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Glassy State of Pharmaceuticals. II.¹⁾ Bioinequivalence of Glassy and Crystalline Indomethacin²⁾

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Bioinequivalence of glassy and crystalline indomethacin was investigated in rabbits. *In vivo* absorption studies were carried out by determining the plasma levels of indomethacin following oral and rectal administrations in rabbits. The *AUC* (area under the concentration–time curve) and the maximum plasma level following the administration of the glassy preparation were larger than those in the case of the crystalline preparation. The amounts of indomethacin absorbed were calculated by the deconvolution method. It was shown that the amounts absorbed following oral and rectal administrations of the glassy preparation were larger than those in the case of the crystalline preparation. These results indicated that the bioavailability of the glassy indomethacin was better than that of the crystalline indomethacin.

Keywords—glassy state; indomethacin; bioavailability; stability; X-ray analysis; deconvolution

In the previous paper,¹⁾ the glassy state of indomethacin was confirmed by detection of the jump of heat capacity and the anomalous endothermic peak in the differential scanning calorimeter (DSC) curve, and the relaxation process below the glass transition temperature (T_g) was traced in terms of the area under the anomalous endothermic peak in the DSC curve. It was also reported that the rate of dissolution of glassy indomethacin was far greater than that of crystalline indomethacin. Further, the rate of crystallization of pulverized glassy indomethacin followed first-order kinetics and the degree of crystallization reached a maximum of 60% after 2 months.

It was expected that the glassy indomethacin would show better bioavailability than crystalline indomethacin because the dissolution rate of the glass is faster than that of the crystals as described in the previous paper.¹⁾ In the present paper, *in vivo* absorption studies of glassy and crystalline indomethacin were carried out in rabbits in order to compare the bioavailability of the two forms.

Experimental

Materials—Indomethacin (Sigma Chemical Co., Ltd.) was of reagent grade. Other materials were of special reagent grade except sodium bicarbonate and lactose, which were of JP X1 grade. Glassy indomethacin was prepared by cooling the melt as reported in the previous paper.¹⁾ Physical mixtures of the glass and the crystals with lactose were prepared by mixing the two ingredients as follows: 200–250 mesh (63–74 μm) fraction of glassy or crystalline indomethacin was collected by using standard sieves after pulverizing the drug in an agate mortar. Then, each powder was mixed with lactose (200–250 mesh) in 1:4 weight ratio by using a spatula.

X-Ray Diffraction—A Rigaku Denki Geigerflex instrument was used for the present investigation. The X-ray source was Cu- K_α with an Ni filter (voltage 35 kV, current 10 mA), and a scintillation counter was used as a detector.

DSC—DSC curves were measured with a DSC-2 (Perkin-Elmer). The measurement conditions were the same as those reported in the previous paper.¹⁾

Infrared (IR) Absorption Spectra—IR spectra were measured on a model 295 infrared spectrophotometer (Hitachi). The measurements were made by the KBr disc method.

Thin Layer Chromatography (TLC)—The conditions of TLC were the same as those reported in the previous paper¹⁾ and the spots were visualized under ultraviolet (UV) light.

Dissolution Studies—The dissolution behavior of glassy and crystalline indomethacin was studied by the beaker method with 100 ml of 0.02 M phosphate buffer (pH 7.2) maintained at 37 °C as a solvent. The samples were prepared as reported in the previous paper.¹⁾ Concentration of indomethacin in the solvent was determined spectrophotometrically at 317 nm with a Hitachi 340 spectrophotometer.

Wettability Test—The penetration rate of liquid into glassy and crystalline indomethacin powder (200–250 mesh) beds and into the glassy and crystalline preparations was measured by the powder method. Distilled water and distilled water saturated with lactose were used as penetrating liquids.

Animal Studies—Male rabbits (2.5–3.0 kg) were used after fasting for about 18 h.

i) **Intravenous Administration:** Indomethacin was dissolved in water containing sodium bicarbonate (weight ratio of indomethacin and sodium bicarbonate, 5 : 2) and indomethacin solutions of 7.5, 15, and 30 mg/ml were used as sample solutions.

Three dosage levels of indomethacin (5, 10, and 20 mg/kg body weight) were administered intravenously. Blood samples were taken from an ear vein at appropriate intervals into a heparinized syringe.

ii) **Oral and Rectal Administrations:** A two-way cross-over design was employed for the oral and rectal administrations. A dose of 20 mg/kg body weight as indomethacin was enclosed in a No. 0 gelatin capsule, and then administered orally or rectally. Blood samples were taken from an ear vein at appropriate intervals into a heparinized syringe.

Determination of the Plasma Levels of Indomethacin—Plasma level of indomethacin was determined according to Nogami's modification³⁾ of Hucker's fluorescence method.⁴⁾ Fluorescence intensity was measured with a Hitachi MPF-4 spectrofluorometer (activation maximum, 295 nm; fluorescence maximum, 375 nm uncorrected).

Calculation of Amount Absorbed—The amount of indomethacin absorbed following oral or rectal administration was calculated by the least-squares deconvolution method developed by Pedersen.⁵⁾

Results and Discussion

Preparation and Characterization of Glassy and Crystalline Indomethacin

Glassy indomethacin was prepared by cooling the melt, and the glassy state was confirmed by detection of the glass transition temperature in the DSC curve and a halo in the X-ray diffraction pattern.¹⁾ Crystalline indomethacin used was γ -type crystals; it was characterized by measurements of the X-ray diffraction pattern, melting point (by DSC) and IR spectrum.⁶⁾ Decomposition products of glassy and crystalline indomethacin were not detectable by TLC.

Dissolution Rates of Glassy and Crystalline Indomethacin

Figure 1 shows the dissolution profiles of glassy and crystalline indomethacin in

Fig. 1. Dissolution Profiles of Glassy and Crystalline (γ -Type) Indomethacin Determined by the Beaker Method in Phosphate Buffer (pH 7.2)

●, glassy indomethacin; ○, crystalline indomethacin.

Fig. 2. The Rate of Crystallization of Pulverized Glassy Indomethacin in Water

phosphate buffer (pH 7.2). Glassy indomethacin dissolved about 4 times faster than the crystalline material.

Crystallization of Glassy Indomethacin in Water

If crystallization of the glass occurred during dissolution, the dissolution and subsequent absorption of the glass might be affected. Thus, crystallization of the glass in water was investigated.

After being pulverized and mounted on a glass plate for X-ray diffraction measurement, glassy indomethacin was immersed in distilled water for a given period of time and then dried under vacuum in a desiccator containing P_2O_5 . The X-ray diffraction pattern was measured immediately after the sample was dried. The crystallinity of the sample was determined by Hermans' method.⁷⁾ The crystallization process of the sample immersed in water is shown in Fig. 2 as a plot of crystallinity against time.

After standing for 1.5 h in water, the glass began to crystallize and then crystallization proceeded rapidly. It took 10 h to reach maximum crystallization and at this stage indomethacin had 50% crystallinity. It was considered that if glassy indomethacin was dissolved and absorbed rapidly after administration, *in vivo* absorption might not be influenced by the crystallization of the glass.

Some Physicochemical Properties of Sample Powders

Wettability of the Sample Powders—The *in vivo* absorption rate of the drug after the administration of powder dosage forms is influenced in the first place by the wettability of the sample. Thus, the wettability of powder beds of glassy and crystalline indomethacin was examined. Figure 3 shows the penetration of water into the powder beds in terms of a plot of the square of the penetration length against time. Water did not penetrate into the glassy indomethacin powder bed, and crystalline indomethacin was hardly wetted. Thus, the contact angle as a main factor governing the wettability was measured according to the method reported in the previous paper⁸⁾ and values as large as 95° (glassy indomethacin) and 84° (crystalline indomethacin) were obtained. Improvement of the wettability of glassy and

Fig. 3. Penetration of Liquid into the Powder Beds

●, mixture of glassy indomethacin and lactose (the penetrating liquid was distilled water saturated with lactose); ○, mixture of crystalline indomethacin and lactose (the penetrating liquid was distilled water saturated with lactose); ▲, glassy indomethacin (the penetrating liquid was distilled water); △, crystalline indomethacin (the penetrating liquid was distilled water).

Fig. 4. Crystallization of Glassy Indomethacin Mixed with Lactose at Room Temperature

a) immediately after mixing; b) after standing for 5 h; c) after standing for 30 h; d) after standing for 56 h; e) after standing for 180 h.

a) Indicates diffraction peaks of indomethacin crystals.

crystalline indomethacin was attempted by mixing them with lactose. As shown in Fig. 3, the wettabilities were improved and a linear relationship was found between the square of the penetration length and time for the two preparations.

Crystallization of Glassy Indomethacin at Room Temperature—The crystallization of the glass at room temperature was followed by the X-ray diffraction method. Since indomethacin gave a very stable glass, devitrification did not occur for several years in the laboratory at room temperature. It was considered, however, that crystallization of the glass in the preparation might be induced by pulverizing and by mixing the sample with lactose.

Figure 4 shows the X-ray diffraction patterns of the glassy preparation after standing for a given period of time at room temperature. Only diffraction peaks due to crystalline lactose were observed in the X-ray diffraction pattern immediately after mixing the glass with lactose. After the sample had been left for 30 h, slight diffraction due to indomethacin crystals was observed in the vicinity of 12° (2θ). Subsequently, several diffraction peaks due to indomethacin crystals appeared and the intensity of the peaks increased gradually as crystallization proceeded.

These results indicated that the crystallization of the glass was scarcely induced by pulverizing or by mixing the sample with lactose in the initial stage. Therefore, the following experiments were run within 1 h after pulverization of the glass.

In Vivo Absorption Studies

Figure 5 shows the mean plasma levels of indomethacin following bolus intravenous administration of indomethacin solution. Each curve could be adequately approximated by a biexponential equation (Eq. 1), and the pharmacokinetic parameters obtained are shown in

Fig. 5. Mean Plasma Levels Following Intravenous Administration of Indomethacin Solution

○, 20 mg/kg ($n=3$); ●, 10 mg/kg ($n=4$); ◐, 5 mg/kg ($n=3$).

TABLE I. Pharmacokinetic Parameters Obtained from the Plasma Levels Following Intravenous Administration

Parameter	Dose		
	5 mg/kg	10 mg/kg	20 mg/kg
a_1 ($\mu\text{g/ml}$)	27.31 ± 3.65	37.68 ± 6.92	93.77 ± 13.25
b_1 (/min)	0.1222 ± 0.0056	0.1034 ± 0.0048	0.1335 ± 0.0104
a_2 ($\mu\text{g/ml}$)	9.59 ± 2.87	23.97 ± 4.50	39.27 ± 3.18
b_2 (/min)	0.0132 ± 0.0008	0.0146 ± 0.0024	0.0154 ± 0.0005
k_{12} (/min)	0.0578 ± 0.0055	0.0365 ± 0.0034	0.0581 ± 0.0048
k_{21} (/min)	0.0421 ± 0.0101	0.0504 ± 0.0107	0.0521 ± 0.0077
k_{10} (/min)	0.0423 ± 0.0072	0.0311 ± 0.0053	0.0407 ± 0.0016
V_1 (ml/kg)	136 ± 8	163 ± 9	153 ± 15
V_2 (ml/kg)	176 ± 31	131 ± 37	173 ± 18
$AUC_{0 \rightarrow \infty}$ ($\mu\text{g/ml min}$)	939 ± 148	2057 ± 214	3261 ± 282

Each value represents the mean $\pm$ S.E. of 3–4 rabbits.

Fig. 6. Mean Plasma Levels Following Oral Administration of Glassy and Crystalline Indomethacin

●, glassy indomethacin; ○, crystalline indomethacin. Vertical lines represent the S.E. of the mean for six experiments.

Fig. 7. Mean Plasma Levels Following Rectal Administration of Glassy and Crystalline Indomethacin

●, glassy indomethacin; ○, crystalline indomethacin. Vertical lines represent the S.E. of the mean for six experiments.

Table I.

$$c^*(t) = a_1 \exp(b_1 t) + a_2 \exp(b_2 t) \quad (1)$$

where $c^*(t)$ is plasma level time course of indomethacin and a_1 , a_2 , b_1 and b_2 are constants.

Figures 6 and 7 show the plasma levels following oral and rectal administration of the glass and crystal preparations. Maximum plasma level and area under the concentration–time curve (AUC) following administration of the glass were significantly larger than those of the crystals ($p < 0.05$). These results indicate that glassy and crystalline indomethacin are bioinequivalent.

Calculation of Absorption Rate and Amount Absorbed

Absorption rate and the amount absorbed were calculated by the least-squares deconvolution method developed by Pedersen.⁵⁾ The theory of this method is as follows. The response, $c^*(t)$, (plasma level time course of indomethacin following intravenous administration) to unit impulse input is expressed by Eq. 1. The rate of input (absorption rate), $f(t)$, is approximated to a polynomial function and expressed as Eq. 2.

$$f(t) = \sum_{i=0}^N x_i t^i \quad (2)$$

where x_i are the polynomial coefficients and the highest degree of the polynomial function (N) is the number of data points minus one. The response, $c(t)$, (plasma level time course of indomethacin following oral or rectal administration) to an arbitrary input can be expressed by Eq. 3. Substituting Eq. 3 into Eqs. 1 and 2 yields Eq. 4.

$$c(t) = \frac{1}{q^*} \int_0^t f(t-u) c^*(u) du \quad (3)$$

$$c(t) = \frac{1}{q^*} \int_0^t \sum_{i=0}^N x_i (t-u)^i [a_1 \exp(b_1 u) + a_2 \exp(b_2 u)] du \quad (4)$$

where q^* is an intravenous bolus dose. When Eq. 4 is integrated and rearranged, Eq. 5 is obtained.

$$c(t) = \sum_{i=0}^N x_i \theta_i(t) \quad (5)$$

where,

$$\theta_i(t) = \frac{1}{q^* i!} \sum_{j=1}^2 \frac{a_j}{b_j^{i+1}} \left[\exp(b_j t) - \sum_{k=0}^i \frac{(b_j t)^k}{k!} \right]$$

The polynomial coefficients x_i of the input function were calculated by multiple linear regression analysis and the cumulative amount of input (amount absorbed) was calculated from the integrated form of $f(t)$. Figures 8 and 9 show the cumulative amount of input following oral and rectal administrations of glassy and crystalline preparations, respectively. In both cases, the amounts of input following the administration of the glassy preparation were larger than those of the crystalline preparation.

Fig. 8. Percent Absorbed Following Oral Administration of Glassy and Crystalline Indomethacin Calculated by the Deconvolution Method

●, glassy indomethacin; ○, crystalline indomethacin.

Fig. 9. Percent Absorbed Following Rectal Administration of Glassy and Crystalline Indomethacin Calculated by the Deconvolution Method

●, glassy indomethacin; ○, crystalline indomethacin.

References and Notes

- 1) Part I: E. Fukuoka, M. Makita and S. Yamamura, *Chem. Pharm. Bull.*, **34**, 4314 (1986).
- 2) This work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982.
- 3) M. Moriyama, M. Saito, S. Awazu, M. Hanano and H. Nogami, *Yakugaku Zasshi*, **91**, 1217 (1971).
- 4) H. B. Huckler, A. G. Zacchei, S. V. Cox, D. A. Brodie and N. H. R. Cantwell, *J. Pharmacol. Exp. Ther.*, **153**, 237 (1966).
- 5) P. V. Pedersen, *J. Pharm. Sci.*, **69**, 312 (1980).
- 6) H. Yamamoto, *Chem. Pharm. Bull.*, **16**, 17 (1968).
- 7) P. H. Hermans and A. Weidinger, *J. Appl. Phys.*, **19**, 491 (1948).
- 8) E. Fukuoka, S. Kimura and M. Yamazaki, *Chem. Pharm. Bull.*, **31**, 1030 (1983).