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In Vivo and In Vitro Release of Indomethacin from Water-Soluble and Fatty Base Suppositories

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Abstract

The plasma concentration of indomethacin was measured after the rectal administration of water-soluble and fatty base suppositories in rats. The results were compared with the in vitro indomethacin release from suppositories determined by Paddle method using three different types of membranes: cellulose membrane, artificial sausage membrane and natural sausage membrane. The plasma concentrations of indomethacin during the first 4h after the rectal administration were higher in rats that received water-soluble base suppositories than in those that received fatty base types. When either a cellulose membrane or an artificial sausage membrane of cow protein was used in the Paddle method, the amount of indomethacin released from fatty base suppositories was significantly higher than that from water-soluble base ones. However, the results were reversed when a natural sausage membrane of pig colon was used. The discrepancy in the in vitro experiments using water-soluble base suppositories seemed to be due to the difference of pore size of membrane used. Careful consideration should be given to the membrane used in the Paddle method especially when this method is employed to examine the release of poorly soluble drugs like indomethacin in both water-soluble and fatty base suppositories.

KEYWORDS: indomethacin, suppository, in vitro, cellulose membrane, sausage membrane, in vivo bioavailability

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In Vivo and *In Vitro* Release of Indomethacin from Water-Soluble and Fatty Base Suppositories

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The plasma concentration of indomethacin was measured after the rectal administration of water-soluble and fatty base suppositories in rats. The results were compared with the *in vitro* indomethacin release from suppositories determined by Paddle method using three different types of membranes: cellulose membrane, artificial sausage membrane and natural sausage membrane. The plasma concentrations of indomethacin during the first 4h after the rectal administration were higher in rats that received water-soluble base suppositories than in those that received fatty base types. When either a cellulose membrane or an artificial sausage membrane of cow protein was used in the Paddle method, the amount of indomethacin released from fatty base suppositories was significantly higher than that from water-soluble base ones. However, the results were reversed when a natural sausage membrane of pig colon was used. The discrepancy in the *in vitro* experiments using water-soluble base suppositories seemed to be due to the difference of pore size of membrane used. Careful consideration should be given to the membrane used in the Paddle method especially when this method is employed to examine the release of poorly soluble drugs like indomethacin in both water-soluble and fatty base suppositories.

Key words : **indomethacin, suppository, *in vitro*, cellulose membrane, sausage membrane, *in vivo* bioavailability**

Witepsol and Macrogol have generally been used as bases for suppositories. Witepsol, a fatty base, is a mixture of triglycerides of fatty acid with various side chains (C_8-C_{18}). Macrogol, a water-soluble base, is a mixture of polyethyleneglycols with various molecular weights (400-6,000). When fatty bases are stored at high temperature (30°C) for 1 or 2 days, a polymorphism is caused which decreases the release of ingredients and *in vivo* bioavailability (1). Recently, we confirmed that the release of indomethacin from a fatty base suppository stored at low temperature was higher than that stored at high temperature, in both the *in vitro* experiment by the Paddle method using cellulose membrane and the *in vivo* bioavailability test in anesthetized rats (2). In the preliminary study, however, we found that the *in vitro* releases of indomethacin from

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water-soluble and fatty base suppositories determined by the Paddle method using a cellulose membrane did not correlate with those measured in the *in vivo* bioavailability test in rats. In the present study, therefore, we investigated the influence of various membranes used in the Paddle method on the release of indomethacin from water-soluble and fatty base suppositories.

Materials and Methods

Materials. Commercial suppositories of indomethacin donated from six pharmaceutical companies were used (Table 1). Products A, B, C and D were water-soluble base suppositories, and products E and F were fatty base ones. They were stored at 4°C. Pure indomethacin was donated from Sumitomo Chemical Co. (Takarazuka, Japan). All chemicals used were of analytical or reagent grade, obtained from Wako Pure Chemical Industries (Osaka, Japan).

In vivo experiment. Male Wistar rats (supplied by Charls River Lab., Japan) weighing 200–210 g were used as subjects and fasted for 16 h before and during the experiment. Water was freely given. Indomethacin suppositories were cut to a size corresponding to 40 mg/kg, administered intrarectally, and the anuses were closed with alkyl-α-cyanoacrylate monomer (Aron Alpha A, Sankyo Co., Tokyo). At various times after the administration, 60 μl blood samples were drawn into heparinized capillaries from the tail vein. The blood indomethacin level was determined using high performance liquid chromatography with UV detection as described previously (2).

In vitro release experiment. The release of indomethacin from the suppositories was measured by the Paddle method described in Japanese Pharmacopoeia-XI (1986) with modifications using various types of membrane tubes instead of a basket (3). Membranes used were the cellulose membrane (cellophane tubing seamless) of 10 cm in length, 19 mm in diameter and 45 μm in thickness (Wako Pure Chemical Industries); the artificial sausage membrane made of cow protein of 5 cm in length, 19 mm in diameter and 60 μm in thickness (Apex Japan, Tokyo); and the natural sausage membrane of pig colon of 5 cm in length (Ito-Ham Foods Inc., Nishinomiya, Japan), which was partially cut from the mucosal tissue and preserved in salt. Each membrane tube included a suppository with 3 ml of artificial plasma (pH 7.5±0.1), which was consisted of $Na_2HPO_4 \cdot 12H_2O$ (41.2 g) and KH_2PO_4 (2.8 g) in 1,000 ml of distilled water. Artificial plasma was also used as dissolution medium. The rate of rod rotation was 25 rpm.

Electron microscopy. The cellulose membrane, artificial sausage membrane of cow protein and natural sausage membrane of pig colon were used as the specimens. For scanning electron microscop, specimens were dried to the critical point, coated with white gold palladium in an ion coater and observed by JSM T-20 type microscope with 19 KV acceleration voltage.

Pharmacokinetic and statistical analysis. The *in vitro* data were evaluated by two-way analysis of variance. In the *in vivo* study, the pharmacokinetic parameters such as the maximum plasma concentration (Cmax), the absorption rate constant (Ka), the elimination rate (Kel), the elimination half-life (T½) and the volume of distribution (Vd) were obtained from the plasma indomethacin concentration-time curve for each animal, using a personal computer program for nonlinear least squares regression of Marquardt's method (4). The area under plasma drug concentration-time curve (AUC) was determined by the method of Yamaoka *et al.* (5). The statistical analysis was performed by Student's *t* test. Statistical significance

Table 1 Preparations of indomethacin suppositories

Products	Lot no.	Bases	Weight(g) (Mean and SEM)	Labeled amount in a suppository (mg)
A	TE 904	Water-soluble	1.6±0.0	50
B	520131	Water-soluble	1.5±0.0	50
C	LE 091 F	Water-soluble	1.5±0.0	50
D	21 C 6 A	Water-soluble	1.5±0.0	50
E	61301	Fatty	1.2±0.0	50
F	5 F 74	Fatty	0.8±0.0	50

was evaluated at the $p < 0.05$ level.

Results

In vivo bioavailability test. Fig. 1 shows changes in the plasma indomethacin concentration after the rectal administration of water-soluble (product A) and fatty (product E) base suppositories in rats. The plasma indomethacin concentration at 0.5 to 2 h after the administration was significantly higher in rats treated with the water-soluble base suppositories than in those treated with the fatty base suppositories. There was a significant difference ($p < 0.05$) in the AUC_{0-2} values between two groups (Table 2). The Tmax value was significantly smaller ($p < 0.01$) in rats treated with water-soluble base suppositories than in those treated with fatty base ones. However, there were no significant differences in other pharmacokinetic parameters between both groups.

Influence of various membranes on the in vitro indomethacin release. Fig. 2 shows the release of indomethacin from 6 kinds of commercial suppositories contained in cellulose membrane. In all of the suppositories, the indomethacin releases increased linearly for 90 min after the start of the experiment. The indomethacin releases from fatty base suppositories (products E and F) were considerably higher than those from water-soluble base suppositories (products A, B,

Table 2 Comparison of pharmacokinetic parameters in rats after the administration of indomethacin suppositories of either water-soluble or fatty base

Parameter	Water-soluble base (A) (n = 8)	Fatty base (E) (n = 5)
Ka (hr^{-1})	3.55 ± 1.11	0.88 ± 0.34
Kel (hr^{-1})	0.12 ± 0.03	0.18 ± 0.07
Vd (l)	0.11 ± 0.02	0.11 ± 0.04
T½ (hr)	9.0 ± 2.5	11.8 ± 4.5
AUC_{0-2} ($\mu g \cdot hr/ml$)	133 ± 20*	68 ± 6
AUC_{0-12} ($\mu g \cdot hr/ml$)	720 ± 86	624 ± 275
Tmax (hr)	1.4 ± 0.2**	3.8 ± 0.6
Cmax ($\mu g/ml$)	92.9 ± 12.9	63.4 ± 11.5

Each value is the mean and SEM.
*$p < 0.05$, **$p < 0.01$, significantly different from the corresponding value in the group of fatty base suppositories.

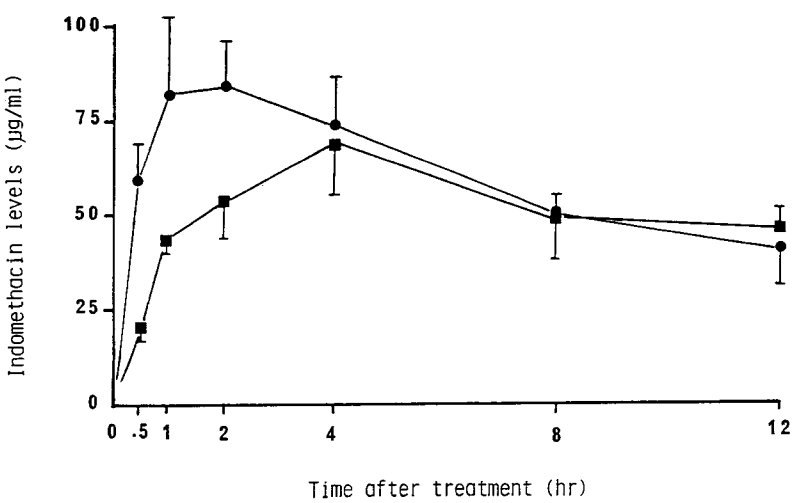

Fig. 1 Changes in plasma indomethacin concentrations after rectal administration of indomethacin suppositories. Water-soluble base (product A, ●). Fatty base (product E, ■). Each point and vertical bar represent the mean and SEM of eight (●) or five (■) animals.

C and D). Among the fatty base suppositories, product E showed a significantly higher ($p < 0.01$) release than product F. Among the water-soluble base suppositories, the release from product B was significantly lower ($p < 0.01$) than from any other products.

When examined using the artificial sausage membrane of cow protein, the indomethacin release from a fatty base suppository (product E) was also significantly higher ($p < 0.01$) than from a water-soluble base suppository (product A), as shown in Fig. 3. In both products, the indomethacin releases were almost equal to those determined using cellulose membrane. However, the indomethacin releases through natural sausage membrane of pig colon from water-soluble base suppositories (products A and B) were markedly higher than those from fatty base suppositories (products E and F), as shown in Fig. 4. Althougth the release from fatty base suppositories using the artificial sausage membrane or the natural sausage membrane of pig colon were almost equal to those determined using cellulose membrane, the release from water-soluble base suppositories was approximately 8 times higher than that determined using cellulose membrane. The marked swelling of these membranes in water-soluble base suppositories was observed comparing with those of fatty base ones. The weight of any tested pieces using these membrane were increased approximately 70 % in this experiment.

Electron microscopic observation. The cellulose membrane consisted of minute particles of 5 nm in diameter. There was variability in the electron density. The pore size was 3 nm in a diameter (Fig. 5). The artificial sausage membrane of cow protein was the aggregation of mature collagen arranged in parallel. The pore of 80-350 nm in a diameter was observed between collagen fibers (Fig. 6). The natural sausage membrane of pig colon consisted of both immature and mature collagen, fibrous cell and basement membrane. The pore size was 100-700 nm in a diameter (Fig. 7).

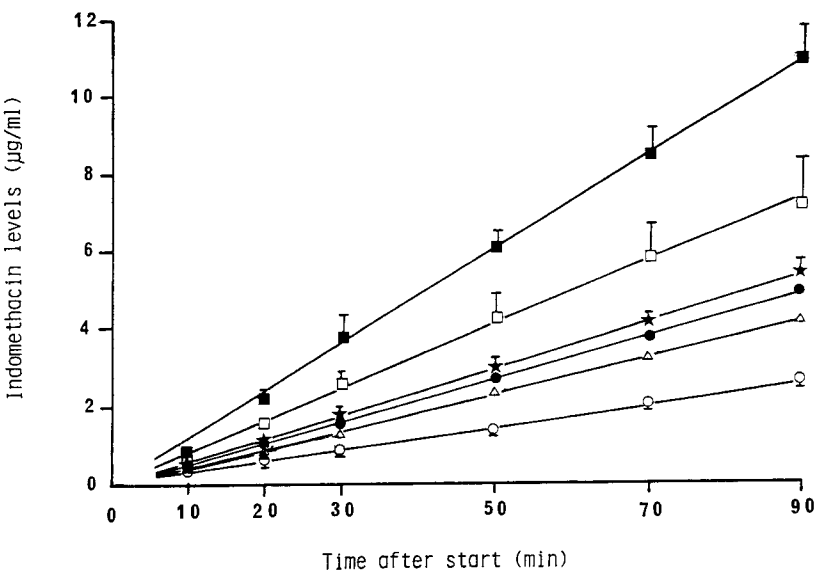

Fig. 2 *In vitro* release of indomethacin from suppositories using cellulose membrane. Water-soluble bases (products A ●, B ○, C △, and D ★). Fatty bases (products E ■ and F □) Each point and vertical bar represent the mean and SEM of five suppositories.

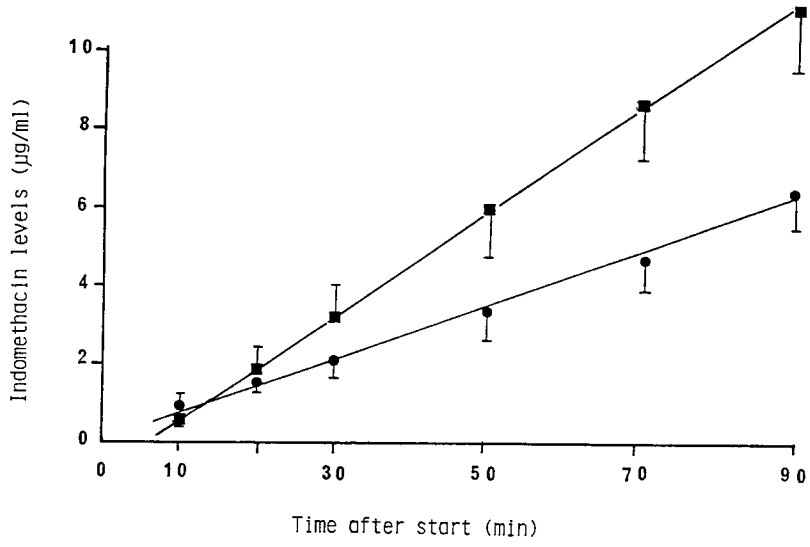

Fig. 3 *In vitro* release of indomethacin from suppositories using artificial sausage membrane of cow protein. Water-soluble base (product A ●). Fatty base (product E ■). Each point and vertical bar reprsent the mean and SEM of three suppositories.

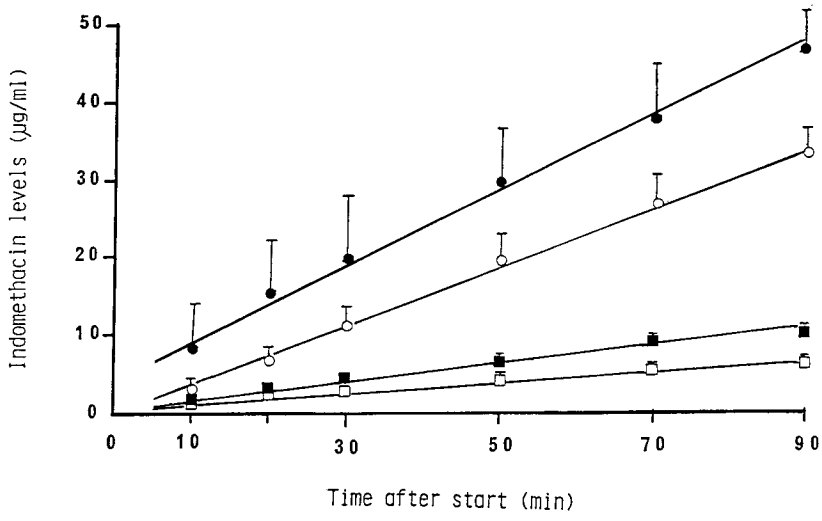

Fig. 4 *In vitro* release of indomethacin from suppositories using natural sausage membrane of pig colon. Water-soluble bases (products A ● and B ○). Fatty bases (products E ■ and F □). Each point and vertical bar represent the mean and SEM of five suppositories.

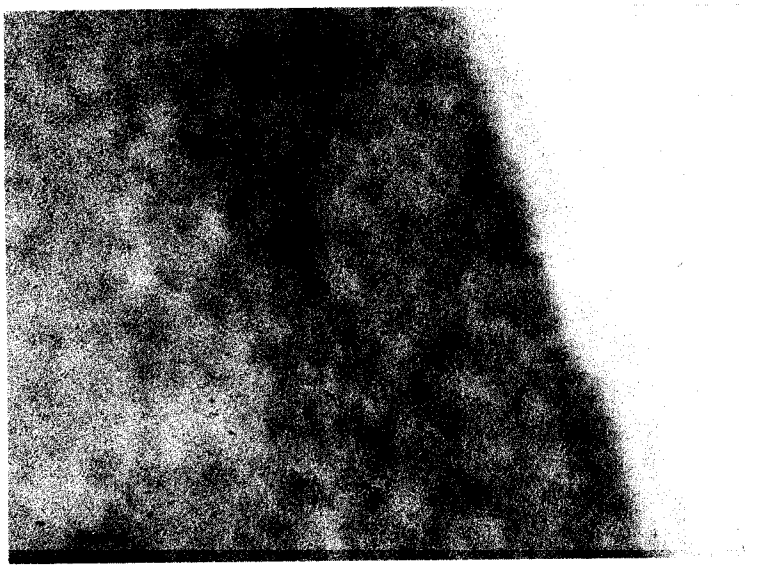

Fig. 5 Electron microscopic observation of cellulose membrane. Bar represents 200 nm.

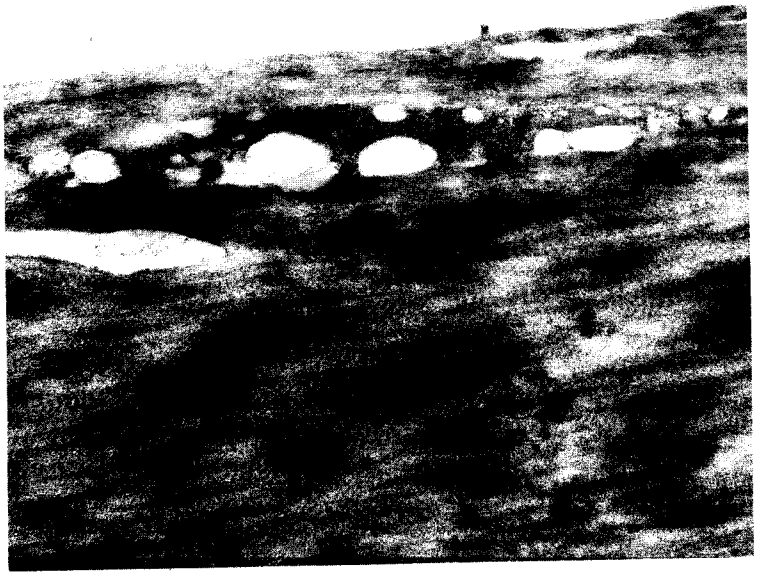

Fig. 6 Electron microscopic observation of artificial sausage membrane of cow protein. Bar represents 200 nm.

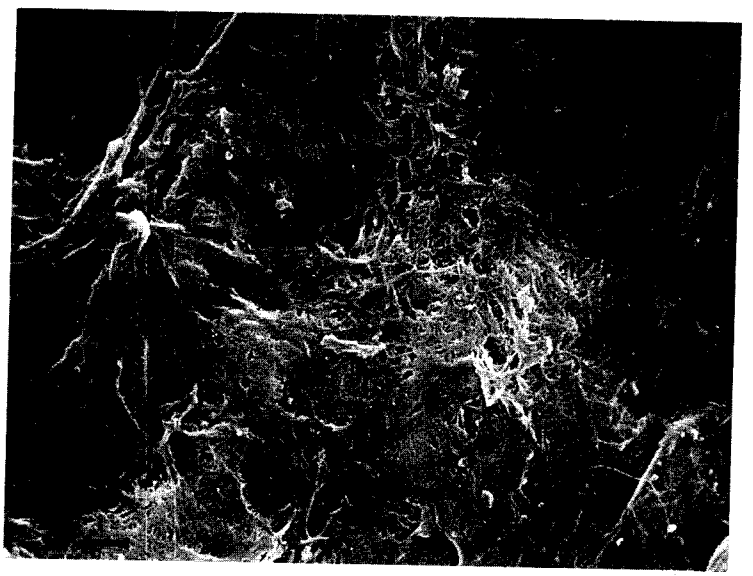

Fig. 7 Electron microscopic observation of natural sausage membrane of pig colon. Bar represents 2,000 nm.

Discussion

A variety of water-soluble and fatty base suppositories has been used for the experiments. In the quality test of these suppositories, there have been only a few reports concerning the influences of various membranes used in the *in vitro* release test. In the present study, the plasma indomethacin concentration after the rectal administration of the suppositories to rats was higher in case of water-soluble bases than fatty bases. However, inconsistent results were obtained in the *in vitro* experiments using the Paddle method of three kinds of membrane. When cellulose membrane or artificial sausage membrane of cow protein was used, the indomethacin release from fatty base suppositories was higher than that from water-soluble base suppositories. When the natural sausage membrane of pig colon was used, a greater amount of indomethacin was released from water-soluble base suppositories than from fatty base ones.

In general, the rate-limiting factors of drug release from fatty base suppositories are considered as a melting rate of bases, a absorption rate of particle to the membrane, a permeation rate through the membrane, solubility of the drug in water and oil: water partition coefficient of the drug (6, 7). Since the solubility of indomethacin in fatty base is low (2.5 mg/ml in Witepsol H-15) (8), most of this compound used in the present study may be dispersed as a free base state in the fatty base suppositories. Therefore, indomethacin is mostly transferred to the artificial plasma in the membrane tube from the sediment. However, since indomethacin is practically insoluble in water, the release rate of this compound may not depend on the pore size of membrane. In fact, in the present *in vitro* tests using three membranes, the absolute amounts of indomethacin released from fatty bases ware almost equal (Figs. 2-4).

The rate-limiting factors of the release of indomethacin from water-soluble base supposi-

tories are the melting rate of the base, and the permeation rate through the membrane (9, 10). Since the solubility of indomethacin on Macrogol 300 is reported to be 125 mg/ml (8), indomethacin may be mostly dissolved in the water-soluble base suppositories used in the present experiment. Polyethyleneglycols (molecular weight, 200-6,000) including indomethacin may diffuse into the dissolution medium through the pores of the membrane after melting. The indomethacin release from water-soluble base suppositories through the natural sausage membrane of pig colon was about 8-fold of that from the cellulose membrane or artificial sausage membrane of cow protein. In observation of electron microscopy, natural sausage membrane of pig colon had numerous pores of 100-700 nm in size. The large pore size of natural sausage membrane may be due to loss of mucosal tissue. The marked swellings of natural sausage membrane tube that was observed may have been due to an elevation of osmotic pressure after dissolution of the water-soluble bases. The increase of the fluid volume in the tube may facilitate the disintegration of the bases and diffusion of indomethacin in polyethyleneglycols into the dissolution fluid through the membrane. In contrast, the pore size of cellulose membrane was remarkably small (about 3 nm, the molecular cut off value 20,000-25,000). The artificial sausage membrane of the cow protein had 80-350 nm pores with low density. No marked swelling was observed in either the cellulose membrane tubes or the artificial sausage membrane tubes in the present experiment. Therefore, it is likely that the water-soluble base might require more time to melt and indomethacin might diffuse through these membranes more slowly. Kameta *et al.* (11) reported in using commercially available indomethacin suppositories that the amount of release measured by the Muranishi method using cellulose membrane with large pore size (3 μm) was markedly higher from water-soluble bases than fatty bases. The *in vivo* fluid volume in the rectum may increase in the water-soluble bases due to an elevation of osmotic pressure. In addition, the weights of water-soluble and fatty base suppositories used were about 1.6 and 1.2 g, respectively. This may cause a much larger expansion of the rectum after the administration of water-soluble bases than after fatty bases. This may explain why plasma concentration of indomethacin was higher when animals were administered with the water-soluble base suppositories than fatty base suppositories in the present experiment. The similar results in the *in vivo* experiment and the *in vitro* test using natural sausage membrane might have derived from the nature of the membrane; the easy transfer of water. A difference in the preparation of suppositories in the manufacturing process may be explain comparatively large variations of indomethacin release in intra-group of water-soluble base suppository or fatty base one.

In conclusion, in the *in vitro* quality test of suppositories, especially of the water-soluble bases, much attention should be given to the kind of membrane used. The natural sausage membrane of pig colon may be more similar to the *in vivo* situation than the cellulose membrane and the artificial sausage membrane of cow protein in respect to the high permeability of water.

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