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Enhanced absorption of indomethacin after oral or rectal administration of a self-emulsifying system containing indomethacin to rats

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Abstract

A self-emulsifying system (SES), a mixture of an oil and a surfactant which forms an oil-in-water emulsion, is expected to improve the in vitro drug dissolution and enhance the in vivo drug absorption. In this study, a poorly water-soluble drug, indomethacin (IDM) was incorporated into the SES to increase bioavailability. The SES with 30% of Tween 85 and 70% of ethyl oleate, EO (w/w) was selected as an optimized formulation (high drug loading, low surfactant concentration, and small particle size). After an oral administration of the SES containing IDM and IDM suspension, (IDM was suspended in methyl cellulose), 22.5 mg/kg as IDM, to rats, the area under the plasma concentration–time curve from time zero to the last measured time in plasma, 12 h ($AUC_{0-12\ h}$) was significantly greater (57% increase) in the SES, suggesting that oral absorption of IDM increased significantly by the SES. After a rectal administration of gelatin hollow type suppositories, filled with the SES containing IDM and IDM powder physically mixed with the SES, 22.5 mg/kg, to rats, the $AUC_{0-12\ h}$ also increased significantly (41% increase) by the SES, suggesting that rectal absorption of IDM also increased significantly by the SES.

Keywords: Indomethacin; SES (Tween 85: ethyl oleate, 3:7); Gelatin hollow type rectal suppository; Rats

1. Introduction

After an oral administration, absorption of drugs into the circulatory system consists of the following sequential rate processes; (1) disintegration of the drugs in the gastrointestinal tracts; (2) dissolution of drugs in the gastrointestinal fluids; and (3) absorption of drugs across gastrointestinal membrane into the circulatory system. For poorly water-soluble drugs, the rate-determining step in the absorption processes is usually dissolution of the drug (Sekikawa et al., 1983).

An improvement of the dissolution characteristics for poorly water-soluble drugs results in higher plasma peak level and greater area under

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the plasma (blood, serum) concentration–time curve from time zero to infinity of the drugs. Numerous methods for the improvement of dissolution characteristics for poorly water-soluble drugs have been reported to enhance bioavailability; micronization, salt formation, formation of solid dispersion with water-soluble carriers, emulsions, and formation of micelles. Among them, oil-in-water emulsion dosage forms have been used for lipophilic drugs. However, emulsions, consisting of two immiscible liquids are thermodynamically unstable, therefore, have a tendency for the two phases to separate (Fahelelbom et al., 1993).

In the absence of water, a mixture of an oil and a non-ionic surfactant forms clear and transparent isotropic solution that is known as a self-emulsifying system (SES) used as a vehicle for drug delivery. The SES system was recently being used to improve characteristics of in vitro dissolution and therefore to enhance in vivo absorption of lipophilic drugs (Charman et al., 1992; Shah et al., 1994). This mixture is known to form a fine oil-in-water emulsion with gentle agitation, when exposed to aqueous media. This property makes the SES a good vehicle for oral delivery of hydrophobic drugs having an adequate oil solubility. Soft gelatin capsules containing SES readily disperse in the stomach to form a fine emulsion; in this case, the gastrointestinal motility can provide the agitating effect necessary for emulsification. For drugs having characteristics of dissolution rate-limited absorption, the SES could enhance the rate and extent of absorption, as well as improve the reproducibility of the plasma (serum, blood) level-time profiles (Myles et al., 1990).

In this study, an unsaturated fatty acid (ethyl oleate, EO) and a nonionic surfactant (Tween 85) were used to formulate a SES for indomethacin (IDM), a poorly water-soluble drug. To characterize various SESs (with different mixtures of EO and Tween 85) containing IDM, phase separation studies were conducted and the particle size was measured using photon correlation spectroscopic method. Based on the studies above, the SES containing 30% of Tween 85 and 70% of EO (w/w) was selected as an optimized formulation for IDM. Finally, in order to examine the SES as a potential vehicle for enhancing the absorption of IDM, the SES (30% of Tween 85 and 70% of EO) containing IDM was administered orally or rectally to rats.

2. Materials and methods

2.1. Materials

IDM (Whail, Seoul, South Korea), EO (Aldrich Chemical, Milwaukee, WI), Tween 85 (polyoxyethylene 20) and sorbital trioleate (Nikko Chemical, Tokyo, Japan), methanol and acetonitrile (HPLC grade, Merck Chemical, Darmstadt, Germany), and acetic acid (HPLC grade, Fisher Chemical, Pittsburgh, PA) were used in the present study. Other chemicals were of reagent grade, therefore, used without further purification.

2.2. Preparation of a SES

Various SESs having different mixtures of EO and Tween 85 (9.5:0.5, 9.0:1.0, 8.5:1.5, 8.0:2.0, 7.5:2.5, 7.0:3.0, 6.5:3.5 and 6.4:4.0, w/w) were prepared by adding EO and Tween 85 into a glass test tube followed by vortex-mixing for 1 min.

2.3. Phase separation study

Each SES (0.05 ml) was added to a glass test tube containing 5 ml of 0.1 N HCl, pH 7.4 phosphate buffer, or distilled water at 25°C. After 1 min vortex-mixing, each mixture was stored for a period of 2 h and phase separation was observed visually. Mixtures that phase separation was not considerable, during the 2 h period, were used in the subsequent study.

2.4. Measurement of particle size

The particle sizes of the resulting emulsion were measured with a photon correlation particle size analyzer (Photal LPA-3000/3100, Ostuka Electris, Tokyo, Japan) at 25°C and scattering angle of 90° ($n = 4$).

2.5. Measurement of viscosity

The viscosity of the various emulsions was measured with a viscometer (Brookfield Eng. Labs., Model DV-11 +, Brookfield, MA) with a LV # 2 disc spindle at 25°C.

2.6. Determination of a partition coefficient of IDM

The partition coefficient (PC) of IDM between EO and buffer phases was determined at 37°C. Two millilitres of each buffer (pHs of 5.5, 6.5, or 7.4) containing IDM was added to 2 ml of EO. The mixture was shaken at 37°C for 24 h. It took approximately 18 h to reach an equilibrium of IDM between the buffer and EO phases. After centrifugation, 1 ml of an oil phase was collected, diluted with 10 ml of methanol, and then IDM concentration in the oil phase was determined by the reported HPLC method (Ohnishi et al., 1986). Amount of IDM dissolved in buffer phase was calculated by subtracting the amount of IDM in EO phase from the initial amount of IDM added to buffer phase. The PC of IDM between EO and buffer phases was calculated by the following equation:

$$PC = C_o/C_b$$

where C_o is the IDM concentration measured in the EO phase and C_b is the IDM concentration calculated in the buffer phase.

2.7. Determination of solubility of IDM in various SESs

After excess IDM was added to various SESs ($n = 6$, each), the resulting mixture was sonicated with an ultrasonic cleaner (Branson Ultrasonics, Danbury, CT) for 20 min. Each mixture was vigorously shaken at 37°C until reaching an equilibrium of IDM between EO and Tween 85. It took $\approx$ 12 h to reach an equilibrium. After reaching an equilibrium, each mixture was centrifuged at 3000 rpm for 10 min and filtered through a 0.22 μm membrane filter (Millipore, Bedford, MA). The filtrate (0.1 ml) was diluted with 10 ml of methanol and IDM concentration in various SESs was determined by the reported HPLC method (Ohnishi et al., 1986).

2.8. Release study

IDM fine powder, 15 mg, physically mixed with 0.4 ml of the SES (Tween 85: EO, 3:7, w/w) or the SES (0.4 ml) containing IDM, 15 mg as IDM ($n = 4$, each), was placed in the dialysis bag (mw cut-off of 17,000, Sigma Chemical, St Louis, MO). The dialysis bag was immersed into a 200 ml of each buffer (pHs of 5.5, 6.5, or 7.4) in a water-bath kept at 37 ± 0.5°C with continuous magnetic stirring. An aliquot (0.8 ml) of sample was collected from each buffer at designated times and analyzed for IDM by the reported HPLC method (Ohnishi et al., 1986). An equivalent volume (0.8 ml) of fresh buffer was added to compensate the loss caused by each sampling.

2.9. Animals

Male Sprague–Dawley rats of 8 weeks of age were purchased from Dai-Han Laboratory of Animal Development (Seoul, South Korea). They were housed for 2 weeks until body weight became 250 g in a specific pathogen-free room (College of Pharmacy, Ewha Womans University, Seoul, South Korea) with 12 h light and 12 h dark cycle and temperature of 22 ± 2°C. The rats were randomly divided into each experimental group.

2.10. Pretreatment of rats

In the early morning, after overnight fasting with free access of water, the left femoral artery was cannulated with polyethylene tube (PE-50, Clay Adams, Parsippany, NJ), under light ether anesthesia (Kim et al., 1996, 1998). The exposed areas were surgically sutured. Each rat was allowed 2 h to recover from anesthesia before the study began. Each rat was held in supine position during the entire experimental period by tying the four feet on a plate.

2.11. Oral study

The SES (Tween 85: EO, 3:7, w/w) containing IDM was employed in the oral study. The SES, 0.68 ml/kg (22.5 mg/kg as IDM, $n = 7$) or 4 ml/kg of IDM suspension (IDM was suspended in 0.5%, w/v, methyl cellulose, 5.625 mg/ml, $n = 6$) was administered orally using a feeding tube (Solco, Seoul, South Korea). Blood samples (0.25 ml) were collected via the left femoral artery at 0 (to serve as a control), 5, 10, 30, 60, 90, 120, 240, 360, 480, 600, and 720 min. Blood samples were centrifuged immediately and an aliquot of plasma sample was stored in the -20°C freezer, until HPLC analysis of IDM (Ohnishi et al., 1986).

2.12. Rectal study

A mixture of gelatin, glycerin, and distilled water (4: 2: 4, w/w/w) was heated at 70°C to dissolve gelatin and the mixture was stored in a water-bath for 48 h at 50°C. After complete removal of bubbles, the mixture was smeared to the surface of a stainless rod (diameter of 0.4 cm of hollow). By removing the rod after solidification, a gelatin hollow type (0.2 cm^3 of hollow) rectal suppository was obtained (diameter, 0.6 cm; length, 1.5 cm; and weight, 0.3 g). The hollow was filled with 5.625 mg of IDM powder or 0.15 ml of SES (Tween 85: EO, 3:7, w/w) containing 5.625 mg of IDM. The two gelatin hollow type rectal suppositories (containing SES with IDM, $n = 6$ or IDM powder, $n = 6$) were administered rectally (22.5 mg/kg as IDM) and glue was applied to anus to prevent leaking of suppository. The other procedures were similar to those of the oral study.

2.13. HPLC analysis of IDM

The concentrations of IDM in the samples above were analyzed by slight modification of the reported HPLC method (Ohnishi et al., 1986). The mobile phase, acetonitrile-1 M acetic acid (1:1, v/v), was run through a reversed phase column (Bondapak C_{18}; 3.9 mm, id. $\times$ 30 cm, *l*; Waters; Milford, MA) at a flow rate of 1.3 ml/min. The column effluent was monitored by a UV detector (Shimadzu, Tokyo, Japan) set at 320 nm.

2.14. Pharmacokinetic analysis

The area under the plasma concentration–time curve from zero time to the last measured time, 12 h in plasma ($AUC_{0-12\ h}$) was calculated by the trapezoidal rule method (Kim et al., 1993); this method employed 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 rising plasma level phase.

2.15. Statistical analysis

A P value of less than 0.05 was considered to be statistically significant, using unpaired t-test

3. Results and discussion

3.1. Characterization of SESs

The SESs containing 0–10% (w/w) of Tween 85 did not form an emulsion, however, the SESs containing higher than 15% of Tween 85 rapidly formed an emulsion which was apparently stable (no phase-separation for 2 h). When the Tween 85 concentrations were 25–30%, phase separation did not occur for 24 h.

3.2. Solubility of IDM in SESs

The solubility of IDM in various SESs increased with increasing concentrations of Tween 85; the values were 4.42 ± 0.582, 19.5 ± 15.8, 22.6 ± 1.74, 29.1 ± 0.303, 39.7 ± 1.49, 47.9 ± 1.21, and 54.0 ± 1.87 mg/ml for EO to Tween 85 ratios (w/w) of 10:0, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, and 6:4 ($n = 6$), respectively.

3.3. Determination of particle size

The mean particle sizes of various SESs with or without IDM in various media are shown in Fig. 1. All SESs with or without IDM formed emulsions, having mean particle size of less than 150 nm. Particle sizes of SESs with or without IDM, decreased with increasing concentrations of Tween 85 (w/w); a minimum particle size was

obtained from the mixtures having 25% (in pH 7.4 phosphate buffer and distilled water) or 30% (in 0.1 N HCl) of Tween 85. Thereafter, the particle sizes of SESs with or without IDM, increased with increasing concentrations of Tween 85 in all three media studied (Fig. 1). Generally, the mean particle sizes of SESs containing IDM tended to be, (or were significantly) larger than those of SESs without IDM in all three media studied (Fig. 1).

3.4. Determination of viscosity

The effects of concentrations of Tween 85 on the viscosity of SESs were also examined. The viscosity of SESs increased with increasing concentrations of Tween 85; the values were 11.7, 13.5, 18.9, and 20.7 cp for concentrations of Tween 85 of 20, 30, 40, and 50%, respectively. Therefore, the formation of larger particle sizes of SESs with increasing concentration of Tween 85, such as more than 25–30% (Fig. 1) could be due to increase in viscosity which promotes the formation of emulsions having large droplets. It was reported in tolbutamide (Sano et al., 1990) that the particle diameter of the agglomerates obtained by the quasi-emulsion solvent diffusion method depends on the size of the initially formed quasi-emulsion droplets, which in turn depended on the viscosity of the emulsion. In the present study, the SES containing 30% of Tween 85 and 70% of EO was selected as an optimized formulation for IDM (high drug loading, low surfactant concentration, and small particle size), therefore, the SES was used in the subsequent studies.

3.5. Partition coefficient of IDM between EO and various buffers

Since the release of IDM from SES depends on the diffusion rate of the drug from oil to aqueous phase, the PC of IDM between EO and various buffer pHs was determined to identify optimum aqueous medium. The PC of IDM decreased with increasing pHs of buffer. The values were 26.7, 2.27, and 1.49 for buffer pHs of 5.5, 6.5, and 7.4, respectively, and this could be due to increased water-solubility of IDM in the higher pH since IDM is a weakly acidic drug having a p*K*a of 4.2. The solubility of IDM in buffer pHs of 5.5, 6.5, or 7.4 was 0.073, 0.571, or 0.943 mg/ml, respectively.

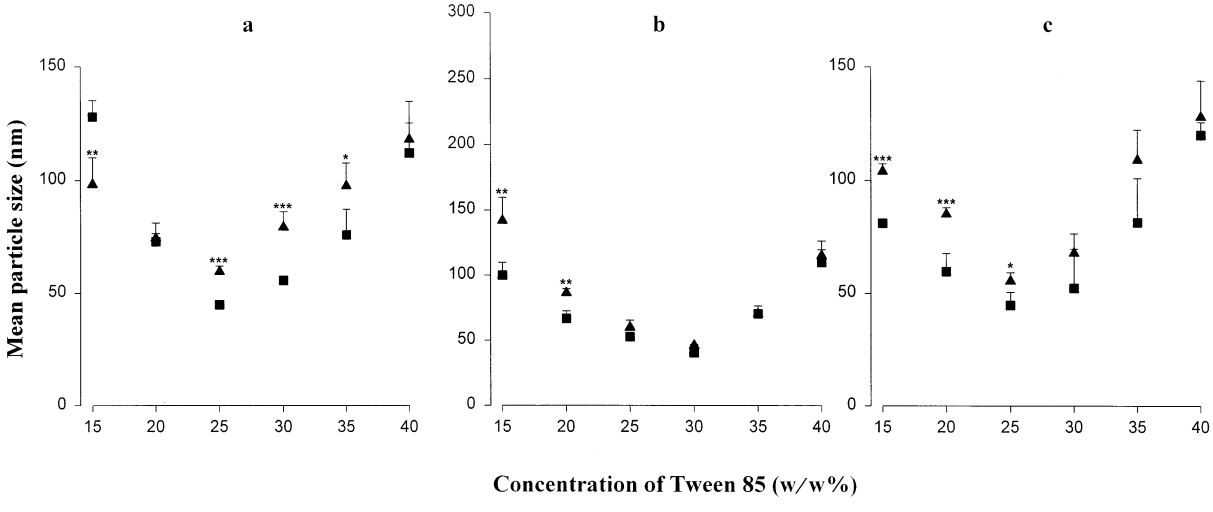

Fig. 1. Effects of concentration of surfactant (Tween 85) on mean particle sizes of various SESs (■) and SESs containing IDM (▲) in various media at 25°C ($n = 4$, each). (a) pH 7.4 phosphate buffer; (b) 0.1 N HCl; and (c) distilled water. Bars represent S.D. $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

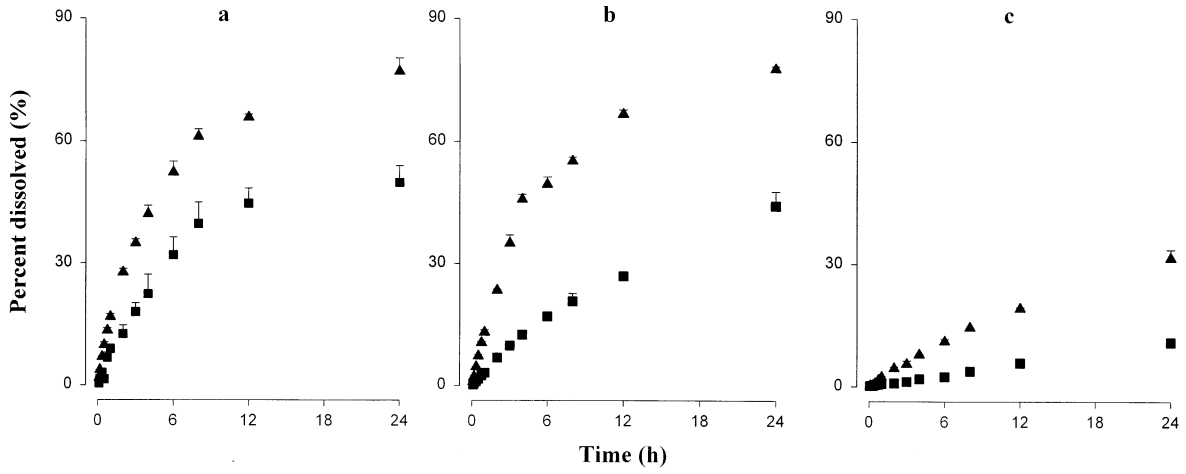

Fig. 2. Dissolution profiles of IDM from IDM powder physically mixed with SES (Tween 85: EO, 3:7) (■) and the SES containing IDM (▲) in various buffer solutions at 37°C ($n = 4$, each). (a) pH 7.4 phosphate buffer; (b) pH 6.5 phosphate buffer; and (c) pH 5.5 acetate buffer. Bars represent S.D.. Each point was significantly different ($P < 0.05$) except at 0.08 and 1.16 h in pH 5.5 acetate buffer.

3.6. Release of IDM from the SES

The release of IDM from the SES (Tween 85: EO, 3:7) was evaluated in buffer solutions having pHs of 5.5, 6.5, or 7.4; the percentages of IDM released from the SES were significantly higher than those of IDM powder physically mixed with 0.4 ml of the SES in all three buffers studied (Fig. 2). Again the percentages of IDM released in pH 7.4 and 6.5 buffers, from both IDM powder physically mixed with the SES and the SES containing IDM increased significantly compared with those in pH 5.5 buffer (Fig. 2), and this also could be due to increased solubility of IDM, at high pHs as mentioned earlier.

3.7. Oral study

After an oral administration of the SES (Tween 85: EO, 3:7) containing IDM to rats, the plasma concentrations and peak plasma concentration of IDM (49.5 ± 2.31 vs 28.9 ± 3.82 μg/ml) were significantly higher than those after IDM suspended in methyl cellulose (Fig. 3). The significantly higher plasma concentrations of IDM, after an oral administration of SES containing IDM, resulted in a significantly greater $AUC_{0-12\ h}$ (375 ± 41.1 vs 239 ± 60.0 μg h/ml). The data above indicated that the absorption of IDM from the SES containing IDM, increased significantly (57% increase based on $AUC_{0-12\ h}$) in rats compared with IDM suspended in methyl cellulose. This could be due to increased water-solubility of IDM in the SES as mentioned earlier, and absorption enhancement of IDM by surfactant, which increases permeability. However, the peak times in plasma concentration of IDM were not significantly different (Fig. 3) between two oral dosage forms.

3.8. Rectal study

After a rectal administration of gelatin hollow type suppository, filled with the SES (Tween 85: EO, 3:7) containing IDM, the plasma concentrations of IDM were significantly higher up to 8 h and peak plasma concentration of IDM was also significantly higher (44.2 ± 5.67 vs 25.6 ± 1.59 μg/ml) than those after IDM powder (Fig. 4). The significantly higher plasma concentrations of IDM after a rectal administration of the SES resulted in a significantly greater $AUC_{0-12\ h}$ (362 ± 86.6 vs 257 ± 28.8 μg h/ml). The data above also indicated that the absorption of IDM

after a rectal administration of the SES increased significantly (41% increase based on $AUC_{0-12\ h}$) compared with IDM powder. This again could be due to increased water-solubility of IDM in the SES and absorption enhancement of IDM by surfactant which increases permeability as mentioned earlier. It was quite surprising that the $AUC_{0-12\ h}$ values of IDM after oral and rectal administration were comparable, 375 (oral administration) versus 362 (rectal administration) μg h/ml for the SES, and 239 (oral administration) versus 257 (rectal administration) μg h/ml for IDM powder. The above data suggested that the extent of absolute bioavailabilities could be similar between an oral and a rectal administration. Again, the peak time of plasma concentration of IDM were not significantly different (Fig. 4) between two rectal suppositories.

4. Conclusions

The SES (30% of Tween 85 and 70% of EO) was selected as an optimized formulation (high drug loading, low surfactant concentration, and small particle size) for IDM, a poorly water-soluble drug. After an oral administration of the SES containing IDM and IDM suspension (IDM powder was suspended in methyl cellulose), 22.5 mg/kg as IDM, to rats, the AUC_{0-12} h was significantly greater (57% increase) in the SES, suggesting that oral absorption of IDM increased significantly by the SES. After a rectal administration of gelatin hollow type suppository filled with the SES containing IDM and IDM powder, 22.5 mg/kg, to rats, the $AUC_{0-12\ h}$ also increased significantly (41% increase) by the SES, suggesting that rectal absorption of IDM also increased significantly by the SES.

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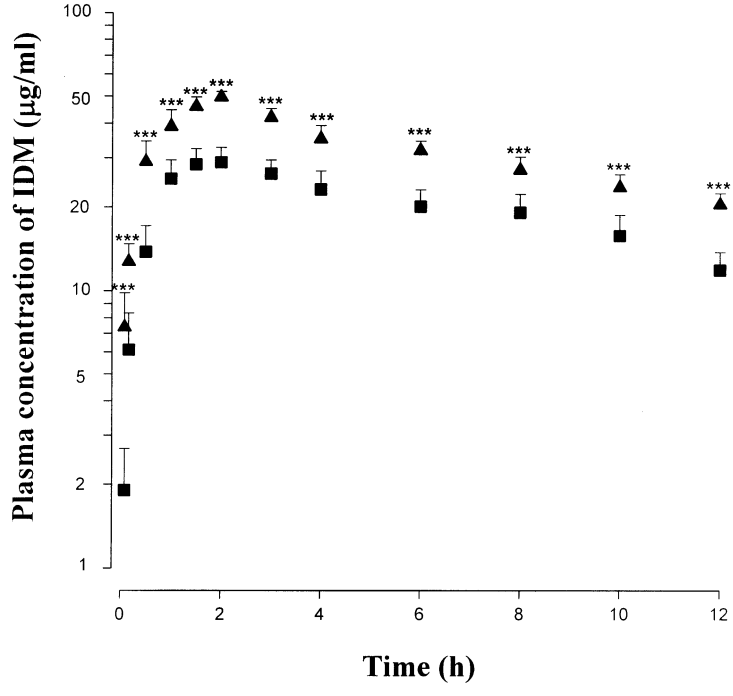

Fig. 3. Mean arterial plasma concentration–time profiles of IDM after oral administration of IDM suspended in methyl cellulose (■, $n = 6$) and SES (Tween 85: EO, 3:7) containing IDM (▲, $n = 7$), 22.5 mg/kg as IDM, to rats. Bars represent S.D. ***$P < 0.001$.

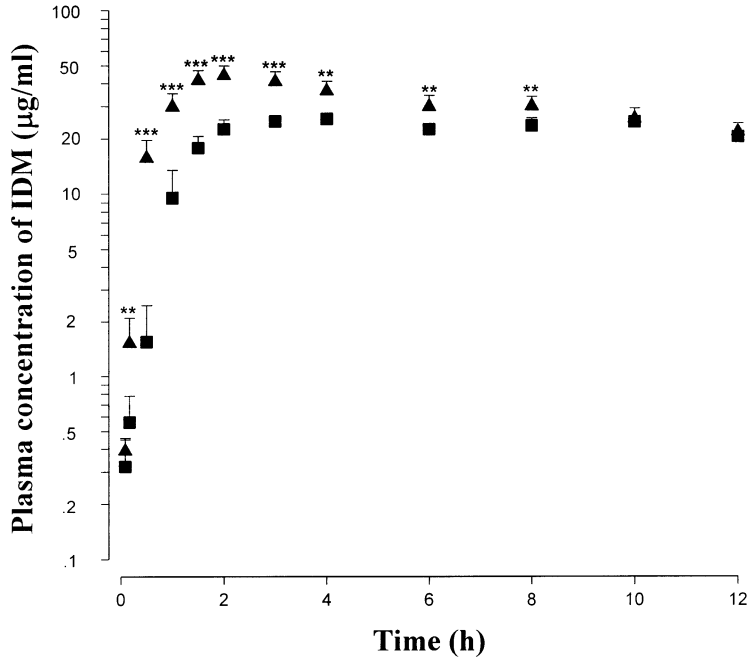

Fig. 4. Mean arterial plasma concentration-time profiles of IDM after rectal administration of gelatin hollow type suppository filled with IDM powder (■, $n = 6$) and SES (Tween 85: EO, 3:7) containing IDM (▲, $n = 6$), 22.5 mg/kg as IDM, to rats. Bars represent S.D. $^{**}P < 0.01$, $^{***}P < 0.001$.

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