/v) was used as a mobile phase and delivered at the flow rate of 1 ml/min. The Effluent was monitored at 328 nm and the limit of quantity of YH439 using HPLC method is 0.05 μg/ml.

***In Vitro* Release Study** The release property of YH439 from the various formulations was studied in pH 1.2 HCl solution according to the USP XXII basket method. Twenty-five milligrams of YH439 as a powder form and added to 5% suspension of Ca-carboxymethylcellulose (CMC), 1 g of Gelucire and 1 g of microemlusion system prepared in this work, were dissolved in 500 ml of dissolution medium at 37±0.5 °C and stirred at 150 rpm. The released amounts were assayed by the same HPLC method described above.

Determination of Particle Size The particle size of thermal reversible microemulsion was measured using a laser particle analyzer (LPA-3000, Photal Otzuka Electronics, Japan).

Caco-2 Cell Culture The human colon adenocarcinoma cell line, Caco-2, was grown as monolayers in Dulbecco's modified Eagle's medium, 10% fatal bovine serum, 1% non-essential amino acid solution, 100 units/ml penicillin, and 0.1 mg/ml streptomycin at 37 °C in an atmosphere of 5% CO_2 and 90% relative humidity. Stock cultures were grown in 75-cm^2 tissue culture flasks and were split when the cell growing 80 to 90 confluence using 0.02% ethylenediaminetetraacetic acid (EDTA) and 0.05% trypsin. The Coco-2 cells (passage number 35—40) were seeded on permeable polycarbonate inserts (1-cm^2, 0.4-mm pore size; Corning Costar Co., Cambridge, MA, U.S.A.) in 12 Transwell® plates at density of 3.0×10^5 cells/cm^2. The inserts were fed by complete media every other day for the first week and then daily until they were used for the transport experiments. The integrity of the cell monolayers was evaluated by measuring transepithelial electrical resistance (TEER) values with an EVOM™ epithelial volt/ohm-meter (World Precision Instruments, Sarasota), after 21 d, matured into confluent monolayers exhibiting a TEER >500 $\Omega\cdot cm^2$ before use in transport experiments. Cell monolayer integrity was evaluated *via* either measurement of [^{14}C]manitol (0.4 μCi/ml) paracellular passive P_{app}, monitoring the change in TEER over the course of the experiment or a combination of both.[15] Caco-2 cellular damage was assessed by measuring spectrophotometrically the concentration of lactate dehydrogenase (LDH) in the original supernatant after incubating samples containing YH439 from 0.5 to 1.0 μM. Results found no significant difference changes of LDH before and after treatment with samples.

Permeability Calculations and Statistical Analysis The accumulated amount of radiolabeled probe appearing in the apical (AP) compartment over time, dQ/dt, was used to calculate the apparent permeability (P_{app}) using the following equation: $P_{app}=dQ/dt\times1/(A\times C_0)$, where A is the area of the filter (1 cm^2) and C_0 is the initial concentration of radiolabeled probe substrate in the donor compartment. In all basolateral (BL)-AP transport experiments, sink conditions were maintained as defined by >80% of compound remaining in the donor compartment at the end of the experiment. P_{app} values were therefore calculated using the slope of the steady-state rate constant dQ/dt. Statistical analyses were performed by comparison of the means of control BL-AP P_{app} values *versus* BL-AP P_{app} values determined in the presence of each test compound using an unpaired *t*-test (GraphPad InStat, version 3.05; GraphPad Software Inc., San Diego, CA, U.S.A.). A p value of <0.05 was taken as the minimum level of statistical significance.

Pharmacokinetic Study Male Sprague-Dawley rats weighing 250±25 g were used for *in vivo* study. After anesthetizing the rats with diethyl ether, the femoral vein and artery were cannulated with 23 gauge-polyethylene cannula. All of the incisions were covered with wet cotton and the cannula was flushed with 0.1 ml of 25-unit heparin saline solution to prevent the blood clotting. After the rats recovered from anesthesia, the thermal reversible micoemulsion, Gelucire® formulation and Ca-CMC suspension equivalent to 15 mg/kg of YH439 were administered orally to rats through an oral sonde. In the meanwhile, the microemulsion equivalent to 2.5 mg/kg of YH439 was given intravenously to rats *via* the femoral vein. The assay method of YH439 in rat plasma was previously reported by our laboratory[16] using the HPLC system. In brief, firstly, separation was performed using an octadecylsilica column (250×4.6 mm, 4 μm particle size, YMC, Kyoto, Japan) with a guard column (3.2×15 mm, 7 μm particle size, XPERTEK, St. Louis, MO, U.S.A.) at ambient temperature. The mobile phase used a mixture of acetonitrile and water (3 : 2, v/v) and delivered with a flow rate of 1.2 ml/min. The YH439 in the eluent was monitored at 328 nm. A mixture of ethyl acetate and hexane (3 : 1, v/v) for extraction containing capsazepine as an internal standard was added to rat plasma. The organic phase was evaporated under nitrogen gas. The residue was reconstituted in acetonitrile, and then an aliquot was injected into the HPLC system. All animal experiments are strictly following rules of IACUC of Chinese Academy of Medical Sciences.

The non-compartmental pharmacokinetic parameters, area under the drug concentration–time curve (*AUC*) was calculated using the trapezoidal rule.[17] The maximal plasma concentration of drug (C_{max}) and the time to reach maximal plasma concentration (T_{max}) were obtained by a graph plotted the concentration of drug as a function of time. The data between different formulations were compared for statistical significance by the one-way analysis of variance (ANOVA). The statistical significance of means among different formulations was then compared by multiple range method of least significant difference. All results were expressed as mean of S.D.

Results and Discussion

In order to prepare a thermal reversible microemulsion system for oral delivery of poorly water-soluble YH439, fatty acids modified with PEG 400 were selected as a lipid matrix. The Cremophor RH40® was selected as a surfactant since it has been know that non-ionic surfactants are less affected by pH and ionic strength changes.[18] The Neobee M-5® containing medium-chain triglycerides derived from coconut oil, was used as an oil. Many reports have been proving that the Neobee M-5® improves the intestinal absorption of co-formulated drugs.[19,20]

Solubility of YH439 in Various Composition The solubility of YH439 in Ca-PEG, Pa-PEG, Cremorphor RH40®, Neobee M-5® and Gelucire® were measured as listed in Table 1. In Pa-PEG and Gelucire, YH439 was dissolved as 25.04±3.15 and 23.11±5.13 (mg/g), respectively. These were about twice than others. Due to the long chain of palmic acid and PEG 400, the Pa-PEG enhanced the solubility of YH439.

Effect of the Composition Ratio on Melting Point and Particle Size The melting point of mixture of Pa-PEG and Ca-PEG was increased with increasing the composition ratio of Ca-PEG to Pa-PEG as shown in Fig. 1. At the ratio of Ca-PEG to Pa-PEG was 1 : 3, the melting point was close to the body temperature as 36 °C. Thus, firstly, the ratio of Ca-PEG to Pa-PEG was fixed at 1 : 3, then the effect of Cremorphor RH40® as a surfactant and Neobee M-5® as an oil on melting point of thermal reversible microemulsions was examined and the optimal composition ratio was determined for the thermal reversible microemulsion system. The melting point of thermal reversible microemulsions was raised up with increasing the amount of oil in the formulation as shown in Fig. 2a. Whereas, the melting point of microemulsions decreased with increasing the amount of surfactant. When the microemulsion was composed of lipid matrix, surfactant and oil with a ratio of 5 : 4 : 1, its melting point was close to the

Table 1. The Solubility of YH439 in Various Composition

Composition	Solubility of YH439 (mg/g) (n=5)
Cremophor RH40®	12.05±4.21
Neobee M-5®	10.42±2.63
Gelucire®	23.11±5.13
Ca-PEG	13.12±2.13
Pa-PEG	25.04±3.15

body temperature as 37 °C. Thus this would be considered as the pre-concentration of microemulsion since the solid state could change to liquid state around 37 °C and rapidly form fine droplets microemulsion in gastrointestinal tract.

The effect of each component of microemulsion systems on the resultant droplet size was investigated as shown in Fig. 2b. In the range of surfactant with more than 0.2 g and oil with less than 0.2 g, small droplet size (<100 nm) of microemulsion was obtained in this system. The droplet size of microemulsion was significantly reduced with increasing the amount of surfactant. At the composition ratio of lipid matrices : Cremorphor RH40® : Neobee M-5® with 5 : 4 : 1, the smallest droplet with 28 nm of mean size was obtained. The smaller sizes of droplets will have a larger interfacial surface areas per unit volume and correspondingly large free energy contribution from the liquid–liquid interfacial tension. As reducing the size of droplets, a larger interfacial surface areas per unit volume could be produced.

Thus from the results, the optimal physical properties including melting point and particle size of microemulsion were produced by the composition ratio of lipid matrices : Cremorphor RH40® : Neobee M-5® with 5 : 4 : 1. This formulation would be used as a formulation of the thermal reversible microemulsion system for further study in the work.

Release Profile of YH439 from Thermal Reversible Microemulsion The YH439 was released from various formulations including powder form, 5% of Ca-CMC suspension, Gelucire® and the thermal reversible microemulsion prepared in this work. The powder form of YH439 was released less than 5% of initial amount and after suspending in the 5% of Ca-CMC, the YH439 was released upto 20%. However, by additions of the Gelucire® formulation and the microemulsion to YH439, 90% of YH439 was released until 180 min as shown in Fig. 3. The systems of Gelucire® and microemulsion remarkably improved the release property compared with powder form and 5% of Ca-CMC suspension. On the other hand, the fast release pattern was observed in the microsemulsion system compared with the Gelucire® formulation. This is desirable property since a slow dissolution rate of formulation might retard the absorption of drugs in the gastrointestinal tract.

The Transport of YH439 in Microemulsion, Gelucire® Formulation and 5% Ca-CMC Suspension across the Caco-2 Cell Monolayer The apparent permeability (P_{app}) of YH439 from the apical to the basolateral and basolateral to apical were calculated according the equation mentioned above. P_{app} of YH439 from the apical to the basolateral and basolateral to apparent direction were 2.08×10^{-5} cm/s, 4.71×10^{-5} cm/s for 0.63 μM, YH439. It was suggested YH439 which was transported in the basolateral to apical direction is larger than from apical to basolateral direction in the present of a transport system as shown in Fig. 4. It has been reported that the efflux of YH439 which is found in Caco-2 cells does not appear to influence the bioavailability of YH439 (Evidenced by the sufficiently high permeability in the absorption direction).[14] YH439 transported from microemulsion formulation was larger than Gelucire® formulation and 5% Ca-CMC sus-

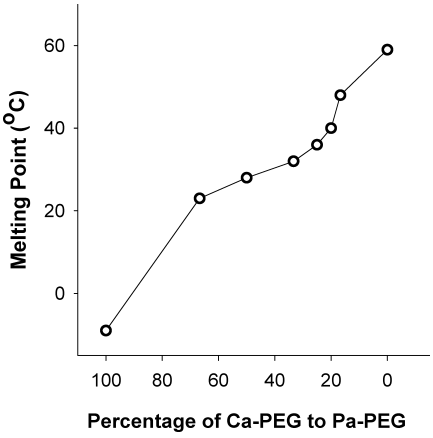

Fig. 1. The Change of Melting Point Was Function of Altering the Composition Ratio of Capric-PEG 400 to Palmitic-PEG 400

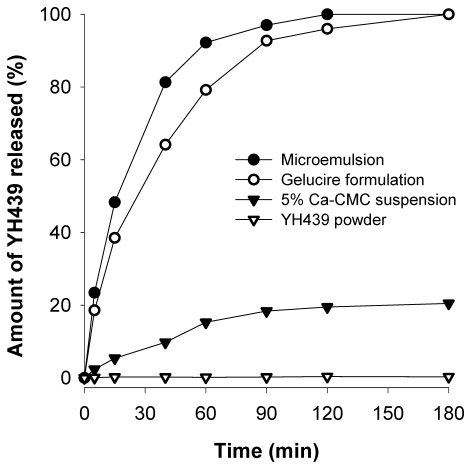

Fig. 3. Release Profiles of YH439 from the Thermal Reversible Microemulsion, Gelucire® Formulation, 5% Ca-CMC Suspension and Powder State of YH439

The thermal reversible microemulsion was composed of lipid matrices, Cremorphor RH40® and Neobee M-5® with a ratio of 5 : 4 : 1.

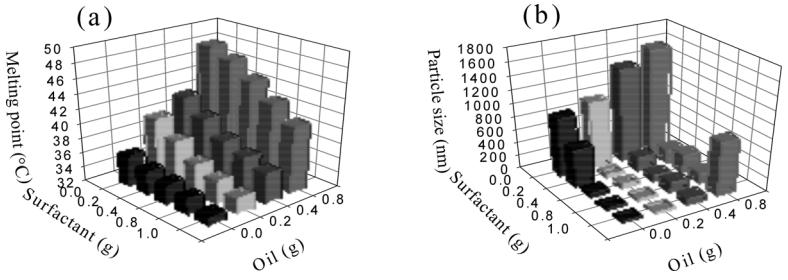

Fig. 2. Effect of Oil and Surfactant on Changing Melting Point (a) and Particle Size (b) of Microemulsion System

The ratio of Capric-PEG 400 to Palmitic-PEG 400 was fixed at 1 : 3 and the lipid mixture of Capric-PEG 400 and Palmitic-PEG 400 was equivalent to 1 g.

pension as shown in Fig. 4. The results were expected to respond to *in vivo* animal experiments.

Pharmacokinetic Analysis *In vivo* study was undertaken to examine the effect of a thermal reversible microemulsion system of YH439 on gastrointestinal (G.I.) absorption after oral administration equivalent to 15 mg/kg of YH439 to rats. The plasma concentration of YH439 after oral administration of YH439 in the thermal reversible microemulsion formulation to rats increased compared with in the Gelucire® formulation and 5% Ca-CMC suspension as shown in Fig. 5. $AUC_{0—24}$ of YH439 in the thermal reversible microemulsion formulation increased 1.17-fold and 6.91-fold compared with that of Gelucire® formulation and 5% Ca-CMC suspension (28.14 *vs.* 24.01 μg·h/ml and 28.14 *vs.* 4.07 μg·h/ml) as listed in Table 2. The thermal reversible microemulsion formulation of YH439 also enhanced C_{max} of YH439 by 1.59-fold and 7.29-fold compared with Gelucire® formulation and 5% Ca-CMC suspension (2.26 *vs.* 1.42 μg/ml and 2.26 *vs.* 0.31) (Table 2). However, the significant difference was not observed in T_{max} value (Table 2). These results indicated that the thermal reversible microemulsion formulation of YH439 considerably increased the bioavailability of YH439 compared with Gelucire formulation and 5% Ca-CMC suspension. The enhanced bioavailability was very probably due to the increasing drug dispersion in the GI tract. Therefore, the increased solubility and enhanced bioavailability of YH439 from a thermal reversible microemulsion could result in improved drug efficacy.

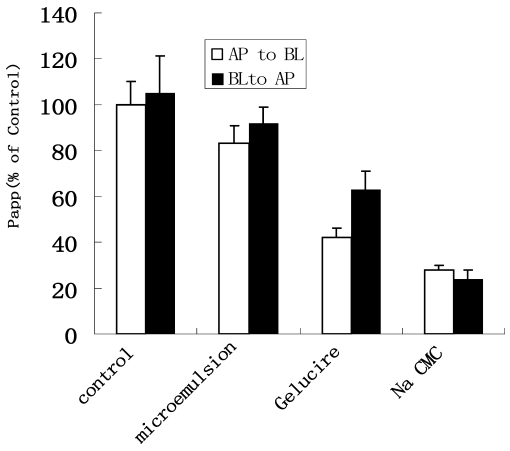

Fig. 4. Effect of YH439 Transport from Apical to Basolateral and Basolateral to Apical in the Microemulsion Formulation, Gelucire® Formulation, and 5% Ca-CMC Suspension (n=4)

Conclusion

The thermal reversible microemulsion system of YH439 was prepared from a lipid mixture of Ca-PEG and Pa-PEG, Cremophor RH40® and Neobee M-5® at the ratio of 5 : 4 : 1. The thermal reversible microemulsion promoted to increase the solubility of a poorly water-soluble YH439 and to enhance its bioavailability after orally administration to rat. The increased stability of poorly water-soluble YH439 by thermal reversible microemulsion system could lead to rapidly disperse as fine droplets inclusion drug in the gastrointestinal tract. Therefore, the thermal reversible microemulsion system could provide a useful dosage form for oral intake of

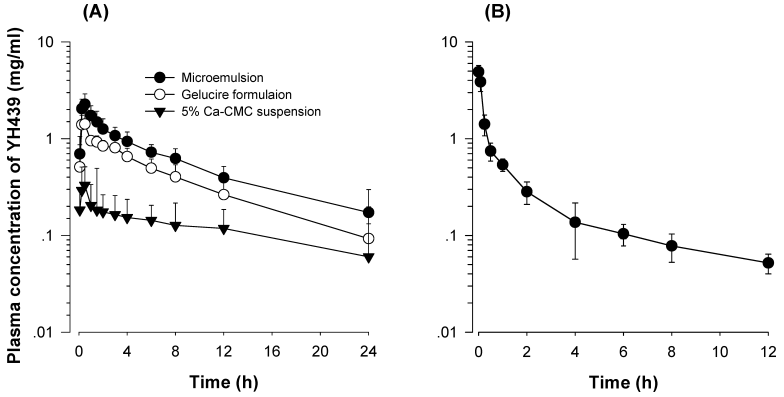

Fig. 5. Plasma Concentration of YH439 after Oral Administration of Thermal Reversible Microemulsion, Gelucire® Formulation and 5% Ca-CMC Suspension to Rats at the Dose of 15 mg/kg (A) and after Intravenous Administration of Thermal Reversible Microemulsion Equivalent to 2.5 mg/kg as YH439 to Rats (B)

The thermal reversible microemulsion was composed of lipid matrices, Cremorphor RH40® and Neobee M-5® with a ratio of 5 : 4 : 1. Each group used 6 rats.

Table 2. Plasma Concentration of YH439 after Oral Administration of Thermal Reversible Microemulsion, Gelucire® Formulation and 5% Ca-CMC Suspension to Rats at the Dose of 15 mg/kg and after Intravenous Administration of Thermal Reversible Microemulsion Equivalent to 2.5 mg/kg as YH439 to Rats (Each Group n=6)

Parameters	Intravenous	Oral		
		Microemulsion	Gelucire®	Suspension
C_{max} (mg/ml)	—	2.26±0.47[a,b]	1.42±0.10[a]	0.31±0.85
T_{max} (min)	—	30	30	30
AUC (mg·h/ml)	6.11±0.28	28.14±2.89[a]	24.01±2.78[a]	4.07±0.32
Absolute bioavailability (F%)[c]	—	76.75[a,b]	65.49[a]	11.10

a) $p<0.001$ by the AVOVA when compared to suspension. *b*) $p<0.05$ by the AVOVA when compared to suspension. *c*) $F=[AUC_{oral}/Dose_{oral}]/[AUC_{i.v.}/Dose_{i.v.}]$.

water-insoluble YH439.

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References

1) Susan A. C., William N. C., Mark C. R., Terry D. W., *Pharm. Res.*, **9**, 87—93 (1992).
2) Constantinides P. P., Yiv S. H., *Int. J. Pharm.*, **115**, 225—234 (1995).
3) Höter D., Dressman J. B., *Adv. Drug Deliv. Rev.*, **25**, 3—14 (1997).
4) Ritschel W. A., *Methods Find. Exp. Pharmacol.*, **13**, 205—220 (1991).
5) Eccleston G. M., "Encyclopedia of Pharmaceutical Technology," Vol. 9, ed. by Swarbrick J., Boylan J. C., Marcel Dekker Publishers, New York, 1992, pp. 375—421.
6) Sarciaux J. M., Acar L., Sado P. A., *Int. J. Pharm.*, **120**, 127—136 (1995).
7) Kim C. K., Ryuu S. A., Park K. M., Lim S. J., Hwang S. J., *Int. J. Pharm.*, **147**, 131—134 (1997).
8) Gao Z. G., Choi H. G., Shin H. J., Park K. M., Lim S. J., Hwang K. J., Kim C. K., *Int. J. Pharm.*, **161**, 75—86 (1998).
9) Pouton C. W., *Adv. Drug Deliv. Rev.*, **25**, 47—58 (1997).
10) Shah N. H., Carvajal M. T., Patel C. I., Infeld M. H., Malick A. W., *Int. J. Pharm.*, **106**, 15—23 (1994).
11) Lee I. J., Jeong K. S., Roberts B. J., Kallarakal A. T., Fernansezsalguero P., Gonzalez F. J., Song B. J., *Mol. Pharmacol.*, **49**, 980—988 (1996).
12) Castillo T., Koop D. R., Kamimura S., Triadafilopoulos G., Tsukamoto H., *Hepatology*, **16**, 992—996 (1992).
13) Lee W. I., Yoon W. H., Park J. H., Lee J. W., Shim C. K., Lee M. G., *Biopharm. Drug Dispos.*, **16**, 775—789 (1995).
14) Kim S. H., Park K. J., Yoon W. H., Lee J. W., Kim N. D., Lee M. G., *Res. Commun. Mol. Pathol. Pharmacol.*, **91**, 233—244 (1996).
15) Park H. W., Chung S. J., Lee M. G., Shim C. K., *Arch. Pharm. Res.*, **24**, 584—589 (2001).
16) Gao Z. G., Lee M. K., Oh U., Suh Y. G., Kim C. K., *J. Liq. Chromatogr. Relat. Technol.*, **23**, 1865—1872 (2000).
17) Gibaldi M., Perrier D., "Pharmacokinetics," Marcel Deller, New York, 1982.
18) Constantinides P. P., *Pharm. Res.*, **12**, 1561—1572 (1995).
19) Constantinidines P. P., Scalart J. P., Lancaster C., Marcello J., Marks G., Ellens H., Smith P. L., *Pharm. Res.*, **11**, 1385—1390 (1994).
20) Swenson E. S., *Adv. Drug Deliv. Rev.*, **8**, 39—92 (1992).