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Studies on Sustained-Release Dosage Forms. I. Preparation and Bioavailability of Indomethacin Suppositories[1)]

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Microencapsulation of indomethacin (mc-IM) was carried out by using a gelatin. Sustained-release suppositories containing only mc-IM, and containing both mc-IM and intact IM were prepared by the fusion method.

Plasma concentrations after rectal administration of the sustained-release suppository containing only mc-IM reached a plateau level and did not rise above 5 μg/ml. No difference in bioavailability could be seen between this sustained-release suppository and the conventional suppository.

The sustained-release suppository prepared by mixing mc-IM with intact IM rapidly gave a desirable plasma concentration and maintained an effective plasma level of the drug for a considerable period of time, that is, the maximal plasma concentration (C_{max}) was below 5 μg/ml and the time for which a plasma level above 2 μg/ml was maintained was 6 h.

On the basis of these results, the combined dosage form should represent a convenient therapy with reduced risk of side-effects and reduced frequency of administration.

Keywords——indomethacin; microcapsule; suppository; *in vitro* release; sustained-release dosage form; rectal administration

The aims in designing a sustained-release dosage form are to obtain rapidly a desirable blood concentration of the drug, to maintain such a concentration at a roughly constant level for a suitable period of time, to reduce the frequency of drug administration, and to reduce the incidence and intensity of side-effects.[2)] Many pharmaceutical means for the preparation of sustained-release dosage forms have been investigated, and it was reported that microencapsulation is a useful method.[3)]

Indomethacin (IM) is an anti-inflammatory and anti-pyretic drug commonly used for relief of pain and stiffness in rheumatic disease, but IM is capable of producing gastrointestinal side-effects. Recently, a microencapsulated form of IM without adverse effect on the stomach was reported by Takeda *et al.*[4)] using soybean oil.

Rectal administration of IM is commonly used in an attempt to minimize the gastrointestinal side-effects of IM, and, when used at night, to relieve the morning pain and stiffness of rheumatoid arthritis. However, it was reported that the high plasma level of IM had an adverse effect on the nervous system.[5)]

In the present study, we prepared sustained-release suppositories of IM in an attempt to obtain desirable plasma concentrations, and investigated their bioavailability and utility.

Experimental

Materials——IM was a gift from Sumitomo Chemical Co., Ltd., and gelatin (lot, G16481; isoelectric point, pH 7.1) was supplied by Nitta Gelatin Co., Ltd. All other chemicals were reagent grade commercial products.

Preparation of Microcapsules of IM——Microencapsulation of IM (mc-IM) was carried out according to the method of Tanaka *et al.*[6]

Preparation 1: IM (8 g) was mixed with 40 ml of 30% gelatin solution and then the mixture was poured into a stainless steel vessel containing 120 ml of liquid paraffin (heated previously to 60 °C and stirred at 20—30 rpm). After 5 or 6 min, the vessel was cooled quickly to less than 5 °C, and kept at the same temperature for 10 min. Sixty ml of cold isopropanol was added to the vessel for dehydration, and then the microcapsules were separated. The microcapsules were washed twice with cold isopropanol and dried. The microcapsules were hardened by immersing 1 g of microcapsules in 10 ml of 10% formalin–isopropanol and by standing the mixture in a refrigerator for 24 h.

Preparation 2: A 20% gelatin solution was used and the preparation was carried out in the same manner as described above.

IM contents in microcapsules were assayed spectrophotometrically at 318 nm (Hitachi spectrophotometer, model 200-20) after complete destruction of the microcapsules with a pestle and mortar and dissolution of the residue in 0.2 M phosphate buffer (pH 7.2). The microcapsules of particle size from 177 μm to 590 μm were used in the subsequent experiments. The contents of IM were 34.03 ± 0.98 w/w% (mean $\pm$ S.E.) in preparation 1, and 38.56 ± 0.85 w/w% (mean $\pm$ S.E.) in preparation 2.

Preparation of Suppositories of IM——The conventional suppositories were prepared using intact IM and macrogol 4000 as a base by the fusion method. The sustained-release suppositories were prepared by using mc-IM (preparation 1 or 2) in a similar manner. The combined sustained-release suppositories were prepared by mixing mc-IM with intact IM (85 : 15). The content of IM in one suppository was 6.25, 12.5 or 25 mg.

Dissolution of IM from Microcapsules and Release from Suppositories——Dissolution of IM from microcapsules was tested according to the method of the J.P.X with stirring at 100 rpm at 37 °C; the dissolution medium was 1000 ml of 0.2 M phosphate buffer (pH 7.2). After 25 mg of intact IM or mc-IM had been dispersed in the dissolution medium, aliquots of the solution were removed at appropriate times and filtered through a Millipore filter (pore size 0.45 μm), then assayed spectrophotometrically at 318 nm.

Release of IM from suppositories was measured according to a modification of the method of Muranishi *et al.*[7] The test solution was the same medium as described above. Each suppository was placed in a cylindrical filter paper (Toyo No. 82). The filter paper with suppository was placed in medium at a constant temperature (37 °C). The filter paper was rotated at 100 rpm. Dissolved drug was continuously assayed with a spectrophotometer by circulating the solution through a flow cell.

Animal Experiments——White male rabbits weighing 2.9—3.4 kg were fasted for 24—36 h prior to the experiments but allowed free access to water. After administration of the suppository, blood samples were collected from the ear vein at regular intervals. The plasma samples were frozen and stored at -5 °C until assay.

Measurements of IM in Plasma——The high-performance liquid chromatography (HPLC) reported by Kazmi *et al.*[8] was applied with a slight modification as follows: 0.5 ml of plasma was placed in a test tube and 1 ml of 1 N HCl and 6 ml of benzene–cyclohexane (85 : 15) solution were added. After being shaken for 10 min. The mixture was centrifuged at 5000 rpm for 10 min. Five ml of the organic phase was transferred to another test tube, and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 100 μl of methanol and 50 μl (25 μg) of diazepam solution (internal standard) was added and mixed. A 20 μl sample was injected into the HPLC apparatus (Shimadzu LC-3A with a Shimadzu SPD-2A detector). The conditions for analysis were as follows: column, 25 cm/4 mm i.d.; packing, Partisil 10 ODS; mobile phase, acetonitrile–0.1 M acetic acid (3 : 2); flow rate, 0.8 ml/min; wavelength, 318 nm; sensitivity, 0.005 a.u.f.s.

Computer Analysis——The curve-fitting program used was the MULTI program written in BASIC[9] for an NEC N4700 minicomputer system. The least-squares algorithm used was the simplex method at the preliminary fitting, and the converged values were further analyzed by using the SALS program.

Results and Discussion

In Vitro Dissolution of IM from Microcapsules and Release from Suppositories

The dissolution rate profiles of microcapsules are shown in Fig. 1. Each point represents the average of at least three experiments. As shown in Fig. 1, the rate of dissolution of IM from mc-IM was significantly slower than that of intact IM. The intact IM dissolved completely within 45 min. The dissolution rate of preparation 1 was slower than that of preparation 2. The percentage of the drug dissolved within 2 h was 60% for preparation 1, and 80% for preparation 2.

Figure 2 shows the release patterns of IM from suppositories. The rates of release from the sustained-release suppositories were slower than those of the conventional suppositories. No significant difference in release rate from suppositories between preparations 1 and 2 could

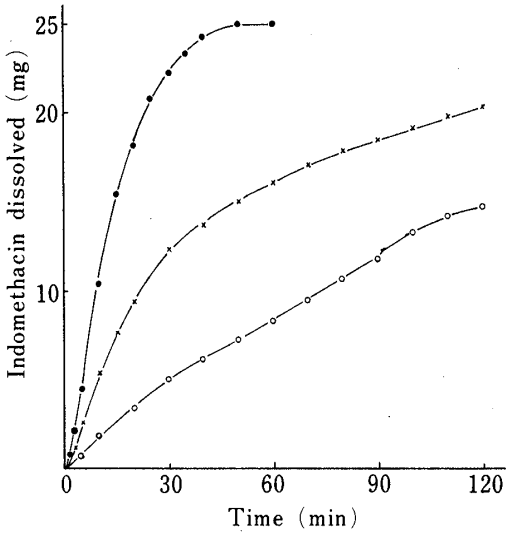

Fig. 1. Dissolution of Indomethacin in 0.2 M Phosphate Buffer Solution (pH 7.2, 1000 ml)

●, intact IM (25 mg); ○, preparation 1 (25 mg content of IM); ×, preparation 2 (25 mg content of IM).

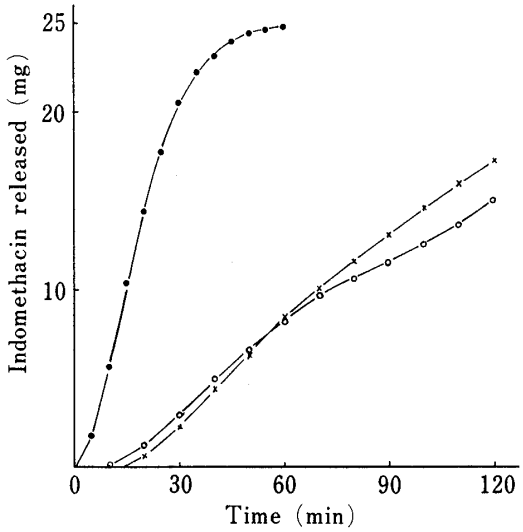

Fig. 2. Release of Indomethacin from Suppositories of 25 mg Drug Content

●, conventional suppository; ○, sustained-release suppositories prepared by using preparation 1; ×, sustained-release suppositories prepared by using preparation 2.

be seen. Therefore, preparation 1 was used for the study of sustained-release suppositories.

Plasma Concentrations of Drug after Rectal Administration

The plasma levels of drug after rectal administration are shown in Fig. 3. The absorption of IM from conventional suppositories was very rapid, and the plasma level reached a peak of 17.8 μg/ml at 45—60 min after administration.

On the other hand, the sustained-release suppositories containing only mc-IM produced a more gradual rise and a more sustained plateau of plasma concentration of IM than did the conventional suppositories. The peak level was 4.17 μg/ml at 2 h after administration. The mean area under the plasma curve [AUC] was calculated from the time course of plasma concentration by use of a linear trapezoidal equation (from zero to ten h) with extrapolation to infinite time. $[AUC]_0^\infty$ values after administration of sustained-release suppositories were almost equal to those of conventional suppositories at corresponding doses as shown in Table I. Therefore, no difference in extent of bioavailability could be seen between sustained-release suppositories and conventional suppositories.

Marmont *et al.*[5] reported that the clinical effect of IM increased arithmetically with increase of dose, but side effects of IM increased geometrically with dose. Alvan *et al.*[10] also described the adverse effect of IM on the nervous system at plasma concentrations exceeding 5 μg/ml, and Baber *et al.*[11] found that the mean plasma concentration at the steady-state in those patients experiencing headache was higher than the corresponding mean for all the patients.

The sustained-release suppositories containing only mc-IM gave a plateau level of drug in plasma after administration without producing a sharp peak of plasma concentration. Therefore, it was considered that this suppository, although it showed a lag time of about 30 min, is a favorable dosage form for reducing the incidence of side-effects and the frequency of administration.

Table I summarizes the relationship between dose and $[AUC]_0^\infty$. Plasma level–time curves after administration of three doses (6.25, 12.5 and 25 mg) were similar in pattern, and no dependency on dose could be seen.

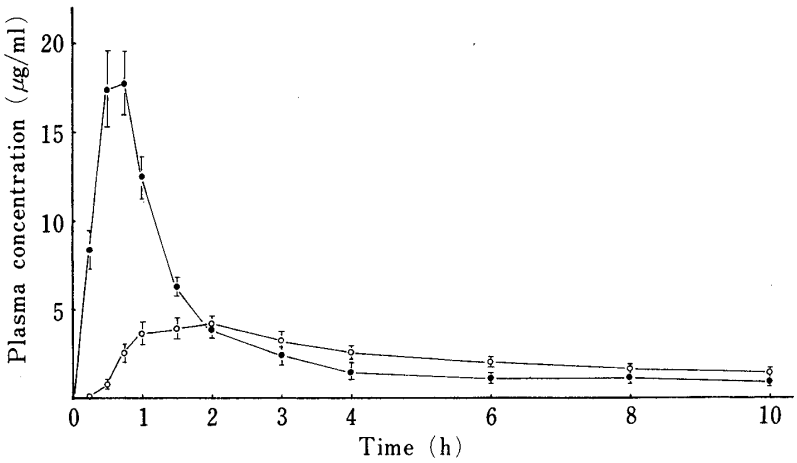

Fig. 3. Plasma Concentrations of Indomethacin after Rectal Administration

●, conventional suppositories (25 mg content of IM); ○, sustained-release suppositories prepared by using preparation 1 (25 mg content of IM).
Each point represents the average ± S.E.

TABLE I. Relationship between Dose and $[AUC]_0^\infty$

	Conventional sup.			Sustained-release sup.		
Dose (mg)	6.25	12.5	25	6.25	12.5	25
$n^{a)}$	6	6	6	6	6	9
Body weight (kg)	3.27 ± 0.09	3.17 ± 0.23	2.96 ± 0.08	3.24 ± 0.11	2.90 ± 0.12	3.42 ± 0.13
$[AUC]_0^\infty$ μg·h/ml$^{b)}$	7.77 ± 0.18	14.47 ± 0.60	31.48 ± 1.37	7.60 ± 0.19$^{c)}$	15.42 ± 0.56$^{c)}$	31.52 ± 0.63$^{c)}$
$[AUC]_0^\infty$/dose h/ml ($\times 10^{-3}$)	1.24	1.16	1.26	1.22	1.23	1.26

Each value represents the average ± S.E.
a) The numbers of rabbits used.
b) Calcd by use of the trapezoidal rule (from zero to ten h) with extrapolation to infinite time. The sustained-release suppositories showed no significant difference (*c*) $p < 0.01$) from the conventional suppositories of corresponding dose.

Determination of the Most Suitable Mixing Ratio of mc-IM and Intact IM

The most suitable sustained-release dosage form is that which would rapidly give the desired plasma concentration, and sustain this plasma level. For this purpose, we considered the combination of mc-IM with intact IM. The most suitable mixing ratio was determined by curve-fitting of plasma levels obtained after administration of the dosage forms separately and then by synthesizing curves for the mixtures.[12)]

Ziel *et al.*[13)] reported that the dosage which produced a reduction of 50% in formation of prostaglandin E_2 *in vitro* was 2 μg/ml in the case of IM, but few clinical trials have been carried out relating plasma concentration of IM to therapeutic effect. We therefore assumed that the minimum therapeutic level of IM was 2 μg/ml.

The plasma level $\{C_r(t)\}$ after administration of conventional suppositories (25 mg content of IM) was calculated by means of the least-squares method as follows:

$$C_r(t) = -34.29e^{-4.47(t-0.13)} + 4.30e^{-0.28(t-0.13)} + 29.95e^{-1.37(t-0.13)} \quad (1)$$

where 0.13 is the lag time (h). The plasma level $\{C_s(t)\}$ after administration of sustained-release suppositories (25 mg content of IM) was as follows:

$$C_s(t) = -1.61e^{-5.29(t-0.44)} + 2.58e^{-0.37(t-0.44)} - 4.47e^{-1.77(t-0.44)} + 3.49e^{-0.13(t-0.44)} \quad (2)$$

where 0.44 is the lag time (h).

Plasma level–time curves at three dose levels were similar in pattern and the plasma concentrations at each time after administration were proportional to the dose of the drug. Therefore, we calculated the plasma level of IM equivalent to 1 mg dose of the drug in conventional suppositories and in sustained-release suppositories, and then determined the theoretical plasma level according to the following equation:[12]

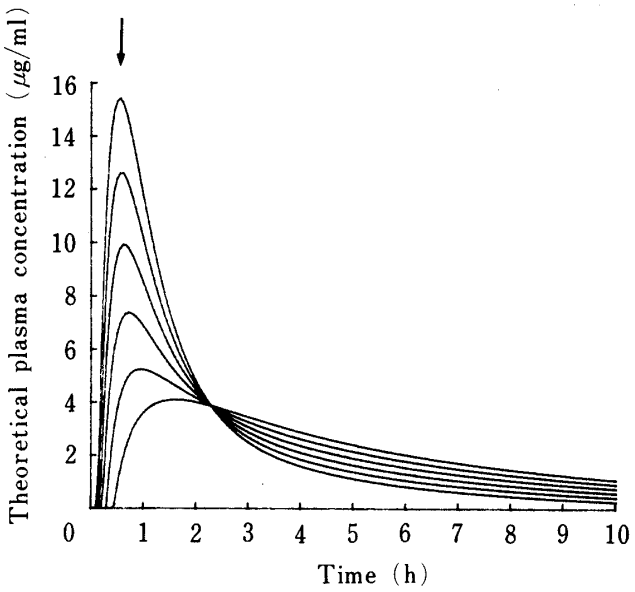

Fig. 4. Plasma Concentration–Time Curves Showing the Effect of Various Combinations of Intact IM with Microencapsulated IM

$$C_p(t)=25\cdot\left\{\frac{F\cdot C_s(t)/25+(100-F)\cdot C_r(t)/25}{100}\right\}$$

$C_p(t)$: theoretical plasma level after administration of combined suppository.
25: dose (mg).
$C_s(t)$: plasma level after administration of sustained-release suppository.
$C_s(t)=-1.61e^{-5.29(t-0.44)}+2.58e^{-0.37(t-0.44)}-4.47e^{-1.77(t-0.44)}+3.49e^{-0.13(t-0.44)}$.
$C_r(t)$: plasma level after administration of conventional suppository.
$C_r(t)=-34.29e^{-4.47(t-0.13)}+4.30e^{-0.28(t-0.13)}+29.95e^{-1.37(t-0.13)}$.
F (w/w%): fraction of mc-IM (0, 20, 40, 60, 80 and 100% from the top).

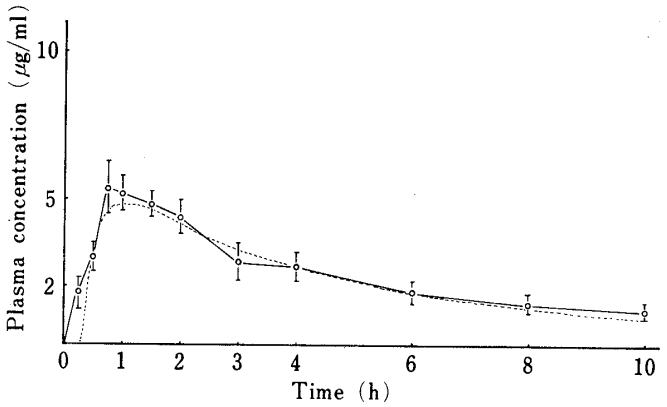

Fig. 5. Plasma Concentrations after Administration of Combined Suppositories (F=85 w/w%)

The dashed line indicates the theoretical curve.
Each point represents the average ± S.E. of six experiments.

$$C_p(t)=25\cdot\left\{\frac{F\cdot C_s(t)/25+(100-F)\cdot C_r(t)/25}{100}\right\} \quad (3)$$

where $C_p(t)$ is the theoretical plasma level, 25 is the dose (mg) and F is the fraction (w/w%) of mc-IM.

A detailed pharmacokinetic analysis for IM after rectal administration will be described in another paper.

Figure 4 shows the simulated curves of plasma level at various mixing ratio of mc-IM and intact IM. It was found that the suppository consisting of 85% mc-IM and 15% intact IM was most suitable, that is, the maximal plasma concentration (C_{max}) was below 5 μg/ml and the time for which a plasma level above 2 μg/ml was maintained was 6 h.

Figure 5 shows the theoretical curve and the experimental values for this combined suppository. The experimental values were satisfactory for our purposes.

From these results, it was concluded that this sustained-release suppository should provide a more convenient therapy with less risk of side-effects and should reduce the necessary frequency of administration of the drug.

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References and Notes

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