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Evaluation of sustained release suppositories prepared with fatty base including solid fats with high melting points

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Abstract

To prepare the sustained release suppositories, solid fats such as polyglycerol ester of fatty acids (PGEFs) or beeswax were utilized with a fatty suppository base, Witepsol H15. PGEFs such as decaglycerol heptabehenate (HB750) and hexaglycerol pentastearate (PS500), and beeswax have relatively high melting points. The addition of PGEFs or beeswax to Witepsol H15 increased the apparent viscosity of suppository bases at 37 °C without any large change in the melting point of Witepsol H15. Moreover, the apparent viscosity of a mixed base with HB750, PS500 or beeswax at 37 °C was significantly correlated with the amount of each solid fat in a mixed base. The release of acetaminophen (AAP), a model drug, from suppositories was delayed by HB750, PS500 or beeswax, and an excellent correlation was observed between the apparent viscosity of these mixed bases and Higuchi's rate constants in each mixed base suppository, suggesting that these solid fats could regulate the drug release from the mixed base suppositories by changing their viscosity. In the in vivo absorption study in rats, several suppositories made from Witepsol H15–HB750 or Witepsol H15–beeswax mixed bases prolonged the rectal absorption of AAP without reducing AUC. In conclusion, by using solid fats such as HB750 and beeswax with relatively high melting points, it is possible to control the rate of drug release from fatty base suppositories for maintaining the plasma concentration of drugs for longer time periods.

Keywords: Fatty suppository; Polyglycerol ester of fatty acid; Beeswax; Viscosity; Solid fat with high melting point; Controlled release

1. Introduction

The rectal route for drug administration is attractive and useful because it can avoid hepatic first-pass effect, decrease gastrointestinal side effects, and avoid undesirable effects of meals on drug absorption (de Boer et al., 1979, 1982). However, in the case of drugs that are rapidly eliminated from the systemic circulation, frequent administration would be needed to maintain the therapeutic plasma concentration. To reduce the frequency of dosing, several approaches have been performed to prepare the controlled release suppositories by using various additives such as lecithin (Nishihata et al., 1985), sucrose fatty acid ester (Nakajima et al., 1990) and carboxyvinyl polymer (Azechi et al., 2000; Yahagi et al., 2000). Various hydrogel formulations were also investigated (Morimoto et al., 1989; Miyazaki et al., 1998). Furthermore, we reported that a mixed base of hexaglycerol pentastearate (PS500), a solid fat, with Witepsol H15 has the good property of sustained release for brilliant blue (Saito et al., 1994). PS500, one of polyglycerol

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ester of fatty acids (PGEFs), has relatively high melting point, and it can be mixed uniformly with fatty suppository bases. Moreover, it was reported that the solid fat such as PS500 is available as a micro-matrix base (Akiyama et al., 1993).

In the present study, we, therefore, tried to prepare the sustained release suppositories by employing several solid fats such as PGEFs (decaglycerol heptabehenate (HB750), PS500) and beeswax. Acetaminophen (AAP), a typical antipyretic analgesic, was chosen as a model drug, because the increase in the area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) would lead to the maintenance of the therapeutic effect of AAP. Then, the physicochemical properties of the suppositories such as viscosity, melting behavior and in vitro drug release profile were evaluated, and the in vivo drug absorption behavior was also investigated after rectal administration into rats.

2. Materials and methods

2.1. Materials

Witepsol H15 was obtained from Mitsuba Trading Co. Ltd. (Tokyo, Japan). PS500, HB750 (Sakamoto Yakuhin Kogyo Co. Ltd., Osaka, Japan), beeswax (Croda Japan KK, Osaka), hydrogenated caster oil (Lubliwax®, Kawaken Fine Chemicals Co. Ltd., Tokyo) and glycerol ester of fatty acid (GMS, Nikko Chemicals Co. Ltd., Tokyo) were used as solid fats. AAP was purchased from Iwaki Pharmaceutical Co. Ltd. (Tokyo). Other reagents were analytical or special reagent grade commercial products.

2.2. Preparation of suppositories

Suppositories were prepared according to the fusion method. Briefly, a solid fat was mixed well with Witepsol H15 at 60–80 °C. AAP, pulverized by a hummer-type mill (Sample Mill AP-S type, Hosokawa Micron Japan, Osaka; screen diameter 1.0 mm), was added to the melted bases at 2% (w/w) and dispersed homogeneously. The resulting mixture was then poured into plastic molds and allowed to cool at room temperature. Prepared suppositories were stored at 4 °C until use.

2.3. Differential scanning calorimetry (DSC) analysis

DSC thermograms were obtained using a DSC 2920 system (TA Instruments, New Castle, DE, USA). A solid fat and Witepsol H15 (2:8, w/w) were melted and mixed at 60 °C, and solidified at 4 °C. The resulting mixed base was milled, weighed in an aluminum pan and heated at 2 °C/min to obtain a DSC thermogram. An empty pan was set as a reference for each mixed base examined.

2.4. Measurement of viscosity

The apparent viscosity of bases (approximately 1 g) was measured with a cone and plate viscometer (Visconic® model E, Toki Sangyo Co. Ltd., Tokyo) at 37 °C.

2.5. In vitro release study

The release test was carried out by PTSW-type rotating dialysis cell method (Fig. 1; Pharma Test, Hainburg, Germany) (Saito et al., 1994). The dissolution phase was 900 ml of phosphate buffer (pH 7.3) and 3 ml of phosphate buffer was placed in the rotating cell. The cell was rotated at 25 rpm and the dissolution phase was maintained at 37 °C. In this study, a Teflon stirring bar ($\phi = 6\,\text{mm} \times 30\,\text{mm}$) was put in the rotating cell in order to shear the melted suppository. The drug concentrations in the dissolution phase were assayed spectrophotometrically at 250 nm. The release profile was analyzed based on Higuchi's model

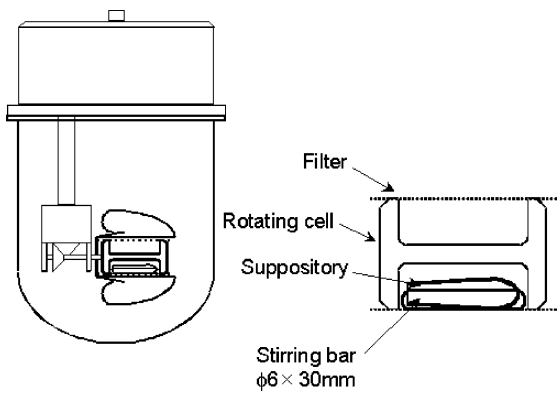

Fig. 1. Schematic representation of in vitro release testing apparatus: PTSW type rotating dialysis cell method with a stirring bar.

(Higuchi, 1963; Schwartz et al., 1968; Azechi et al., 2000), where the cumulative amount of drug released (Q) versus time profile can be described partly by the following equation:

$$Q = k_H \cdot t^{1/2}$$

where k_H means the Higuchi's rate constant. The value of k_H was obtained as a slope of the linear portion of the Q versus $t^{1/2}$ plot for each mixed base by the least squares method.

2.6. In vivo absorption study

Male Wistar rats (Japan SLC, Hamamatsu, Japan), maintained at 20–26 °C and 40–70% humidity, were allowed free access to standard laboratory chow (CLEA Japan Inc.) and water prior to the experiments. Rats weighing 180–220 g were assigned randomly to each experimental group. Prior to the experiment, the fecal content in the rectal canal was reduced by fasting for 24 h. The suppositories ($\phi = 3\,\text{mm} \times 10\,\text{mm}$) containing AAP (2%, w/w; 20 mg/kg) were prepared by the fusion method and inserted to the rectum of rats under ether anesthesia. After dosing, the anus was glued together in superglue for surgery in order to prevent a leak of suppository. Blood samples (0.3 ml each) were collected periodically from the jugular vein using heparinized syringes. Our investigations were performed after approval by our local ethical committee at Dainippon Pharmaceutical Co. Ltd. and Okayama University, and in accordance with "*Principles of Laboratory Animal Care* (*NIH publication #85–23*)".

2.7. Analytical method

The plasma concentration of AAP was assayed by HPLC with an internal standard of 2-acetaminophenol according to the method reported in our previous paper (Shimono et al., 2003). HPLC system consists of Shimadzu HPLC system (Model LC-10ADVp pump, Model SIL-10ADVp auto injector, Model SPD-10AVp detector set at 247 nm) equipped with a 6.0 mm × 150 mm ODS column (YMC-Pack ODS-A, YMC, Kyoto). A mixture of pH 3.0 phosphate buffer/acetonitrile/methanol (10/1/0.5, v/v) was delivered as the mobile phase at a flow rate of 1.0 ml/min. The coefficient of variation for the standard curve ranged from 0.1 to 8.7% and the squared correlation coefficient was over 0.999.

2.8. Pharmacokinetic analysis

The AUC and MRT of AAP from 0 to 24 h were calculated by the trapezoidal method. The highest concentration of AAP observed in plasma was employed as C_{max} value, and the time for C_{max} was defined as T_{max}.

2.9. Statistical analysis

Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunnet's method or Student's *t*-test.

3. Results and discussion

3.1. DSC thermograms and viscosity of mixed bases of Witepsol H with solid fats

Fig. 2 shows the representative DSC thermograms of Witepsol H15–solid fat mixed bases, and endothermic peaks of solid fats and the mixed bases are listed in Table 1. An endothermic peak of Witepsol H15 appeared at 29.6 °C. When Witepsol H15 was mixed with PS500, the main endothermic peak derived from Witepsol H15 was hardly shifted and the dissociation of secondary peak (43.5 °C) at the higher temperature side of the main endothermic peak was obtained (Fig. 2A) as observed in our previous study (Saito et al., 1994). In the cases of HB750 (Fig. 2B) and beeswax (Fig. 2C), the main endothermic peak derived from Witepsol H15 was slightly shifted to higher temperatures, but secondary peaks (56.2 and 54.3 °C, respectively) were clearly separated from the main endothermic peak. Hydrogenated caster oil (Lubliwax) did not change the endothermic peak derived from Witepsol H15, but secondary peak was observed at a high temperature (78.1 °C) (Table 1). A mixed base of Witepsol H15 with glycerol ester of fatty acid (GMS) gave a very complicated profile of thermogram, although the main endothermic peak derived from Witepsol H15 was hardly changed

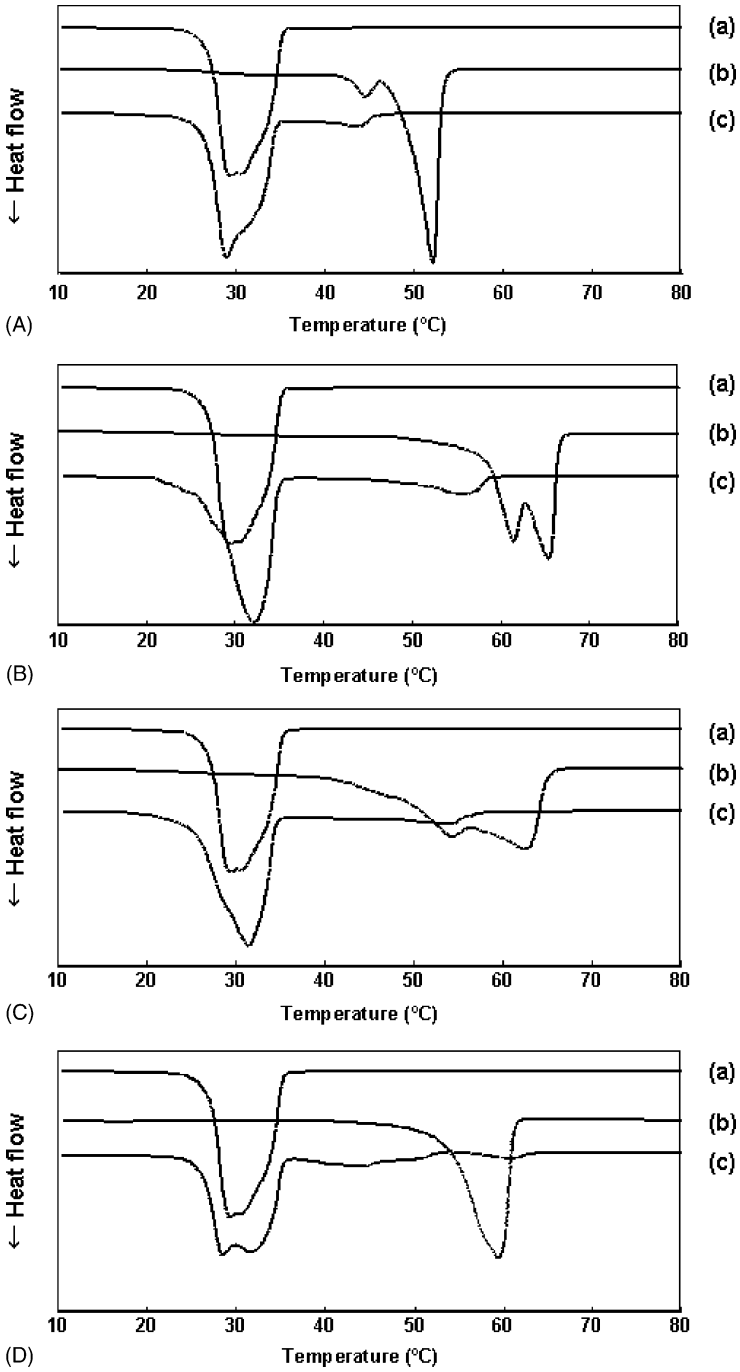

Fig. 2. DSC thermograms of mixed bases. (A) Witepsol H15–PS500 mixed base; (a) Witepsol H15, (b) PS500 and (c) Witepsol H15–PS500 mixed base containing 20% PS500. (B) Witepsol H15–HB750 mixed base; (a) Witepsol H15, (b) HB750 and (c) Witepsol H15–HB750 mixed base containing 20% HB750. (C) Witepsol H15–beeswax mixed base; (a) Witepsol H15, (b) beeswax and (c) Witepsol H15–beeswax mixed base containing 20% beeswax. (D) Witepsol H15–GMS mixed base; (a) Witepsol H15, (b) GMS and (c) Witepsol H15–GMS mixed base containing 20% GMS.

Table 1
Temperature of endothermic peaks (°C) in DSC thermograms for mixed bases of Witepsol H15 with solid fats

Base or solid fat	Original peak	Mixed base with Witepsol H15	
		Main peak	Secondary peak
Witepsol H15 alone	29.6	–	–
Hexaglycerol pentastearate (PS500)	52.2	29.0	43.5
Decaglycerol heptabehenate (HB750)	65.5	32.2	56.2
Beeswax	62.5	31.5	54.3
Hydrogenated caster oil (Lubliwax)	86.3	29.1	78.1
Glycerol ester of fatty acid (GMS)	59.5	28.6	44.3, 60.8

(Fig. 2D). From the results of DSC analysis, a mixed base with Lubliwax or GMS was considered inadequate for the preparation of suppository, because its melting point is too high or the complicated profile might be leading to its instability for Lubliwax or GMS, respectively. Therefore, the mixed base with PS500, HB750 or beeswax was employed for further investigations.

The apparent viscosity was measured for the three mixed bases at 37 °C and its relationship with the content of solid fats were examined (Fig. 3). The linear relationship was observed for each mixed base and the squared correlation coefficient values for PS500, HB750 and beeswax were 0.933, 0.997 and 0.991, respectively. Every correlation was statistically significant ($P < 0.05$). These results show that the apparent viscosity of the mixed base of Witepsol H15 can be regulated quantitatively by the addition of PS500, HB750 or beeswax into Witepsol H15. Furthermore, it has been found that the use of HB750 or beeswax makes it possible to prepare more viscous base than that of PS500, which could be related to high secondary endothermic peaks of Witepsol H15–HB750 and Witepsol H15–beeswax bases (Fig. 2).

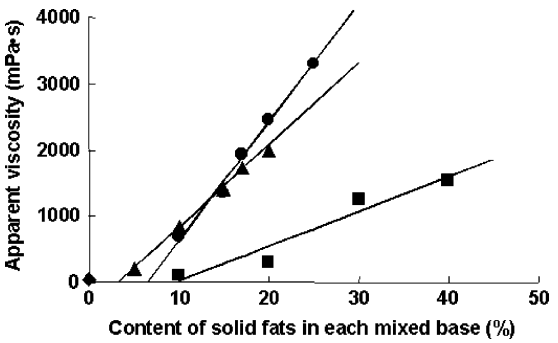

Fig. 3. Apparent viscosity of mixed bases as a function of solid fats content. Apparent viscosity of mixed bases was measured at 37 °C. Straight lines were obtained by least squares method and the values of squared correlation coefficient were 0.933, 0.997 and 0.991 for PS500, HB750 and beeswax mixed bases, respectively ($P < 0.05$). (◆) Witepsol H15; (■) PS500; (▲) HB750; (●) beeswax.

3.2. Drug release from suppositories of Witepsol H15–solid fats mixed bases

Fig. 4 shows the release profiles of AAP from suppositories made from various mixed bases. The release rate of AAP from the suppository decreased with an increase in the weight fraction of solid fats for each mixed base. The release rates from Witepsol H15–PS500 mixed bases (Fig. 4A) were obviously higher than those from the other mixed bases (Fig. 4B and C), even though Witepsol H15–PS500 mixed bases contain the relatively higher content of the solid fat, PS500. In the case of beeswax, the release rates of AAP from the mixed bases decreased most markedly with the increase in the weight fraction of the solid fat (Fig. 4C).

Azechi et al. (2000) reported that the Fickian diffusion model could be applied for the release of diclofenac from the polymer-containing suppositories, although Saito et al. (1994) reported that the Jander's equation could also be applied to the release data. Based on the results obtained by the testing method as shown in Fig. 1, the rate of drug release from suppositories could be expressed by Fickian diffusion model (Higuchi's model), in which the drug release from the suppository is regulated by the drug diffusion through the melted base (Higuchi, 1963; Schwartz et al., 1968). In Fig. 5A, the logarithmic values of k_H obtained were

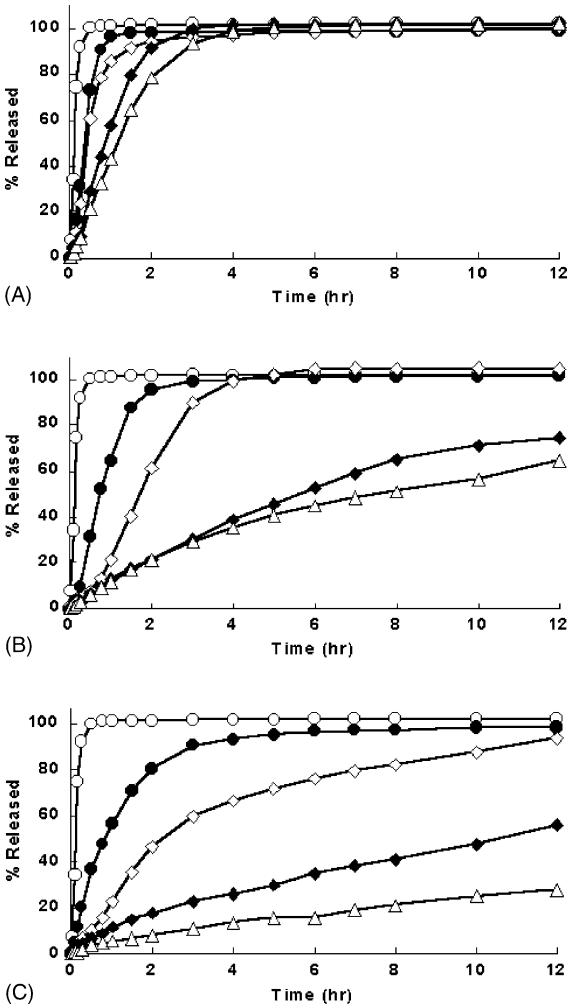

Fig. 4. Release profiles of acetaminophen from suppositories at 37 °C. In vitro release study was performed according to the method described in Section 2. Each value represents the mean of six experiments. (A) Witepsol H15–PS500 mixed base: (○) Witepsol H15 alone; (●) 10% PS500; (◇) 20% PS500; (◆) 30% PS500; (△) 40% PS500. (B) Witepsol H15–HB750 mixed base: (○) Witepsol H15 alone; (●) 5% HB750; (◇) 10% HB750; (◆) 15% HB750; (△) 20% HB750. (C) Witepsol H15–beeswax mixed base: (○) Witepsol H15 alone; (●) 10% beeswax; (◇) 15% beeswax; (◆) 20% beeswax; (△) 25% beeswax.

plotted against the weight fraction of solid fats for each suppository, showing a significant linear correlation between the two parameters. This result indicates that the rate of release can be controlled by changing the content of solid fats for each mixed base suppository.

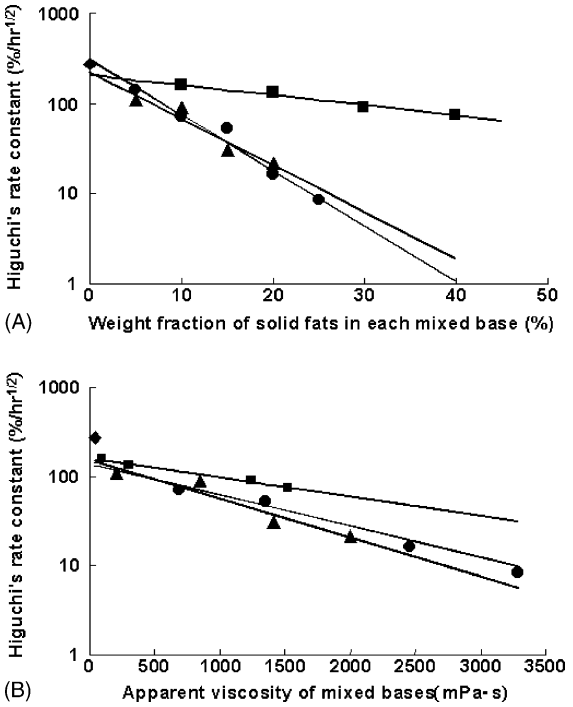

Fig. 5. Relationship between Higuchi's rate constant (k_H) and (A) weight fraction of solid fats in mixed bases or (B) apparent viscosity of mixed bases. The value of k_H was obtained based on the results shown in Fig. 4 according to the method described in Section 2. The solid lines represent the least squares linear regression lines and the values of squared correlation coefficient were as follows: (A) PS500: 0.980; HB750: 0.930; beeswax: 0.899 ($P < 0.05$). (B) PS500: 0.988; HB750: 0.919; beeswax: 0.982 ($P < 0.05$). (◆) Witepsol H15 alone: (■) PS500; (▲) HB750; (●) beeswax.

Fig. 5B shows the relationship between k_H and the apparent viscosity, and the results clearly indicate that the release of AAP from the suppositories examined decreases with the increase in viscosity of bases. Iwamoto et al. (1987) suggested that the drug release from the suppository containing carboxyvinyl polymer was regulated by the viscosity in the suppository that was increased by the addition of carboxyvinyl polymer. As shown in Fig. 3, the viscosity of suppositories increases as the content of solid fats increases, which could be due to the increase in the content of un-melted solid fat at 37 °C. Therefore, taken collectively, it is indicated that solid fats such as PS500, HB750 and beeswax can regulate the drug release from the mixed base suppositories by changing their viscosity.

Table 2
Pharmacokinetic parameters for acetaminophen after rectal administration of mixed base suppositories of Witepsol H15 with HB750 or beeswax in rats

Base	T_{max} (h)	C_{max} (μg/ml)	MRT (h)	AUC, 0–24 h (μg/ml h)
Witepsol H15 alone	0.3 ± 0.1	4.35 ± 2.00	1.4 ± 0.4	4.89 ± 1.44
With 10% HB750	2.0 ± 1.2	0.79 ± 0.21**	5.0 ± 0.4**	4.26 ± 0.41
With 15% HB750	3.3 ± 2.7**	0.70 ± 0.37**	6.5 ± 1.7**	3.82 ± 0.80
with 20% HB750	1.2 ± 0.4	0.37 ± 0.13**	7.5 ± 0.6**	2.35 ± 0.61**
With 10% beeswax	2.0 ± 1.2	0.89 ± 0.31**	5.0 ± 1.6**	4.27 ± 0.51
With 15% beeswax	3.3 ± 1.3**	0.69 ± 0.16**	6.1 ± 0.9**	4.54 ± 0.83
With 20% beeswax	2.6 ± 1.4*	0.72 ± 0.14**	6.0 ± 0.8**	4.05 ± 0.41
With 25% beeswax	2.0 ± 1.2	0.42 ± 0.17**	6.3 ± 1.6**	2.65 ± 0.68**

Dose of acetaminophen was 20 mg/kg. Results are expressed as the means ± S.D. of 5–7 experiments.
* $P < 0.05$ compared with the corresponding parameter for Witepsol H15 alone.
** $P < 0.01$ compared with the corresponding parameter for Witepsol H15 alone.

3.3. Absorption of AAP from suppositories of mixed bases with HB750 or beeswax

Fig. 6 shows the plasma concentration of AAP after rectal administration of Witepsol H15–HB750 (Fig. 6A) or Witepsol H15–beeswax suppositories (Fig. 6B). Pharmacokinetic parameters obtained are summarized in Table 2. An early and high peak in the plasma level of AAP was observed after rectal administration of the suppository made from Witepsol H15 only, but the plasma concentration decreased rapidly. On the other hand, plasma concentrations of AAP after rectal administration of Witepsol H15–HB750 or Witepsol H15–beeswax suppositories were relatively lower at the early period. However, from around 2 h after dosing, the plasma concentrations of AAP were explicitly higher than those observed for a Witepsol H15 suppository and the higher levels were sustained for longer time periods. MRT values of all the mixed base suppositories examined were significantly longer than that of Witepsol H15 suppository. In the case of Witepsol H15–HB750 suppositories, 10 and 15% HB750 gradually increased MRT, but did not change AUC of AAP. Although 20% HB750 increased MRT more, AUC was significantly decreased. On the other hand, every Witepsol H15–beeswax suppository examined gave similar values of MRT and AUC except AUC was significantly decreased in the case of 25% beeswax. Therefore, Witepsol H15–HB750 mixed base containing 10 or 15% HB750, or Witepsol H15–beeswax mixed base containing 15% beeswax could be the best preparation in the present study.

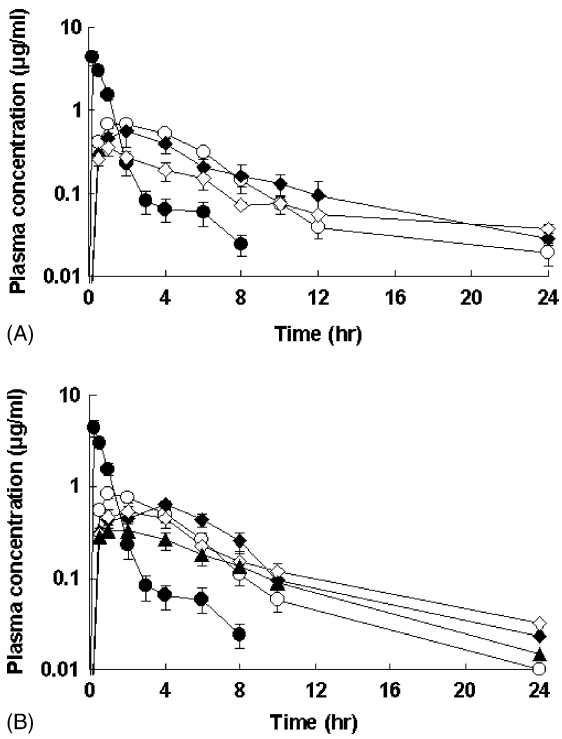

Fig. 6. Plasma concentration of acetaminophen after rectal administration of suppositories made from (A) Witepsol H15–HB750 or (B) Witepsol H15–beeswax mixed bases in rats. Results are expressed as the means ± S.E. of 5–7 experiments. (A) Witepsol H15–HB750 mixed bases: (●) Witepsol H15 alone; (○) 10% HB750; (◆) 15% HB750; (◇) 20% HB750. (B) Witepsol H15–beeswax mixed bases: (●) Witepsol H15 alone; (○) 10% beeswax; (◆) 15% beeswax; (◇) 20% beeswax; (△) 25% beeswax.

These results on in vivo absorption behavior would mostly reflect the physicochemical properties examined in in vitro studies. Larger amount of solid fats caused the attenuation of the rectal absorption of AAP, although the absorption was successfully sustained for a long time. This might be explained by a following reason that the suppository vehicles were too hard to release the drug as much as others did in the rectum.

In conclusion, we prepared the controlled release suppositories consisting of a fatty suppository base, Witepsol H15, and solid fats such as PS500, HB750 and beeswax. The addition of solid fats increased the apparent viscosity of the mixed bases at 37 °C, leading to the reduction of drug diffusivity within the melted base, and the subsequent reduction of drug release rate. Several suppositories made from Witepsol H15–HB750 or Witepsol H15–beeswax mixed bases successfully prolonged the rectal absorption of AAP without reducing AUC.

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