J.Pharm.Dyn., **6**, 9–17 (1983)

STUDIES ON ASPIRIN DERIVATIVES WITH VERY LITTLE SIDE EFFECT. IV.[1] INHIBITORY EFFECT OF ASPIRIN-ISOPROPYLANTIPYRINE (AIA) ON SEVERAL EXPERIMENTAL THROMBOSES

SHIGERU AONUMA, YASUHIRO KOHAMA, SHIGEKO FUJIMOTO, MASATO NOMURA AND EMI YAMAHATA

Faculty of Pharmaceutical Siences, Osaka University, Yamadaoka 1-6, Suita, Osaka 565, Japan

(Received July 21, 1982)

The effect of a new aspirin derivative, aspirin-isopropylantipyrine (AIA), with potent platelet anti-aggregant activity, on several experimental thromboses was evaluated and compared with that of aspirin. AIA (50 mg/kg, *s.c.*) as well as aspirin (50 mg/kg, *s.c.*) significantly inhibited thrombus formation in extracorporeal shunt model of rats. AIA (50 mg/kg, *s.c.*) significantly shortened the duration of apnea and respiratory distress induced by a rapid injection of adenosine 5′-diphosphate in rats, while aspirin (50 mg/kg, *s.c.*) did not. Inhibitory effect of AIA (50 mg/kg, *s.c.*) on arachidonic acid-induced mortality in mice was less than that of aspirin (50 mg/kg, *s.c.*). AIA and aspirin (10 mg/kg/d × 10, *s.c.*) had no effect on laurate-induced arterial occlusive disease in rats. AIA (200 μM) showed weak and reversible inhibition of prostaglandin I_2 generation in isolated rat aorta strip, while aspirin (200 μM) showed irreversible inhibition.

AIA (50 and 200 μM inhibited Ca^{2+}-, K^+- or norepinephrine-induced contraction on isolated rat aorta strip. AIA (200 μM) had no effect on malondialdehyde formation, cyclic AMP level and adenylate cyclase activity in rat platelets. AIA (100 μM) inhibited arachidonic acid-induced contraction on rat fundus strip by about 50%, while aspirin (100 μM) did not. These results strongly suggest that anti-thrombotic activity of AIA was originated at least from its anti-aggregant effect on platelets, differing from aspirin.

Keywords—aspirin derivative; aspirin; experimental thrombosis; prostaglandin I_2; Ca^{2+}-induced aorta contraction

In recent years, anti-platelet drugs have been applied to thromboses, because of their lower side effect than thrombolytic or anti-coagulant agents. Pathogenesis of thrombosis involves many factors, among which platelet aggregation plays a great part of initial events in thrombus formation on arterial vessels. Previously, we reported the discovery of a new aspirin derivative, *N*-3′a-propyphenazonyl-2-acetoxybenzamide (aspirin-isopropylantipyrine, AIA), which possessed potent platelet anti-aggregant activity,[2] very little gastric ulcerogenecity and very slight acute toxicity, compared with aspirin.[3] In this paper, in anticipation of a preventive effect of AIA on thrombosis, its inhibitory effect on several experimental thrombotic models was investigated together with its action mode on the platelet function.

MATERIALS AND METHODS

Materials and Animals—AIA was synthesized in our laboratory as described previously by the authors.[3] Adenosine 5′-diphosphate disodium salt (ADP), adenosine 5′-triphosphate disodium salt (ATP), ethylene glycol bis (β-aminomethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) and thiobarbituric acid (TBA) were purchased from Nakarai Chemicals Ltd. Sodium laurate, Somnopentyl (sodium pentobarbital) and sodium fluoride (NaF) were purchased from Wako Pure Chemical Ind. Ltd. Arachidonic acid (from porcine liver), phos-

phocreatine disodium salt hydrate, creatine phosphokinase (Type I from rabbit muscle), papaverine, norepinephrine bitartrate and bovine serum albumin (BSA) were products of Sigma Chemical Co., U.S.A. Isopropylantipyrine (IA) obtained from Toho Shinyaku Ltd., aspirin from Yoshitomi Pharmaceutical Co. and 1,1,3,3-tetraethoxypropane from Tokyo Kasei Kogyo Ltd. Cyclic AMP kit and liquid scintillation cocktail, ACS II, were products of Amersham, U.K. AIA or aspirin suspended in 5% acacia was injected subcutaneously to animals in the measurement of biological activity *in vivo*. The *in vitro* samples were dissolved in 10% ethanol. The animals used in this study were Wistar male rats weighing 300—400 g, ddy male mice weighing 20—25 g and male rabbit weighing 2.5—3.5 kg.

Experimental Thrombotic Models——Extracorporeal Shunt Model in Rats: The extracorporeal shunt model was made in rats as described by Umetsu and Sanai.[4] Rats were anesthetized with sodium pentobarbital (65 mg/kg, *i.p.*). Upon tracheostomy, a tube was inserted after exposure of the trachea. Extracorporeal shunt was a series of 3 polyethylene tubings which consisted of two polyethylene tubes (Hibiki, No.4, 12.5 cm length, 1.3 mm out diameter) at both ends and one polyethylene tube (Hibiki, No.7, 6 cm length, 2.3 mm out diameter) threaded with a white silken thread (Kanebo Co., No.50, 5 cm length) in the middle. After the shunt was filled with a heparin solution (100 IU/ml), one end was inserted into a left jugular vein, and the heparin solution (100 IU/ml/kg) was slowly injected *via* the shunt. The detached end of tube was inserted into a right carotid artery to establish the circulation of blood. Samples were injected 1 h before the blood was put into the shunt circulation. Twenty min after blood circulation, the blood flow was stopped with an artery clip. Then, the middle tubing was taken away and from which the thread coated with thrombus was carefully pulled out. Immediately, the wet weight of thrombus was measured. Subsequently, 0.2 ml of heparin solution (100 IU/ml) was quickly injected from free end of tubing at the venous side. Another new polyethylene tube containing a silken thread was connected between venous and artery tubings. Then, the blood circulation was reestablished. By the same procedure, the third circuit of blood was established. The total thrombus weight for 60 min on three experiments was determined and used as an indicator of thrombus formation.

ADP-induced Respiratory Distress and Arrhythmia in Rats: Rats were anesthetized with sodium pentobarbital (65 mg/kg, *i.p.*) and inserted with a tracheostomy tube, as described above. ADP (6 mg/ml/kg) was rapidly injected into the jugular vein. The test agent was injected 1 h before ADP injection. The changes in respiratory rate and electrocardiogram (lead II) were monitored and recorded on a Polygraph system (Nihon Koden, RM-6000). Duration of apnea, respiratory inhibition which is less than 90% of normal respiratory rate, and arrhythmia were measured.

Arachidonic Acid-induced Mortality in Mice: According to the method of Griffett *et al.*,[5] arachidonic acid (60 mg/10 ml/kg, *i.v.*) was administered 1 or 4 h after injection of the test agent, and the mortality was observed.

Laurate-induced Arterial Occlusive Disease in Rats: According to the method of Ashida *et al.*,[6] 0.1 ml of sodium laurate solution (10 mg as acid/ml) was injected into the right femoral artery of a rat. Daily injections of the test agent were started 1 day before laurate injection. The degree of gangrane at 6th day and mummification at 10th day after operation was graded into 0 to IV according to the severity of the lesions.

Prostaglandin (PG)I_2 Generation: According to the method of Harada *et al.*,[7] PGI_2 generation from endogenous arachidonic acid was measured on isolated rat aorta. Briefly, thoracic aorta strip was incubated in 1 ml of Tris-buffered saline (pH 7.4) at 37°C. The amount of PGI_2 which was released into the medium during 10 min incubation, was bioassayed as an inhibitory activity against the rabbit platelet aggregation induced by ADP *in vitro*. AIA (200 μM) and

aspirin (200 μM) had no effect on the primary aggregation induced by ADP *in vitro*.[2]

Action Mode —Malondialdehyde (MDA) Formation: The procedure for measurement of MDA was essentially the same as that described by Ashida and Abiko.[8] The washed rat platelets were finally suspended in a solution of 25 mM Tris-HCl buffer (pH 7.5) containing 130 mM NaCl, 0.1% glucose and 0.3 mM EDTA. The suspension (0.47 ml, 3×10^9 platelets/ml) was preincubated at 37°C for 2 min, incubated with the test solution (20 μl) for 5 min, and then incubated with an arachidonic acid solution (10 μl, 0.1 mM final concentration) for 5 min. The reaction was stopped by adding a TBA reagent (0.5 ml).[9] Then, the mixture was heated in a boiling water bath for 10 min. After centrifugation, OD (at 532 nm) of the supernatant was measured. A standard curve of MDA was prepared by using 1,1,3,3-tetraethoxypropane.[10] A blank was prepared for each test specimen.

Contraction of Fundus Strip: According to the method of Splawinski *et al.*,[11] rat fundus strip was mounted in an organ bath containing 50 ml of Krebs-bicarbonate buffer bubbled with 95% O_2–5% CO_2 at 37°C. The resting tension was 2 g. The test solution (0.5 ml) was added to the organ bath 3 min before addition of arachidonic acid.

Cyclic AMP Level: The pellet of washed rat platelets[8] which was prepared as described by Ashida and Abiko, was suspended in a solution of 15 mM Tris-HCl buffer (pH 7.5) containing 139 mM NaCl. The suspension (0.5 ml, 3×10^9 platelets/ml) was incubated with a sample solution (20 μl) at 37°C for 10 min, and the mixture was heated in a boiling water bath for 2 min. After centrifugation, the supernatant was lyophilized and assayed for cyclic AMP using the assay kit. The sample for radioactivity was added to ACS II (10 ml) and the radioactivity was counted with a Liquid scintillation spectrometer (Beckmann, LS-150).

Adenylate Cyclase Activity: The pellet of washed rat platelets[8] was suspended in a solution of 25 mM Tris-HCl buffer (pH 7.5) containing 130 mM NaCl. The suspension (10^{10} platelets/ml) was sonicated at 150 mA for 30 s. The reaction mixture (0.5 ml final volume) consisting of 25 mM Tris-HCl buffer (pH 7.5), 5 mM $MgCl_2$, 4 mM phosphocreatine, 0.2 mg creatine phosphokinase/ml, 1 mg BSA/ml, 0.4 mM ATP, 0.25 mM papaverine, 100 μl of platelets lysate and 20 μl of sample solution. The mixture was incubated at 30°C for 10 min. The reaction was stopped by rapid heating in a boiling water bath for 2 min. After centrifugation, the supernatant was assayed for cyclic AMP as described above.

Contraction of Thoracic Aorta: According to the method of Schümann *et al.*,[12] the spinal strip of rat aorta was mounted in an organ bath containing 10 ml of Krebs-bicarbonate buffer bubbled with 95% O_2–5% CO_2 at 37°C. The contraction was recorded *via* a Force-displacement transducer (Nihon Koden, TB-611T) on the RM-6000. The dose response curve for KCl or norepinephrine was determined in the buffer. The dose-response curve for $CaCl_2$ was determined in a calcium-free and potassium-rich buffer (30 mM KCl) after the strip was washed 3 times with a calcium-free buffer containing 1.0 mM EGTA. Increasing doses of KCl, norepinephrine or $CaCl_2$ were added every 10 min until the maximum response at each concentration of agonist was reached. The test solution was added 5 min before the addition of agonist.

RESULTS AND DISCUSSION

Inhibitory Effect on Thromboses

In extracorporeal shunt model, AIA (50 mg/kg, *s.c.*) significantly inhibited the thrombus formation, and its inhibitory action was as strong as that of aspirin (50 mg/kg, *s.c.*), as shown in Table I. AIA was found to inhibit the thrombus formation which originated from platelet aggregation *in vivo*, as expected from its inhibitory action on the platelet aggregation induced by collagen *ex vivo* and *in vitro*.

The effect of AIA on respiratory distress and arrhythmia which were the index of ADP-induced pulmonary thrombosis and were fol-

lowed by platelet fall,[12] was shown in Table II. Following a rapid injection of ADP, duration of apnea and respiratory inhibition was significantly blocked by the pretreatment of AIA (50 mg/kg, *s.c.*). ADP-induced arrhythmia was not significantly influenced by AIA. Aspirin (50 mg/kg, *s.c.*) had no effect on respiratory distress and arrhythmia. We reported previously that AIA did not inhibit the primary platelet aggregation induced by ADP *in vitro*.[2] Therefore, the

TABLE I. *Effect of AIA on Extracorporeal Shunt Model in Rats*

Sample[a]	Dose (mg/kg, *s.c.*)	Wet weight of thrombus (mg)[b]	Inhibition (%)
Control	–	54.5±0.67	–
AIA	50	32.8±4.66**	39.8
Aspirin	50	34.4±5.96*	36.9

a) Sample was injected 1 h after the loop circulation.
b) Each value represents the mean ± s.e. (n=3).
**p< 0.05, **p< 0.01: versus control.*

TABLE II. *Effect of AIA on ADP-induced Respiratory Distress and Arrhythmia in Rats*

Sample[a]	Dose (mg/kg, *s.c.*)	Duration time (s)[b]		
		Apnea	Respiratory depression	Arrhythmia
Control	–	49.6±14.4	103.1±13.6	131.5±11.0
AIA	50	14.2± 1.6*	62.4±12.0*	105.1± 7.2
Aspirin	50	27.7±13.2	90.1±11.9	121.1±14.1

a) Sample was injected 1 h before ADP injection (6 mg/kg, i.v.).
b) Each value represents the mean ± s.e. (n=8).
**p< 0.05: versus control.*

TABLE. III. *Effect of AIA on Arachidonic Acid-induced Mortality in Mice*

Sample[a]	Time (h) after injection	Dose (mg/kg, *s.c.*)	Ratio of dead mice to tested mice	Mortality (%)
Control	1	–	7/7	100
Control	4	–	6/7	86
AIA	1	50	2/7*	29
AIA	4	50	6/7	86
Aspirin	1	50	0/7**	0
Aspirin	4	50	0/7**	0

a) Sample was injected 1 or 4 h before arachidonic acid injection (50 mg/kg, i.v.).
**p< 0.05, **p< 0.01: versus control.*

TABLE IV. *Effect of AIA on Laurate-induced Arterial Occlusive Disease in Rats*

Sample[a]	Dose (mg/kg/d, *s.c.*)	Morbidity[b]									
		Gangrane					Mummification				
		0	I	II	III	IV	0	I	II	III	IV
Control	–			2	3	3				4	4
AIA	10	1		1	3	3	2			1	5
Aspirin	10		2	1	2	3	1		1	2	4

a) Daily administrations of sample were started at 1 d before laurate injection (1 mg as acid/rat).
b) Normal appearance, 0; the affected region was limited to the nail parts, I; to the fingers, II; to the whole paw, III; and extended to the lower leg, IV. Value in each grade represents the number of rats.

inhibitory action of AIA on ADP-induced respiratory distress *in vivo* is considered to be due to the effect on other process of thrombotic formation than platelet aggregation, *i.e.* vascular functions.

The effect of AIA on arachidonic acid-induced mortality was shown in Table III. The arachidonic acid-induced mortality is considered to be caused by the platelet aggregation which was induced by the promotion of PG biosynthesis.[5,14] AIA (50 mg/kg, *s.c.*) significantly decreased the mortality at 1 h after the injection, but it did not affect that at 4 h. Aspirin (50 mg/kg, *s.c.*) abolished the mortality at 1 and 4 h after the injection.

In laurate-induced arterial occlusive disease in rats, the injected laurate may precipitate with platelets on the peripheral vascular bed and lead to endothelial damage.[6] As shown in Table IV, daily injections of AIA or aspirin (10 mg/kg/d, *s.c.*) showed no effect on gangrane on 6th day and mummification on 10th day.

PGI_2 which was generated in endothelial cells of blood vessels, inhibits the initial step of thrombus formation by its potent anti-aggregant action on platelet.[15] Therefore, the inhibition of PGI_2 generation would be a drawback to the anti-thrombotic effect of drugs. It is well-known that aspirin inhibits the PGI_2 generation in vessels. The effect of AIA on PGI_2 generation from endogenous arachidonic acid in isolated aorta strip was shown in Fig. 1, in comparison with aspirin. In this assay system, aspirin (200

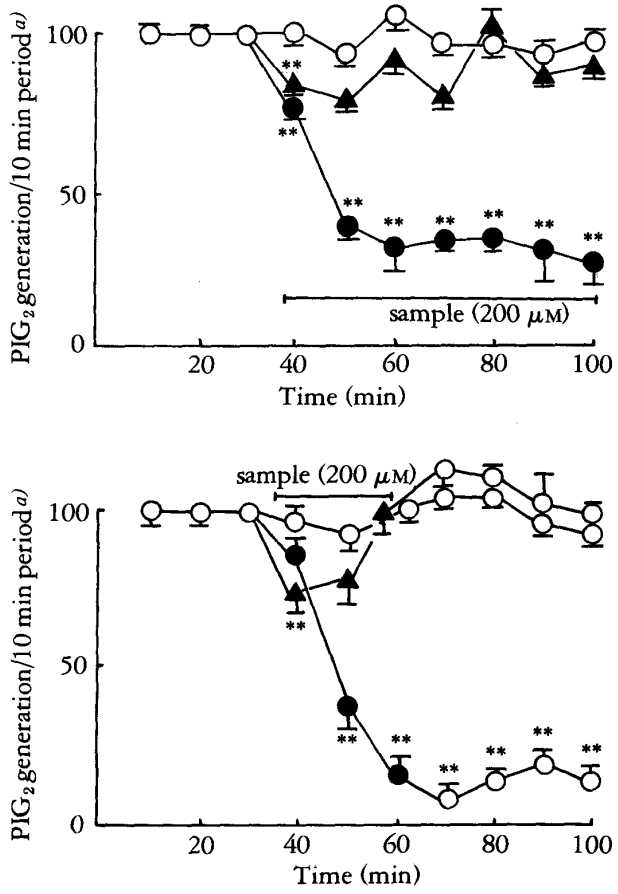

FIG. 1. *Effect of AIA on PGI_2 Generation in Isolated Aorta Strip of Rat*
a) The amount of PGI_2 in the medium was expressed as the percentage of control value immediately before sample addition.
*—○— control, —▲— AIA (200 μM) —●— aspirin (200 μM) Each point represents the mean ± s.e. (n=3). **p< 0.01: versus control.*

μM) strongly inhibited PGI_2 generation by about 70%, while AIA (200 μM) weakly but significantly inhibited by about 10% during the first 10 min period. In the subsequent incubation periods, AIA was indistinguishable from control, whether or not AIA was present. After the removal of aspirin from incubation medium, the generation of PGI_2 in the strip which was pretreated with aspirin was still inhibited. AIA was recognized to be a more preferable antithrombotic agent than aspirin.

Action Mode

Aspirin shows an anti-platelet activity by inhibiting irreversibly platelet cyclooxygenase. The cyclooxygenase inhibition in the vessel wall inhibits the generation of PGI_2 which has the most potent platelet anti-aggregant activity among natural sources. To elucidate the action mechanism of AIA on PG biosynthesis, three approaches have been attempted. AIA (200 μM) inhibited by about 56% of the platelet aggregation which was induced by arachidonic acid *in vitro*, and aspirin (200 μM) did by about 64%.[2] AIA (50 mg/kg) also decreased by about 70% of arachidonic acid-induced mortality in mice only at 1 h after the injection, while aspirin (50 mg/kg) completely abolished it at 1 and 4 h, although both drugs (50 mg/kg) displayed much

TABLE. V. *Effect of AIA on MDA Formation in Rat Platelets in Vitro*

Sample[a]	Final concentration (μM)	MDA formation[b] (pmol/10^8 platelets/5 min)
Control	–	187±7
AIA	200	183±4
Aspirin	200	57±2**

a) Sample was incubated with platelets suspension for 5 min before arachidonic acid addition.
b) Each value represents the mean ± s.e. (n=3).
***p< 0.01: versus control.*

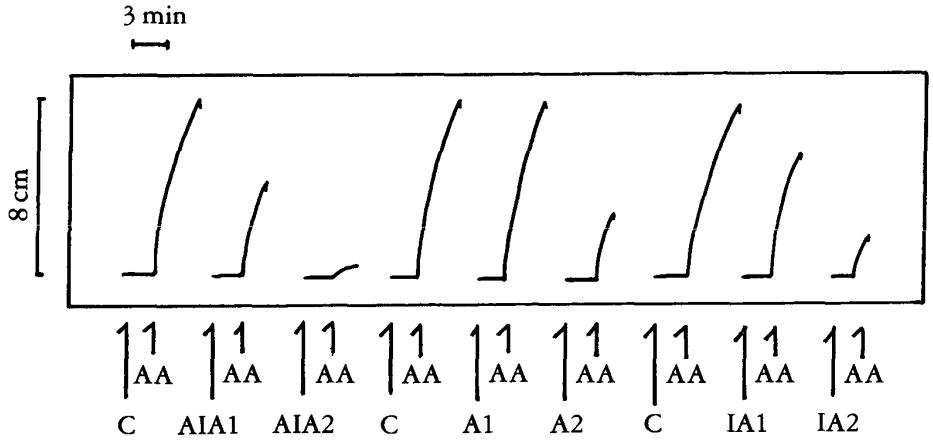

FIG. 2. *Effect of AIA on Arachidonic Acid-induced Contraction of Rat Fundus Strip*
AA: arachidonic acid (100 μM), C: control, AIA 1: (100 μM), AIA 2: (1000 μM), A1: aspirin (100 μM), A2: aspirin (1000 μM), IA 1: (100 μM), IA 2 (1000 μM).

the same inhibitory activity in extracorporeal shunt model. In the PGI_2 generation from endogenous arachidonic acid in isolated aorta strip, AIA (200 μM) showed a very slight inhibition of about 10%, and aspirin (200 μM) exerted an irreversible and strong inhibition of about 70%. Consequently, the inhibitory effect of AIA on PG biosynthesis was weaker than that of aspirin. These results may be explained by the fact that AIA was a compound substituted with 3-aminomethyl-IA on carboxyl group of aspirin. Further, in the present experiment, two new approaches have been applied on this problem. The first was the effect of AIA on MDA formation,

TABLE. VI. *Effects of AIA on Cyclic AMP Level and Adenylate Cyclase Activity in Washed Platelets of Rats*

Sample	Final concentration (μM)	Cyclic AMP level[a] (pmol/ml PRP)	Cyclic AMP formation[b] (pmol/10^8 platelets/10 min)
Control	–	4.05±0.36	19.2±0.17
AIA	200	3.78±0.24	23.8±2.20
Aspirin	200	4.74±0.24	19.0±1.61

a) Each value represents the mean ± s.e. (n=6).
b) The lysate of washed platelets was used. Each value represents the mean ± s.e. (n=3). Washed platelets formed 193.7± 12.1 pmol/ 10^8 platelets/ 10 min ($p< 0.001$: versus control) in the presence of NaF (4 mM).

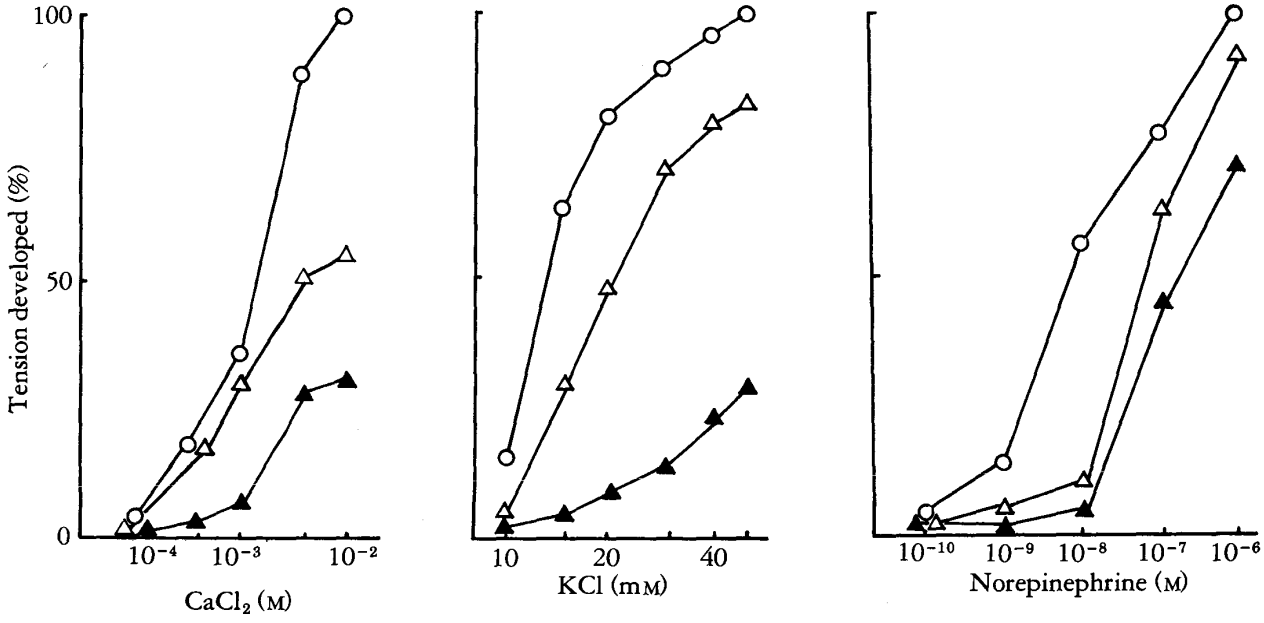

FIG. 3. *Effect of AIA on $CaCl_2$-, KCl- or Norepinephrine-induced Contraction of Thoracic Aorta Strip of Rats*

In each preparation, the maximum tention developed by the agonist in the control solution was taken as 100%.

—○— control, —△— AIA (50 μM), —▲— AIA (200 μM). Each point represents the mean value of 2 experiments.

colorimetric measurement of which is a useful assay for studies on effects of activators, cofactors or inhibitors on the pathway from exogenous arachidonic acid to MDA by PG synthetase. As shown in Table V, AIA (200 μM) did not inhibit the MDA formation, while aspirin (200 μM) significantly inhibited by about 60%. The second was the effect of AIA on fundus strip contraction which was induced by PG formed from exogenous arachidonic acid. In this experiment, the contraction of fundus strips might be influenced by a relaxation effect of drug. As shown in Fig. 2, AIA (100 μM) inhibited about 50% of contraction which was induced by arachidonic acid, while aspirin (100 μM) did not. At 1 mM concentration, the difference of both drugs in potency was evident. Since IA (100 μM), a constituent of AIA molecule, showed about 30% inhibition in the arachidonic acid-induced contraction of fundus strip, as shown in Fig. 2, the amount of anti-contractile effect of AIA might be due to relaxation effect of AIA on muscle. It can not be ruled out that contraction of contractile protein in platelets relates to the aggregation and the release reaction of platelets,[16] and that the increase of cytoplasmic Ca^{2+} concentration plays an important role in the initiation of platelet activation.[17] So the effect of AIA on cyclic AMP level and adenylate cyclase activity of platelets and Ca^{2+}-, K^+- or norepinephrine-induced contraction, which are related to the contraction of muscle and the platelet aggregation, was investigated. Elevation of cyclic AMP level of platelets shows depression of platelet aggregation. As shown in Table VI, AIA (200 μM) had no effect on cyclic AMP level of platelets, and did not show any significant increase on adenylate cyclase activity of platelets lysate, like that seen with aspirin (200 μM). Aspirin has no effect on several agonist-induced contractions of smooth muscle.[3,18] The effect of AIA on the dose response curve for Ca^{2+}, K^+ or norepinephrine on the rat thoracic aorta was shown in Fig. 3. AIA (200 μM) was evidently an inhibitor to three agonist-induced contractions. The mean $\pm$ s.e. ($n=4$) of pD_2 value was 4.09 ± 0.13 for Ca^{2+}, 3.88 ± 0.13 for K^+ and 3.13 ± 0.15 for norepinephrine, respectively.

These results strongly suggest that antithrombotic activity of AIA is due to anti-platelet action which was originated from its anti-aggregant effect in platelets, differing from aspirin.

Acknowledgment The authors wish to thank Toho Shinyaku Ltd., for supplying some materials.

REFERENCES

1) Part III: S.Aonuma, Y.Kohama and S.Fujimoto: Studies on aspirin derivatives with very little side effect. III. Absorption, distribution, excretion and metabolism of tritium-labeled aspirin-isopropylantipyrine (AIA) in rats, *J. Pharm. Dyn.*, **5**, 252–258 (1982).
2) S.Aonuma, Y.Kohama, S.Fujimoto and T.Makino: Studies on aspirin derivatives with very little side effect. II. Potent platelet anti-aggregant activity and no mutagenecity of aspirin-isopropylantipyrine (AIA), *J. Pharm. Dyn.*, **4**, 803–811 (1981).
3) S.Aonuma, Y.Kohama, Y.Komiyama and S.Fujimoto: Gastric ulcerogenic and biological activities of *N*-3′ a-propyphenazonyl-2-acetoxybenzamide, *Chem. Pharm. Bull.*, **28**, 1237–1244 (1980).
4) T.Umetsu and K.Sanai: Effect of 1-methyl-2-mercapto-5-(3-pyridyl)-imidazole (KC-6141), an anti-aggregating compound, on experimental thrombosis in rats, *Thrombos. Haemostas.* (Stuttg.), **39**, 74–83 (1978).
5) E.Griffett, S.Kinnon, A.Kumar, D.Lecker, G.Smith and E.Tomich: Effects of 6-(*p*-4-phenylacetylpiperazine-1-yl)phenyl-4,5-dihydro-3(2*H*)-pyridazione (CCI 17810) and aspirin on platelet aggregation and adhesiveness, *Br. J. Pharmacol.*, **72**, 697–705 (1981).
6) S.Ashida, M.Ishihara, H.Ogawa and Y.Abiko: Protective effect of ticlopidine on experimentally induced peripheral arterial occlusive disease in rats, *Thrombos. Res.*, **18**, 55–67 (1980).
7) Y.Harada, K.Tanaka and M.Katori: Acceleration of endogenous PGI_2 generation from isolated rat aortae by MK-447, *Jpn. J. Pharmacol.*, **31**, 845–848 (1981).
8) S.Ashida and Y.Abiko: Mode of action of ticlopidine in inhibition of platelet aggregation in rat, *Thrombos. Haemostas.*, **41**, 436–449 (1979).
9) R.J.Frower, H.S.Cheung and D.W.Cushman: Quantitive determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase, *Prosta-*

glandins, **4**, 325–341 (1973); M.Okuma, M.Steiner and M.Baldini: Studies on lipid peroxide in platelets. 1. Method of assay and effect of storage, *J. Lab. Clin. Med.*, **75**, 283–296 (1970).
10) F.Masuo and Y.Kimura: Spectrophotometric determination of malondialdehyde in aqueous solution, *Nippon Kagaku Zasshi*, **81**, 434–437 (1960).
11) J.Splawinski, A.Nies, B.Sweetman and J.Oates: The effects of arachidonic acid, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ on the longitudinal stomach strip of the rat, *J. Pharm. Exp. Ther.*, **187**, 501–510 (1973).
12) H.J.Schümann, B.D.Görlitz and J.Wagner: Influence of papaverine, D-600 and nifedipine on the effects of noradrenaline and calcium on isolated aorta and mesentric artery of the rabbit, *Naunyn-Schmideberg's Arch. Pharmacol.*, **289**, 409–418 (1975).
13) N.Mashimo, T.Motomiya, N.Kikutani, C.Sakakibara and S.Yamazaki: The role of platelet reactivity in thrombogenesis (II): Decrease of platelet aggregation and release reaction in spontaneously hypertensive rats (SHR), *Blood and Vessel,* **10**, 463–467 (1979).
14) C.Kohler, W.Wooding and L.Ellenbogen: Intravenous arachidonate in the mouse: A model for the elevation of antithrombotic drugs, *Thrombos. Res.*, **9**, 67–80 (1976); M.J.Silver, W.Hoch, J.J.Koksis, C.M.Ingerman and J.B.Smith: Arachidonic acid causes sudden death in rabbit, *Science,* **183**, 1085–1087 (1974).
15) S.Ashida and Y.Abiko: Effect of ticlopidine and acetylsalicylic acid on generation of prostaglandin I_2-like substance in rat arterial tissue, *Thrombos. Res.*, **13**, 901–908 (1978).
16) R.J.Haslam and J.A.Lynham: Relationship between phosphorylation of blood platelet proteins and secretion of platelet granule constituents. 1. Effects of different aggregation agent, *Biochem. Biophys. Res. Commun.*, **77**, 714–722 (1977).
17) P.Massini, R.Käser-Glanzmann and E.F.Lüscher: Movement of calcium ions and their role in the activation of platelets, *Thrombos. Haemostas.* (Stuttg.), **40**, 212–218 (1978).
18) B.Samuelsson and P.Paoletti (ed.), "Advances in Prostaglandin and Thromboxane Research," Japan Medical Center, Inc., Tokyo, 1976, pp. 127–131.