Gastric Ulcerogenic and Biological Activities of N-3'a-Propyphenazonyl-2-acetoxybenzamide

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A new derivative of aspirin, N-3'a-propyphenazonyl-2-acetoxybenzamide (aspirin-isopropylantipyrine, AIA) was synthesized, and its gastric ulcerogenic and biological activities were investigated together with those of related compounds. AIA (100, 200 mg/kg, *p.o.*, *i.p.*) showed much less gastric ulcerogenic activity than aspirin in pylorus-ligated rats. The effect of AIA on the stomach was also weaker than those of salicylate-IA (SIA), salicyloyl leucine (SL), salicyloyl methionine (SM) and IA. AIA (200 mg/kg, *p.o.*) did not injure the stomachs of adjuvant arthritis rats given the drug daily for 21 days, unlike aspirin. However, AIA (100 mg/kg, *i.p.*) did not improve the gastric ulcerations induced by pylorus ligation, histamine, acetic acid and stress in rats. AIA showed analgetic activity (100, 200 mg/kg, *s.c.*) in the acetic acid method and the D'Amour-Smith method in mice, antipyretic activity (100 mg/kg, *s.c.*) in rats treated with *E. coli*, inhibitory activity (100 mg/kg, *s.c*) on carrageenin edema formation in rats and inhibitory activity (200 mg/kg/day×21, *p.o.*) on adjuvant arthritis in rats. SL (100 mg/kg, *s.c.*) showed analgetic and antipyretic activities, while SIA (100 mg/kg, *s.c*) showed only analgetic activity, and SM (100 mg/kg, *s.c*) did not show any activity. These compounds were inactive on carrageenin edema formation, unlike AIA and aspirin. AIA (10^{-4} g/ml) inhibited the contraction of isolated ileum preparations stimulated by histamine, acetylcholine and barium chloride, but had no effect on anaphylactic shock of the ileum sensitized by egg albumin. AIA had no effect on increased vascular permeability in mice, rabbit blood pressure and the beating of perfused frog heart. The acute toxicity of AIA in mice was much less than that of aspirin, as shown by the LD_{50} values which were more than 5 g/kg *p.o.*, *s.c.* and 3.68 g/kg *i.p.* for AIA.

Keywords――N-3'a-propyphenazonyl-2-acetoxybenzamide; aspirin; isopropylantipyrine; gastric ulcerogenic activity; analgetic activity; anti-inflammatory activity

Aspirin is the most extensively employed analgetic, antipyretic and anti-inflammatory agent. However, it is well-known that the ingestion of aspirin may result in epigastric distress and nausea,[2] and therefore care is required in its use for long periods in rheumatoid arthritis. Further, this drug is not only gradually decomposed by moisture but also becomes discolored on combination with other drugs. It was considered that these defects of aspirin derive from the carboxyl or hydroxyl group of the molecule *in vitro* or *in vivo*. In the present paper, in order to develop a new aspirin derivative with very little gastric ulcerogenic activity, isopropylantipyrine (propyphenazone, IA), which does not lose its pharmacological activities on substitution of the 3-methyl group, as previously reported by the authors,[3] was chosen as a blocking agent for the carboxyl group of aspirin. Aspirin-IA (N-3'a-propyphenazonyl-2-acetoxybenzamide, AIA) was synthesized, and its gastric ulcerogenic, analgetic, antipyretic and anti-inflammatory activities were investigated in comparison with those of related compounds.

1) Location: *1-33-1, Yamadakami, Suita, Osaka.*
2) H. Salter, *Am. J. Dig. Dis.*, **13**, 38 (1968).
3) *a*) S. Aonuma, Y. Kohama, Ing-Jun Chen, S. Yashiki, S. Nakajin, and J. Sugatani, *Yakugaku Zasshi*, **95**, 151 (1975); *b*) S. Aonuma, Y. Kohama, Ing-Jun Chen, S. Yashiki, and H. Egawa, *Yakugaku Zasshi*, **96**, 1313 (1976).

Experimental

Syntheses of AIA and Related Compounds——A solution of bromo-IA[3a] (0.01 mol) and hexamine (0.0105 mol) in $CHCl_3$ (18 ml) was refluxed for 2.5 hr. After removing $CHCl_3$ *in vacuo*, the residue was hydrolyzed with C_2H_5OH–HCl (1 : 1, v/v, 60 ml) for 36 hr at room temperature. The solvent was removed by evaporation, and the residue was suspended in $CHCl_3$ (50 ml) and saturated with dry NH_3 gas. The resulting precipitate, NH_4Cl, was removed by filtration. After the removal of NH_3 in the filtrate by evaporation, 3-aminomethyl IA ($IANH_2$) was purified by silica gel column chromatography (Wako gel C-200) with a mixed solvent of $CHCl_3$ and CH_3OH. Yield, 80%. Dicyclohexylcarbodiimide (DCC, 0.011 mol) was added to a solution of aspirin (0.01 mol) in $CHCl_3$ (50 ml), and the mixture was stirred for 30 min at 0°. $IANH_2$ (0.01 mol) was added to the mixture, and the reaction was allowed to continue for 24 hr at room temperature. After filtration to remove DCurea, the solvent was removed by evaporation, and AIA was separated by silica gel column chromatography with a mixed solvent of $CHCl_3$ and CH_3OH. AIA was recrystallized from ethylacetate to give white crystals, mp 141—142°. Yield, 78%. *Anal.* Calcd for $C_{23}H_{25}N_3O_4$: C, 67.81; H, 6.14; N, 10.32. Found: C, 67.82; H, 6.07; N, 10.29. AIA gave a single spot, which was different from aspirin and IA on thin layer chromatography (TLC) with $CHCl_3$–CH_3OH (9 : 1, v/v) and ether.

Salicylate-IA (N-3′a-propyphenazonyl-2-hydroxybenzamide, SIA) was synthesized by hydrolyzing AIA with 1 N NaOH for 5 min. After the hydrolysis, the solvent was evaporated off, and SIA was crystallized from ether and petroleum ether to give white crystals, mp 191—193°. Yield, 59%. *Anal.* Calcd for $C_{21}H_{23}N_3O_3$: C, 69.04; H, 6.30; N, 11.51. Found: C, 68.70; H, 6.37; N, 11.19. SIA gave a single spot which was different from aspirin, IA and AIA on TLC with $CHCl_3$–CH_3OH (9 : 1, v/v) and ether. Salicyloyl leucine (SL) and salicyloyl methionine (SM) were prepared by the method of Vigneron.[4]

Samples and Animals——AIA, SIA, aspirin, SL, SM and IA suspended in 5% acacia were used in the measurements of biological activities, except for the experiments *in vitro* using isolated organ preparations as described below. As a control, 5% acacia was used. The animals were male rats (Wistar, 150—200 g body weight), mice (ddy, 20—25 g), guinea pigs (Hartley, 300—350 g) and frogs (150—200 g).

Measurements of Biological Activities   Gastric Ulcerogenic Activities——Rats were fasted for 24 hr before use, according to the method of Okabe *et al.*[5] Samples were orally administered just after pyloric ligation. After 7 hr, the stomachs were opened along the greater curvature, and gastric juices were collected. The lengths of lesions in the glandular portion were measured and the lengths (mm) were summed to obtain an ulcer index for each rat. The volume, acidity and peptic activity of the gastric juice were determined. An area of hemorrhage or ulceration, 2 mm or greater in size, was taken as another indicator of positive ulcerogenic response, giving an ulcer % which was the percentage of rats with ulcer per rats treated.[6] According to the method of Dass *et al.*,[6] gastric ulceration was produced by intraperitoneal injection of the sample. Samples were injected just after ligation of the pylorus and rats were sacrificed 5 hr later. The stomachs were removed and examined to determine the ulcer index and ulcer %.

Analgetic Activity——Acetic Acid Method[7]: Samples were injected subcutaneously, 30 min prior to intraperitoneal injection of 0.7% acetic acid (10 ml/kg). The total number of writhings was counted for a 10 min period from 10 min after the irritant injection.

D′Amour-Smith Method[8]: The prolongation time of the pain threshold of mouse tail was determined at 15 min intervals for 15 min using an analgesic meter (Kyoto Keisoku Kogyo, MW-7000) from 15 min after subcutaneous injection of the sample.

Antipyretic Activity——Rats received a sterillized *E. coli* suspension (1 platinum loop/2 ml saline, 0.5 ml/100 g, *i.v.*) as a pyrogen, and 2 hr later samples were injected subcutaneously. The temperature was taken at 1 hr intervals before and after sample injection.

Effect on Carrageenin Edema Formation——Inhibitory effects on carrageenin edema formation in rats were determined as described by Winter *et al.*[9] Samples were administered subcutaneously 30 min prior to subplanter injection of 0.05 ml of 1% carrageenin solution. The swelling rate of edema in the foot pad of the hind paw was determined just after the irritant injection and at 1 hr intervals for 6 hr.

Effect on Increased Vascular Permeability——Using the method of Fujimura,[10] samples were injected subcutaneously into mice, then 1 hr later 0.5% trypan blue (0.1 ml/10 g) was injected intravenously. After 15 min, the vascular permeability in the ear induced xylene was determined.

Effect on Form of Adjuvant Arthritis——Using the method of Person *et al.*,[11] rats were injected in one foot pad with 0.1 ml of Freund′s adjuvant (Difco. Lab.) on day 0, and the body weight and rear paw volume

4) M. Vigneron, Fr. Patent M2137 (1963) [*C.A.*, **60**, 10786 (1968)].
5) S. Okabe, K. Takeuchi, K. Nakamura, and K. Takagi, *Jpn. J. Pharmacol.*, **24**, 363 (1964).
6) M. Dass, M.B. Gupta, and K.P. Bhargava, *Indian. J. Med. Res.*, **65**, 273 (1977).
7) R. Koster, M. Anderson, and E.J.de Beer, *Fed. Proc.*, **18**, 412 (1959).
8) F.E. D′Amour and D.L. Smith, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1941).
9) C.A. Winter, E.A. Risley, and G.W. Nuss, *J. Pharmacol. Exp. Ther.*, **141**, 369 (1963).
10) H. Fujimura, *Nippon Yakurigaku Zasshi*, **64**, 379 (1968).
11) C.M. Person, B.H. Waksman, and J.T. Sharp, *J. Exp. Med.*, **113**, 485 (1961).

were determined periodically. Samples were given orally from day 0 to day 20. Animals were sacrificed on day 21, and the organ weights were measured. Stomachs were fixed in 1% formalin, and the lengths of lesions in the glandular portion were measured to obtain the ulcer index of each rat.

Effect on Isolated Ileum Preparations of Guinea Pig and Rat——A guinea pig or rat isolated ileum preparation was suspended in a 100 ml organ bath filled with Tyrode's solution (temperature: guinea pig, $32°$; rat, $28°$). Samples suspended in Tyrode's solution were applied to the organ bath. The tension developed in the ileum was recorded on a kymograph. Histamine, acetylcholine and barium chloride were used as spasmogenics. Similarly, guinea pig isolated ileum sensitized with egg albumin was used to test the effects of samples on anaphylactic shock.

Effects on Rabbit Blood Pressure and Frog Isolated Perfused Heart——A rabbit anesthetized with urethane was given the sample intravenously. Arterial blood pressure was determined with a mercury manometer. The effect on isolated perfused heart was determined by Yagi-Straub's method.[12]

Anti-gastric Ulcerogenic Activity——Anti-gastric ulcerogenic activities on Shay's ulcer,[13] histamine-induced ulcer,[14] stress-induced ulcer[15] and acetic acid-induced ulcer[16] were determined.

LD_{50}——LD_{50} was determined by Van der Warden's method.[17]

Results

Gastric Ulcerogenic Activity

Table I shows the gastric ulcerogenic activities of AIA and related compounds orally administered to pylorus-ligated rats. The ulcer indices of rats treated with AIA, SIA, SL, SM, or IA were significantly decreased as compared with that of rats given aspirin. The AIA group showed the lowest ulcer index, even at a dose of 200 mg/kg. The SM group showed a relatively high value among related compounds. Taking the ulcer % as another indication of positive ulcerogenic response, the value for the AIA group was low, those of IA, SIA, and SL were medium, and those of aspirin and SM were high, corresponding to the ulcer indices. Gastric juices of the same animals used in this experiment were analyzed to determine the volume, acidity and peptic activity, as shown in Table II. The gastric volume of rats treated with AIA or SM was significantly increased compared with those of control and aspirin groups, and acidity of rats treated with aspirin, SL or SM was significantly decreased from the control value. As compared with the aspirin group, AIA, IA, and SIA significantly increased the acidity. The effects of all the compounds on peptic activity were in the range of the control. To investigate the gastric ulcerogenic effects without direct contact of the drug with the gastric mucosa, AIA was injected intraperitoneally into rats, and was compared with aspirin. As

TABLE I. Gastric Ulcerogenic Activities of AIA and Related Compounds
Orally Administered to Pylorus-ligated Rats (7 hr after ligation)

Sample	Dose (mg/kg, $p.o.$)	Ulcer index (mean $\pm$ s.e.)	No. of rats with ulcer/ No. of rats treated	Ulcer %
Aspirin	100	32.3 ± 5.7	9/10	90
AIA	100	$0.7 \pm 0.5^{a)}$	1/10	10
AIA	200	$1.4 \pm 0.5^{a)}$	2/10	20
IA	100	$3.3 \pm 0.3^{a)}$	5/10	50
SIA	100	$4.6 \pm 2.1^{a)}$	4/10	40
SL	100	$2.7 \pm 0.9^{a)}$	2/5	40
SM	100	$13.0 \pm 4.9^{b)}$	4/5	80

a) $p < 0.01$: significantly different from aspirin.
b) $p < 0.05$: significantly different from aspirin.

12) G.B. West, "Hormone Assay," ed. by C.W. Emmens, Academic Press Inc., New York, 1950, pp. 98—99.
13) H. Shay, S.A. Komarov, S.S. Fels, D. Meranze, M. Gruentein, and M. Siplet, Gastroenterology, 5, 43 (1945).
14) F. Buchner, P. Siebert, and P.J. Malloy, Beitr. Path. Anat., 81, 391 (1928).
15) K. Takagi and S. Okabe, Jpn. J. Pharmacol., 18, 9 (1968).
16) K. Takagi, S. Okabe, and R. Saziki, Jpn. J. Pharmacol., 19, 418 (1969).
17) Van der Warden, Arch. Exptl. Pathol. Pharmakol., 195, 387 (1940).

shown in Table III, the ulcerogenic indicators of AIA (200 mg/kg) were 1.8 ulcer index and 40 ulcer %, which were clearly lower than the values of 25.4 and 90, respectively, of aspirin.

Analgetic Activity

Table IV shows analgetic activities determined by the acetic acid method. AIA (100 mg/kg) significantly decreased the number of writhings, like aspirin (50, 100 mg/kg), SIA (100 mg/kg) and SL (100 mg/kg). This effect of AIA was a strong as those of aspirin (50 mg/kg) and IA (50 mg/kg).[3a] SM (100 mg/kg) slightly increased the number of writhings. In the D'Amour-Smith method, as shown in Fig. 1, AIA (200 mg/kg) significantly prolonged the heat pain threshold time of mouse tail, and its analgetic effect was stronger than that of aspirin

TABLE II. Effects of AIA and Related Compounds Orally Administered on Gastric Secretion in Pylorus ligated Rats (7 hr after ligation)

Sample	Dose (mg/kg, $p.o.$)	No. of rats	Volume[a] (ml)	Acidity[b] (μEq/ml)	Peptic activity[b] (mg as tyrosine/ml)
Control	0	10	3.42 ± 0.29	91.4 ± 6.9	64.0 ± 2.2
Aspirin	100	10	3.40 ± 0.13	63.2 ± 3.8^{c}	62.4 ± 4.1
AIA	100	10	$4.60 \pm 0.44^{d,e}$	96.2 ± 1.8^{f}	58.6 ± 2.2
IA	100	10	2.84 ± 0.31	89.8 ± 4.4^{f}	62.9 ± 1.5
SIA	100	10	3.51 ± 0.53	87.3 ± 4.4^{f}	62.4 ± 0.9
SL	100	5	4.48 ± 0.53	63.7 ± 7.1^{d}	61.0 ± 2.1
SM	100	5	$6.21 \pm 0.12^{c,f}$	53.0 ± 4.3^{c}	60.9 ± 1.3

a) Mean±s.e. per 100 g body weight.
b) Mean±s.e.
c) $p < 0.01$: significantly different from control.
d) $p < 0.05$: significantly different from control.
e) $p < 0.05$: significantly different from aspirin.
f) $p < 0.01$: significantly different from aspirin.

TABLE III. Gastric Ulcerogenic Activity of AIA Intraperitoneally Administered in Pylorus ligated Rats (5 hr after ligation)

Sample	Dose (mg/kg, $i.p.$)	Ulcer index (mean ± s.e.)	No. of rats with ulcer/ No. of rats treated	Ulcer %
Aspirin	200	25.4 ± 4.5	9/10	90
AIA	200	1.8 ± 1.2^{a}	4/10	40

a) $p < 0.01$: significantly different from aspirin.

TABLE IV. Analgetic Effects of AIA and Related Compounds in Mice Determined by the Acetic Acid Method

Sample	Dose (mg/kg, $s.c.$)	No. of mice	No. of writhings (mean ± s.e.)	Inhibitory %
Control	0	8	32.2 ± 3.7	—
Aspirin	50	8	17.2 ± 4.0^{a}	46.6
Aspirin	100	8	15.3 ± 2.4^{b}	52.5
AIA	50	8	22.1 ± 3.3	31.4
AIA	100	8	18.3 ± 1.7^{b}	43.2
SIA	50	8	25.1 ± 3.3	22.2
SIA	100	8	20.8 ± 3.1^{a}	35.4
SL	100	8	14.3 ± 2.9^{b}	55.6
SM	100	8	38.8 ± 4.6	−20.5

a) $p < 0.05$: significantly different from control.
b) $p < 0.01$: significantly different from control.
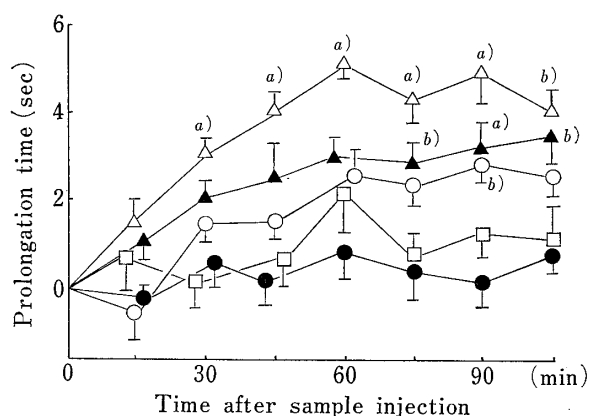
Fig. 1. Effects of AIA and Related Compounds on the Prolongation of Heat Pain Threthold Time of the Mouse Tail

Each point represents the mean±s.e. (n=8).
—●— control, —○— AIA (100 mg/kg,$s.c.$), —△— AIA mg/kg,$s.c.$), —▲— aspirin (200 mg/kg, $s.c.$), —□— SIA (200 mg/kg,$s.c.$).
  a) $p<0.01$: significantly different from control.
  b) $p<0.05$: significantly different from control.

Fig. 2. Antipyretic Actions of AIA and Related Compounds in Rats Treated with E. coli

Each point represents the mean of 5 rats.
—●— control, —○— AIA (100 mg/kg, $s.c.$), —▲— aspirin (100 mg/kg, $s.c.$), —△— SIA (100 mg/kg, $s.c$), —■— SL (100 mg/kg, $s.c$), —□— SM (100 mg/kg, $s.c.$).

(200 mg/kg). AIA (100 mg/kg) showed a similar effect, but SIA (200 mg/kg) did not in this method.

Antipyretic Activity

As shown in Fig. 2, AIA (100 mg/kg) showed an antipyretic effect on rats treated with *E.coli*, but its effect was weaker than those of SL and aspirin (100 mg/kg). All the samples (100 mg kg) showed no effect on the rectal temperature of normal rats.

Effect on Carrageenin Edema Formation

As shown in Fig. 3, AIA (100 mg/kg) significantly inhibited carrageenin edema formation 2, 3, and 4 hr after irritant injection. This inhibitory effect was weaker than that of aspirin
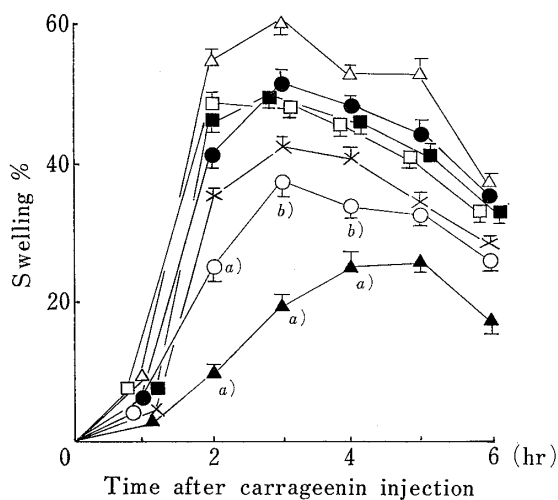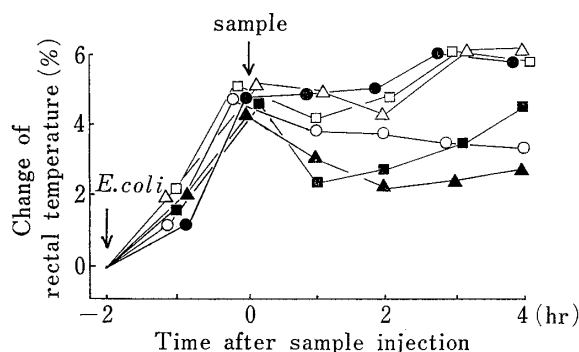

Fig. 3. Effects of AIA and Related Compounds on Carrageenin Edema Formation in Rats

Each point represents the mean±s.e. (n=5).
—●— control, —○— AIA (100 mg/kg, $s.c.$), —▲— aspirin (100 mg/kg, $s.c$), —×— aspirin (50 mg/kg, $s.c$), —△— SIA (100 mg/kg, $s.c$), —■— SL (100 mg/kg, $s.c.$), —□— SM mg/kg, $s.c$).
  a) $p<0.01$: significantly different from control.
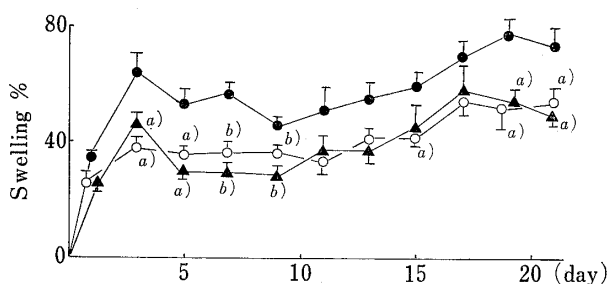 b) $p<0.05$: significantly different from control.

Fig. 4. Effect of AIA on the Form of Adjuvant Arthritis Produced by Freund's Adjuvant in Rats

Each point represents the mean±s.e. (n=5).
—●— control, —○— AIA (200 mg/kg/day, $p.o.$), —▲— aspirin (200 mg/kg/day, $p.o.$).
  a)$<p$ 0.05: significantly different from control.
  b)$<p$ 0.01: significantly different from control.

at a dose of 100 mg/kg but stronger than that of aspirin at 50 mg/kg. SIA, SL and SM had no effects.

Effects on Form of Adjuvant Arthritis

As shown in Fig. 4, AIA (200 mg/kg) inhibited both the primary edema and the secondary inflammation as effectively as aspirin (200 mg/kg). At day 21, there were no differences in organ weights of the thymus, spleen, adrenals, kidneys and liver among the groups (Table V).

TABLE V. Gastric Ulceration and Organ Weight of Adjuvant
Arthritic Rats Given AIA Orally for 21 Days

Sample	Dose (mg/kg, $p.o.$)	Ulcer index	Organ weight per 100 g body weight				
			Thymus (mg)	Spleen (mg)	Adrenal (mg)	Kidney (mg)	Liver (g)
Control	0	0.5 ± 0.5	161 ± 10	188 ± 4	17.4 ± 0.7	914 ± 26	3.65 ± 0.10
Aspirin	200	$32.9 \pm 13.7^{a)}$	143 ± 26	234 ± 32	17.4 ± 1.1	882 ± 3	3.25 ± 0.17
AIA	200	0.4 ± 0.3	171 ± 16	214 ± 15	16.8 ± 1.2	908 ± 28	3.52 ± 0.11

Data are means ± s.e.
a) $p < 0.01$: significantly different from control.

TABLE VI. Effect of AIA on the Increased Vascular
Permeability Induced by Xylene in Mice

Sample	Dose (mg/kg, $s.c.$)	No. of mice	Vascular permeability transmission (%)[a]
Control	0	7	39.5 ± 2.0
Aspirin	100	7	$66.2 \pm 9.7^{b)}$
AIA	100	7	40.5 ± 2.2

a) Mean ± s.e.
b) $p < 0.05$: significantly different from control.

Effect on Increased Vascular Permeability

As shown in Table VI, aspirin (100 mg/kg) significantly inhibited increased vascular pemeability, but AIA (100 mg/kg) had no effect.

Effect on Isolated Ileum Preparations of Guinea Pig and Rat

As illustrated in Fig. 5, AIA (10^{-4} g/ml) was found to have effective relaxing action on the histamine-induced contraction of guinea pig ileum. However, aspirin (10^{-4} g/ml) had no effect. AIA and aspirin had no effect on anaphylactic shock of guinea pig ileum. As illustrated in Fig. 6, AIA (10^{-4} g/ml) showed relaxing action on rat ileum stimulated by acetylcholine or barium chloride, as well as IA (10^{-5} g/ml). Aspirin (10^{-4} g/ml) had no effect.

Effect on Rabbit Blood Pressure and Frog Isolated Perfused Heart

AIA (1 mg/kg) and aspirin (1 mg/kg) showed no effect on blood pressure. AIA (10^{-4} g/ml) and aspirin (10^{-4} g/ml) had no effect on inotropism and chronotropism of the heart.

Anti-gastric Ulcerogenic Activity

AIA (100 mg/kg) had no effect on various ulcerations in rats, as shown in Table VII.

LD_{50}

As shown in Table VIII, the acute toxicity of AIA was much less than that of aspirin in mice orally, subcutaneously and intraperitoneally.
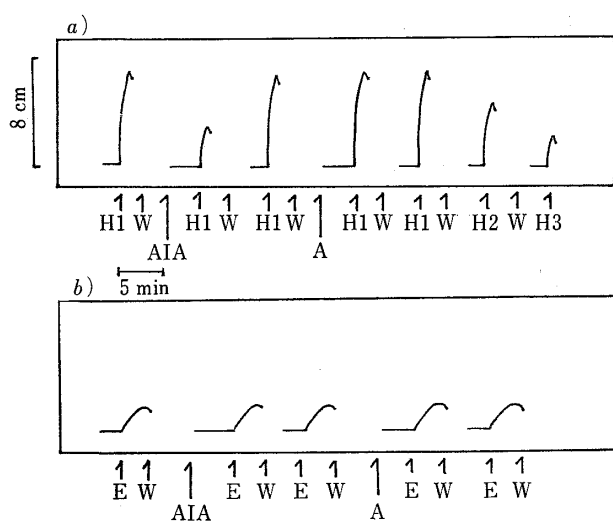
Fig. 5. Effect of AIA on Histamine and Anaphylactic Shocks of Guinea Pig Isolated Ileum Preparations

a): normal ileum, *b*) ileum sensitized with egg albumin, W: wash, H1: histamine (4×10^{-9}g/r l final concentration), H2: (3×10^{-9} g/ml), H3: (10^{-9} g/ml), AIA: (10^{-4} g/ml), A: aspirin (10^{-4} g/ml), E: egg albumin (5×10^{-6} g/ml).
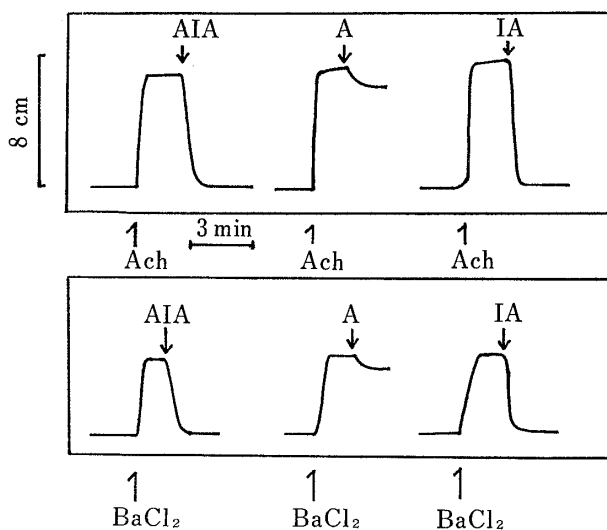
Fig. 6. Effect of AIA on Acetylcholine- and $BaCl_2$-induced Contractions of Rat Isolated Ileum Preparations

Ach: acetylcholine (10^{-8} g/ml final concentration), $BaCl_2$: (3×10^{-5}g/ml), AIA: (10^{-4} g/ml), A: aspirin(10^{-4} g/ml), IA: (10^{-5} g/ml).

TABLE VII. Anti-ulcerogenic Activity of AIA

Ulceration	Ulcer index (mean $\pm$ s.e., $n = 8$)	
	Control	AIA (100 mg/kg, $i.p.$)
Pylorus ligation	4.3 ± 0.3	3.5 ± 0.4
Histamine	3.2 ± 0.2	3.2 ± 0.2
Stress	10.1 ± 2.0	11.0 ± 5.0
Acetic acid	30.3 ± 9.5	44.5 ± 8.7

TABLE VIII. LD_{50} Values of AIA and Aspirin in Mice

Sample	LD_{50} (g/kg)		
	$p.o.$	$s.c.$	$i.p.$
Aspirin	1.16 ± 0.07	1.02 ± 0.11	0.76 ± 0.09
AIA	> 5.0	> 5.0	3.68 ± 0.07

Discussion

Substitutions on the carboxyl or hydroxyl group of salicylate serve only to change the potency or toxicity of the compound. It was reported that derivatives having carboxamide instead of the carboxyl group, salicylamide,[18] salicyloyl amino acid,[4] ethenzamide[19] and MOGM,[20] showed analgetic and antipyretic activities with weaker gastric ulcerogenic activi-

18) G. Levy and T. Masuzawa, *J. Pharm. Sci.*, **55**, 222 (1966); E.R. Hart, *J. Pharmacol. Exp. Ther.*, **89**, 205 (1947).
19) E.M. Barin, *J. Pharm. Pharmacol.*, **4**, 872 (1952); C. Davison, J. Wangler, and P.K. Smith, *J. Pharmacol. Exp. Ther.*, **136**, 226 (1962).
20) H. Fujimura, *Nippon Yakurigaku Zasshi*, **62**, 11 (1966).

ties, and that their carboxamide bonds could not be readily cleaved *in vivo*. AIA synthesized here by coupling IANH$_2$ on the carboxyl group of aspirin showed much lower gastric ulcerogenic activity than aspirin in pylorus-ligated rats on single oral or intraperitoneal administration, and even in adjuvant arthritis in rats on oral administration daily for 21 days. The gastric ulcerogenic activity of AIA was also weaker than those of SIA, SL, and SM, having the hydroxyl group. AIA, IA, and SIA did not change the acidity in gastric juices from the control level, unlike aspirin, SL and SM which carry the carboxyl group. Therefore, the safety of AIA in the stomach is probably due to the absence of direct stimulation to gastric mucosa and of back diffusion of gastric acid by this drug. In addition, it is possible that AIA has different reactivities from aspirin on prostaglandin[21] in the stomach, because while AIA intraperitoneally administered clearly showed less gastric ulcerogenic activity than aspirin, AIA showed activities in the biological assays *in vivo* almost as strong as those of aspirin.

AIA showed analgetic activity in the acetic acid method and the D'Amour-Smith method, and showed antipyretic activity and inhibitory effect on carrageenin edema formation comparable to those of aspirin. SIA, which lacks the 2-acetyl group of AIA, showed analgetic activity in the acetic acid method, but did not effect the heat pain threshold, elevation of temperature by *E.coli*, or carrageenin edema formation. SL showed analgetic and antipyretic activities, but did not inhibit carrageenin edema formation. SM did not show any activities. The different actions found among these compounds may results from differences in their absorption properties, metabolism and prostaglandin biosynthesis inhibitory effects *in vivo*.[22] AIA also possessed inhibitory activity on adjuvant arthritis and anti-spasmodic activity, which are pharmacological activities of aspirin and/or IA. In the biological activities of AIA tested in the present experiments, no apparent additive effects of aspirin and IA groups in the molecule of AIA were found, except for analgetic activity in the D'Amour-Smith method. However, AIA showed much weaker acute toxicity than aspirin and IA.[3a] Thus, AIA is an analgesic-antipyretic with anti-inflammatory activity and very little gastric ulcerogenic activity and toxicity, and can presumably be used in combination with other basic drugs because it lacks a carboxyl group, unlike aspirin.

21) G.L. Kauffman and M.I. Grossman, *Gastroenterology*, **75**, 1099 (1978).
22) R.J. Flower, R. Gryglewski, K. Herbachzyniska-Cedro, and J.R. Vane, *Nature New Biol.*, **238**, 104 (1972); R.J. Flower, *Pharm. Rev.*, **26**, 33 (1974).