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RESEARCH PAPER

Release Property of Progesterone from a Mixed-Base Suppository Consisting of Witepsol® W35 and Witepsol® E85

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ABSTRACT

The mechanism of drug release from progesterone suppositories that consist of two types of hard fat (Witepsol® W35 and Witepsol® E85) was investigated. The strength, the thermodynamic characteristics, the surface structures, the drug release property, methylene blue penetration into suppositories, and change of surface structure after the dissolution test were employed for detecting characteristics of progesterone suppositories. The formulation with a mixing ratio of Witepsol W35 and Witepsol E85 at a 1:1 ratio showed the maximum strength value. The peak temperature of the suppositories showed a tendency to increase with increases in the ratio of Witepsol E85. The maximum height of the profiles measured with laser microscopy, from 20.8 μm to 29.2 μm, reached a maximum after 3 h of the dissolution test. When the suppositories were immersed in pH 7.4 phosphate buffer containing 0.5% methylene blue at 37°C, the penetrating area increased with time. The weight of the suppositories also increased with time. According to these findings, it was suggested that the release

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of drug from a mixed type of suppository containing progesterone was via the matrix and pores.

Key Words: *Drug release; Hospital preparation; Progesterone; Suppositories; Witepsol*

INTRODUCTION

Progesterone suppositories prepared as a hospital preparation as therapeutic agents for sterility, infertility, and external fertilization have shown clinical efficacy (1). We previously examined the vaginal administration of progesterone to improve the quality of life for patients (2) and developed various progesterone suppositories with continuous release properties that maintained a constant concentration of progesterone in plasma (3,4). However, proper selection criteria and preparation methods are required because an easy preparation method and safety of the suppository are essential for clinical use. We reported that a suppository base containing two kinds of hard fat (Witepsol®) with different characteristics was superior in safety and usefulness to others.

In this suppository, diclofenac sodium was used as a model drug and was gradually released from the pores produced by penetrating water into the suppository (5,6).

In the present study, progesterone was employed as an active ingredient, and the mechanism of drug release from the suppositories prepared with the mixed base was investigated in detail.

EXPERIMENTAL

Materials

Progesterone was purchased from Wako Pure Chemicals Industries (Tokyo, Japan). Witepsol W35 and Witepsol E85 were used for the suppository base and were from Mitsuba Trade Company, Limited (Tokyo, Japan). All other reagents used were analytical grade.

Preparation of the Suppositories

Table 1 shows the formulations of progesterone suppositories used in this study. The suppositories were prepared by the fusion method as reported previously (3). Briefly, Witepsol W35 and Witepsol E85 were melted at 50°C; progesterone was added, and the mix was stirred until clear. This solution was refrigerated and solidified after pouring into a plastic suppository mold. Suppositories were used in experiments after storing for least 2 days in the dark at a cool temperature (4°C).

Physical Characteristics of the Suppositories

Strength

The strength of the suppository was represented as the fracture stress using a Fudoh Rheo Meter RT-3005D (Reotech Co., Ltd., Japan) and apparatus no. 13 under compression at 60 mm/min, and the value of the suppository strength was the average of six experiments.

Thermodynamic Characteristics

Peak temperature and heat of fusion of the suppositories were measured using a differential scanning calorimeter (Thermoflex-DSC8230D, Rigaku Co., Ltd., Japan) with TAS200 analysis software. The value of the peak temperature and the heat of fusion were calculated for an average of five experiments.

Surface Structure

The surface of the suppository was estimated using a 1LM21 laser microscope (Lasertec Co., Ltd., Japan). The value of the height of the profiles

Table 1

Progesterone Suppository Formulation

Formulation	Witepsol W35, g (%)	Witepsol E85, g (%)	Progesterone, g
A	1.30 (100)	0.00 (0)	0.10
B	1.27 (75)	0.13 (25)	0.10
C	0.65 (50)	0.65 (50)	0.10
D	0.13 (25)	1.27 (75)	0.10
E	0.00 (0)	1.30 (100)	0.10

and the 10-point height of irregulations as a surface parameter (Japanese Industrial Standard, JIS) were calculated from an average of eight experiments.

Drug Release Experiments

Drug release of 100-mg progesterone suppositories was determined from a dissolution test (3ed method, JP 13). Phosphate buffer at pH 7.4 was used as a test fluid. The test fluid was taken periodically by Mini-sampler One (Otax Co., Ltd., Switzerland). The concentration of progesterone in the sample was measured by the ultraviolet (UV) method (248 nm) using a UV2200 spectrophotometer (Shimadzu Co., Ltd., Japan). The release parameters were calculated from an average of six experiments.

Change of the Suppository Weight

The weight of each suppository was measured periodically after the suppository was immersed in 50 ml phosphate buffer (pH 7.4 at 37°C). The change of suppository weight was calculated as the ratio of the weight at each measuring time point to the initial weight. The ratio of weight increase was calculated by an average of four experiments.

Methylene Blue Penetration in Suppository

After a suppository was immersed in phosphate buffer pH 7.4 containing 0.5% methylene blue at 37°C, the suppository was removed from this solution and dried at room temperature. The widest section of the suppository was photographed using a charge-coupled device (CCD) camera (SONY Co., Ltd., Japan). This photograph was digitally exchanged, and then the area stained with methylene blue penetrating into the suppository was calculated (7). The ratio of methylene blue penetrating into the suppository was represented as the percentage of the area stained with methylene blue to the total section of the suppository.

Change in the Surface Structure of the Suppository After Dissolution Tests

The suppository, after immersion in phosphate buffer at 37°C, was removed and dried at room temperature. The height of the profiles and 10-point height of irregularities were measured using a scanning confocal laser microscope in the same manner as described previously (6).

Calculation

The dissolution parameters by moment analysis were obtained using a program by Yamaoka et al. (8).

RESULTS AND DISCUSSION

Characterization of Suppositories

The characteristics of the suppositories are shown in Table 2. The strength ranged between 1.8 kg and 2.4 kg based on the various mix weight ratios of Witepsol W35 and Witepsol E85. Formulation C, in which the ratio of Witepsol W35 to Witepsol E85 was 1:1, showed the minimum strength value of 1.8 kg. The strength of the suppository is important when it is administered

Table 2

Characteristics of Mixed-Type Progesterone Suppositories

	Formulation				
	A	B	C	D	E
Strength (kg)	2.42 ± 0.16	2.38 ± 0.36	1.81 ± 0.27	2.09 ± 0.29	2.23 ± 0.46
Peak temperature (°C)	36.5 ± 0.6	38.1 ± 1.0	40.5 ± 0.5	41.3 ± 1.2	42.1 ± 1.0
HF (kcal/g)	27.8 ± 1.8	26.8 ± 2.4	26.2 ± 3.1	32.6 ± 5.4	29.3 ± 1.5
Ry (μm)	29.2 ± 3.6	24.6 ± 3.7	21.7 ± 4.0	20.8 ± 5.2	21.5 ± 5.8
Rz (μm)	29.9 ± 2.4	23.9 ± 1.4	23.1 ± 2.4	24.5 ± 1.5	25.1 ± 1.8

HF, heat of fusion; *Ry*, maximum height of the profiles; *Rz*, 10-point height of irregularities, mean ± SD.

for treatment. The strength of each suppository formulation was considered adequate for treatment and administration.

The peak temperature of the suppository showed a tendency to increase with an increase in the ratio of Witepsol E85. This increase in the peak temperature of the suppository was affected by the ratio of the two suppository bases because the melting temperatures of Witepsol W35 and Witepsol E85 ranged from 33.5°C to 35.5°C and from 42°C to 44°C, respectively. However, the heat of fusion was similar among the formulations and, on clear correlation, was noted between the heat of fusion and mix ratio. This suggested that the total heat of fusion was not significantly changed because Witepsol consisted of a triglyceride mixture containing various saturated fatty acids.

The maximum height of the profiles measured by laser microscopy, which indicated the roughness of the surface of the suppositories, was between 20.8 μm and 29.2 μm. An increase of Witepsol W35 in the mixture induced an increase in this parameter. This tendency was greater when Witepsol W35 was above 50% in the mixture ratio (formulations A–C). The 10-point height of irregulations resulted in formulation A containing Witepsol W35 alone, and this value was 1.3-fold greater than for formulation C. These findings suggested that increased Witepsol W35 induced smoother surfaces.

Drug Release from the Suppositories

Figure 1 shows the drug release profiles from progesterone suppositories by the flow-through cell method. The progesterone released in formulations A and E was approximately 40% and 7%, respectively, at 12 h. The area under the curve of drug release time from 0 to 12 h after the set of dissolution tests (ADT0-12) in formulations A,B,C,D, and E, which implies the parameter of drug release, was 39.5 ± 9.8 mg · h/ml, 25.3 ± 4.8 mg · h/ml, 23.4 ± 4.2 mg · h/ml, 19.5 ± 4.9 mg · h/ml, and 6.0 ± 3.0 mg · h/ml, respectively. Hence, an increase in the ratio of Witepsol E85 induced a decrease in the amount of progesterone released from the suppository. However, the drug release rates at 1 h obtained for each formulation were 4.5 ± 0.6 mg/h (A), 3.6 ± 0.8 mg/h (B), 3.6 ± 0.2 mg/h (C), 3.7 ± 0.6 mg/h (D), and 1.42 ± 0.42 mg/h (E), respectively. These findings indicated that the drug release rate from suppository E during the initial period from the start of the test was approximately 7% lower than from formulation A.

Figure 2 shows the plot of the amount of progesterone released as a function of the square root of time. The plots of the slope values for formulations B, C, and D are similar. These findings suggest that the release of progesterone from mixed-type suppositories agrees with the first-order kinetics law (9).

Change in the Weight of the Suppositories

Figure 3 shows the change in suppository weight at each time point after the suppository was added to the phosphate buffer. The change in weight of formulation A could not be determined since this suppository was immediately melted in phosphate buffer at 37°C. The change in weight of each formulation

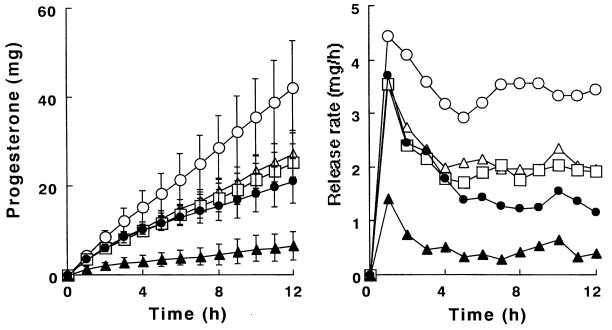

Figure 1. Profiles of drug release rate from progesterone suppositories: ○, formulation A; △, formulation B; □, formulation C; ●, formulation D; ▲, formulation E. Each point represents the mean ± SD ($n = 6$).

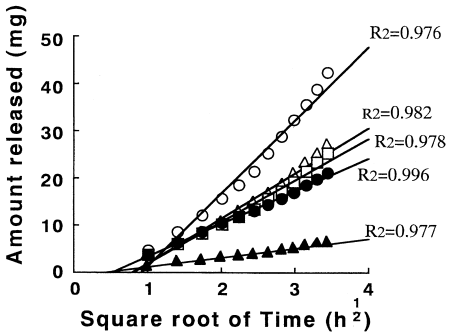

Figure 2. The plot of the amount of progesterone released as a function of the square root of time: ○, formulation A; △, formulation B; □, formulation C; ●, formulation D; ▲, formulation E.

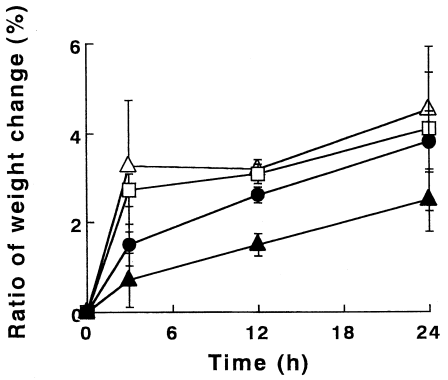

Figure 3. The time course change of weight increased: △, formulation B; □, formulation C; ●, formulation D; ▲, formulation E. Each point represents the mean ± SD ($n = 4$).

except formulation A at 3 h after starting the test was 2.32% (B), 2.56% (C), 0.94% (D), and 0.54% (E). Furthermore, the weight increased with test time, and the values increased at 24 h in formulations B, C, D, and E by 4.53%, 4.10%, 3.81% and 2.50%, respectively. This suggested that the test fluid had penetrated into the suppository. These findings suggested that an increase in Witepsol W35 induced an increase in weight of the suppository due to facilitating water penetration into the suppository.

Penetration of Methylene Blue into the Suppositories

Figure 4 shows the sections of suppositories of formulations B and E after being immersed in pH 7.4 phosphate buffer conforming to dissolved 0.5% methylene blue at 24 h. In addition, the change in the stained area with time of the sections of suppositories of formulations B, C, D, and E is shown in Fig. 5. The ratio of the stained area at 3 h was approximately 3% to 30%, which was equilibrated at 3 h. The findings of the drug dissolution test and the weight change suggested that the drug was easily released from suppositories consisting of fatty acidic triglycerides with less carbon since the solubility of fatty acidic triglycerides was determined based on the number of carbon atoms in glycerides. The triglyceride Witepsol W35 with the small number of carbon atoms was melted more easily in the test fluid compared with the fatty acidic triglyceride with longer chain length. Therefore, the increase in the weight ratio of triglycerides, Witepsol W35, induced more rapid drug release from the suppository by enhancing the penetration of water due to the formation of pores induced by dissolving triglycerides.

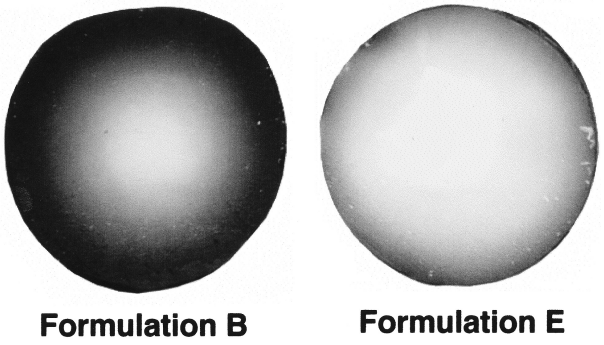

Figure 4. Penetration of methylene blue into suppositories after 24 h setting at 37°C.

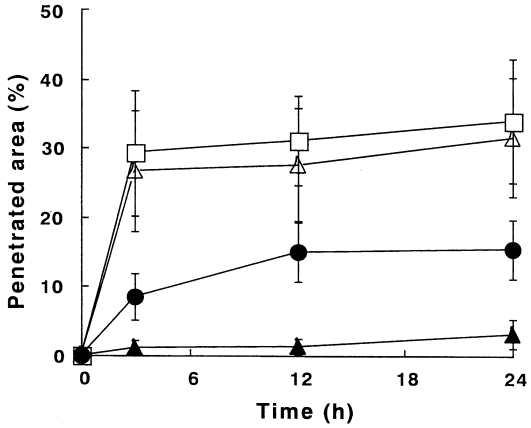

Figure 5. Change in the penetration ratio of methylene blue into suppositories: △, formulation B; □, formulation C; ●, formulation D; ▲, formulation E. Each point represents the mean ± SD ($n = 3$).

Change in the Surface of the Suppositories

It was suggested that the drug was released via the melting of Witepsol W35 and by penetration of water from the pores induced at the surface of the suppository. To confirm this hypothesis, using laser microscopy, we estimated the change in the suppository surface. Figure 6 shows the surface of a suppository at 0 h and 3 h after starting the test. Figure 7 shows the time course changes of the maximum

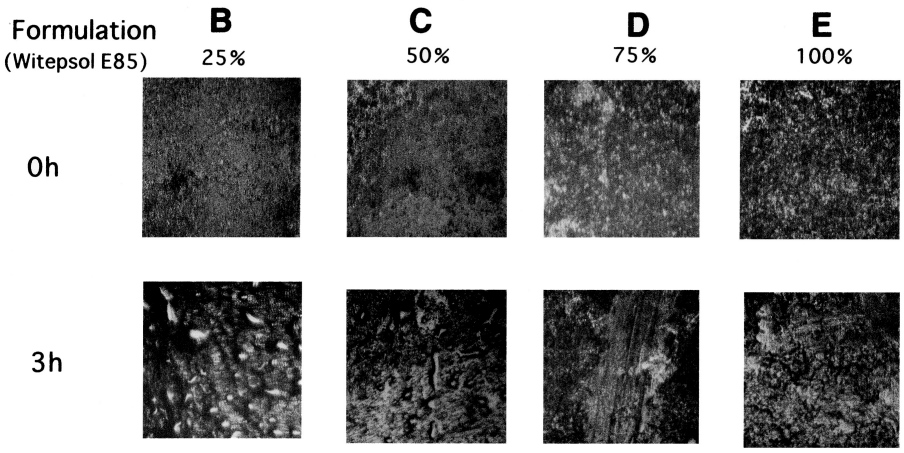

Figure 6. Laser microscopic view of surface after 3 h examination (×20).

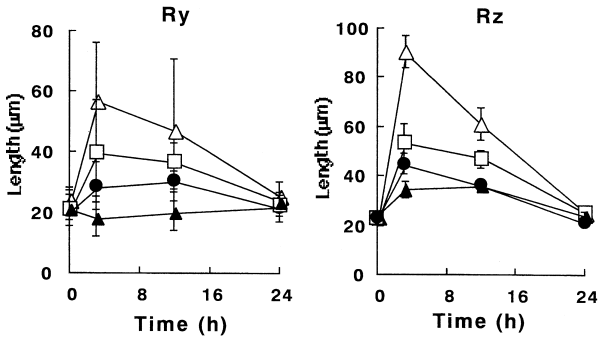

Figure 7. Change in maximum height of the profiles *Ry* and 10-point height of irregulation *Rz*: △, formulation B; □, formulation C; ●, formulation D; ▲, formulation E. Each point represents the mean ± SD ($n = 5$).

height of profiles *Ry* and the 10-point height of irregulations *Rz*, which represents the structure of the surface of the suppository.

The surface of the formulation A suppository containing Witepsol W35 alone could not be observed because it was immediately melted in the phosphate buffer at 37°C. The maximum height of the profiles of the other formulations reached a maximum at 3 h and returned to the initial value after 24 h. However, the 10-point height of irregulation did not show clear differences among the formulations, although the tendency of this parameter was similar to the height differences. These findings suggested that the initial roughness of surface structure was made at the surface of the suppository by dissolution of the Witepsol component, such as Witepsol® 35, but it became smooth due to the formation of pores and the penetration of water compared with the initial state. From these findings, our hypothesis that the mixed-type progesterone suppository released its drug through the matrix after vaginal administration was visually and statistically demonstrated.

In conclusion, we demonstrated that the drug was released from the mixed-type suppository containing hydrophobic drugs such as progesterone by making a matrix with an agent such as diclofenac, as previously reported (5). The formation of a matrix with pores on the surface of the progesterone suppository was confirmed in in vitro experiments. The drug release property, which was evaluated using a dissolution test of the progesterone suppositories, was controllable by altering the ratio of Witepsol W35 to Witepsol E85. The mixed type of progesterone suppository, which discharges a drug over a long period and improves the quality of life, is expected to offer immediate benefits clinically in fields such as in vitro fertilization and embryo transfer. In future studies, it is necessary to investigate in vivo experiments of the mixed type of suppository using Witepsol W35 and Witepsol E85 to clarify its clinical use for luteal support.

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