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Influence of Pharmaceutical Properties on Gastric Lesions Induced in Rats by AD-1590, a Non-steroidal Anti-inflammatory Drug[1)]

TERUTAKA TAKAHASHI,*,[a] MASAAKI MORI,[b] YUKO UEZONO,[a]
HIROKAZU MAKITA,[b] YUTAKA NAKANISHI,[a]
and YU IMASATO[b]

Pharmaceutical Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564, Japan[a] and Ebie 1–5–51, Fukushima-ku, Osaka 553, Japan[b]

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The influence of pharmaceutical properties on the gastric lesions induced by AD-1590 (AD), a non-steroidal anti-inflammatory drug, was investigated in rats.

AD, indomethacin (IM) and aspirin (ASA) were orally administered in powder or suspension form to fasted rats. It was found that the gastric ulcerogenic activity of the powder was stronger than that of the suspension in all cases. Thus, the gastric ulcerogenic activity was influenced by the dosage form.

Solid preparations of AD having various pharmaceutical properties were orally administered to fasted rats, and it was found that the gastric ulceration caused by AD was associated with the residence time of preparations in the stomach rather than the maximum plasma concentration (C_{max}) or extent of bioavailability (area under the plasma concentration-time curve). Preparations having a long residence time caused gastric ulcers. The gastric ulceration caused by AD might be eliminated by improvement of dissolution and disintegration properties of the drug preparation and by suitable control of gastric emptying. Thus, it appears to be possible to evaluate the influence of pharmaceutical properties on gastric lesions induced by non-steroidal anti-inflammatory drugs (NSAIDs) by using small laboratory animals such as rats.

Keywords——AD-1590; solid preparation; gastric lesion; plasma concentration; gastric emptying

AD-1590 (AD), 2-[8-methyl-10,11-dihydro-11-oxodibenz[*b*,*f*]oxepin-2-yl]propionic acid, is a new acid non-steroidal anti-inflammatory drug (NSAID) having potent antipyretic activity.[2,3)] NSAIDs frequently cause gastrointestinal lesions (GI-lesions) as a common side effect.[4)] AD similarly causes GI-lesions in rats.[3)] As GI-lesions are an important clinical problem, it is necessary to investigate the relationship between GI-lesions and drug formulation from an early stage of the formulation study in order to design products which do not cause GI-lesions. In pharmacological and toxicological studies, a small animal such as the rat is used for evaluation of GI-lesions, and a solution or suspension dosage form is usually used because oral administration of solid preparations to small animals may be difficult and troublesome. However, most drug products are solid forms and it is, therefore, necessary to take into account the differences between dosage forms in pharmacokinetic properties, such as dissolution and GI transit time, which influence GI-lesions. Accordingly, solid preparations should be used for the evaluation of GI-lesions in small animals in order to ensure efficacy and GI safety for NSAIDs. However, surprisingly little has been published in this field with regard to the influence of pharmaceutical properties of NSAIDs on GI-lesions in small animals. The purpose of this study was to investigate how GI-lesions induced in rats by AD were influenced by the pharmaceutical properties of the drug. Solid preparations having different pharmaceutical properties were orally administered to fasted rats and the re-

lationship between the pharmacokinetic properties of each preparation and the induction of gastric lesions was clarified.

Experimental

Materials——Powder of AD (Dainippon Pharmaceutical Co., Ltd.) was pulverized and screened to provide fine powder (specific surface diameter, about 10 μm) and coarse powder (gross particle size, 150—350 μm). Fine powder was used for preparation of suspension. Powders of indomethacin (IM) and aspirin (ASA) were of JP X grade and were used after being pulverized. The specific surface diameter of IM powder was about 10 μm and the gross particle size of ASA powder was 50—100 μm. Other materials were commercial products.

Test Preparations——Test preparations for oral administration were as follows: coarse powder; fine powder; suspension; acid-soluble powder (A-powder); acid-insoluble powder (E-powder); rapidly disintegrating tablet (R-tablet); slowly disintegrating tablet (S-tablet).

Suspension were prepared by suspending AD in 0.5% gum tragacanth at a content of 1.5—6 mg/ml. A-powder was prepared by the fusion method. AD–polyethylene glycol 6000 (1 : 9) was heated over a thermostated plate with constant stirring until a clear homogeneous melt was obtained. The melt was quickly poured onto air-cooled aluminum pans, and then pulverized and screened. E-powder was prepared by the solvent method. AD–hydroxypropylmethylcellulosephthalate (1 : 1) was dissolved in mixed solvent (ethanol–dichloromethane, 1 : 1) and then the organic solvent was evaporated off *in vacuo*. The residue was dried *in vacuo* to constant weight, then pulverized and screened. Gross particle size of A-powder and E-powder was about 100 μm.

R-tablet and S-tablet (weight, 60 mg) containing 10 mg of AD were prepared by a simple wet granulation method using hydroxypropylmethylcellulose, low-substituted hydroxypropylcellulose, lactose and magnesium stearate. Calcium carboxymethylcellulose as a disintegrant was contained in R-tablet but not in S-tablet. Small tablets for oral administration were prepared by cutting the original tablet to a size (cube, 1—1.2 × 1—1.2 mm) equivalent to a dose of 6 mg/kg. This tablet size permits oral administration and gastric emptying in rats.

Solution for intravenous administration was prepared by dissolving AD in mixed solvent (propylene glycol–distilled water, 1 : 1) and its content was 2 mg/ml. Suspensions of IM and ASA were prepared with 0.5% gum tragacanth. The contents of IM and ASA in suspension were 3 mg/ml and 16 mg/ml, respectively.

Disintegration Test——The disintegration time was determined according to the JP X method using the basket–rack assembly equipped with a 40 mesh screen instead of 12 mesh. The disintegration times of R-tablet and S-tablet were 30 s and 30 min, respectively.

Dissolution Test——Dissolution tests were carried out according to the JP X method (paddle method, 100 rpm). Each preparation (containing 20 mg as AD) was dissolved in 1000 ml of the JP X disintegration 1st fluid (pH 1.2) and 2nd fluid (pH 6.8) at 37 °C. The concentration of AD in the fluid was determined by ultraviolet spectrophotometry at 314 nm.

Figure 1 shows dissolution profiles for coarse powder, A-powder and E-powder. AD from A-powder was rapidly and completely dissolved in the 1st fluid and its concentration was much higher than the solubility of AD. This supersaturation was maintained for 2 h. It is interesting that the solubility of A-powder, which is a solid dispersion formed from AD and polyethylene glycol 6000, was very much increased in acidic medium. AD from A-powder was also rapidly dissolved in the 2nd fluid. On the other hand, AD from E-powder was not dissolved in the 1st fluid but was rapidly dissolved in the 2nd fluid. This indicates that E-powder has enteric characteristics.

Animals——Wistar-strain male rats, weighing about 200 g, were fasted for 24 h in cages with a wire net floor prior to this experiment.

Administration Apparatus——Figure 2 shows the apparatus for oral administration of solid preparations to rats. This apparatus consists of syringe, sonde and polyethylene tubing (2 mm in diameter, 50 mm in length) for packing solid preparations.

Bioavailability——Each solid preparation equivalent to a dose of 6 mg/kg was packed in the tubing and was orally administered with 0.5 ml of tap water to fasted rats. Suspension was placed in a syringe for administration. Blood specimens were withdrawn from the jugular vein at predetermined times and the plasma samples were frozen and stored (−20 °C) until assay.

The bioavailability was evaluated from the observed maximum plasma concentration of AD (C_{max}), the time to C_{max} (T_{max}) and the area under the plasma concentration–time curve from zero to infinite time (AUC), calculated using the trapezoidal rule and according to the method of Wagner.[5] The mean residence time of a drug in the gastrointestinal tract (MRT_g) was calculated according to the method of Yamaoka *et al.*[6]

Gastric Lesions——Rats were sacrificed by cutting the aorta 7 h after oral administration and the stomach was isolated and cut along the greater curvature. The lesions of the gastric wall were observed macroscopically. The gastric lesions were graded according to the method of Nakamura *et al.*[7]: normal, 0; bleeding and/or erosion of mucosa, 1; less than 5 small ulcers (less than 2 mm in diameter or length), 2; 5 or more small ulcers or one large ulcer (2 mm or more in diameter or length), 3; two or more large ulcers, 4. The gastric lesions with a score of 2 or more were

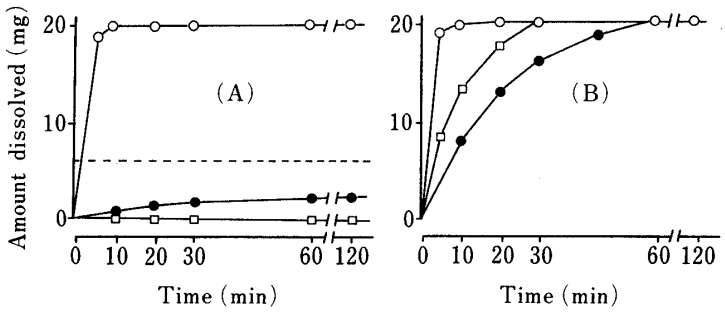

Fig. 1. Dissolution Profiles for Coarse Powder, A-Powder and E-Powder in JP X 1st and 2nd Fluids at 37 °C

(A), JP X 1st fluid; (B), JP X 2nd fluid.
The dotted line shows the solubility of AD in the 1st fluid.
●, coarse powder; ○, A-powder; □, E-powder.

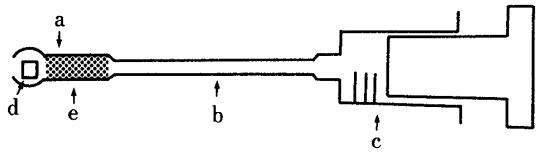

Fig. 2. Apparatus for Oral Administration of Solid Preparations

a, polyethylene tubing; b, sonde; c, syringe; d, small tablet; e, powder.

considered to be ulcers.

The doses of IM (8 mg/kg) and ASA (40 mg/kg) for evaluation of the gastric lesions were decided on the basis of $UD_{50\%}$ of the suspension in rats.[3]

Gastric Emptying——Rats were sacrificed by cutting the aorta 30 min after oral administration of each preparation equivalent to a dose of 6 mg/kg. The stomach was isolated and the blood adhering to the stomach was blotted with filter paper. Then the stomach was cut along the greater curvature, and this specimen was transferred to a beaker with 100 ml of isotonic phosphate buffer of pH 7.0. After being stirred with a magnetic stirrer for 3 h, the extracts were filtered with a filter of 0.4 μm pore size and then the filtrate was subjected to assay.

Assay——The concentration of AD in the plasma and the extracts from the stomach was determined by high-performance liquid chromatography (HPLC). First, 0.2 ml of internal standard (indomethacin 1.0 μg/ml in ethanol), 2.0 ml of phosphate–citrate buffer (pH 3.0) and 5.0 ml of diethylether were added to 0.2 ml of plasma sample or extract. The mixture shaken for 10 min, then 4 ml of the organic layer was separated and evaporated under a nitrogen gas stream at 40 °C. The dried residue was dissolved in 100 μl of methanol and analyzed by HPLC.

HPLC was performed using a μBondapak C_{18} column (Waters Assoc.) with 0.02 M ammonium acetate–acetonitrile–methanol (9 : 4 : 2) as the mobile phase (2.0 ml/min flow rate and 254 nm detection). The detection limit of AD in the plasma was 50 ng/ml.

Results and Discussion

Dosage Form–Gastric Lesions

Table I shows the gastric ulcerogenic activity after oral administration of AD, IM and ASA in powder or suspension form to fasted rats.

The frequency of ulceration with AD suspension increased with increasing dose and $UD_{50\%}$, the dose required to produce ulcers in 50% of the rats used, was estimated to be somewhat more than 12 mg/kg. $UD_{50\%}$ for IM suspension and ASA suspension were estimated to be approximately 8 mg/kg and somewhat more than 40 mg/kg, respectively. These results were in good accordance with those reported by Nakamura *et al.*[3] The frequency of ulceration for AD powder (coarse powder) increased with increasing dose and the $UD_{50\%}$ was estimated to be about 6 mg/kg which is approximately one-half that of the suspension. The score of the gastric lesions at $UD_{50\%}$ of the powder (6 mg/kg) was larger than that of the suspension (12 mg/kg) and moreover, the score in rats having ulcers was 3.2 ± 0.4 for powder and 2.0 ± 0.0 for suspension. Thus, the gastric ulcerogenic activity of powder was stronger than that of suspension, and the activity was influenced by dosage form. In the cases

TABLE I. Gastric Ulcerogenic Activity of AD, IM and ASA after Oral Administration of Powder and Suspension to Fasted Rats

Drug	Dose (mg/kg)	Frequency of ulceration (No. of rats with ulcer[a]/tested)		Mean score ± S.E.	
		Powder	Suspension	Powder	Suspension
AD	3	2/10	0/10	0.6±0.3	0.2±0.1
	6	5/10	1/10	1.6±0.6	0.4±0.2
	12	7/10	4/10	1.9±0.3	1.0±0.2
IM	8	6/6	3/6	2.0±0.0	1.3±0.3
ASA	40	6/6	2/7	3.0±0.3	1.1±0.3

a) Gastric lesions with the score of 2 or more.

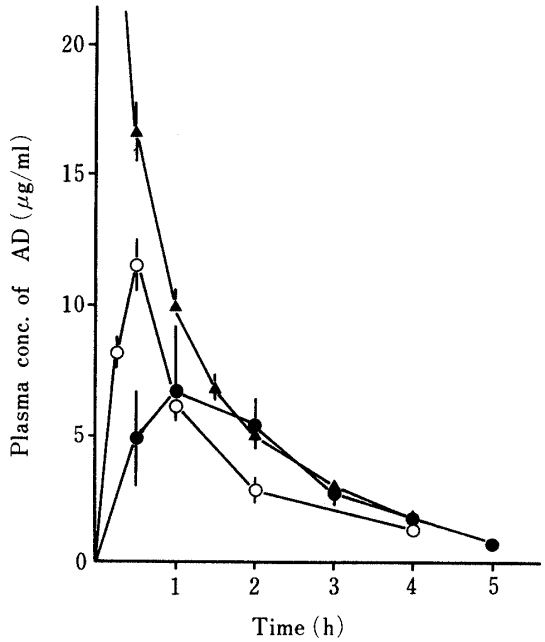

Fig. 3. Comparison of Mean Plasma Concentration–Time Curves between Preparations after Administration of 6 mg/kg of AD to Fasted Rats

▲, injection; ●, coarse powder; ○, suspension. Each point is the mean and the standard error for four (injection), eight (coarse powder) or five (suspension) animals.

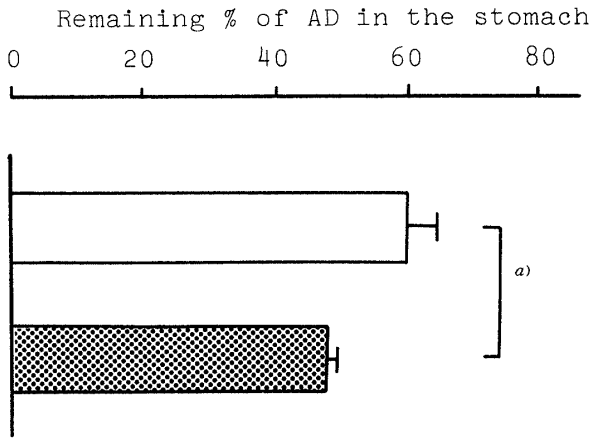

Fig. 4. Comparison of Remaining % of AD in the Stomach between Coarse Powder and Suspension 30 min after Oral Administration of 6 mg/kg of AD to Fasted Rats

□, coarse powder; ▩, suspension. *a*) Significantly different ($p<0.05$). Each column is the mean and the standard error for five animals.

of IM and ASA, as well as AD, the gastric ulcerogenic activity of powder was stronger than that of suspension (Table I).

These results indicate that the gastric ulcerogenic activity of NSAIDs is generally influenced by dosage form. Further, these results point out the importance of considering the dosage form in evaluating gastric lesions induced by NSAIDs.

Figure 3 shows plasma concentration–time curves after oral administration and intravenous administration of 6 mg/kg of AD to fasted rats. Table II summarizes the parameters of bioavailability.

It is evident that AD is absorbed faster from suspension than from powder, as shown in Fig. 3. However, AUC and C_{max} were not significantly different between powder and

TABLE II. Comparison of T_{max}, C_{max} and AUC Values between Coarse Powder and Suspension

Preparation	T_{max} (h)	C_{max} (μg/ml)	AUC (μg·h/ml)
Coarse powder	0.5—2.0	7.50±1.48	19.47±3.43
Suspension	<0.5	11.53±0.48	19.42±0.64
t-Test	—	N.S.	N.S.

N.S., not significantly different ($p>0.05$). Each datum is the mean and the standard error for eight (coarse powder) or five (suspension) animals.

suspension owing to the large interanimal variation in the case of powder (Table II). In the case of intravenous administration, no gastric ulcer was found, though the plasma concentration was much higher than that of powder or suspension (Fig. 3). These results suggest that the gastric ulcer induced by AD is attributable to a local action in the GI tract rather than to a systemic mechanism. Therefore, differences in bioavailability parameters such as C_{max} and AUC may not explain why coarse powder causes gastric ulcers at a lower dose as compared with suspension.

Figure 4 shows remaining % of AD in the stomach 30 min after oral administration of 6 mg/kg of AD as a coarse powder or suspension to fasted rats. Remaining % of coarse powder in the stomach was significantly higher than that of the suspension. This indicates that coarse powder adheres to or resides on the gastric mucosa for longer than the suspension. Rainsford[8] found that gastric ulcers induced by ASA occurred only in the case of tablets when tablet, suspension and solution were administered to domestic pigs, and suggested that this might be attributable to an increase of the local concentration of ASA in the stomach owing to the long residence time of tablets. Similar findings were obtained with AD in rats. Thus, it is considered that the gastric ulcer induction by AD, as well as ASA, is also dependent on the residence time of preparations in the stomach and it is therefore suggested that the gastric ulcerogenic activity of coarse powder was enhanced by a local action arising from contact with the gastric mucosa, besides any systemic mechanism that may be involved.

Preparation–Gastric Lesions

Table III shows the gastric ulcerogenic activities after oral administration of preparations having different pharmaceutical properties to fasted rats (dose, 6 mg/kg). Suspension caused little gastric ulceration, as described above (frequency of ulceration, 1/10). Coarse powder and fine powder, irrespective of particle size (coarse powder, 150—350 μm; fine powder, about 10 μm), caused gastric ulceration at the same frequency. However, the degree of damage was larger in the case of coarse powder than fine powder (see score in parentheses). It was thus found that the gastric ulcerogenic activity was influenced by particle size. E-powder caused little gastric ulceration (frequency of ulceration, 1/12) and A-powder caused none. From the results of the dissolution test in acidic medium (Fig. 1), it is considered that gastric ulcers are not induced by preparations which are rapidly dissolved or not dissolved in the stomach (gastric juice pH 2—3). The gastric ulceration could be reduced by modification of the dissolution properties of AD. R-tablet did not cause gastric ulcers, while S-tablet caused them at the same frequency as fine powder.

From these results, it is clear that particle size, dissolution rate and disintegration time in AD preparations are important factors in relation to the gastric ulcer-inducing activity.

Bioavailability–Gastric Lesions

Figure 5 shows the relationship between the frequency of ulceration and C_{max}, AUC and

TABLE III. Gastric Ulcerogenic Activity after Oral Administration of Different Preparation to Fasted Rats

Preparation	Frequency of ulceration (No. of rats with ulcer[a]/tested)	Mean ± S.E.
Suspension	1/10	0.5±0.3 (2.0)
Coarse powder	5/10	1.6±0.6 (3.2±0.4)
Fine powder	5/10	1.4±0.2 (2.2±0.2)
A-Powder	0/12	0.3±0.1
E-Powder	1/12	0.5±0.5 (2.0)
R-Tablet	0/12	0.4±0.3
S-Tablet	7/12	1.5±0.3 (2.0±0.0)

a) Gastric lesions with the score of 2 or more. Figures in parentheses are the score based only on rats with ulcers.

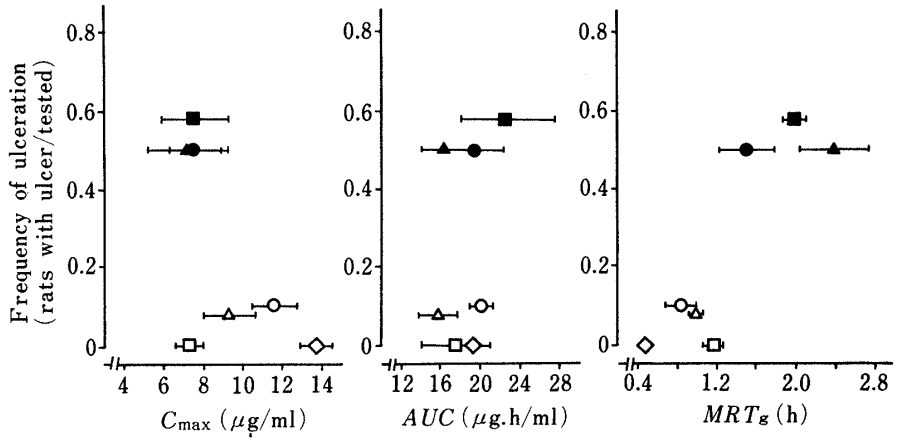

Fig. 5. Relationship between Frequency of Ulceration and C_{max}, AUC and MRT_g after Oral Administration of 6 mg/kg of AD as Different Preparations to Fasted Rats

○, suspension; ◇, A-powder; △, E-powder; □, R-tablet; ▲, fine powder; ●, coarse powder; ■, S-tablet.
Each point is the mean and the standard error for eight (coarse powder) or five (others) animals.

MRT_g.

AUC was similar in the normal group (open symbols), which showed little or no gastric ulceration, and in the ulcer group (closed symbols), in which more than 50% of the rats showed ulcers. These results indicate that AUC, namely the extent of bioavailability, is not particularly associated with the gastric ulceration. The C_{max} values of suspension and A-powder in the normal group tended to be higher than those of other preparations. It seems, however, that C_{max}, namely maximum plasma concentration, is also not particularly associated with the ulceration. Preparations were divided roughly into two groups with regard to MRT_g (mean residence time of preparations in the gastrointestinal tract). One is the normal group having short MRT_g (open symbols) and the other is the ulcer group having long MRT_g (closed symbols). It appeared that the probability of ulceration increases if preparations reside for a long time in the gastrointestinal tract. As AD is an acidic drug having a pK_a of *ca.* 6, it is considered that AD readily dissolves in the small intestinal secretion as compared with the gastric juice. As AD is also readily absorbed from the small intestine, it seems that MRT_g is closely related to the gastric emptying rate of preparations. Figure 6 shows the gastric emptying rate for the different preparations used in this study. The gastric emptying of the group having long MRT_g, namely the ulcer group, tended to be slower than that of the group having short MRT_g, namely the normal group. These results indicate that the gastric

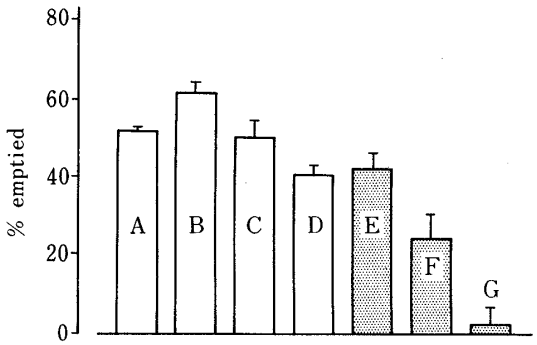

Fig. 6. Remaining % of AD in the Stomach 30 min after Oral Administration of 6 mg/kg of AD as Different Preparations to Fasted Rats

A, suspension; B, A-powder; C, R-tablet; D, E-powder; E, coarse powder; F, fine powder; G, S-tablet. Each column is the mean and the standard error for five animals.

ulcer induction by AD is associated with the residence time of preparations in the stomach rather than with the maximum plasma concentration (C_{max}) or extent of bioavailability (AUC). It is therefore reasonable to suggest that gastric ulceration induced by AD is attributable to a local action exerted by contact of the preparations with the gastric mucosa. On the other hand, E-powder, though having the same residence time in the stomach as coarse powder, did not cause gastric ulcers and did not have such local action. This may reflect insolubility of E-powder in the gastric juice.

The results obtained in this study indicate that AD directly affects the gastric mucosa, and thus it is of importance to design preparations having rapid gastric emptying or having enteric characteristics in order to reduce the gastric lesions. Such a concept for NSAIDs has been generally accepted and demonstrated by studies using large animals such as dogs[9] and pigs.[8] However the influence of the pharmaceutical properties of NSAIDs on GI lesions has not been investigated in small animals. This study has demonstrated that it is possible to evaluate the influence of pharmaceutical properties on the gastric lesions by using rats. It therefore seems that rats can be used as an animal model for evaluating GI-lesions caused by NSAIDs in the early stages of a formulation study, though there might be differences in sensitivity to the side effects of NSAIDs and there are anatomical and physiological differences in the GI tracts between humans and the test animals.

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References and Notes

1) This work was presented at a Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, December 1984.
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