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RESEARCH PAPER

Effect of Sodium Caprate on the Absorption of Progesterone from Mixed Suppositories Administered to the Vagina of Rabbits

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ABSTRACT

The promoting effect of sodium caprate (CAP) added to a progesterone mixed suppository was investigated in vitro and in vivo. The mixed suppository (S50), consisting of Witepsol W35 and ethylene-vinyl acetate copolymers (EVA40Y and EVA250); and a control suppository of Witepsol W35 (C50) were prepared by the fusion method. One or five percent of CAP was added to S50 and C50 containing 50 mg of progesterone. The release of progesterone from C50 was not influenced by the addition of CAP and there was no change in plasma level profile of progesterone after administration to the vagina of rabbits. On the other hand, S50 added with CAP showed rapid elevation of the plasma level of progesterone without any change in the sustained-release characteristics.

INTRODUCTION

The significance of the progesterone supplementation in luteal phase defects has been reported for patients with infertility or sterility (1). The most common method of progesterone replacement is by intramuscular injection, although a certain amount of discomfort is inevitable with intramuscular injection. We have previously reported newly developed progesterone suppositories (2,3). Polyethyleneglycol (PEG) has been used as the base of progesterone suppositories but PEG is known to cause erosion and bleeding because of its irritating

effects on the mucosal membrane. Witepsol, a popular suppository base, is less of an irritant on the mucosal membrane. However, Witepsol has a tendency to release the drug rapidly because it melts quickly at body temperature. Therefore, the blood levels of progesterone cannot be maintained at the levels required for effective progesterone replacement therapy.

Lately, we developed a new type of suppository (the mixed suppository) (4) in which Witepsol and ethylene–vinyl acetate copolymers are used as the base. However, the initial concentration after administration was lower than the therapeutic level although the sustained-release characteristics were superior to the Witepsol suppository.

Sodium caprate (CAP) (5,6) is known to modify the barrier function of the biomembrane against drug permeation transiently and reversibly. In this study, the effect of CAP on the in vitro release and in vivo absorption of progesterone from the mixed suppository was investigated to evaluate a new suppository formulation for use in progesterone replacement therapy.

EXPERIMENTAL

Materials

The progesterone used as the active ingredient was purchased for Wako Pure Chemicals Industry Ltd. Witepsol W35 (Dynamit Nobel Co. Ltd., Tokyo, Japan) was used as the base of the suppository. Ethylene–vinyl acetate copolymers (EVA40Y and EVA250), which were added as a component of the base of the mixed suppository, were supplied by Mitsui–Du Pont Chemical Co. Ltd (Tokyo, Japan). Sodium caprate (CAP; Kanto Chemical Co. Ltd., Tokyo, Japan) was used as an absorption promoter. Other reagents used were of analytical grade.

Preparation of the Suppositories

The formulations of the progesterone suppositories prepared in this study are shown in Table 1. The content of progesterone was fixed at 50 mg/suppository. The progesterone suppository based on Witepsol (C50) and that with 1% added CAP (C′50) were prepared as controls. Mixed suppositories (S50) consisting of Witepsol, EVA40Y, and EVA250 as a base, and 1% or 5% of CAP (S′50, S″50) were prepared. All the suppositories were prepared by the fusion method and were stored in a refrigerator until the experiment.

Measurement of Characteristics of the Suppositories

Stress of Penetration

The stress of penetration was measured at 40°C under the pressing speed of 60 mm/min using a Rheometer RT-3005D (Fudo Co. Ltd., Tokyo, Japan) and a disk (10 mm diameter). Measurement was repeated three times and the mean value was used.

Strength

Samples were compressed using a spherical attachment of 10 mm in diameter at the rate of 60 mm/min, and the stress required to break the suppository was measured as the strength using the rheometer described above. The mean value of three measurements was used.

Table 1

The Formula of Progesterone Suppositories

	Progesterone	CAP	Witepsol	Mixed Base	Total Weight (g)
Control					
C-50	0.05	—	1350	—	1400
C′-50	0.05	0.014	1336	—	1400
Mixed suppositories					
S-50	0.05	—	—	1350	1400
S′-50	0.05	0.014	—	1336	1400
S″-50	0.05	0.070	—	1280	1400

Note: CAP: sodium caprate; mixed base: Witepsol, EVA40Y, EVA250 (85.9%:8.7%:5.4%).

Thermal Characteristics

Differential scanning calorimetry (DSC) was carried out using a Thermoflex DSC8230 (Rigaku Denki Co. Ltd., Tokyo, Japan). Both endothermic peak temperature and heat of fusion were measured. The mean value was calculated after five measurements.

Drug Release Properties In Vitro

Release tests of suppositories were performed by the beads rotatory cell method (7). Details of this method were previously reported by the authors. The following conditions for the test were applied: test fluid, 900 ml of saline added with 1% sodium lauryl sulfate; temperature, 37.0 ± 0.5°C; rotating speed, 25 rpm; membrane filter, Millipore Type SS (pore size, 3 μm). Forty glass beads and 40 nylon beads 2 mm in diameter, and 5 ml of test fluid were set in the cell together with a suppository. The test fluid (5 ml) was collected at appropriate intervals for 24 hr, and equal volumes of fluid were immediately added to the beaker. The absorbance of samples was measured at 248 nm and progesterone levels were determined from the calibration curve. The release test was repeated three times and the mean value was used.

In Vivo Absorption Study Using Rabbits

After a suppository was administered to the vagina of rabbits (mean body weight 2.5 kg), blood samples were collected at appropriate intervals. Blood samples were treated with heparin sodium and centrifuged (3000 rpm and 5 min) to obtain plasma. The plasma level of progesterone was measured using a commercially available radioimmunoassay kit (Progesteron Kit Daiichi II, Nihon DPC Co., Tokyo, Japan).

Calculation

Drug release parameters were obtained from drug release profiles. The peak plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), the area under the plasma concentration–time curves from 0 to 5 hr ($AUC_{0\text{-}5}$), calculated by the trapezoidal rule, were calculated using Yamaoka's program (8) on an NEC PC-9801FA.

RESULTS AND DISCUSSION

Characteristic Values of the Suppositories

The physicochemical properties of the progesterone suppositories are shown in Table 2. The mean value of stress on penetration of Witepsol suppositories at 40°C, C50 and C'50 were 3.26 g and 3.13 g, respectively, suggesting that the addition of CAP did not affect the characteristics in the melted state. On the other hand, the value of stress on penetration of the mixed suppositories, S50 and S'50 was 35.1 g and 25.3 g, respectively. The stress on penetration of the mixed suppository (S50) was about 10 times greater than the Witepsol suppository (C50). The viscosity of the melted suppository affects the topical residing property and the bioavailability of the suppository; therefore, the larger the stress is, the better the topical residence of the suppositories. In this regard, S50 and S'50 are preferable compared with C50 and C'50.

The mechanical strength of C50 and C'50 was 3.66 g and 4.63 g, respectively, and the addition of CAP led to an increase in mechanical strength. On the other hand, the strength of S50 and S'50 was 2.34 g and 2.21 g, respectively, showing no significant difference between these two suppositories. The results showed that the addition of CAP did not decrease the strength of the suppositories.

Table 2

Characteristics of Progesterone Suppositories

	Witepsol Suppositories		Mixed Suppositories	
	C-50	C'-50	S-50	S'-50
Stress on penetration at 40°C (g)[a]	3.26 ± 0.03	3.13 ± 0.09	35.1 ± 2.5	25.3 ± 2.5
Strength (kg)[a]	3.66 ± 0.07	4.63 ± 0.53	2.21 ± 0.10	2.34 ± 0.12
DSC peak temp. (°C)	38.3 ± 0.7	39.8 ± 1.2	38.2 ± 0.9	37.5 ± 1.1
DSC heat of fusion (kJ/g)[b]	7.32 ± 0.51	6.06 ± 0.53	3.48 ± 1.06	4.31 ± 1.45

[a]Mean ± *SD* (n = 3).
[b]Mean ± *SD* (n = 5).

No remarkable difference was observed in the endothermic peak temperature in the DSC of the suppositories, irrespective of the addition of CAP in the formulations. On the other hand, the heat of fusion decrease slightly in Witepsol suppositories with the addition of CAP; however, this did not change in the mixed suppositories.

Drug Release Properties In Vitro

The progesterone release profiles of the suppositories determined by the beads rotatory cell method are shown in Figs. 1 and 2. The percent of progesterone released at 24 hr in the Witepsol and in the mixed suppositories was approximately 46.9% and 29.8%, respectively, and the latter showed a sustained-release property.

The released amount of the mixed suppository increased slightly with the addition of CAP. A correlation was observed between the 16% release time ($T_{16\%}$, Y), a parameter estimated from the log-normal distribution, and the combinating rates of CAP (X). $T_{16\%}$ (Y) was predicted by the linear function of X.

$$Y = 11.623 - 1.3850X \qquad (r^2 = 0.997)$$

This tendency should be explained by the decreased rate of EVAs which are related to the sustained release property, according to increased rate of CAP in the suppository.

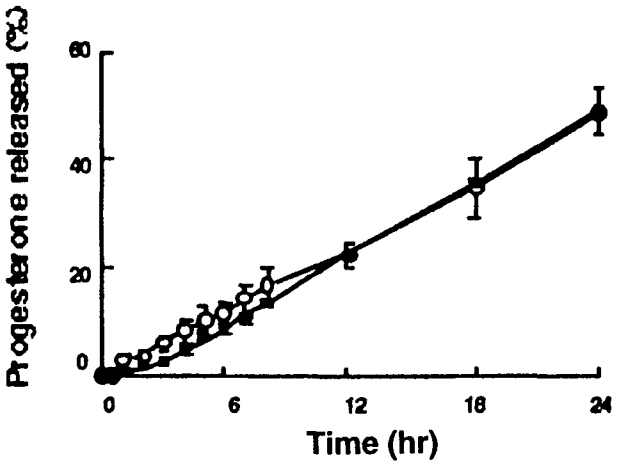

Figure 1. Effect of sodium caprate on release of progesterone from suppositories: —○—, C50; —●—, C'50. Each point represents the mean ± *SD* (n = 3).

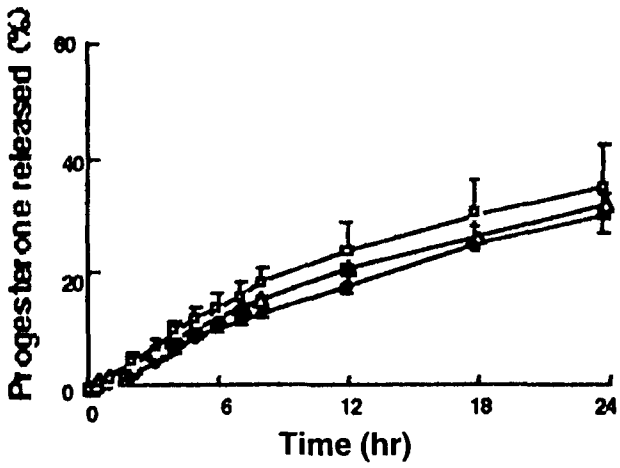

Figure 2. Effect of sodium caprate on release of progesterone from suppositories: —●—, S50; —Δ—, S'50; —□—, S"50. Each point represents the mean ± *SD* (n = 3).

Drug Absorption In Vivo

The plasma progesterone profiles after vaginal administration of suppositories to rabbits are shown in Figs. 3 and 4. In the Witepsol suppository (Fig. 3), the plasma concentration of progesterone in the initial period was high and the maximum plasma concentrations (C_{max}) of C50 and C'50 were 38.4 and 36.8 ng/ml, respectively; and T_{max} was 0.5 hr in both suppositories. At 3 hr after the administration, the plasma concentration declined to less than 10 ng/ml. No absorption-enhancing effect of CAP was observed in the Witepsol suppositories, possibly because the drug was released before effect of CAP became apparent. In the cases of the mixed suppositories, the plasma progesterone profiles showed sustained plasma concentrations of progesterone at 5 hr after administration were kept at a level of more than 10 ng/ml. The plasma progesterone concentration of S'50 and S"50 at 1 hr became approximately 1.2 and 2 times of that in S50, respectively, indicating the absorption-promoting effect of CAP. The pharmacokinetic parameters ($AUC_{0\text{-}5}$ and MRT) for Witepsol suppositories and mixed suppositories are shown in Table 3. Mixed suppositories showed larger $AUC_{0\text{-}5}$ and MRT values than the Witepsol suppositories, suggesting the sustained release of progesterone in vivo. The $AUC_{0\text{-}5}$ values of S'50 and S"50 were 93.5 ng·hr/ml and 168.9 ng·hr/ml, respectively. There was no statistical difference among AUC and MRT values

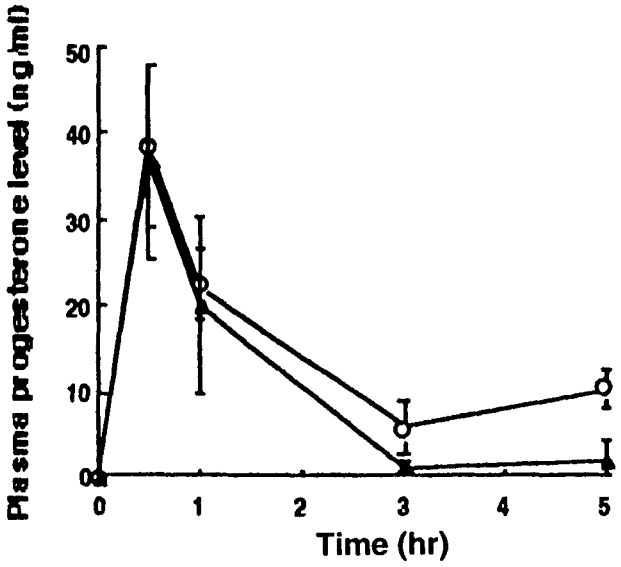

Figure 3. Plasma concentration of progesterone after vaginal adminnistration of suppositories (50 mg) to rabbits: —○—, C50; —▲—, C′50. Each point represents the mean ± *SE* (n = 3).

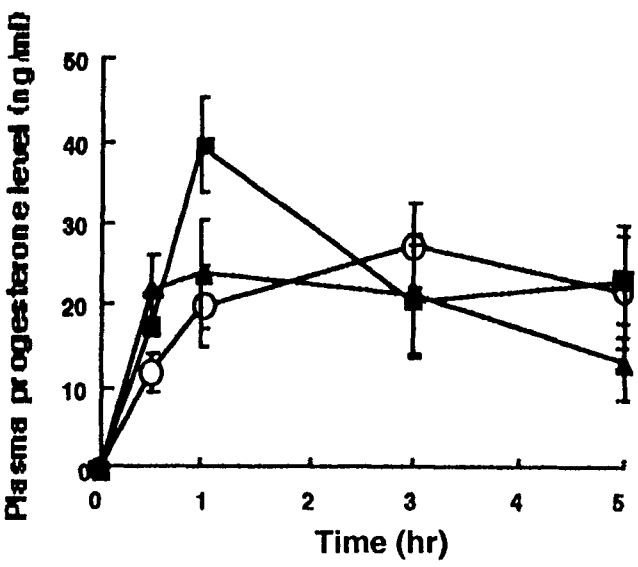

Figure 4. Plasma concentration of progesterone after vaginal administration of suppositories (50 mg) to rabbits: —○—, S50; —▲—, S′50, —■—, S″50. Each point represents the mean ± *SE* (n = 3).

for the mixed suppositories, but the addition of CAP brought about the higher initial concentration of progesterone compared with the mixed suppository without the addition of CAP (C50).

CONCLUSION

The absorption-promoting effect of CAP added into the mixed suppository of progesterone was investigated in vitro and in vivo. Though the in vitro release characteristics were not influenced by the addition of CAP, the initial plasma concentration in vivo was increased. The $AUC_{0\text{-}5}$ was increased about 1.6 times by adding 5% of CAP to the mixed suppository.

These results suggest that CAP promoted the vaginal absorption of progesterone in rabbits via biomembrane perturbation at the administration site, as reported already by Murakami et al. (5). In progesterone replacement therapy, it is important to maintain the appropriate levels of plasma progesterone continuously.

In this study, it was confirmed that the addition of CAP to the mixed suppository increased the initial plasma levels of progesterone compared with the ordinary mixed suppository, without losing the sustained release characteristics in vivo.

Table 3

Parameters for Progesterone Suppositories In Vivo

	C50	C′50	S50	S′50	S″50
$AUC_{0\text{-}5}$ (ng/hr/ml)	68.8 ± 12.8	48.1 ± 19.6	106.5 ± 4.4	93. 5 ± 11.0	168.9 ± 73.1
MRT (hr)	1.79 ± 0.17	0.93 ± 0.23	2.81 ± 0.40	2.33 ± 0.40	2.89 ± 0.86

Note. Each value represents the mean ± *SE* (n = 3).

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