Indomethacin Sustained-Release Suppositories Containing Sugar Ester in Polyethylene Glycol Base

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Indomethacin (IM) sustained-release suppositories were prepared by the fusion method using sugar ester and polyethylene glycol 4000 (PEG). The suppositories were evaluated by *in vitro* release testing, X-ray analysis and *in vivo* absorption testing in rabbits. X-ray analysis showed that IM was amorphous in PEG-base suppositories. In a release test, slow-release was obtained when the sugar ester content of a suppository was 60%. The IM plasma level following the administration of the suppository was well sustained in the absorption test. The main slow-release mechanism is considered to be the release of IM from the matrix composed of sugar ester and PEG, which is represented by the Higuchi equation. A good correlation between the release test and the absorption test was obtained. It is considered that the amorphous state of IM in this type of sustained-release suppository would enhance the release and absorption of IM in the rectum of the rabbit, whose rectal fluid volume is small.

Keywords indomethacin; sugar ester; suppository; sustained-release suppository; polyethylene glycol; amorphous; absorption

In previous papers,[1,2] we reported that indomethacin (IM) sustained-release suppositories were obtained when approximately 30% of hydrogenated soybean lecithin (HL) or sucrose fatty acid ester (sugar ester) was added to a Witepsol® H-15 (H-15) base.

The mechanism of the sustained-release of IM from these suppositories is thought to be that HL or sugar ester makes the suppositories hard and that IM is dissolved gradually from the surface of the suppositories because sugar ester has properties similar to HL: for example, they have two acyl chains and almost the same fatty acid component. There was, however, a difference between the suppositories containing HL and those containing sugar ester concerning the correlation between the release test using the Muranishi method[3] and the absorption test in rabbits. The suppositories containing HL showed a good correlation, that is, the IM slow-release suppositories caused a sustained-plasma level of IM. On the contrary, the suppository containing sugar ester did not show a good correlation; that is, the suppositories containing more than 52.5% of sugar ester showed slow-release profiles, but the plasma level of IM was not sustained and the area under the concentration–time curve (*AUC*) was considerably lower. On the other hand, the suppositories containing 30.0% of sugar ester did not show a slow-release profile, but the plasma level of IM was well sustained and the *AUC* was adequate compared with the control suppositories.

The difference between the suppositories containing HL or sugar ester concerned the IM crystallinity in them. Those with HL contained IM in the amorphous state, while those with sugar ester contained IM in the crystal state. Then, in this paper, we tried to prepare sustained-release suppositories containing sugar ester in which IM existed in amorphous state, in order to investigate the correlation of the release test and the absorption test.

Polyethylene glycol is widely used as a suppository base and is also used to make a solid dispersion[4] in order to enhance the solubility of drugs. Ohnishi *et al.*[5] reported on IM sustained-release suppositories prepared with solid matrices or solid dispersions composed of several kinds of polymer and polyethylene glycol.

In the present study we used sugar ester, not a polymer but a surfactant, and polyethylene glycol 4000 (PEG) to prepare sustained-release suppositories with IM in the amorphous state.

The suppositories containing sugar ester were evaluated by *in vitro* release testing, X-ray analysis, thermal analysis and *in vivo* absorption testing in rabbits.

Experimental

Materials The sources of the materials used in this work were as follows: IM from Sumitomo Chemical Co., Ltd. (Osaka, Japan), PEG from Sanyo Kasei Co., Ltd. (Tokyo, Japan), sugar ester (DK ester F-20w (SE) and F-10 (SE-2)) from Dai-ichi Kogyo Seiyaku Co., Ltd. (Kyoto, Japan). The ester value of the SE and SE-2 was 2.08 and 4.85, respectively. The fatty acid of sugar ester was derived from tallow, and its components in the SE and SE-2 were palmitic acid and stearic acid, 30% and 70%, respectively.[6] All other chemicals were reagent-grade commercial products.

Preparation of IM Suppositories Suppositories were prepared as follows: PEG (19.5—49.5 g) and SE or SE-2 (0—30 g) were fused in a beaker in an oil bath at 80 °C. Then, IM (0.5 g) was added and dissolved in the fused bases. The fused bases 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. The samples were stored at 5 °C and were used for experiments within 1 week.

X-Ray Diffraction The X-ray diffraction patterns of the fused mixtures (IM, SE and PEG) were measured to investigate their crystallinity.

The fused mixtures were prepared in the same manner as the suppositories. The crystallinity of the IM in the bases was measured with a Rigaku Geigerflex RAD-C2 (Rigaku Denki Co., Ltd., Tokyo, Japan) under the following measurement conditions: source, Cu-K_α radiation; filter, Ni filter; voltage, 40 kV: current, 30 mA; scanning speed, 4° (2θ)/min.

Calorimetric Study A differential scanning calorimeter (Du Pont Instrument Co., thermal analyzer 1090B, Wilmington, U.S.A.) was used for examining the thermotropic properties of suppositories with a heating rate of 10 K/min. Samples weighing about 5 mg were used.

Release of IM from Suppositories The release of IM was measured using the following three methods: 1) the Muranishi method, as described

TABLE I. Formulae of Suppositories Containing SE and SE-2

Rp.	IM (mg)	SE (mg)	SE-2 (mg)	PEG (mg)
1	10	0	—	Total 1000
2		500	—	
3		600	—	
4	10	—	300	
5		—	400	
6		—	450	
7		—	500	

previously[1]; 2a) the modified Thomas method[7] with a Visking tube: A suppository was put in a Visking tube, which was then placed in 120 ml of the test solution in a flask. The flask was incubated at 37 °C in a shaker (EYELA shaker SS-8, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and agitated at 30 rev./min; 2b) the no-Visking-tube method: A suppository was placed in 120 ml of the test solution in a flask without a Visking tube. The other procedures were the same as described in 2a).

In each test, the IM concentration was assayed spectrophotometrically at 318 nm. Each value represents the mean ± S.E. ($n=3$).

Animal Experiments Male albino rabbits, each weighing 2.8—3.6 kg, were fasted for 48 h before the experiments, but were 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 (2 ml) was taken from the rabbits by cardiac puncture at appropriate time intervals. The plasma was obtained by centrifugation at 3000 rpm for 10 min. *AUC* was calculated by means of the trapezoidal method, from 0 to 10 h.

Assay of IM in Plasma Plasma (0.7 ml) was pipetted into a glass-stoppered centrifuged tube containing 2 ml of 0.2 M citrate buffer (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. Eight milliliters of the ethyl acetate was pipetted into another centrifuged tube and evaporated to dryness under reduced pressure.

The residue was dissolved in 250 μl of the mobile phase. A 20 μl sample was injected into a high-performance liquid chromatography apparatus (Hitachi 655-12 liquid chromatograph with a Hitachi 655A variable-wavelength ultraviolet (UV) monitor). The conditions for 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, v/v); flow rate, 0.5 ml/min; wavelength, UV at 260 nm; column temperature, 50 °C.

Results and Discussion

Crystallinity of IM in Bases The crystallinity of IM in the suppository bases and in the fused mixture of IM, SE and PEG was investigated by X-ray diffractometry. Figure 1 shows the X-ray diffraction spectra of various samples. Since the characteristic diffraction peak ($2\theta=11.5°$) of IM crystals did not overlap the peaks derived from PEG, it was regarded as being the characteristic peak of IM crystals in the bases. As described previously,[1] due to the low sensitivity of IM, an increased amount was used. The fused mixture of PEG and IM did not show the characteristic peak, and neither did the fused mixture of PEG, SE and IM. Thus, IM in a suppository containing SE that was prepared as described in the experimental section was amorphous, like the IM in the suppository containing HL, as described previously.[1]

Thermotropic Properties of Suppositories and Bases Figure 2 shows the differential scanning calorimetry pattern of the suppositories and bases. PEG, SE and the suppository without SE showed an endothermic peak at about 61 °C, while the suppository with SE showed a small shoulder peak at a rather high temperature. It seemed that the interaction among SE, PEG and IM or between SE and PEG caused a shoulder peak like that of the suppository containing HL.[1]

Release of IM from Suppositories Figures 3 and 4 show the effect of the SE-2 and SE content on the release of IM from suppositories, using the Muranishi method. The release rates of IM from the Rp. 4 and 5 suppositories (SE-2, 300 and 400 mg) were quite rapid, almost the same as for the Rp. 1 (control). The Rp. 6 and 7 suppositories (SE-2, 450, 500 mg), however, showed extremely slow-

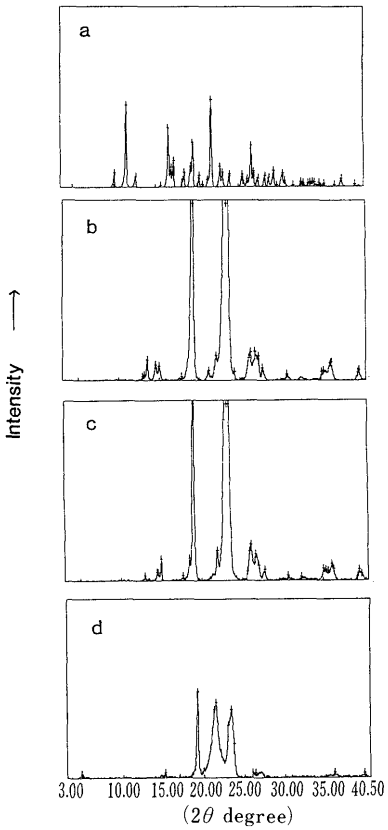

Fig. 1. X-Ray Diffraction Patterns

Alone: a, IM; b, PEG. Fused mixture: c, PEG : IM = 9 : 1 (w/w); d, PEG : SE : IM = 4 : 5 : 1 (w/w).

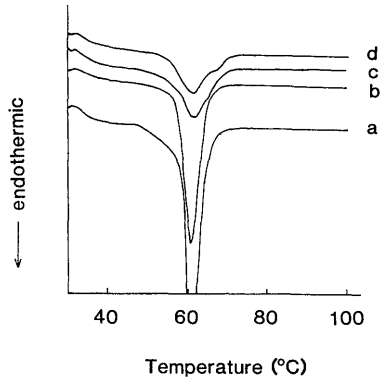

Fig. 2. Differential Scanning Calorimetry Thermograms

a, PEG; b, PEG : IM = 99 : 1 (w/w); c, SE; d, PEG : SE : IM = 39 : 60 : 1 (w/w).

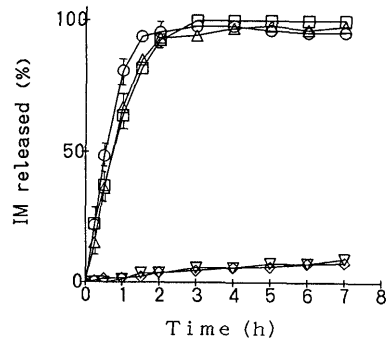

Fig. 3. Release Profiles of IM from Various Suppositories Using the Muranishi Method

SE-2 content: □, 0 mg (Rp. 1); ○, 300 mg (Rp. 4); △, 400 mg (Rp. 5); ▽, 450 mg (Rp. 6): ◇, 500 mg (Rp. 7).

release profiles and inhibited the release of IM. Further, the Rp. 2 suppository (SE, 500 mg) showed a rather slow-release profile like the Rp. 3 (SE, 600 mg). The Rp. 3 suppository did not melt or disintegrate entirely, and the shape was maintained (macroscopic observation) while testing although the size decreased gradually. IM seemed to be released gradually from the surface and the matrix of the suppositories because the content of SE was sufficient to make the suppositories hard and to regulate its release. In the case of SE-2, the suppositories inhibited rather than slowed the release of IM. A smaller amount of SE-2 inhibited the release of IM from the suppositories than of SE because SE-2 has higher ester value (lower hydrophile-lipophile balance) and higher hydrophobicity.

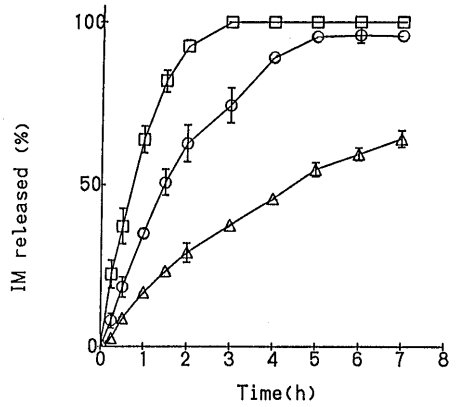

Fig. 4. Release Profiles of IM from Various Suppositories Using the Muranishi Method

SE content: □, 0 mg (Rp. 1); ○, 500 mg (Rp. 2); △, 600 mg (Rp. 3).

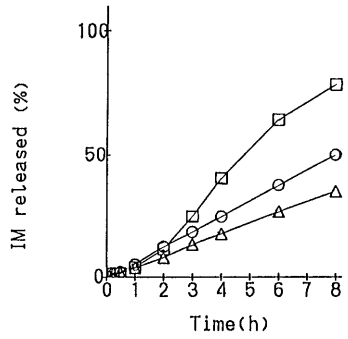

Fig. 5. Release Profiles of IM from Various Suppositories Using the Visking Tube Method

Abbreviations are the same as in Fig. 4.

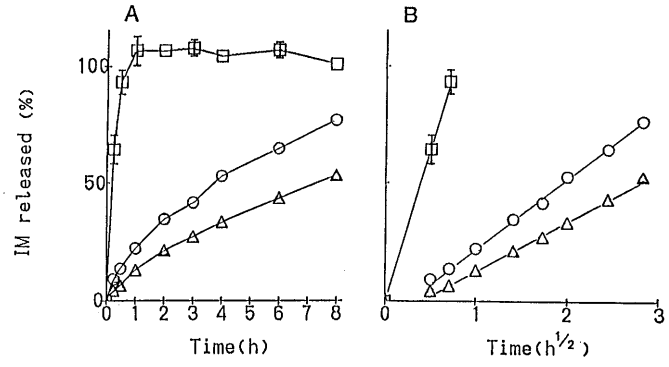

Fig. 6. Release Profiles of IM from Various Suppositories Using the No-Visking-Tube Method

Abbreviations are the same as in Fig. 4. The unit of the horizontal axis: A, h; B, the square root of h.

Figure 5 shows the results of the release test using the Visking tube method. As PEG was not released through the Visking tube membrane, the release of IM from the Rp. 1 suppository was reduced by the cellulose membrane barrier. The Rp. 2 and 3 suppositories also showed slow-release profiles.

Figure 6A shows the results of the release test in the no-Visking-tube method. The Rp. 1 suppository showed a fast-release IM profile and dissolved immediately in the test solution, while the Rp. 2 and 3 showed slow-release profiles although the Visking tube was not used. Neither of the latter two suppositories melted or disintegrated entirely during testing, but showed almost the original shape even after testing (macroscopic observation). Figure 6B shows the results of the release test when the unit of the horizontal axis of Fig. 6A represents the square root of h. The Rp. 2 and 3 suppositories are shown as a straight line in Fig. 6B.

These data indicate that the mechanism of the slow-release of IM was as follows: solid dispersed IM and PEG were gradually dissolved in the test solution from the matrix containing SE. An apparent leaching type of release mechanism, proposed by Higuchi[8] in Eq. 1, may be applied.

$$Q=[D\pi(2A-\pi C_s)C_s t/\tau]^{1/2} \quad (1)$$

where Q = the amount of drug released after time t per unit of exposed area; D = the diffusitivity of the drug in the permeating fluid; τ = the tortuosity factor of the capillary system; A = the total amount of drug present in the matrix per unit volume; C_s = the solubility of the drug in the permeating fluid, and π: the porosity of the matrix.

The release-rate of IM from the Rp. 2 and 3 suppositories was faster by the Muranishi method than by the no-Visking-tube method. The reason was considered to be that the stirring stress or force exerted on the suppository was greater by the Muranishi method; thus, the release of dissolution of IM from the surface of the suppository seemed to be enhanced.

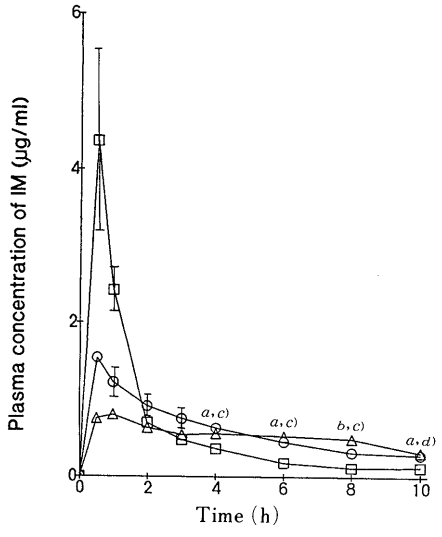

Fig. 7. Plasma Concentration of IM after Rectal Administration of Various Suppositories

Abbreviations are the same as in Fig. 4. Each value represents the mean ± S.E. (n=3—4). *a*) $p<0.01$ in Rp. 3 *vs.* Rp. 1. *b*) $p<0.05$ in Rp. 3 *vs.* Rp. 1. *c*) $p<0.05$ in Rp. 2 *vs.* Rp. 1. *d*) Rp. 1 *vs.* Rp. 2 (not significant, $p>0.05$).

TABLE II. Pharmacokinetic Parameters of IM Following Administration of Various Suppositories

Rp.	IM (mg)	SE (mg)	PEG (mg)	C_{max} (μg/ml)	T_{max} (h)	AUC_0^{10} (μg·h/ml)
1	10	0	Total 1000	4.37±1.15	0.5	6.46±0.72[a,b]
2		500		1.56±0.11	0.5	6.16±0.57[a]
3		600		0.80±0.09	1.0	5.39±0.46[b]

Each value of C_{max} and AUC_0^{10} represents the mean ±S.E. ($n=3$—4). *a*) Rp. 1 *vs.* Rp. 2 (not significant, $p>0.05$). *b*) Rp. 1 *vs.* Rp. 3 (not significant, $p>0.05$).

Nishihata *et al.*[9] reported on the sustained-release suppositories of sodium diclofenac containing lecithins. They also concluded that the slow-release mechanism of diclofenac from the suppositories was as presented by Higuchi Eq. 1. As reported in our previous paper,[1] the suppositories containing HL gradually became smaller while keeping their approximate shape in the cylindrical cell of the Muranishi method (macroscopic observation). Thus, we considered that the main slow-release mechanism was the gradual dissolution of IM from the surface of the suppositories containing HL. Here, both mechanisms were considered in the Muranishi method, that is, the slow-release from the matrix of the suppositories, and the gradual dissolution from the surface of the suppositories.

Absorption Studies on Suppositories Containing SE Figure 7 and Table II show the results of the absorption testing of IM from the suppositories containing SE. The Rp. 2 suppository depressed the rapid absorption of IM earlier during the testing than the Rp. 1, and the plasma level of IM was sustained up to 8 h. On the other hand, the IM plasma level of the Rp. 3 suppository was well sustained, the AUC value was not as low ($p>0.05$) as the Rp. 1 suppository, and the T_{max} was 1 h, although that of other suppositories was 0.5 h. A good correlation was obtained between the release tests in the Muranishi method and the animal test.

As described previously,[2] in the H-15 base suppositories containing SE, a good correlation was not obtained between the release test of the Muranishi method and the absorption test. The reason was thought to be that IM was in a crystalline state in the H-15 base containing SE, but in the amorphous state in the PEG base containing SE. Amorphous IM in this type of slow-release suppository seemed to have a positive effect on the release and absorption of IM in the rectum of a rabbit because the volume of rectal fluid is small[10] and the force of the peristalsis against the suppository was less than that of the release machine of the Muranishi method. As described previously,[1] the H-15 base suppositories containing HL in which IM was amorphous, also showed a good correlation in the release and absorption tests.

In conclusion, we were able to prepare a sustained-release suppository when 600 mg of SE was added to a PEG base. The main mechanism of the slow-release of IM seemed to be as represented by the Higuchi Eq. 1. A good correlation was obtained between the release test in the Muranishi method and the absorption test, and the amorphous state of IM in PEG base seemed to partly contribute to this good correlation.

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