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Release Rates of Indomethacin from Commercial Witepsol Suppositories and the Bioavailabilities in Rabbits and Pigs

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Releases of indomethacin from commercial witepsol suppositories were investigated by JP XI paddle, Muranishi and dialysis tubing methods. The drug was scarcely released from most of the suppositories by the paddle method at 37 °C although it was released at 39 °C. The release behaviors of suppositories differed greatly between the Muranishi and dialysis tubing methods. The melting points of suppositories correlated significantly with the release rates by the dialysis tubing method but not with those by the Muranishi method. The release rates by the Muranishi method were delayed by increasing the fluid volume in the cylindrical cell and the releases by the dialysis tubing method were inhibited by adding aqueous medium into the tubing. The bioavailabilities from two and three of the products were estimated in rabbits and pigs, respectively, and the results correlated well with the release rates determined by the dialysis tubing method but poorly with those by the Muranishi method.

Keywords——indomethacin; suppository; bioavailability; release rate; witepsol; rabbit; pig; rectal absorption; dissolution; bioequivalence

The bioavailabilities of drugs from suppositories are considered to depend on the drug release rates, but no *in vitro* method to estimate the release has been officially stipulated. As the standard method, a simple procedure is required which would show superior reproducibility and a high correlation between drug release and bioavailability. Indomethacin suppositories have been frequently used as an anti-inflammatory agent for patients in whom oral treatment is undesirable. The release rates and bioavailabilities of indomethacin suppositories have been investigated in humans[1,2] and animals,[3,4] but the *in vitro–in vivo* correlation was not fully clarified. This study was undertaken to investigate the release behavior of indomethacin from commercial witepsol suppositories by three different methods, the JP XI paddle, Muranishi[5] and dialysis tubing methods,[6-8] and the relation of the results with the bioavailabilities in rabbits and pigs.

Experimental

Formulation——Six brands of witepsol suppositories containing 50 mg of indomethacin marketed in Japan and one experimental suppository prepared with 700 mg of witepsol W-35, 50 mg of the H-85 and 50 mg of indomethacin were used for the *in vitro* study. Two brands of suppositories showing different release behaviors were employed for the study of rabbits and three brands for the study of pigs (however, the lot numbers differed from those used for the *in vitro* studies).

Melting Point——Test suppositories were filled in a glass capillary tube (1.0 i.d. × 120 mm) to 1 cm depth by pushing the tube into the suppository, and the melting points were determined according to method 2 of the melting

point determination of JP XI. The temperature at which the sample rose in the capillary tube was taken as the melting point.

Release Rate——Suppositories stored in a refrigerator (5 °C) were taken out at least 2 h before the experiment and their release rates were determined by JP XI paddle, Muranishi and dialysis tubing methods, at 37.0±0.2 C using pH 7.2 buffer for the JP XI dissolution test of indomethacin capsules. a) Paddle method: A test suppository was dropped in 900 ml of the medium and the release rate was determined according to the JP XI paddle method at 100 rpm. The drug concentration in the medium was spectrophotometrically determined after filtration of 2 ml of the sample solution with a 0.5 μm membrane filter. b) Muranishi method[5]: A TMS-103 apparatus (Toyama Sangyo Co., Ltd.) was employed in the study; it consists of a plastic cylindrical cell with a metallic net bottom and a glass vessel. The release rate of a test suppository was determined in 500 ml of the fluid, which was agitated with a magnetic stirrer at 100 rpm. A membrane filter (Millipore SSWP, pore size 3.0 μm) was fastened to the bottom of the cylindrical cell, which was attached to the glass vessel so that the bottom of the cell was 3 mm below the fluid surface. The suppository in the cylindrical cell was stirred with a steel rod positioned at 2 mm above the cell bottom at 10 rpm. The drug concentration in the medium was determined by passing the solution into the flow cell of a spectrophotometer. In a modified Muranishi method the cylindrical cell was fixed so that the cell bottom was 15 mm below the medium surface and the steel rod was rotated at 5 mm above the bottom of the cell. c) Dialysis tubing method: A modified Thomas method[6-8] was employed in this study using a JP XI paddle apparatus (Fig. 1). Dialysis tubing (Union Carbide 18/32) was washed with boiling water for 5 min and then with distilled water several times and one end was tied tightly. After removal of the water remaining in the tubing, a test suppository was dropped into the tubing, which was immersed in 900 ml of the medium with a 5.7 g lead weight so that the tied end of the tubing was 8 cm below the fluid surface and 2 cm from the wall of the vessel. The paddle was rotated at 1.5 cm above the bottom of the vessel at 50 rpm. The drug concentration in the medium was determined spectrophotometrically as described in the case of the Muranishi method.

Bioavailability Test——a) Rabbit study: Six male albino rabbits weighing 2.7—3.2 kg were used in this study. A test suppository was rectally administered to each rabbit after fasting overnight and the anus was glued with surgical paste, Aron Alpha® (Sankyo Co., Ltd.). Blood samples were taken from the ear vein at intervals up to 4 h after the administration of suppositories and the serum samples were stored frozen until assayed. The suppositories were administered at a one-week interval according to a randomized block design. The bioavailabilities were estimated from the observed maximum concentration of indomethacin in serum (C_{max}), time to C_{max} (T_{max}) and areas under the serum concentration–time curves from zero to 4 h (AUC_{4h}) and zero to infinity (AUC_{∞}) which were calculated by means of a trapezoidal rule and the method of Wagner,[9] respectively. b) Pig study: Six LWH female pigs (3.5 months in age) were used; their weights at the beginning and end of the cross-over study were 17.6—21.6 (mean = 19.3) and 24.2—31.6 kg (mean = 28.3), respectively. A test suppository was rectally administered to each pig after fasting overnight with a device made of a steel rod (2 mm × 11 cm), to the top of which the suppository was fixed. Blood samples were taken from the jugular vein at intervals up to 3 h after the administration of suppositories and the plasma samples were stored frozen until assayed. The suppositories were administered every week according to a Latin-square cross-over design.

Assay——The serum and plasma concentrations of indomethacin were determined by the reported high performance liquid chromatographic (HPLC) method[10] with some modification. To 0.2 ml of rabbit serum, 0.2 ml of pH 5 sodium acetate buffer (0.2 M) containing 3 μg/ml of griseofulvin as an internal standard and 6 ml of ether were added. The mixture was shaken for 10 min, then 5 ml of the organic layer was taken and evaporated under nitrogen gas flow. The residue was dissolved in 200 μl of methanol and the indomethacin concentration was determined by HPLC using a 4 mm i.d. × 25 cm column of Develosil ODS (Nomura Chemical Co., Ltd.). HPLC conditions: mobile phase, acetonitrile/pH 4.6 sodium acetate (0.01 M) = 1/1; flow rate, 0.7 ml/min; detection wavelength, 254 nm; the plasma concentrations of the drug in pigs were determined by the same procedure as above after extraction of a mixture of 1.0 ml of the plasma and 0.2 ml of pH 5 acetate buffer (1 M) with 6 ml of ether.

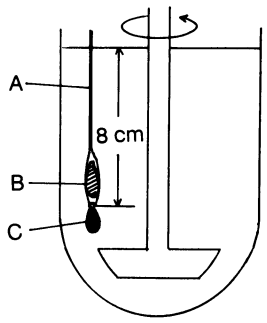

Fig. 1. Dialysis Tubing Method

A, dialysis tubing; B, suppository; C, 5.7 g lead weight.

Results

Release Rate

Table I shows the melting points of suppositories. The maximum difference (observed between suppositories B and E) was 1.3 °C.

a) Paddle Method ——Figure 2 shows the releases of indomethacin from suppositories determined by the paddle method at 37 and 39 °C. Except for products B and D the drug was hardly released at 37 °C from the suppositories, which floated on the medium in an almost intact state without melting or disintegrating. The findings suggest that the heat conductance from the medium into the suppositories was poor. On the other hand, suppositories B and D were broken into small fragments and released the drug, although the release from both suppositories reached a plateau at about 20% of the drug released. When the medium temperature was raised from 37 to 39 °C, the release rates of suppositories were accelerated; products B, D and E showed rapid dissolutions and product F showed slow dissolution (Fig. 2). The rise of the temperature presumably promotes the melting of suppositories, leading to the acceleration of drug release.

b) Muranishi Method——Figure 3 shows the release profiles of indomethacin suppositories determined by the Muranishi method. The drug was rapidly released from suppositories B and C but slowly from A and F. When the cylindrical cell was lowered so that the cell bottom was 1.5 cm below the fluid surface (modified Muranishi method), the release

TABLE I. Melting Points of Six Different Witepsol Suppositories of Indomethacin

	Suppository					
	A (001J05)[a]	B (K03405)	C (TM002S)	D (3NRC1)	E (40170)	F (02102)
mp (°C)	36.3±0.1[b]	37.6±0.1	36.5±0.1	36.6±0.1	36.3±0.1	37.5±0.1

a) Lot No. *b*) Means±S.D. ($n=4$).

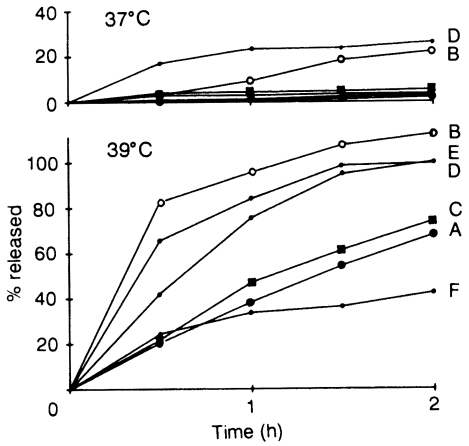

Fig. 2. Releases of Indomethacin from Six Different Witepsol Suppositories Determined by the Paddle Method at 37 °C (Upper) and 39 °C (Lower)

Medium, 900 ml of pH 7.2 buffer; stirring rate, 100 rpm.

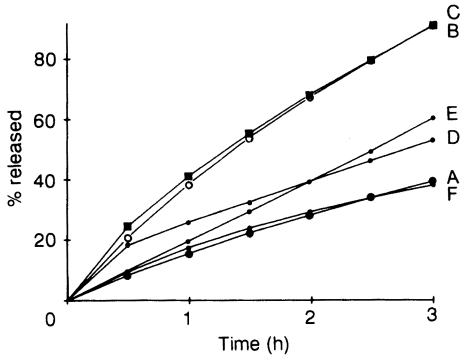

Fig. 3. Release of Indomethacin from Six Different Witepsol Suppositories Determined by the Muranishi Method

The cylindrical cell was fixed so that the cell bottom was 3 mm below the fluid surface and the steel rod was stirred at 10 rpm at 2 mm above the cell bottom.

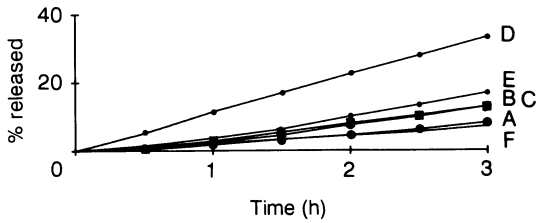

Fig. 4. Releases of Indomethacin from Six Different Witepsol Suppositories Determined by a Modified Muranishi Method

The cylindrical cell was fixed so that the cell bottom was 15 mm below the fluid surface and the steel rod was rotated at 10 rpm at 5 mm above the cell bottom.

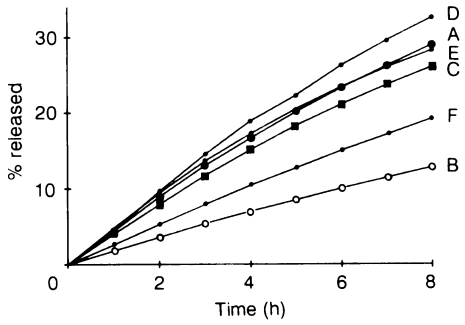

Fig. 5. Releases of Indomethacin from Six Different Witepsol Suppositories Determined by the Dialysis Tubing Method

Medium, 900 ml of pH 7.2 buffer; stirring rate, 50 rpm.

rates from the suppositories were markedly reduced except from product D (Fig. 4), as previously shown.[11] By lowering the cylindrical cell, the volume of the test fluid in the cell was increased and suppositories floated on the fluid. This greatly decreased the frequency with which the stirring rod hit suppositories in the cell, mechanically promoting the disintegration of the suppositories. Therefore, the delay in the drug release due to lowering of the cylindrical cell can probably be ascribed to reduced disintegration of the suppositories and decreased agitation efficiency of the test fluid in the cell caused by the increase of the fluid volume.

c) Dialysis Tubing Method——Figure 5 shows the release of indomethacin from suppositories determined by the dialysis tubing method. The ranking (D > A > E > C > F > B) in the release rate among the products was quite different from that (C, B > E > D > A > F) determined by the Muranishi method (Fig. 3). When observed during the test, suppositories A, D and E appeared to disperse well along the dialysis tubing while suppository B was less well dispersed, which indicates that the drug release depended on the spreading characteristics of suppositories in the dialysis tubing.

Relation between Melting Point and Release Rate

Table II shows the correlation coefficients between the melting points of suppositories and their release rates shown as percent released in 2—8 h, determined by the paddle, Muranishi and dialysis tubing methods. The melting points correlated significantly with the release rates determined by the dialysis tubing method but not with those by the paddle and Muranishi methods. The relation between the melting points and release rates by the dialysis tubing method is shown in Fig. 6. A close correlation of the melting points with the release rates obtained by the dialysis tubing method was also found in previous studies on sodium phenobarbital[6] and sulpyrine suppositories.[12] The findings suggest that drug release from suppositories in the dialysis tubing method depends on their melting characteristics.

Bioavailability

Three brands (A, B and C) of the suppositories were selected for *in vivo* studies: suppository A showed rapid release by the dialysis tubing method but slow release by the Muranishi method, while suppository B showed opposite characteristics. Suppository C gave fast release by both methods. Rabbits were administered suppositories A and B. Pigs were given products A, B and C which, however, differed in lot number from those used for the *in vitro* study.

Figure 7 shows the mean serum concentrations of indomethacin after rectal adminis-

TABLE II. Correlation Coefficients between Melting Points of Witepsol Suppositories of Indomethacin and Release Rates

Method	Parameter[a]	r
Paddle at 37 C	R_{2h}	0.313
Paddle at 39 C	R_{2h}	−0.084
Muranishi	R_{3h}	0.161
Modified Muranishi	R_{3h}	−0.275
Dialysis tubing	R_{8h}	−0.890[b]

a) R_{2h}, R_{3h} and R_{8h} mean the amount of the drug released in 2, 3 and 8 h, respectively. *b*) $p<0.05$.

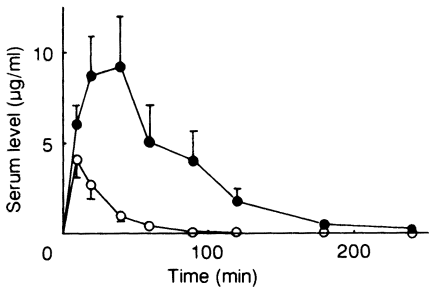

Fig. 7. Mean Serum Levels of Indomethacin after Rectal Administration of Suppositories A (●) and B (○) to Rabbits ($n=6$)

The vertical lines show S.E.

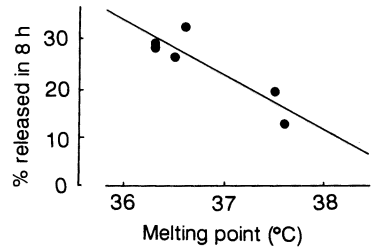

Fig. 6. Relation between the Melting Points and Drug Release Rates (% Released in 8 h) of Indomethacin Suppositories Determined by the Dialysis Tubing Method

$r=-0.890$.

TABLE III. Mean C_{max}, T_{max} and AUC after Rectal Administration of Two Different Witepsol Suppositories of 50 mg of Indomethacin to Rabbits ($n=6$)

	Suppository		Paired
	A (001J05)[a]	B (K03405)	t-test
C_{max} (μg/ml)	10.8±2.4[b]	4.1±1.0	$p<0.10$
T_{max} (min)	23.3±5.5	10.0±0.0	$p<0.10$
AUC_{4h} (h·μg/ml)	12.4±3.7	2.1±0.6	$p<0.05$
AUC_{∞} (h·μg/ml)	14.9±4.1	2.2±0.6	$p<0.05$

a) Lot No. *b*) Means±S.E.

tration of suppositories A and B to rabbits and Table III lists the *in vivo* parameters. Compared with product A, suppository B gave considerably lower serum concentrations. The mean C_{max}, AUC_{4h} and AUC_{∞} of formulation B were 38, 17 and 15% of those of product A, respectively, and the differences in the parameters of AUC were statistically significant at $p<0.05$. The *in vivo* results agreed with the *in vitro* releases determined by the dialysis tubing method but not with those by the paddle and Muranishi methods. On the other hand, T_{max} after dosing with product B was smaller than that after suppository A. This was probably owing to insufficient absorption of the drug from suppository B.

Table IV shows the release rates (percent released in 3 and 6 h) of suppositories A, B and C employed for the study in pigs by the Muranishi and dialysis tubing methods. Figure 8 shows the mean plasma concentrations of indomethacin after administration of those suppositories to pigs and Table V lists the *in vivo* parameters. Suppository C, showing the fastest release by the dialysis tubing method, provided the highest C_{max} and AUC_{∞} which were almost twice those of the least available product B showing the slowest release. However, the differences were statistically not significant. The *in vivo* parameters corrected for the body weights of pigs also gave similar results (data not shown). T_{max} of poorly available products tended to be smaller than that of the highly available one, as observed in the study in rabbits, although the differences were statistically not significant.

Table VI shows the correlation coefficients between the *in vivo* parameters and release rates determined by the Muranishi and dialysis tubing methods, respectively. AUC_{∞} correlated significantly with the release rates determined by the dialysis tubing method but

TABLE IV. Release Rates of Three Different Witepsol Suppositories of Indomethacin Employed for the *in Vivo* Study of Pigs

Method	Parameter[a]	Suppository A (002J19)[b]	B (K06007)	C (TY005S)
Muranishi	R_{3h} (%)	26.9	46.8	60.6
Dialysis tubing	R_{6h} (%)	25.3	21.9	29.1

a) R_{3h} and R_{6h} mean the amount of the drug released in 3 and 6 h, respectively. *b*) Lot No.

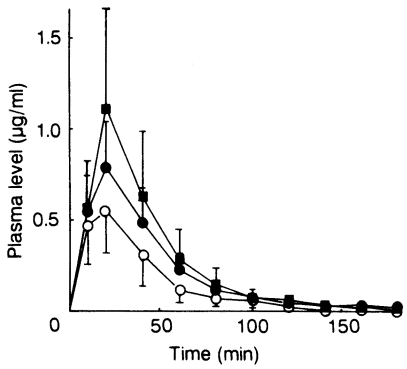

Fig. 8. Mean Plasma Levels of Indomethacin after Rectal Administration of Suppositories A (●), B (○) and C (■) to Pigs ($n=6$)

The vertical lines show S.E.

TABLE V. Mean C_{max}, T_{max} and AUC after Rectal Administration of Three Different Witepsol Suppositories of 50 mg of Indomethacin to Pigs ($n=6$)

	Suppository A (002J19)[a]	B (K06007)	C (TY005S)	ANOVA[c]
C_{max} (μg/ml)	0.89± 0.24[b]	0.59±0.22	1.12±0.55	NS
T_{max} (min)	15.0 ± 2.2	13.3±2.1	16.7±2.1	NS
AUC_{3h} (min·μg/ml)	37.8 ±12.4	24.6±11.7	48.0±26.2	NS
AUC_{∞} (min·μg/ml)	38.2 ±12.4	24.6±11.7	52.6±30.0	NS

a) Lot No. *b*) Means±S.E. *c*) Analysis of variance. NS: not significant at $p<0.05$.

TABLE VI. Correlation Coefficients between *in vivo* Parameters in Pigs and Release Rates (R_{6h} by the Dialysis Tubing Method and R_{3h} by the Muranishi Method)

	C_{max}	AUC_{3h}	AUC_{∞}
Muranishi	0.059	0.062	0.151
Dialysis tubing	0.994	0.994	0.999[a]

a) $p<0.01$.

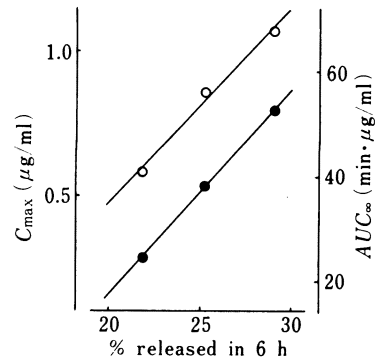

Fig. 9. Relations of Drug Release Rates (% Released in 6 h) from Three Suppositories Determined by the Dialysis Tubing Method with Their Mean C_{max} (○) and AUC_{∞} (●) in Pigs

○, $r=0.994$; ●, $r=0.999$.

poorly with those by the Muranishi method. The *in vitro–in vivo* correlation obtained by the dialysis tubing method is shown in Fig. 9. The findings suggests that as well as suppository B, product F, showing slow release by the dialysis tubing method, may also give poor bioavailability.

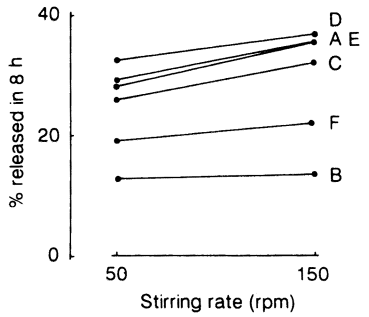

Fig. 10. Effects of the Stirring Rate on the Drug Release Rates (% Dissolved in 8 h) from Indomethacin Suppositories Determined by the Dialysis Tubing Method

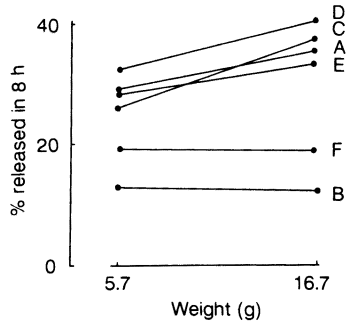

Fig. 11. Effects of Lead Weight on the Drug Release Rates (% Dissolved in 8 h) of the Indomethacin Suppositories Determined by the Dialysis Tubing Method

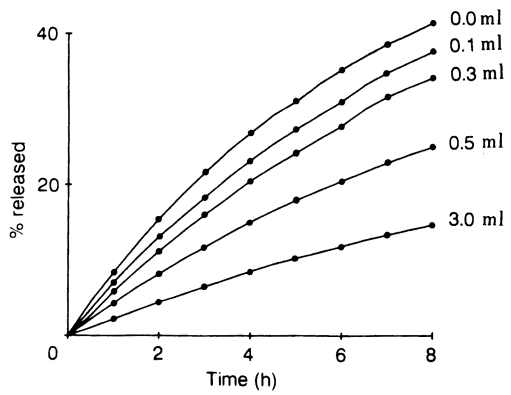

Fig. 12. Effects of Fluid Volume (0—3 ml) Added into the Dialysis Tubing on the Release of Indomethacin from the Experimental Witepsol Suppository Determined by the Dialysis Tubing Method

Medium, 900 ml of pH 7.2 buffer; stirring rate, 50 rpm.

Factors Affecting Drug Release in the Dialysis Tubing Method

The dialysis tubing method provided a good *in vitro–in vivo* correlation. Therefore, the effects of *in vitro* conditions in the dialysis tubing method on the release of indomethacin from suppositories were investigated. The increase of the agitation rate from 50 to 150 rpm and the alteration of the weight hanging on the tube from 5.7 to 16.7 g did not significantly affect the drug release behavior, as shown in Figs. 10 and 11, though the releases from some products including C tended to be accelerated by the increase of the weight. The drug release rates from suppositories did not differ whether the dialysis tube was washed with boiling water or not before use (data not shown). However, the addition of the test fluid into the dialysis tubing significantly retarded the release of indomethacin as shown in Fig. 12, and the effect increased with increase of the fluid volume. The delays in the releases can probably be attributed to the reduction in the effective surface area of the suppository for drug release, because the melted or semi-melted oily suppository did not spread well along the dialysis tubing, especially in the presence of a large quantity of the medium, when the melted suppository floated on the aqueous medium and the dispersion of the melted product along the tubing was inevitably limited.

The delay in the drug release caused by addition of even a small quantity of the fluid into the tubing indicates that much attention should be paid to the removal of water remaining in the dialysis tubing before its use or to the fluid volume when aqueous fluid is intentionally added into the dialysis tubing.

Discussion

The release of indomethacin from suppositories was investigated by three different methods, the JP XI paddle, Muranishi and dialysis tubing methods, which were selected from among many available methods[13,14] for the following reasons. The JP XI paddle method is the most familiar and does not employ any membranes. The Muranishi method is frequently employed in our country and the apparatus is commercially available. The dialysis tubing method is static, whereas the paddle and Muranishi methods are dynamic, and it can provide *in vitro* data reflecting bioavailabilities.[3,8,12] The release behavior of indomethacin from suppositories differed greatly among the three methods. One of the main reasons for this is the differences in the physical force affecting the suppository: in the JP XI paddle method the hydrodynamic flow affects the suppositories, while in Muranishi method the stirring steel rod must promote the disintegration of suppositories and in the dialysis tubing method the pressure of test fluid and the tension of the weighted tubing would enhance the spreading of melted suppositories along the dialysis tubing. These differences among the methods must contribute to the differences in the drug release behavior, although other factors including the membrane permeability of the drug should also be taken into consideration.

When the bioavailabilities were estimated, AUC_∞ values of the least available suppositories were 15% in rabbits and 50% in pigs with respect to those of the most available products, which suggests that bioequivalence studies are required on other commercial suppositories. It is clearly necessary to establish *in vitro* methods reflecting the *in vivo* availabilities.

The bioavailabilities in rabbits and pigs correlated well with the *in vitro* release rates determined by the dialysis tubing method. A previous study on aminopyrine in rabbits also showed high *in vitro–in vivo* correlations for the modified Thomas method but poor correlation for the Muranishi method.[8] The findings suggest that static methods such as the dialysis tubing mthod are preferable to dynamic methods, although the bioavailabilities in humans may differ from those in rabbits and pigs, and further studies on other suppositories are required.

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References

1) Y. Kameda, N. Yasojima and N. Mizuno, *Iyaku Jyanaru*, **19**, 1379 (1983).
2) J. M. Aiache, M. Islasse, E. Beyssac, S. Aiache, R. Renoux and J. P. Kantelip, *Int. J. Pharmaceut.*, **39**, 235 (1987).
3) N. J. Vidras, V. E. Reid, N. R. Bohidar and F. M. Plakogiannis, *J. Pharm. Sci.*, **71**, 945 (1982).
4) C. C. Huang, N. Nambu and T. Nagai, *Yakuzaigaku*, **44**, 141 (1984).
5) S. Muranishi, Y. Ohkubo and H. Sezaki, *Yakuzaigaku*, **39**, 1 (1979).
6) W. H. Thomas and R. McCormack, *J. Pharm. Pharmacol.*, **23**, 490 (1971).
7) H. Yoshino, M. Kobayashi and M. Samejima, *Yakuzaigaku*, **41**, 102 (1981).
8) K. Tanabe, S. Itoh, S. Yoshida, Y. Furuichi, M. Sawanoi, M. Yamazaki and A. Kamada, *Yakuzaigaku*, **44**, 155 (1984).
9) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, 1975, p. 344.
10) G. G. Skellern and E. G. Saloe, *J. Chromatogr.*, **114**, 483 (1975).
11) H. Nakahara, S. Okada and H. Isaka, *Yakuzaigaku*, **46**, 276 (1986).
12) K. Tanabe, Y. Furuichi, R. Inaba, S. Itoh, M. Sawanoi, M. Yamazaki and A. Kamada, *Yakuzaigaku*, **45**, 61 (1985).
13) K. Tanabe and M. Sawanoi, *Iyaku Jyanaru*, **17**, 1273 (1981).
14) H. Ogata, "Suppositories from Dosage Forms to Clinical Applications," ed. by S. Muranishi, Nanzando Co., Ltd., Tokyo, 1985, p. 93.