

**Influence of Inclusion of Nonsteroidal Antiinflammatory Drugs with  
 $\beta$ -Cyclodextrin on the Irritation to Stomach of  
Rats upon Oral Administration<sup>1,2)</sup>**

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Nonsteroidal antiinflammatory drugs are generally very slightly soluble in water and sometimes cause an adverse reaction due to a stimulant property to stomach upon oral administration. The present study was attempted to investigate the effect of inclusion with  $\beta$ -cyclodextrin ( $\beta$ -CD) on the irritation to stomach of three kinds of drugs, *i.e.*, indomethacin (IMC), flufenamic acid (FFA) and phenylbutazone (PBZ) using Wistar male rats. The samples of the inclusion compounds or the intact ones were administered to stomach of rats, and the degree of injury of mucosa of stomach was observed by a dissection microscope, being evaluated by the six grades of numerical marks from 0 to 4.0. Any significant difference was not observed between the freeze-dried inclusion compounds and intact drugs of both IMC and FFA. There observed no significant difference between the two dosages of IMC. In the case of PBZ, however, there was a significant difference between the freeze-dried inclusion compound and intact drug by *t*-test at 5% level, suggesting that owing to its basic property the inclusion compound was effective in reducing the stomach injury due to the drug.

**Keywords**—inclusion compound;  $\beta$ -cyclodextrin; indomethacin; flufenamic acid; phenylbutazone; injury of stomach; Wistar male rat

It was reported previously that the freeze-drying method was successful in obtaining the inclusion compounds of antiinflammatory drugs with cyclodextrin (CD),<sup>4,5)</sup> and also that the bioavailability of some antiinflammatory drugs was enhanced when administered orally to rabbits and dogs in the form of powdered freeze-dried inclusion compounds with  $\beta$ -cyclodextrin ( $\beta$ -CD).<sup>6)</sup>

In pharmaceutical and toxicological points of view, CD seems to have various merits as a drug additive when used in the form of such the inclusion compounds. In the same sense, CD may afford a promising means for reducing an adverse drug effect. In this connection, nonsteroidal antiinflammatory drugs, which are generally very slightly soluble in water and sometimes cause an adverse reaction due to a stimulant property to stomach upon oral administration. All the freeze-dried inclusion compounds of these drugs dissolved well in aqueous media.<sup>7)</sup> Even if it takes a fairly long period for the inclusion compound to dissolve

- 1) This paper forms Part XI of "Pharmaceutical Interaction in Dosage Forms and Processing." The preceding paper, Part X: K. Takayama, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 2965 (1978).
- 2) This work was partly presented 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) N. Nambu, M. Shimoda, Y. Takahashi, H. Ueda, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 2952 (1978).
- 7) N. Nambu, M. Shimoda, Y. Takahashi, M. Tsai, H. Ueda, and T. Nagai, unpublished data.

in gastric juice, there may be little chance for the drug itself to touch the stomach wall directly, making less an irritation or injury to stomach upon oral administration.

Based on the above consideration, the present study was attempted to investigate such the effect of inclusion with  $\beta$ -CD on the irritation to stomach of three kinds of nonsteroidal antiinflammatory drugs, *i.e.*, indomethacin (IMC), flufenamic acid (FFA) and phenylbutazone (PBZ), using Wistar male rats. As a result, it was found that the inclusion compound was effective in reducing the injury of stomach in the case of phenylbutazone.

### Experimental

**Materials and Samples Administered**—As reported in the previous report,<sup>5)</sup>  $\beta$ -CD was used after recrystallization from water. Very pure compounds of nonsteroidal antiinflammatory drugs, all of which conformed to the registered standards, were as follows: indomethacin, mp 153—154°; flufenamic acid, mp 133—136°; and phenylbutazone, mp 105°. Following the previous report,<sup>5)</sup> the powdered inclusion compounds were prepared by freeze-drying. The formation of powdered inclusion compounds of drugs with  $\beta$ -CD was confirmed by various physico-chemical analyses.<sup>4,5)</sup> All the sample powders administered to rats were of 100—200 mesh (74—149  $\mu$ ).

**Administration of Samples to Wistar Male Rats and Evaluation of Stomach Injury**—Five healthy Wistar male rats weighing 150 to 200 g were used for each sample after fasting for 8 hr. The rats were administered compulsorily to stomach through a cannula for oral use with quite very fresh aqueous suspensions containing the respective drugs as shown in Table I. Inclusion compounds contained the same amount

TABLE I. The Amount and the Concentration of the Samples Administered to Wistar Male Rats

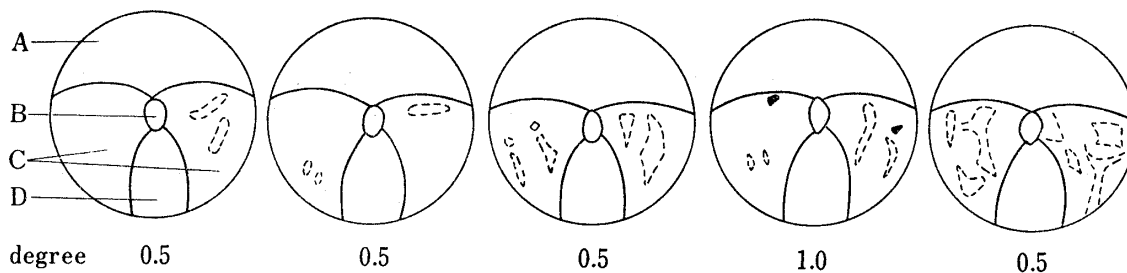
	IMC		IMC		FFA		PBZ	
	Intact drug	Inclusion compound	Intact drug	Inclusion compound	Intact drug	Inclusion compound	Intact drug	Inclusion compound
Amount administered (mg/kg)	25	148.8	12.5	74.4	100	757.6	100	694.4
Concentration in suspensions (mg/ml)	5	29.7	2.5	14.9	20	151.5	20	138.8

of the respective drugs as the intact ones. The amounts administered were determined by a preliminary experiment from such a viewpoint to be able to observe a difference in the effect among the samples. The rats were fasted after administration except for giving water. Seventeen hours after administration, the rats were injected 2% Evan's Blue dissolved in physiological saline in the dose of 0.5 ml per 150 g weight through tail vein or femoral vein. After one hour the rats were killed by sucking ether, and the abdomen was opened. The stomach was excised accompanying with both a part of duodenum and 15 to 20 mm of esophagus. The duodenum just under pylorus was closed by a hemostat, and the stomach was swelled by putting 10 to 12 ml physiological saline through the esophagus by a syringe with an end point cut needle and was closed by a hemostat near the cardia. After fixing the tissues by soaking in 5% formaldehyde solution for about five minutes, the stomach was incised along greater curvature and washed out with physiological saline. The degree of injury of mucosa was observed by a dissection microscope in 5 to 10 magnification and sketched. The degree of injury was evaluated by the following numerical marks.

- 0: normal (no injury, bleeding and latent injury)
- 0.5: latent injury or wide-spread bleeding
- 1.0: slight injury (2 or 3 small dotted injuries)
- 2.0: severe injury (continuous lined injury or 5 to 6 dotted injuries)
- 3.0: very severe injury (several continuous lined injuries)
- 4.0: wide-spread lined injury or widened injury

### Result and Discussion

In comparison between the inclusion compound and the intact drug of PBZ, Fig. 1 shows the schematic expression of the inside of stomach of five rats for each sample after administration. In the case of IMC, the degree of injury was rather high in spite of the form of

freeze-dried inclusion compound with  $\beta$ -cyclodextrin

## intact drug

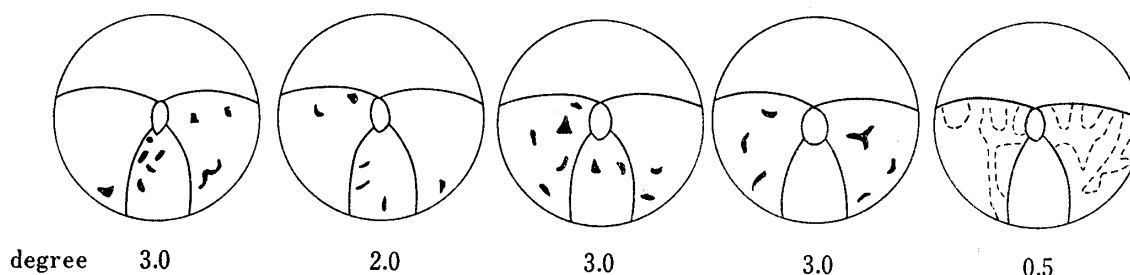


Fig. 1. Degree of Injury of Stomach of Wistar Male Rats Incised along Greater Curvature after Administration of Phenylbutazone

A: pars proventricularis; B: cardia; C: pars grandularis D: pyloric region.

Dotted line: latent injury; simple solid point or line: slight injury; heavy solid depiction: severe injury.

TABLE II. Degree of Injury of the Stomach of Wistar Male Rats

Drug	Amount administered (mg/kg)	Degree of injury	
		Intact drug <sup>a)</sup>	Inclusion compound <sup>a)</sup>
IMC	25	4.0±0	4.0±0
IMC	12.5	3.8±0.2	3.4±0.4
FFA	100	1.0±0.3	1.4±0.3
PBZ	100	2.3±0.5	0.6±0.2 <sup>b)</sup>

a) Average±standard error.

b) Significant by *t*-test at 5% level.

samples, and thus the examination was carried out in two dosages. Table II shows the degree of injury of rat stomach. Any significant difference was not observed between the freeze-dried inclusion compounds and intact drug of both IMC and FFA. No significant difference was observed between the two dosages of IMC. In the case of PBZ, however, there was a significant difference between the freeze-dried inclusion compound and the intact drug by *t*-test at 5% level, showing that the inclusion compound was effective in reducing the stomach injury due to the drug.

The reason is not obvious why only the inclusion compound of PBZ was effective in reducing the injury of stomach upon oral administration. Anyhow, this result is very interesting and further investigations should be made to make clear the reason. One thing which may be considered as the reason is the intrinsic property of the drug itself. However, it seems reasonable to say that such effect may appear on the basis of the combination of

various factors. In this connection, according to the bioavailability study using rabbits reported previously,<sup>6)</sup> such an exceptional result was obtained that the bioavailability was not enhanced by the inclusion compound of IMC contrary to the general property of inclusion compound. Owing to its basic property suggested in this study, an inclusion compound was effective in reducing the stomach injury as mentioned already, and thus some modification of the method of administration might be useful to develop such effectiveness.

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