

Bioavailability of Powdered Inclusion Compounds of Nonsteroidal Antiinflammatory Drugs with β -Cyclodextrin in Rabbits and Dogs^{1,2)}NAOKI NAMBU,^{3a)} MICHITOSHI SHIMODA,³⁾ YASUYUKI TAKAHASHI,^{3b)}
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Freeze-dried inclusion compounds of nonsteroidal antiinflammatory drugs with β -cyclodextrin were administered orally to rabbits and beagle dogs in comparison with simply freeze-dried drugs. The antiinflammatory drugs used were flufenamic acid, ibuprofen, ketoprofen and indomethacin. Freeze-dried inclusion compounds showed higher levels of blood concentration and cumulative urinary excretion compared with simply freeze-dried drugs, except for indomethacin. In general, freeze-dried inclusion compounds of drugs with β -cyclodextrin showed a high dissolution rate and high bioavailability. As an exceptional case, no enhancement of the bioavailability was observed in rabbits for the inclusion compound of indomethacin compared with the simply freeze-dried drug. A double maxima phenomenon in blood concentration was observed in the cases of flufenamic acid and indomethacin.

Keywords—bioavailability; rabbit; beagle dog; β -cyclodextrin; inclusion compound; flufenamic acid; ibuprofen; ketoprofen; indomethacin

It was reported previously that the freeze-drying method was suitable for obtaining inclusion compounds of antiinflammatory drugs with cyclodextrins (CD); this was confirmed by various physico-chemical analyses.^{4,5)} All the drugs tested were included in almost a 1:1 molecular ratio. The dissolution curves were obtained by a stationary disk method,⁶⁾ and all of these freeze-dried inclusion compounds were found to dissolve well in aqueous media.⁴⁾ In addition to these inclusion compounds with CD, mixtures, coprecipitates, and freeze-dried samples of antiinflammatory drugs with polyethylene glycol and polyvinylpyrrolidone all dissolved well in aqueous media.⁷⁾ However, CD seemed preferable to these kinds of water-soluble synthetic polymers because of the low hygroscopicity and high fluidity of the powdered inclusion compounds. Moreover, CD may have an advantage over the others as regards safety as an additive. In this point of view also, CD seems to afford a promising means for pharmaceutical preparations.

The authors had a chance to know that the bioavailability of ibuprofen increased when administered orally to rabbits in the form of the powdered inclusion compounds with β -cyclodextrin (β -CD) mentioned above.⁷⁾ Following this finding, the present study was initiated to investigate the bioavailability of the powdered inclusion compounds with β -CD of four kinds of nonsteroidal antiinflammatory drugs, *i.e.*, flufenamic acid (FFA), ibuprofen (IPF), ketoprofen (KPF) and indomethacin (IMC), using rabbits and beagle dogs.

- 1) This paper forms Part IX of "Pharmaceutical Interaction in Dosage Forms and Processing." The preceding paper, Part VIII: Y. Machida and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 1652 (1978).
- 2) This work was presented in part at the 1st International Conference of Pharmaceutical Technology, Paris, May/June 1977.
- 3) Location: a) Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan; b) Kanade-378, Oi-machi, Kanagawa 258, Japan.
- 4) T. Nagai and N. Nambu, *FIP Abstracts* (Dublin, 1975), p. 55.
- 5) M. Kurozumi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 3062 (1975).
- 6) Y. Hamada, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 1205 (1975).
- 7) N. Nambu, M. Shimoda, Y. Takahashi, M. Tsai, H. Ueda, and T. Nagai, unpublished data.

Experimental

Materials and Freeze-Dried Samples— β -CD was used after recrystallization from water. Very pure nonsteroidal antiinflammatory drugs, which all conformed to the registered standards, were as follows: flufenamic acid, mp 133—136°; ibuprofen, mp 74—75°; ketoprofen, mp 93°; indomethacin, mp 153—154°. Following the previous report,⁵⁾ the powdered inclusion compounds were prepared by freeze-drying. The formation of powdered inclusion compounds of drugs with β -CD was confirmed by various physico-chemical analyses.^{4,5)} Simply freeze-dried drug powders were also prepared for comparative experiments to exclude the effects of freeze-drying itself.⁵⁾ All the sample powders administered in the bioavailability study were of 100—200 mesh (74—149 μ).

Bioavailability Study in Rabbits and in Beagle Dogs—Three healthy male albino rabbits weighing 3.0 to 3.5 kg were used after fasting for 24 hr in each comparative experiment. The drugs were administered to the stomach of each rabbit through a sonde, using fresh suspensions containing 100 mg as FFA, 200 mg as IPF, 100 mg as KPF, or 50 mg as IMC.

In the case of beagle dogs, six healthy males weighing 10.2 to 11.5 kg were used after fasting for 24 hr in each experiment. The cross-over method was used with an interval of one week. The dogs were administered 100 mg of KPF orally as a powder wrapped in two pieces of oblate.

Two and two-tenths milliliters of heparinized blood sample was collected from the ear vein of rabbits and from the cephalic vein of beagle dogs at appropriate times and immediately centrifuged at 3000 rpm to obtain plasma samples.

Determination of the Concentrations of Drugs in Blood and in Urine—Blood concentrations of drugs were determined by the following methods. It was confirmed initially that the recovery of drugs by the methods employed here was more than 95%.

The concentration of FFA was determined according to the method described by Hattori *et al.*,⁸⁾ using a Hitachi 204 fluorescence spectrophotometer.

The concentration of IPF was determined using a Shimadzu GC-5AP₃ gas chromatograph equipped with a hydrogen ionization detector. The column was of glass tubing, 2 m long by 3.0 mm internal diameter, packed with 3% OV-17 on Chromosorb W, AW-DMCS, 80—100 mesh. Operating conditions were: injection port, 230°; column oven, 160°; and detector, 230°. Gas flows were: nitrogen, 60 ml/min; hydrogen, 50 ml/min; and air, 1 l/min. One ml of the plasma sample, 4 ml of 0.5 N hydrochloric acid and 10 ml of benzene were shaken together for 30 min, and then centrifuged. Eight ml of the benzene layer was evaporated down at 40°. The residue was dissolved in 2 ml of diazomethane/ether, left to stand for 1 hr, and then evaporated down to remove the ether. The residue was dissolved in 0.1 ml of acetone and a 2.5 μ l aliquot was injected into the chromatograph. The concentration of IPF was obtained by reference to a standard curve.

The concentration of KPF was determined in a way similar to that used for IPF. Operating conditions were: injection port, 270°; column oven, 240°; and detector, 270°. The other conditions were the same as those for IPF. One ml of the plasma sample, 4 ml of 0.1 N hydrochloric acid and 10 ml of benzene were shaken together for 30 min. Then, the procedure followed that in the case of IPF, though the final sample injected into the chromatograph was more diluted in order to get an appropriate chromatogram.

The concentration of IMC was determined according to the method described by Aoyama *et al.*,⁹⁾ using a Shimadzu GC-5AP₃ gas chromatograph equipped with an electron capture detector.

Urine concentrations of IMC and FFA were determined in the same way as the blood concentrations after incubating 0.5 ml of the urine sample and 2 ml of 0.2 M acetate buffer solution (pH 5.0, containing 1000 μ /ml of β -glucuronidase) for 16 hr at 37°. IPF and KPF were determined by gas chromatography starting with 0.5 ml of the urine sample, according to the methods described by Sakai *et al.*,¹⁰⁾ and by Kageyama *et al.*,¹¹⁾ respectively.

Results and Discussion

Bioavailability Study of FFA, IPF, KPF and IMC in Rabbits

Figure 1 shows the blood concentration curves with standard error for FFA after oral administration. The freeze-dried inclusion compound gave a high blood concentration curve compared with the simply freeze-dried drug. Two phases, or double maxima, were observed

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9) T. Aoyama, S. Iguchi, and W. Tseng, *Yakuzaiigaku*, **27**, 53 (1967).

10) Y. Sakai, T. Yamamoto, M. Inoue, H. Inoue, T. Kimura, A. Komori, and T. Funakoshi, *Clin. Repts.* (Tokyo), **4**, 1763 (1970).

11) T. Kageyama, S. Sugawara, and H. Sato, *RIUMACHI*, **13**, 245 (1973).

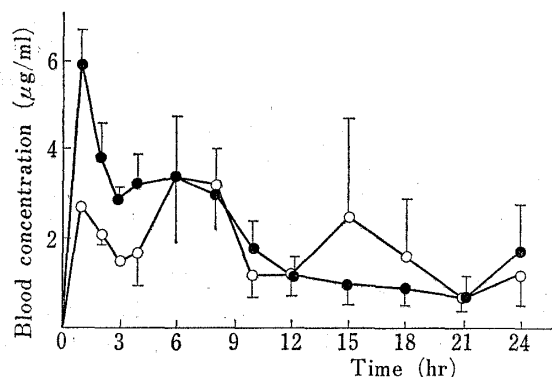


Fig. 1. Blood Concentration Curves with Standard Error for FFA in Rabbits after Oral Administration of 100 mg of FFA

●: FFA/ β -CD freeze-dried inclusion compound.
○: freeze-dried FFA.

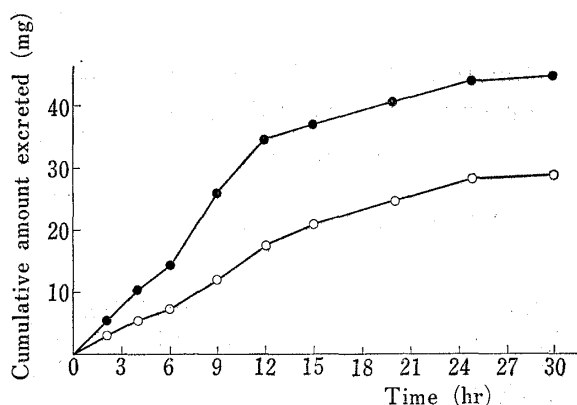


Fig. 2. Average Cumulative Urinary Excretion Curves for FFA in Rabbits after Oral Administration of 100 mg of FFA

●: FFA/ β -CD freeze-dried inclusion compound.
○: freeze-dried FFA.

on the curves. This phenomenon may be a property of the drug itself, and may be related to entero-hepatic circulation of the drug; a similar effect was observed in the case of IMC, as will be mentioned later. Further investigations are required to elucidate the mechanism on the basis of studies of absorption, distribution, metabolism and excretion (ADME).

Figure 2 shows the average cumulative urinary excretion curves for FFA. The cumulative amount excreted was high for the freeze-dried inclusion compound compared with the simply freeze-dried one. This result corresponds to the blood concentration curve in Fig. 1. The recovery in urine of FFA administered was 44.5% for the freeze-dried inclusion compound and 28.5% for the simply freeze-dried one. The path of the unrecovered drug requires further investigation by ADME studies as is also the case for IPF, KPF and IMC.

Figure 3 shows the blood concentration curves with standard error for IPF after oral administration. In this case also, the freeze-dried inclusion compound gave a high level. No double maxima phenomenon was observed.

Figure 4 shows the average cumulative urinary excretion curves for IPF. These urinary excretion curves also correspond to the blood concentration curves. The recovery in the

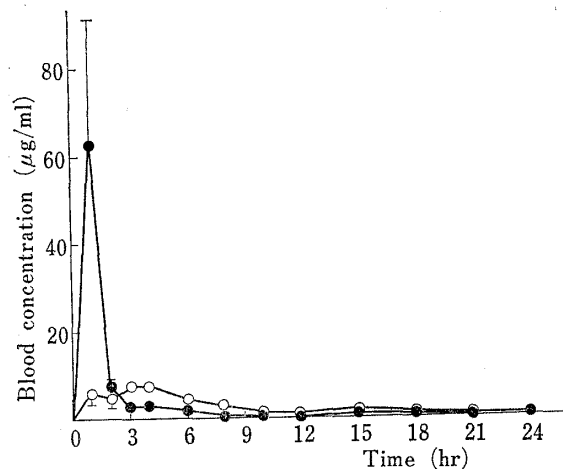


Fig. 3. Blood Concentration Curves with Standard Error for IPF in Rabbits after Oral Administration of 200 mg of IPF

●: IPF/ β -CD freeze-dried inclusion compound.
○: freeze-dried IPF.

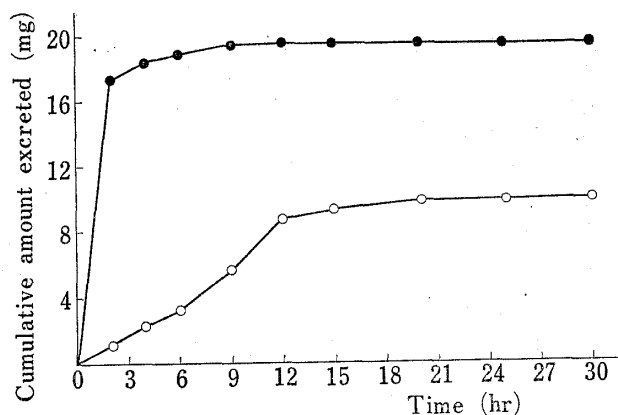


Fig. 4. Average Cumulative Urinary Excretion Curves for IPF in Rabbits after Oral Administration of 200 mg of IPF

●: IPF/ β -CD freeze-dried inclusion compound.
○: freeze-dried IPF.

urine of IPF administered was 9.7% for the freeze-dried inclusion compound and 5.0% for the simply freeze-dried one.

Figure 5 shows the blood concentration curve with standard error for KPF after oral administration. The freeze-dried inclusion compound gave a high level, and no double maxima phenomenon was observed.

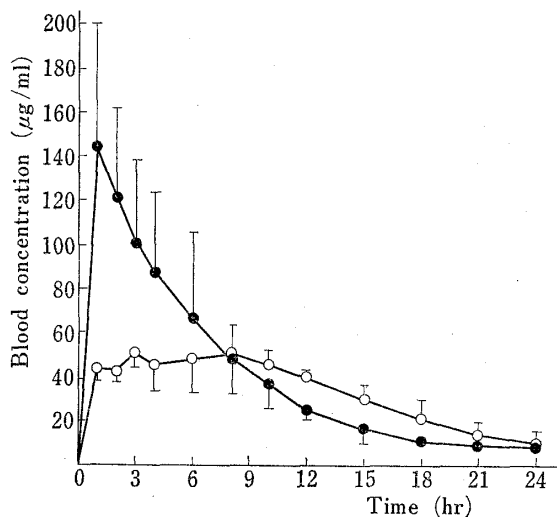


Fig. 5. Blood Concentration Curves with Standard Error for KPF in Rabbits after Oral Administration of 100 mg of KPF

●: KPF/β-CD freeze-dried inclusion compound.
○: freeze-dried KPF.

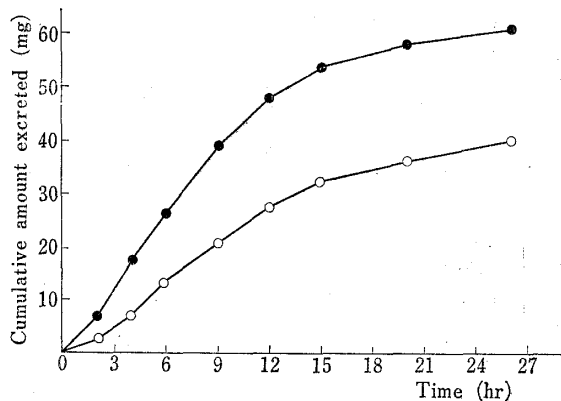


Fig. 6. Average Cumulative Urinary Excretion Curves for KPF in Rabbits after Oral Administration of 100 mg of KPF

●: KPF/β-CD freeze-dried inclusion compound.
○: freeze-dried KPF.

Figure 6 shows the average cumulative urinary excretion curves for KPF. The result was similar to those of FFA and IPF for urinary excretion. The recovery in the urine of KPF administered was 60.5% for the freeze-dried inclusion compound and 40.0% for the simply freeze-dried one.

Figure 7 shows the blood concentration curves with standard error for IMC after oral administration. In this case, there was no significant difference in blood concentration curves between the freeze-dried inclusion compound and the simply freeze-dried one. In the initial stage, the level was rather high for the simply freeze-dried one compared with the

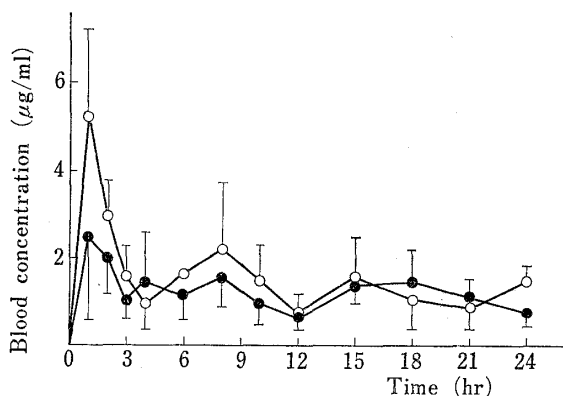


Fig. 7. Blood Concentration Curves with Standard Error for IMC in Rabbits after Oral Administration of 50 mg of IMC

●: IMC/β-CD freeze-dried inclusion compound.
○: freeze-dried IMC.

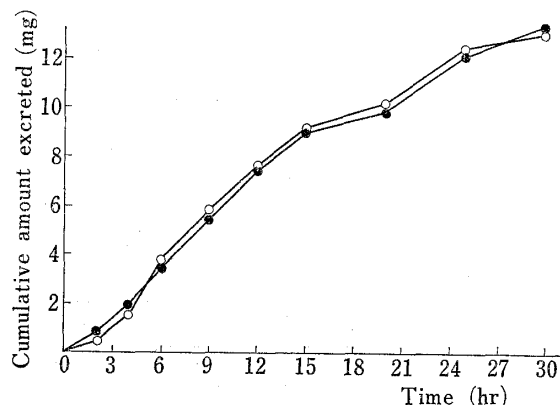


Fig. 8. Average Cumulative Urinary Excretion Curves for IMC in Rabbits after Oral Administration of 50 mg of IMC

●: IMC/β-CD freeze-dried inclusion compound.
○: freeze-dried IMC.

freeze-dried inclusion compound. Double maxima in the blood concentration curves were observed, as in the case of FFA. In this connection, the drugs were considered to be classifiable into two groups, *i.e.*, FFA/IMC and IPF/KPF. Further investigations will be necessary to determine the reason for this.

Figure 8 shows the average cumulative urinary excretion curves of IMC. The urinary excretion of IMC corresponds to the blood concentration curves. The recovery in urine of IMC administered was 26.5% for the freeze-dried inclusion compound and 26.0% for the simply freeze-dried one. The freeze-dried inclusion compound was not effective in increasing the bioavailability of IMC.

On the other hand, the dissolution rate was high in the case of the freeze-dried inclusion compound of IMC.^{4,7)} Therefore, it seemed possible that dissolution was not the rate-determining step in the absorption of IMC in this case. Some modification of the method of administration might be useful to enhance the bioavailability of the inclusion compound. In any case, detailed investigation of individual drugs seems to be necessary to extend the usefulness of inclusion compounds.

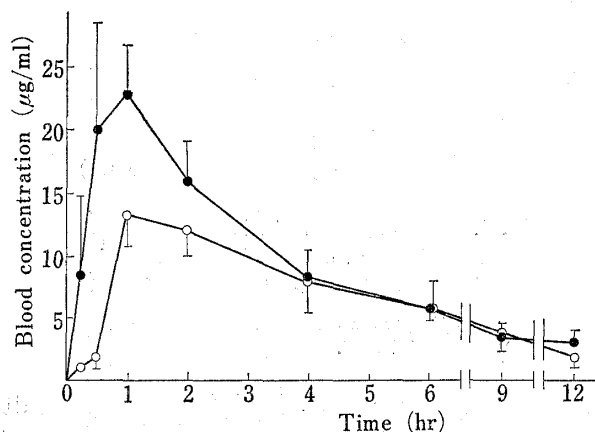


Fig. 9. Blood Concentration Curves with Standard Error for KPF in Beagle Dogs after Oral Administration of 100 mg of KPF

●: KPF/ β -CD freeze-dried inclusion compound.
○: freeze-dried KPF.

Bioavailability Study of KPF in Beagle Dogs

In order to confirm the results in rabbits, a bioavailability study was carried out using beagle dogs. KPF was chosen for these experiments because the rise of blood level in rabbits produced by KPF was the highest among the four drugs. Fig. 9 and Table I show that the freeze-dried inclusion compound was remarkably effective in increasing the bioavailability of KPF in beagle dogs as well. This result seems significant, perhaps indicating that some general property of freeze-dried inclusion compounds affects the bioavailability of drugs (the case of IMC in rabbits may be exceptional). On measuring the

TABLE I. Blood Concentration with Standard Error and *t*-Value for KPF in Beagle Dogs at Various Times after Oral Administration of 100 mg of KPF

Time (hr)	β -CD freeze-dried inclusion compound ^{a)} ($\mu\text{g/ml}$)	Freeze-dried drug ^{a)} ($\mu\text{g/ml}$)	<i>t</i> -Value
0.25	8.40 \pm 6.56	0.58 \pm 0.75	10.38 ^{b)}
0.5	20.03 \pm 8.40	1.85 \pm 0.88	20.54 ^{b)}
1	22.88 \pm 3.94	13.43 \pm 0.74	12.69 ^{b)}
2	15.88 \pm 3.18	12.00 \pm 0.65	6.01 ^{b)}
4	8.40 \pm 2.05	7.88 \pm 0.60	0.87
6	5.88 \pm 2.10	5.88 \pm 0.50	0
9	3.55 \pm 1.12	3.90 \pm 0.39	0.91
12	3.28 \pm 1.02	2.18 \pm 0.38	2.87

^{a)} Average \pm standard error.

^{b)} Significant as determined by the *t*-test at the 0.1% level.

area under the curve up to 4 hr after administration with a planimeter, the ratio was found to be 1.52: 1.00 for the inclusion compound of KPF against the simply freeze-dried drug.

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