BIOPHARMACEUTICAL CHARACTERISTICS OF A SUPPOSITORY BASE CONTAINING POLY (OXYETHYLENE)-POLY (OXYPROPYLENE) COPOLYMER, UNILUBE. I. EFFECTS OF A SUPPOSITORY BASE CONTAINING UNILUBE 70DP-950B ON RELEASE AND RECTAL ABSORPTION OF AMINOPYRINE IN RABBIT

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The rectal absorption of aminopyrine (AP) from a three-component-system suppository base (TCS), which is a mixture of oleaginous base, water-soluble base and the poly-(oxyethylene) poly-(oxypropylene) block copolymer, Unilube®, was investigated. Albino rabbits were used as the animal model. The bioavailability of oleaginous base and water-soluble base (polyethylene glycol 4000) were 44.3% and 82.6%, respectively. The time to reach maximum plasma concentration ($T_{\max}$) of the oleaginous and the water-soluble bases were 21.0 ± 8.2 min and 33.0 ± 16.4 min, respectively. When TCS was used, its $T_{\max}$ was 30 min and increasing the amount of Unilube in the base from 5% (w/w) to 20% (w/w) decreased the bioavailability from 100.4% to 47.2%. The softening temperature of TCS was 55–57 °C. Collectively, TCS containing 5% of Unilube gave significantly higher bioavailability than the other bases without softening until 57 °C. Also, the results of the two different release tests were compared with the results of an animal experiment in this paper.

Keywords — poly (oxyethylene) poly (oxypropylene) copolymer; aminopyrine; rectal absorption; suppository base; rabbit; release test; aminopyrine plasma concentration

INTRODUCTION

At the present time, many kinds of suppository bases are used. Suppository bases are broadly divided into three classes, oleaginous, water-soluble and emulsifying bases. It has been reported that the water-soluble and the emulsifying suppository bases have an irritating effect on the rectal mucosal membrane.¹⁾ Although a semi-synthesized triglyceride which is one of oleaginous bases does not irritate the rectal mucosal membrane,¹⁾ it has the following drawbacks, 1) it must be stored in a cool place because of its low melting point, in the range of 32–35 °C and 2) the release of the active ingredient tends to change during storage. On the other hand, water-soluble bases have an advantage in that they are convenient to store because of its higher melting point (45–55 °C).

The three-component-system suppository base (TCS),* which is a mixture of an oleaginous

base, a water-soluble base and Unilube, is classified as an emulsifying base. Unilube is poly-(oxyethylene) poly (oxypropylene) block copolymer. It is reported that Unilube* does not show irritation in ocular tests in rabbits and in rectal tests in rats. The purpose of this paper is to characterize the biopharmaceutical behavior of this suppository base using aminopyrine (AP) as the active component. In this experiment, the ratio of the oleaginous base to the water-soluble base was fixed at 1:1 and the ratio of Unilube was changed from 5% (w/w) to 20% (w/w) in order to study the effect of Unilube. Plasma concentration of AP was monitored during the 3-h post-administration period. Experiments concerning some physicochemical properties of suppositories were also performed.

METHOD

Materials — Unilube 70DP-950B®, Phar-

* Nippon Oil & Fats Co., Ltd., "Pharmasol For Suppositories," Tokyo, Japan.

TABLE I. *Composition of Suppository Bases*

Composition (w/w)	Formula number				
	I	II	IIIa	IIIb	IIIc
B115	100	—	47.5	45	40
PEG	—	100	47.5	45	40
Unilube	—	—	5.0	10	20

Each suppository (0.8 g) contained 750 mg of suppository base and 50 mg of aminopyrine.

masol B115® (B115) and polyethylene glycol 4000 (PEG) were obtained from Nippon Oil & Fats Co., Ltd. Unilube 70DP-950B® contains 68% oxyethylene with a nominal molecular weight of about 12800 and has the following general formula*: $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_a(\text{CH}[\text{CH}_3]-\text{CH}_2\text{O})_b(\text{CH}_2\text{CH}_2\text{O})_c\text{H}$ AP was of JPX grade and was used without further purification. All other chemicals were of reagent grade.

Preparation of Suppositories — AP was passed through a No. 200 sieve to obtain particles <74 μm in size. For preparation of TCS three vehicles were melted and homogenously mixed at 65 °C. The AP was then added and the mixture was continuously mixed until it become homogeneous. The mixture was poured into a plastic

mould and allowed to stand for 1 h at about 10 °C in a refrigerator. The content of AP was 50 mg in each suppository (0.8 g). All other suppositories were prepared in the same manner. The composition of the suppositories are shown in Table I.

Animal Experiment — Male albino rabbits, weighing 2.8–3.5 kg ($n=6$), were fasted overnight but were allowed free access to water. For intravenous administration, AP (50 mg in 1.0 ml of saline) was infused over a period of 30 s into the marginal vein of one ear. Blood samples were taken from the other ear at intervals up to the 3-h post-administration period. For rectal administration, the suppository containing 50 mg of AP was administered and blood samples were

FIG. 1. *In Vitro Release Apparatus*

Keys: 1, thermometer; 2, T-shaped stirrer; 3, 50 mM phosphate buffer (pH 7.0, 500 ml); 4, visking cellulose dialysis tubing; 5, millipore membrane; 6, suppository; 7, magnetic stirring bar; 8, plastic closer; 9, weight (2 g); 10, stainless steel stirrer.

* Nippon Oil & Fats Co., Ltd., "Pharmasol For Suppositories," Tokyo, Japan.

taken.

Determination of Plasma Aminopyrine — AP in plasma was analyzed by high-performance liquid chromatography (HPLC).²⁾ The plasma samples (0.2 ml) were extracted with chloroform (7 ml). After the upper aqueous layer was separated by centrifugation the organic layer (5 ml) was transferred into a glass test tube and evaporated to dryness under reduced pressure. The residue was redissolved in 80 μ l of a mobile phase containing phenobarbital (20 μ g/ml) as an internal standard and a sample (up to 50 μ l) injected into an HPLC system comprising a Hitachi 635S pump and μ Bondapak C₁₈ column (300 $\times$ 4 mm, i.d.). The mobile phase was 10 mM KH₂PO₄ (pH 4.3) containing 25% (v/v) of acetonitrile at a flow rate of 1.0 ml/min and peaks were detected by UV absorption at 260 nm using a Hitachi 635M detector. The peak height ratio was measured and the concentration was determined by comparison with known extracted standards.

Release of Aminopyrine from the Suppository — The release of AP from the suppository was determined using the two types of the apparatus shown in Fig. 1.

Method A: A suppository release apparatus (Toyama Sangyo Co., Ltd., Osaka) was used. A 500 ml of 50 mM phosphate buffer, pH 7.0, was

FIG. 2. Apparatus for Measuring Softening Temperature (Method C) and Liquefaction Time (Method D)

Keys: 1, thermometer; 2, glass rod (8 g); 3, suppository; 4, distilled water (5 ml). 5, The temperature of water bath was increased at a rate of 0.5 $^{\circ}$ C/min. 6, The temperature was kept at 37 $^{\circ}$ C.

degassed and warmed to 37 $^{\circ}$ C in the flask. The suppository was placed on a Milipore membrane (MF-SS, pore size 3.0 μ m; Japan Millipore Ltd., Tokyo) at the bottom of the cylinder and stirred at 10 rpm. The temperature of the solution was held at 37 $^{\circ}$ C, with stirring at 150 rpm. A sample of 2 ml was taken at intervals for 3 h and diluted with two volumes of distilled water. Dissolved AP was assayed spectrophotometrically at 254 nm.

Method B: The modification by Tanabe³⁾ of the method of Thomas was used. A dialysis membrane (Visking Cellulose Tubing, 20/32, 15 cm, Union Carbide Co.) was used.

Differential Scanning Calorimetry — A differential scanning calorimeter (SSC/560S, Daini Seikosha Co., Ltd., Tokyo) was used. Samples (10.0–10.3 mg) were prepared in aluminum cups and were tested against an empty cup blank. The test runs were carried out at a heating rate of 2.0 $^{\circ}$ C/min over the range of 5–70 $^{\circ}$ C.

Liquefaction Time and Softening Temperature — The two techniques shown in Fig. 2 were used to determine the liquefaction time and the softening temperature. The apparatus was a modification of Krowczynski's device.⁴⁾ The suppository sat on the constriction and a glass

FIG. 3. Differential Scanning Calorimetry Scan of Various Suppositories

rod (8 g) was placed on the suppository. In method C, to determine the softening temperature, the temperature of the water bath was increased at a rate of 0.5 °C/min. When the rod reached the constriction, the softening temperature was read. In method D, the liquefaction time was determined in a water path kept at 37 °C. The time of liquefaction was read in the same manner as method C.

RESULT AND DISCUSSION

Differential Scanning Calorimetry

The results of the differential scanning calorimetry (DSC) endotherm scan are given in Fig. 3. Base I showed a double peak. The major peak was at 36 °C and the minor peak was at 31.5 °C. Base II showed a single peak at 58 °C. In the case of TCS (IIIa, IIIb, IIIc), two kinds of peaks were observed. The peak observed with the major peak position at 36 °C appeared to be the endotherm peak of B115. With the increase in the content of Unilube, this peak broadened, decreased and became a single peak. The other endotherm peak observed at 58 °C might be of PEG and a sharpening and a decrease of the peak were observed.

Liquefaction Time and Softening Temperature

Table II summarizes the results of the base screening study. The liquefaction times of base I and base II were 5 min and 37 min, respectively. The liquefaction time of the TCS base was prolonged from 19 min (base IIIa) to 61.5 min (base IIIc) with the increase in the content of Unilube. The results of the softening temperature (Table II) show that all of the suppository bases, except base I, did not soften below 55 °C. This finding suggested that a TCS suppository base has a higher softening temperature, as high as PEG and Unilube. Also, with the increase in Unilube content, liquefaction time was delayed. B115 melts at body temperature and is insoluble in rectal fluid. Unilube dissolves in rectal fluid and has a higher melting point. A TCS suppository

base is a mixture of B115, PEG and Unilube. Prolongation of liquefaction time by the addition of Unilube in TCS is probably due to a lower dissolution rate of Unilube than PEG. In this study, the softening temperature of base I was slightly higher than the peak position (36 °C) of base I given by DSC analysis. The reason appeared to be that the heating rate was too fast and that the poor heat transfer characteristics of fats prevented accuracy due to the lag time in the return to an equilibrium.

Release of Aminopyrine from Suppositories

Many *in vitro* methods of drug release from suppositories have been reported. In this study, two different methods were used and compared. The release characteristics *in vitro* of various suppositories by method A are shown in Fig. 4 and the results by method B are given in Fig. 5. In Fig. 4, the amounts of AP released from PEG (base II) was higher than that occurring from B115 (base I). In the case of TCS, although the release amount from base IIIa was higher than that from the other bases (base IIIb and base IIIc) until 60 min. After 90 min the release from base IIIa stopped and finally the release amount from base IIIb and base IIIc became slightly higher than that from base IIIa. In contrast, in Fig. 5, the release rate of base I was much faster than that of base II, but the release amount at the end of the release period did not differ. For the TCS suppository base, only base IIIc showed a slower release than base IIIa and base IIIb. No difference between base IIIa and base IIIb was observed. The difference of the release characteristics of base I and base II between method A and method B resulted from the different pore size of the membrane separated receptor solution from the suppository base. In method A, PEG dissolved in the receptor solution easily and passed through the larger pores (3.0 µm) of the membrane alone or with AP. B115 could not pass through the membrane due to its hydrophobic property. For this reason, the release rate of

TABLE II. *Comparison of Pharmaceutical Properties for Various Suppositories*

	Formula number				
	I	II	IIIa	IIIb	IIIc
Liquefaction time (min)	5	37	19	36.5	61.5
Softening temperature (°C)	39.5	57	57	55	55

FIG. 4. Release Profile of Aminopyrine from Different Suppository Bases by Method A
Keys: ●, base I; △, base II; ○, base IIIa; ▲, base IIIb; □, base IIIc.

FIG. 5. Release Profile of Aminopyrine from Different Suppository Bases by Method B
Keys: ●, base I; △, base II; ○, base IIIa; ▲, base IIIb; □, base IIIc.

PEG might be apparently faster than B115 in method A. In contrast, the pore size of the membrane used in method B is 2.5 nm. PEG could hardly pass through the membrane. The PEG inside the dialysis tubing drag water through the membrane by its own hydrophilic property and gradually dissolved. Thus, the concentrated PEG solution inside the membrane trapped AP and decreased the penetration of AP through the membrane. On the other hand, B115 could not go through the membrane, in the same way as method A and showed a similar release profile in both methods.

Rectal Absorption in Rabbits

The mean plasma concentration of AP after

rectal administration of various suppositories are given in Fig. 6 and the bioavailability parameters are shown in Table III. For base I, relative bioavailability was found to be about 44% of that after intravenous dosing. For base II this value was 82.6%. The lower bioavailability of base I than that of base II was more parallel with the results in Fig. 4 than that shown in Fig. 5. In the case of TCS, base IIIa showed the highest bioavailability (100.4%). This value decreased and the T_{max} tended to delay as the Unilube content increased. These results correlated with the facts that the release amount, in Fig. 4 and Fig. 5, decreased as the Unilube content increased in the TCS suppository bases, even though the dif-

FIG. 6. Plasma Concentration of Aminopyrine after Rectal Administration of Various Suppositories
 Each point represents the mean $\pm$ S.E. of 6 rabbits.
 Keys: ●, base I; △, base II; ○, base IIIa; ▲, base IIIb; □, base IIIc.

ferences in the extent of the release were less significant than that given in the rectal study.

In the case of a water-soluble base, the drug release from the suppository is affected by the amount of aqueous medium. In these release tests the results were not consistent with the *in vivo* results. Since the aqueous medium passed freely through the membrane in method A and fairly well even in method B, significant differences might not be obtained. This explanation is supported by the observation that significant differences were obtained in the results of liquefaction time shown in Table II and that they were in good agreement with the *in vivo* results. In the case of B115, however, it liquefied quickly and the drug remained dissolved in the base. Therefore, it is thought that the delay in the dis-

solution of the suppositories is responsible for the decrease in the bioavailability with the increase of the Unilube content. However, the mechanism of the increase in bioavailability in base IIIa is not clear.

The softening temperature of TCS bases was 55–57 °C and was independent of the Unilube contents in the formulation studied. This higher softening temperature indicate that this TCS base is a convenient suppository base for storage as PEG. In addition, it might be possible to control physical properties of the TCS base and the absorption rate by changing the ratio of the three kinds of components in the TCS suppository base.

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TABLE III. Bioavailability Parameter Values for Aminopyrine Suppositories

Formula No.	AUC ($\mu\text{g} \cdot \text{min}/\text{ml}$)	C_{max} ($\mu\text{g}/\text{ml}$)	T_{max} (min)	F^a
I	342.9 ± 42.4	4.56 ± 0.79	21.0 ± 8.2	0.443
II	639.1 ± 70.6	8.33 ± 2.54	33.0 ± 16.4	0.826
IIIa	776.6 ± 50.8	10.76 ± 1.86	30.0 ± 0	1.004
IIIb	617.0 ± 63.0	8.73 ± 2.37	33.0 ± 16.4	0.798
IIIc	365.1 ± 57.2	4.27 ± 1.44	48.0 ± 26.8	0.472
Intravenous administration	773.4 ± 136.0	—	—	1

a) $F = AUC_{\text{rectal}} / AUC_{\text{intravenous}}$
 Each value represents the mean $\pm$ S.E. of 6 rabbits.

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