Study on Absorption of Indomethacin from Sustained-Release Suppositories Containing Hydrogenated Soybean Lecithin in Rabbits

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The absorption of indomethacin (IM) from suppositories containing hydrogenated soybean lecithin (HL) after rectal administration in rabbits was investigated with the aim of producing sustained-release suppositories. The suppositories were prepared by the fusion method with IM, HL and Witepsol® H-15 (H-15). The IM release rate from the suppositories (IM 10 mg, HL 200 mg, total weight 1 g) was faster than that of the control suppositories without HL.

The release of IM from the suppositories (IM 10 mg, HL 300 mg or 350 mg) showed slow-release profiles. In absorption studies in rabbits, sustained-plasma levels of IM were obtained when suppositories having an HL content of more than 300 mg were administered. The suppositories composed of Witepsol® E-85 (melting point approximately 43 °C), 10 mg of IM, and 200 mg of HL, showed slow-release profiles in the release test, but did not show sustained plasma levels of IM in the absorption test.

These results indicate that sustained-release suppositories able to relese IM gradually from the surface of the suppositories can be obtained when HL, IM and H-15, whose melting point is lower than body temperature, are used in the preparation of the suppositories, provided that the HL content is high enough in relation to IM.

Keywords indomethacin; hydrogenated soybean lecithin; suppository; sustained-release suppository; absorption; rabbit

In the previous report,[1] indomethacin (IM) slow-release suppositories were obtained when hydrogenated soybean lecithin (HL) was added at over 30% (w/w) to Witepsol® H-15 (H-15) and the IM content was 10 mg per suppository. It was considered that IM was dissolved gradually from the surface of the suppositories because HL at a sufficient content to give slow release of IM made the suppositories hard.

In this study, we investigated the absorption of IM from suppositories containing HL after rectal administration in rabbits. Furthermore, the effects of high melting point (mp) suppository bases such as Witepsol® E-85 (E-85, mp approximately 43 °C) on the release of IM from the suppositories in the release test and the absorption of IM from the suppositories in rabbits were investigated.

Experimental

Materials The sources of materials used in this work were as follows: IM from Sumitomo Chemical Co., Ltd., HL from Nikko Chemicals Co., Ltd., H-15 and E-85 from Dynamit Nobel Co., Ltd. All other chemicals were reagent-grade commercial products.

Preparation of Suppositories Suppositories were prepared as follows[1]: H-15 or E-85 (31.5—49.5 g) and HL (0—17.5 g) were fused in a beaker on an oil bath at 80 °C. Next, IM (0.5 g) was added and dissolved in the fused bases. The fused bases containing IM with or without HL were cooled to 70 °C and poured into suppository molds (1.0 ml in volume), which were quickly placed in a refrigerator at 5 °C. Table I shows the formulae of the suppositories.

Release of IM from Suppositories Release of IM was measured using the Muranishi method as described previously[1]: the test solution was a 0.2 M phosphate buffer solution (pH 7.2). A suppository was placed in 3 ml of the buffer solution in a cylindrical cell equipped with a Millipore filter (pore size 3.0 μm, type SS) and stirred with a rod at 25 rpm. The cell was connected with a glass vessel containing 300 ml of the test solution which was stirred with a magnetic stirrer at 100 rpm. At an appropriate time, 1 ml of the releasing fluid was removed and 1 ml of the fresh buffer solution was added to the glass vessel to maintain the original volume. IM concentration was assayed spectrophotometrically at 318 nm.

TABLE I. Formulae of Suppositories

Rp.	IM (mg)	HL (mg)	Base (mg)
1	10	0	H-15 (Total 1000)
2		200	
3		300	
4		350	
5		0	E-85 (Total 1000)
6		200	
7		350	

Animal Experiments White male rabbits, each weighing 2.8—3.6 kg, were fasted for 48 h prior to the experiment, but allowed free access to water. The suppositories (IM, 3 mg/kg) were inserted manually. Retention of the suppositories by the rabbits was ensured by fastening the anus with a clip after insertion. Blood samples (2 ml) were taken by cardiac puncture from rabbits at different time intervals. The plasma was obtained by centrifugation at 3000 rpm for 10 min. The area under the concentration–time curve (AUC) was calculated by means of the trapezoidal method from zero to 10 h.

Assay of IM in Plasma The plasma (0.7 ml) was pipetted into a glass-stoppered centrifuge tube containing 2 ml of 0.2 M citrate buffer solution (pH 3.6) and 10 ml of ethyl acetate. The test tube was mechanically shaken for 10 min and then centrifuged at 3000 rpm for 10 min. An 8 ml aliquot of the ethyl acetate phase was pipetted into another centrifuge tube and evaporated to dryness under reduced pressure. The residue was dissolved in 250 μl of a mobile phase. A 20 μl sample was injected into a high-performance liquid chromatography apparatus (a Hitachi 655-12 liquid chromatograph with a Hitachi 655A variable-wavelength ultraviolet (UV) monitor).

The conditions used during analysis were as follows: column, 15 cm × 4 mm i.d.; packing TSK-LS410 (5 μm) ODS; mobile phase, methanol–water–acetic acid–triethanolamine (74.3 : 25 : 0.5 : 0.2); flow rate, 0.5 ml/min; detection wavelength, UV at 260 nm; column temperature, 50 °C.

Results and Discussion

Release and Absorption Studies on Suppositories (H-15 Base) Containing HL Figure 1A shows the release profiles of IM from H-15 base suppositories having HL contents in the range from 200 to 350 mg. The release rate from the Rp. 2 suppository (HL 200 mg) was fast, but the Rp. 3 and Rp. 4 suppositories (HL 300 and 350 mg, respectively) showed slow-release profiles. The Rp. 3 and Rp. 4 suppositories did not disintegrate or melt entirely, and the shape of the suppositories was maintained (macroscopic observation). As reported previously,[1] HL increased the melting point and hardness of the suppositories. Thus, the

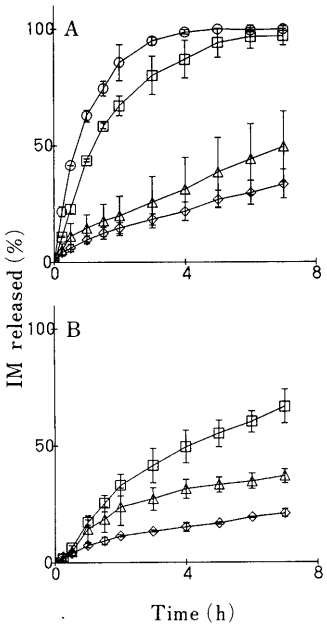

Fig. 1. Release Profiles of IM from Various Suppositories

A: H-15 base suppositories. □, Rp. 1 (IM 10 mg, HL 0 mg); ○, Rp. 2 (IM 10 mg, HL 200 mg); △, Rp. 3 (IM 10 mg, HL 300 mg); ◇, Rp. 4 (IM 10 mg, HL 350 mg).
B: E-85 base suppositories. □, Rp. 5 (IM 10 mg, HL 0 mg); △, Rp. 6 (IM 10 mg, HL 200 mg); ◇, Rp. 7 (IM 10 mg, HL 350 mg).
Each value represents the mean ± S.E. ($n=3$).

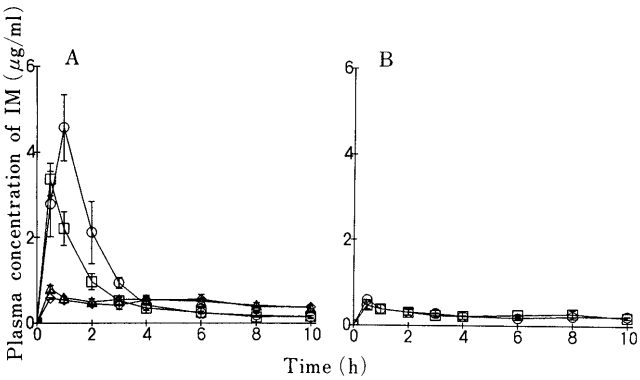

Fig. 2. Plasma Concentration of IM after Rectal Administration of Various Suppositories

A: H-15 base suppositories. □, Rp. 1 (IM 10 mg, HL 0 mg); ○, Rp. 2 (IM 10 mg, HL 200 mg); △, Rp. 3 (IM 10 mg, HL 300 mg); ◇, Rp. 4 (IM 10 mg, HL 350 mg).
B: E-85 base suppositories. □, Rp. 5 (IM 10 mg, HL 0 mg); ○, Rp. 6 (IM 10 mg, HL 200 mg).
Each value represents the mean ± S.E. ($n=3$).

TABLE II. Pharmacokinetic Parameters of Various Suppositories

Rp.	IM (mg)	HL (mg)	Base (mg)	C_{max} (μg/ml)	T_{max} (h)	ACU_0^{10} (h·μg/ml)
1	10	0	H-15 (Total 1000)	3.35 ± 0.38	0.5	6.30 ± 0.71
2		200		4.75 ± 0.77	1.0	9.46 ± 2.31
3		300		0.80 ± 0.08	0.5	4.90 ± 0.32
4		350		0.57 ± 0.01	0.5	4.57 ± 0.56
5	10	0	E-85 (Total 1000)	0.46 ± 0.08	0.5	2.59 ± 0.01
6		200		0.59 ± 0.00	0.5	2.49 ± 0.52

Each value of C_{max} and AUC_0^{10} represents the mean ± S.E. ($n=3$).

HL content of the Rp. 2 suppository was insufficient, whereas the HL contents of the Rp. 3 and Rp. 4 ones were sufficient for the production of slow-release suppositories.

Figure 2A and Table II show the results of the IM absorption study in rabbits. Although there was no significant difference of AUC between Rp. 1 and Rp. 2, the plasma concentration of IM at 1 h after administration of Rp. 2 suppository was significantly higher than that in the case of Rp. 1 suppository ($p<0.05$). This seemed to be correlated to the release test, that is, the release rate of IM from Rp. 2 suppository was faster than that from Rp. 1 suppository. The reasons are considered to be that HL acted as a dispersing agent and a surfactant[3] or an absorption enhancer[4] because the HL content was insufficient to provide a slow-release suppository. On the other hand, the Rp. 3 (HL 300 mg) and Rp. 4 (HL 350 mg) suppositories, whose release rates of IM were slow, showed sustained plasma levels of IM.

As discussed in a previous paper,[1] in cases when the HL content exceeded 300 mg, the hardness of the suppositories increased, and IM was observed to dissolve gradually from the surface of the suppositories. In the absorption test, it was thought that the same phenomena occurred in the rectum of rabbits; thus, a sustained plasma level could be obtained.

In the previous paper,[1] the release test using the Muranishi method was compared with the modified Thomas method,[5] *i.e.* the Visking tubing method. In the modified Thomas method, the release profiles of IM from the Rp. 2, 3 and 4 suppositories showed almost the same slow-release profiles, although the release rate of IM from the Rp. 2 suppository was fast using the Muranishi method. When the two release tests were correlated with the absorption study, the Muranishi method showed better correspondence to the absorption test than the modified Thomas method. This is because the stirring stress or the force imposed on the suppository was greater in the Muranishi method than in the modified Thomas method, and the Visking tubing acted as a cushion barrier in the modified Thomas method. Thus, the Rp. 2 suppository showed a slow-release profile in the modified Thomas method since the Rp. 2 suppository did not melt or disintegrate entirely during testing (macroscopic observation).

Release and Absorption Studies on Suppositories (E-85 Base) Containing HL The hardness and the melting point of the suppositories containing HL were considered to be important factors for the slow-release of IM in the release test and the sustained plasma level of IM in the absorption test. Thus, suppositories were prepared with E-85, which has a higher melting point (approximately 43 °C) than that of H-15 (approximately 34 °C), to investigate the release and the absorption of IM.

Figure 1B shows the release profiles of IM from the suppositories (E-85 base). The Rp. 5 suppository (HL 0 mg) showed a slow-release profile. The Rp. 6 suppository (HL 200 mg) also showed a slow-release profile although the release rate of IM from the Rp. 2 suppository (HL 200 mg, H-15 base) was fast. The release rate of IM from the Rp. 7 suppository (HL 350 mg) was considerably slow. The Rp. 1 suppository (H-15 base) melted fast in the cylindrical cell of the release machine used in the Muranishi method. Although the Rp. 5 (E-85 base) suppository hardly melted, it was disintegrated owing to the stirring stress and shear imposed by the rod of the release machine. Thus, the Rp. 5

suppository released 70% of IM within 7 h. On the other hand, the Rp. 6 suppository hardly melted or disintegrated during testing because HL made the suppository harder. When HL swelled at the surface of the suppository (H-15 base), since the interaction of HL, IM and H-15 seemed to be weakened, H-15 gradually melted. On the other hand, E-85 base suppository containing HL (Rp. 6 suppository) hardly melted (macroscopic observation) because of the high melting point of E-85 even if HL became swollen. IM seemed to be released from the matrix of the suppository (E-85 base). Thus, the release rate of IM from the Rp. 4 suppository did not become slower at the late phase of the release test, whereas the release rate of IM from the Rp. 6 suppository did do so.

Figure 2B and Table II show the results of an absorption study. The *AUC* values of the Rp. 5 and 6 suppositories were approximately 40%, compared with the Rp. 1 suppository (H-15 base, HL 0 mg). Although the Rp. 5 suppository released 70% of IM within 7 h and the Rp. 6 suppository released 40% of IM at 7 h in the release test, the IM plasma levels were almost the same and considerably low. This is because the stirring stress or the force imposed on the suppository by the rod of the release machine using the Muranishi method was considerably strong; there is presumably less stress or force in the rabbit rectum. It was also presumed that the amount of rectal fluid[6] was less than that of the release test solution. It was concluded that only when HL, IM and H-15, whose melting point was lower than body temperature, were used for preparation of the suppository, did the sustained plasma level of IM appear, because IM dissolved gradually from the surface of the suppository and was absorbed in the rabbit rectum. The gradual dissolution of IM can be attributed to the hardness of the suppository because of the interaction of HL, IM and H-15.

References and Notes

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