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Studies on Heart. XXIV.[1] Inhibitory Effect of Antiarrhythmic Peptide (AAP) on Experimental Thromboses

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The antithrombotic action of antiarrhythmic peptide (AAP) was studied by using various *in vivo* thrombosis models.

AAP (1, 10 or 100 mg/kg, *i.v.*, 10 mg/kg, *i.p.* or 100 mg/kg, *p.o.*) significantly inhibited white thrombus formation on a silken thread in the extracorporeal shunt models in rats, its ED_{50} being about 30 mg/kg, *i.v.* AAP (10 mg/kg, *i.v.*) was effective in protecting rats against the decrease in platelet count, incidence of electrocardiographic alterations (T wave inversion and ST segment depression) typical of myocardial ischemia, and development of ectopic beats in the coronary thromboembolism induced by intravenous infusion of adenosine 5′-diphosphate. The peptide (10 mg/kg, *i.p.*) was also effective in preventing thrombus formation in the lung and the decrease of platelet count induced by lactic acidosis in rats, and it (10 mg/kg, *i.v.*) clearly inhibited thromboembolic death induced by rapid intravenous injection of collagen in mice. Daily treatments with the peptide (10 mg/kg/d, *i.p.*) resulted in significant delay of the progression of gangrene and mummification in laurate-induced peripheral arterial occlusive disease in rats. AAP did not affect venous thrombus formation, blood flow through the carotid artery, plasma recalcification time or fibrinolytic activity in rats. It is likely that the potent antithrombotic action of AAP is mainly due to its anti-platelet-aggregating action *in vivo*.

Ticlopidine (100 mg/kg, *p.o.*) also showed a comparatively wide antithrombotic spectrum, like AAP, in the present thrombosis models, but ticlopidine lacked the action against myocardial ischemia, like aspirin (50 mg/kg, *s.c.*).

Keywords——peptide; arrhythmia; thrombosis; platelet; antithrombotic drug; antiarrhythmic drug

An antiarrhythmic peptide (AAP), isolated from bovine atria[2] and identified as Gly–Pro–Hyp–Gly–Ala–Gly,[3] has a protective effect against experimental arrhythmias of cultured myocardial cells, isolated atria and whole hearts induced by several drugs.[2,4] Since the vulnerability of the heart to arrhythmias is increased during acute myocardial ischemia and pulmonary thromboembolism, it is of great advantage for protection against arrhythmias that an antiarrhythmic drug should also possess antithrombotic action. In the present study, the effectiveness of AAP in preventing experimental thromboses *in vivo*, including the extracorporeal shunt model, coronary thromboembolism, pulmonary thromboembolism, peripheral arterial occlusive disease and venous thrombosis was evaluated and compared with those of known antithrombotic drugs, ticlopidine[5] and aspirin.[6]

Experimental

Materials and Animals——Synthetic AAP prepared in our laboratory[1] was used in this experiment. Lauric acid sodium salt, *trans*-4-(aminomethyl)-cyclohexanecarboxylic acid (tranexamic acid, Aldrich Chemical Co., U.S.A.), lactic acid, adenosine 5′-diphosphate disodium salt (ADP, Kojin Co., Ltd.), collagen (Sigma Chemical Co., U.S.A., Type III, acid-soluble from calf skin), thrombin (Mochida Seiyaku Ltd.) and panaldine tablets (Daiichi Seiyaku Co., Ltd.; one tablet contains 100 mg of ticlopidine hydrochloride) were obtained from Nakarai Chemical Ltd., aspirin

was from Yoshitomi Seiyaku Co., and Somnopentyl (sodium pentobarbital, Pitman-Moore Inc., U.S.A) and heparin sodium salt (165 units/mg) were from Wako Pure Chemical Ind. Ltd.

AAP dissolved in saline and adjusted to pH 7.3 was administered *i.v.*, *i.p.*, *s.c.* or *p.o.* to animals. Aspirin (50 mg/kg) or a homogenous powder of panaldine tablet (100 mg as ticlopidine/kg) suspended in 5% acacia was administered *s.c.* 1 h or *p.o.* 3 h, respectively, before all tests. When the *i.v.* injection of saline was used as a control *versus* these drugs, additional saline was injected *i.v.* into these drug-treated rats at the same dose and time as in the case of the saline group. Sample volume for administration was 0.5 ml/kg, *i.v.*, 1 ml/kg, *i.p.* and *s.c.* or 2 ml/kg, *p.o.* Intravenous samples were injected into the right femoral vein *via* a cannula.

Animals were Wistar male rats weighing 300—350 g and ddy male mice weighing 25—30 g, and they were anesthetized with pentobarbital (40 mg/kg,*i.p.*) for operation, injection and infusion except for subsequent daily administrations of sample. If necessary, the anesthesia was maintained throughout the experiment by giving small additional doses of the anesthetic as required.

Extracorporeal Shunt Model——The model was prepared in rats as described by the authors.[6b] Briefly, after heparinization (100 units/kg, *i.v.*), a series of 3 polyethylene tubes, two tubes (Hibiki, No. 4, 12.5 cm length) at both ends and one tube (Hibiki, No. 7, 6 cm length) threaded with a white silken thread (Kanebo Co., No. 50, 5 cm length) in the middle, filled with heparin solution (100 units/ml), was installed between the right carotid artery and left jugular vein. The blood circulation *via* the shunt was established for 20 min and the wet weight of thrombus coated on the thread was measured. Subsequently, the rat was heparinized (50 units/kg, *i.v.*), followed by a second and then a third circuit of blood using another new tube containing a silken thread in each case. The total thrombus weight in 1 h in three experiments was determined and used as an indicator of thrombus formation.

ADP-induced Electrocardiogram (ECG) Alterations——According to the method of Dejana *et al.*,[7] ADP (1 mg/kg) was infused at a constant rate of 0.05 ml/min/330 g body weight for 10 min into a rat femoral vein after heparinization (100 units/kg, *i.v.*). T wave inversion, ST segment depression and ectopic beats on ECG (lead I) were monitored during a 10 min infusion of ADP using a Polygraph system (Nihon Koden, RM-6000). Platelet count was measured on blood samples obtained from the right carotid artery *via* a cannula immediately before, at 1, 5 and 9 min during and 5 min after the infusion, using a Platelet counter (Sysmex, PL-100).

Acute Pulmonary Thromboses——According to the method of Tomikawa *et al.*,[8] pulmonary thrombosis was induced in rats by an infusion of 0.7 M lactic acid (14 ml/kg/h) for 2 h into a femoral vein. The severity of thrombus was represented as the number of total thrombi (stained with hematoxylin–eosin) counted in ten successive fields of four paraffin sections from the lungs under 200-fold magnification. Thrombus count was measured on the samples obtained immediately after the infusion. Platelet count was measured on the blood obtained from the femoral artery immediately before the infusion and at the end of the infusion.

Acute thromboembolic death was induced in mice by rapid intravenous injection of a soluble collagen solution (25 mg/10 ml/kg) according to the method of Tomikawa *et al.*[5] Cardiac arrest was monitored by ECG (lead II) using the Polygraph system.

Venous Thrombosis——According to the method of Kumada *et al.*,[9] a stainless steel coil (Zipperer, Zdarsky Ehrler K. G., West Germany, size 40 (st)) was inserted into the lumen of the inferior vena cava at the site just below the left renal vein branching in the rat. Thrombus size was measured by determining total protein content in the thrombus on the wire and the mural thrombus obtained 48 h after the wire insertion. The protein content was determined by the method of Lowry *et al.*[10] Platelet count was measured on samples obtained from the inferior vena cava immediately before the insertion and at the removal of the wire.

Laurate-induced Arterial Occlusive Disease——According to the method described by the authors,[6b] 0.1 ml of sodium laurate solution (10 mg as acid/ml) was injected into the right femoral artery of the rat. The degree of gangrene and mummification was graded into 0 to IV according to the severity of lesions during 5 d from the 2nd d after the injection of laurate.

Blood Flow——Blood flow through the right carotid artery of the rat was measured with an electromagnetic blood flow meter (Nihon Koden, MFV-1100).

Recalcification Time and Fibrinolytic Activity——Blood was taken from the abdominal artery of the rat with a syringe containing 1/10 volume of 0.027 M NaCl solution with 3.008% sodium citrate, and centrifuged at 3000 rpm for 10 min at 4 °C. One hundred μl of plasma and 100 μl of saline were mixed and 1 min later the clotting time was measured at 37 °C after the addition of 100 μl of 0.025 M $CaCl_2$ solution using a Clotec (Nihon Travenol).

Two hundred μl of citrated plasma was mixed with 10 ml of 0.8% NaCl solution containing 6.8 mM $CaCl_2$ and 0.32 mM tranexamic acid, and the mixture was incubated at 37 °C for 1 h, followed by centrifugation at 3000 rpm for 15 min. The fibrin precipitate was washed with distilled water and the protein content of the precipitate was determined by the method of Lowry *et al.*[10] Euglobulin fraction was prepared by mixing 100 μl of citrated plasma with 2 ml of cold 0.01% acetic acid solution (pH 3.6), followed by centrifugation at 3000 rpm for 10 min after standing at 4 °C for 20 min. The precipitate was dissolved in 200 μl of 0.05 M phosphate buffer (pH 7.4). The euglobulin solution was mixed with 10 μl of thrombin solution (200 units/ml) and the lysis time of the clot was determined at 37 °C. Fibrinolytic activity in plasma was expressed in terms of the fibrinolytic index calculated as the amount (μg) of fibrinogen in 100 μl of plasma used/euglobulin lysis time (ELT, min), as described by Tomikawa *et al.*[8]

Results

Extracorporeal Shunt Model

AAP was intravenously given to rats 1 min before the establishment of blood circulation through the shunt and the weight of thrombus formed on the thread during a total of 1 h was determined. As shown in Table I, AAP significantly inhibited thrombus formation in this model at 1, 10 or 100 mg/kg. The inhibitory action of AAP on the thrombosis increased linearly with dose from 1 to 100 mg/kg, its ED_{50} being about 30 mg/kg.

Table II shows the effect of intraperitoneal, oral or subcutaneous administration of AAP on thrombus formation in the extracorporeal shunt model in rats. AAP at a dose of 10 mg/kg, given *i.p.* 20 min before the blood circulation, strongly inhibited thrombus formation. The peptide at a dose of 100 mg/kg, given *p.o.* 3 h before the test, also significantly inhibited the thrombosis. The peptide in a dose of 50 mg/kg, given *s.c.* 1 h before the test, inhibited the thrombosis slightly, although the effect was not statistically significant. Although it may be difficult to compare directly the inhibitory actions of AAP given by various routes because of

Table I. Effect of Intravenous Injection of AAP on Thrombus Formation on Silken Thread in Rat Extracorporeal Shunt

Sample[a]	Dose (mg/kg, *i.v.*)	Wet weight of thrombus[b] (mg, mean ± S.E. of 6 rats)
Saline	—	41.4 ± 2.1
AAP	1	28.2 ± 4.1[c] (32)
AAP	10	22.2 ± 3.3[d] (46)
AAP	100	18.8 ± 0.4[d] (55)

a) Sample or saline was injected 1 min before the blood circulation for 1 h.
b) Inhibition % of thrombus formation relative to the saline group is given in parenthesis. Data were analyzed by means of Student's *t*-test. *c*) $p < 0.05$, *d*) $p < 0.001$: *versus* saline.

Table II. Effect of Intraperitoneal, Oral or Subcutaneous Administration of AAP on Thrombus Formation on Silken Thread in Rat Extracorporeal Shunt

Sample[a]	Administration			Wet weight of thrombus[b] (mg, mean ± S.E. of 6 rats)	
	Dose (mg/kg)	Route	Time (min)		
Saline	—	*i.p.*	20	40.5 ± 3.2	
AAP	10	*i.p.*	20	14.9 ± 2.4[e]	(63)
Saline	—	*p.o.*	180	41.0 ± 3.6	
AAP	100	*p.o.*	180	29.1 ± 3.8[c]	(29)
5% Acacia	—	*p.o.*	180	42.2 ± 2.5	
Ticlopidine	100	*p.o.*	180	25.7 ± 6.4[c]	(39)
Saline	—	*s.c.*	60	43.1 ± 4.0	
AAP	50	*s.c.*	60	35.8 ± 6.6	(17)
5% Acacia	—	*s.c.*	60	45.9 ± 2.7	
Aspirin	50	*s.c.*	60	26.2 ± 4.2[d]	(43)

a) Sample or vehicle was administered according to the dose, route and time given in the table, before blood circulation for a total of 1 h.
b) Inhibition % of thrombus formation with respect to the vehicle group is given in parenthesis. Data were analyzed by means of Student's *t*-test. *c*) $p < 0.05$, *d*) $p < 0.01$, *e*) $p < 0.001$: *versus* the vehicle.

the different doses and times, it seems that AAP given *i.p.* was most effective in inhibiting thrombus formation in this model, followed by *i.v.*, *p.o.* and *s.c.* in that order.

As positive controls, aspirin and ticlopidine were tested using doses, routes and times known to be effective in inhibiting thromboses.[5,6] Aspirin at a dose of 50 mg/kg, given *s.c.* 1 h before, and ticlopidine in a dose of 100 mg/kg, given *p.o.* 3 h before the test significantly inhibited the thrombosis in this model, as shown in Table II. The inhibitory actions of aspirin and ticlopidine were similar to that of AAP at a dose of 10 mg/kg *i.v.*, and were less than that of AAP at a dose of 10 mg/kg *i.p.*

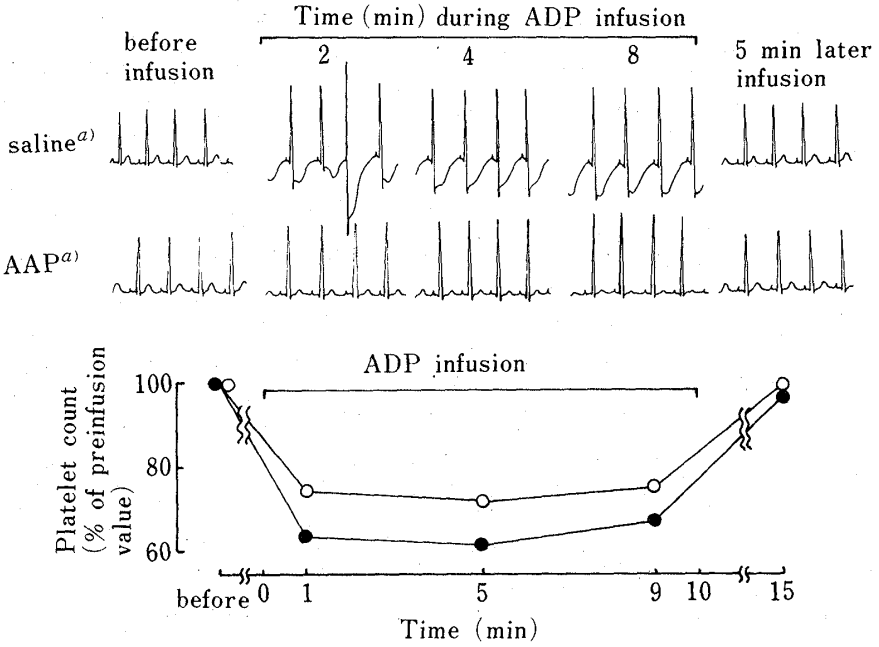

Fig. 1. Examples of ECG (Lead I) Alterations and Platelet Decrease during Intravenous Infusion of ADP in Saline- or AAP-Treated Rat

a) AAP (10 mg/kg) or saline was given *i.v.* 5 min before intravenous infusion of ADP (1 mg/kg) at 0.5 ml/10 min/330 g body weight for 10 min. In the lower figure: (○) AAP-treated rat; (●) saline-treated rat.

TABLE III. Effect of AAP on ECG and Platelet Count during Intravenous Infusion of ADP in Rats

Sample[a)]	Administration			Incidences of ECG (lead I) alterations during ADP infusion[b)]			Platelet count at 5 min during ADP infusion[d)] (% of preinfusion value, mean ± S.E. of 10 or 6 rats)
	Dose (mg/kg)	Route	Time (min)	T wave inversion	ST segment depression	Ectopic beats	
Saline	—	*i.v.*	5	9/10	8/10	7/10	61.2 ± 3.9
AAP	10	*i.v.*	5	2/10[c)]	1/10[c)]	3/10	72.0 ± 2.2[e)]
Ticlopidine	100	*p.o.*	180	4/6	4/6	4/6	75.7 ± 4.5[e)]
Aspirin	50	*s.c.*	60	4/6	4/6	4/6	69.0 ± 4.9

a) Sample or saline was administered according to the dose, route and time given in the table, before the infusion of ADP (1 mg/kg, *i.v.*) at 0.5 ml/10 min/330 g body weight for 10 min. Additional saline was given *i.v.* to the ticlopidine- or aspirin-treated rats 5 min before the infusion.
b) Data are the numbers of rats with ECG alterations per number of rats used. Data were analyzed by means of the X^2-test. *c*) $p<0.01$: *versus* saline.
d) Data were analyzed by means of Student's *t*-test. *e*) $p<0.05$: *versus* saline.

ADP-induced ECG Alterations

In the rats given intravenous infusion of ADP, characteristic ECG alterations, which consisted of T wave inversion, ST segment depression and ectopic beats, were visible in the first lead, and simultaneously the platelet count fell during the infusion, as shown in Fig. 1 and Table III. AAP at a dose of 10 mg/kg, given *i.v.* before the infusion, significantly inhibited the decrease of platelet count and the incidence of T wave inversion and ST segment depression, and tended to inhibit development of ectopic beats, as shown in Fig. 1 and Table III. Ticlopidine (100 mg/kg, *p.o.*) also significantly inhibited platelet decrease and aspirin (50 mg/kg, *s.c.*) tended to inhibit it, although they did not affect the ECG alterations induced by ADP infusion (Table III).

Acute Pulmonary Thromboses

AAP was given to rats at a dose of 10 mg/kg, given *i.p.* 20 min before the infusion of lactic acid for 2 h, and its protective action against thrombus formation in the lungs and thrombocytopenia was studied. As shown in Table IV, AAP was significantly effective in inhibiting thrombus formation and platelet decrease in this model. Ticlopidine (100 mg/kg, *p.o.*) also significantly inhibited thrombus formation and thrombocytopenia (Table IV).

AAP was given *i.v.* at a dose of 10 mg/kg 5 min before rapid intravenous injection of soluble collagen solution, and its protective effect against acute thromboembolic death was

TABLE IV. Effect of AAP on Acute Pulmonary Thrombosis Induced by Lactic Acid in Rats

Sample[a]	Administration			Thrombus count[b] (mean ± S.E. of 6 rats)	Platelet count[b] (% of preinfusion value, mean ± S.E. of 6 rats)
	Dose (mg/kg)	Route	Time (min)		
Saline	—	*i.p.*	20	65.9 ± 8.6	35.0 ± 1.3
AAP	10	*i.p.*	20	35.9 ± 4.5[c] (46)	43.1 ± 1.3[c]
Ticlopidine	100	*p.o.*	180	30.9 ± 3.9[c] (53)	56.2 ± 2.4[d]

a) Sample or saline was administered according to the dose, route and time given in the table, respectively, before intravenous infusion of 0.7 M lactic acid (14 ml/2 h/kg) for 2 h.
b) Thrombus count in a total of 40 microscopic fields of 4 lung sections under 200-fold magnification and platelet count in blood were measured on samples obtained immediately after the infusion. Inhibition % of thrombosis with respect to the saline group is given in parenthesis. Data were analyzed by means of Student's *t*-test.
c) $p<0.01$. *d*) $p<0.001$: *versus* saline.

TABLE V. Effect of AAP on Acute Thromboembolic Death Induced by Collagen in Mice

Sample[a]	Administration			Mortality rate[b]
	Dose (mg/kg)	Route	Time (min)	
Saline	—	*i.v.*	5	8/10
AAP	10	*i.v.*	5	2/10[c]
Aspirin	50	*s.c.*	60	6/10

a) Sample or saline was administered according to the dose, route and time given in the table, before rapid intravenous injection of soluble collagen solution (25 mg/10 ml/kg). Additional saline was given *i.v.* to the aspirin-treated mice 5 min before the injection of collagen.
b) Data are numbers of mice dead within 4 min after the injection of collagen per number of mice used. Data were analyzed by means of the X^2-test. *c*) $p<0.05$: *versus* saline.

studied. As shown in Table V, AAP significantly reduced mortality from collagen-induced thromboembolism. Aspirin (50 mg/kg, *s.c.*) was less effective in this model than AAP. Ticlopidine (300 mg/kg, *p.o.*) was reported to be effective in this model.[5]

Venous Thrombosis

AAP (10 mg/kg, *i.p.*) was given 2 h before and 6, 18, 30 and 42 h after wire insertion into the inferior vena cava and its effect on thrombus formation and the platelet count was studied 48 h after the wire insertion. As shown in Table VI, AAP had no significant effect in this thrombotic model, although it seemed to slightly reduce thrombus formation. Ticlopidine (100 mg/kg, *p.o.*) given 2 h before and 28 h after the wire insertion tended to inhibit the thrombosis, and it was reported to inhibit the thrombosis significantly[9] when it was given 42,

Table VI. Effect of AAP on Venous Thrombosis in Rats

Sample[a]	Administration		Thrombus size[b] (μg protein, mean ± S.E. of 8 rats)	Platelet count[b] (% of preinsertion value, mean ± S.E. of 8 rats)
	Dose (mg/kg)	Route		
Saline	—	*i.p.*	1265 ± 138	86.9 ± 6.7
AAP	10 × 5	*i.p.*	1052 ± 225 (17)	92.5 ± 8.3
Ticlopidine	100 × 2	*p.o.*	984 ± 190 (22)	95.7 ± 8.9

a) Sample or saline was administered according to the dose and route given in the table. Dose schedules of sample or saline were as follows: AAP and saline, 2 h before and 6, 18, 30 and 42 h after the wire insertion; ticlopidine, 2 h before and 28 h after the wire insertion.

b) Total protein content in the thrombus on the wire and the mural thrombus were measured at 48 h and platelet counts in blood were measured on samples obtained immediately before and at 48 h after the wire insertion. Inhibition % of thrombosis with respect to the saline group is given in parenthesis.

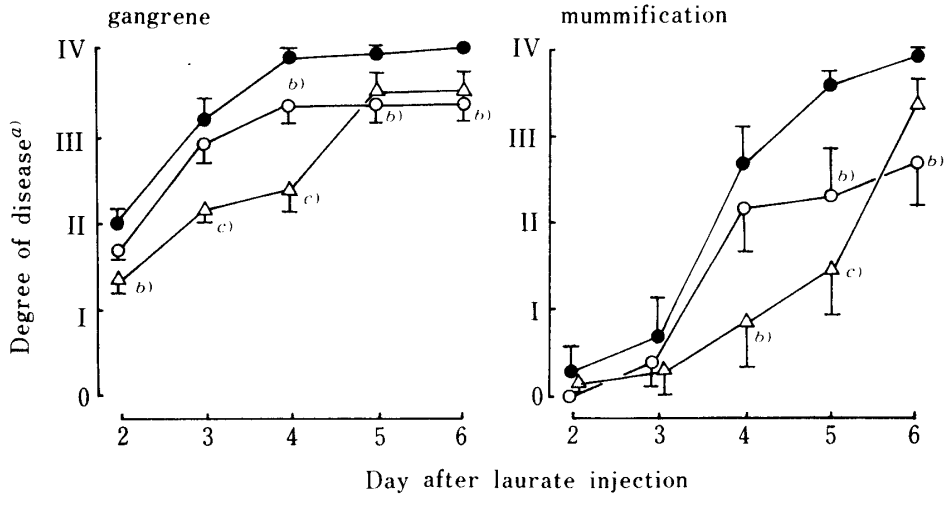

Fig. 2. Effect of AAP on Progression of Arterial Occlusive Disease Induced by Laurate in Rats

Rats received 0.1 ml of sodium laurate solution (10 mg as acid/ml) into the right femoral artery. (○) AAP (5 mg/kg) or (●) saline was given *i.p.* immediately after the laurate injection with subsequent doses twice daily throughout the experiment. (△) ticlopidine (100 mg/kg) was given *p.o.* immediately after the laurate injection with subsequent doses once daily throughout the experiment.

a) Normal appearance, 0; the affected region was limited to the nail parts, I; to the fingers, II; to the whole paw, III; extended to the lower leg, IV. Each point shows the mean ± S.E. of degree of disease in 10 rats. Data were analyzed by means of Student's *t*-test. *b*) $p<0.05$, *c*) $p<0.001$: *versus* saline.

18 and 2 h before and 28 h after the wire insertion. Aspirin given at the same doses and dose schedules as those of ticlopidine was reported to be ineffective in this model.[9] A slight decrease of platelet count 48 h after the wire insertion was seen in the control rats, and AAP and ticlopidine tended to slightly inhibit the platelet decrease.

Laurate-induced Arterial Occlusive Disease

In the control rats which received saline alone after the injection of laurate, gangrene extending from the fingers to the whole paw and to the lower leg was found at 2—4 d and the affected part of the paw then became mummified and fell off at 4—6 d after the injection of laurate, as shown in Fig. 2. AAP (5 mg/kg, *i.p.*) was given immediately after laurate injection with subsequent doses twice daily, and its protective effect against the progression of the disease was studied. As shown in Fig. 2, AAP did not affect the progression of gangrene from the fingers to the whole paw within 3 d, but significantly inhibited the progression of gangrene to the lower leg at 4—6 d. The peptide significantly inhibited progression of mummification from the fingers to the whole paw and to the lower leg at 5—6 d, although it did not affect the progression of mummification to the fingers at 2—4 d. Ticlopidine given once daily at 100 mg/kg, *p.o.*, significantly inhibited the primary progression of gangrene and mummification, but it did not affect the progression of the disease to the whole paw and the lower leg.

Blood Flow

As shown in Fig. 3, AAP given at a dose of 10 mg/kg, *i.v.*, 100 mg/kg, *i.v.* or 100 mg/kg, *i.p.* to rats did not affect blood flow through the carotid artery.

Recalcification Time and Fibrinolytic Activity

AAP in a dose of 10 mg/kg, given *i.p.* 30 min before blood collection, did not affect the plasma recalcification time or fibrinolytic activity, as shown in Table VII.

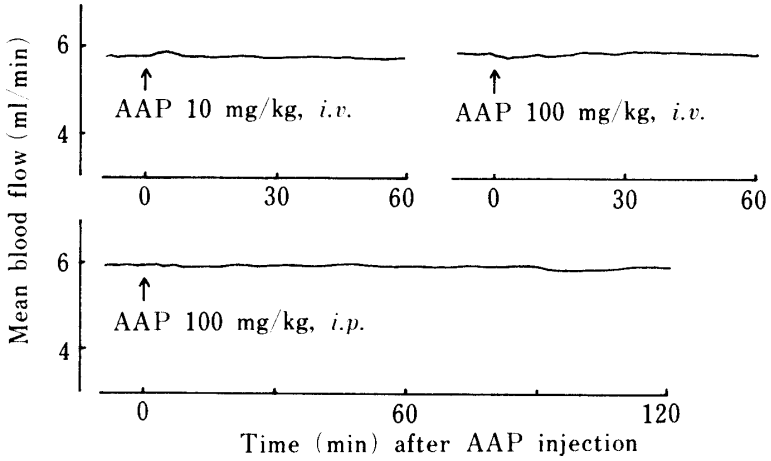

Fig. 3. Effect of AAP on Blood Flow through the Carotid Artery in Rats

TABLE VII. Effect of AAP on Blood Coagulation and Fibrinolysis in Rats

Sample[a]	Dose (mg/kg)	Plasma recalcification time (s, mean ± S.E. of 6 rats)	Fibrinolytic activity[b] (mean ± S.E. of 6 rats)
Saline	—	120.8 ± 11.8	2.24 ± 0.12
AAP	10	117.7 ± 6.1	2.37 ± 0.28

a) Sample or saline was given *i.v.* 30 min before the blood collection.
b) Fibrinogen (μg) in 100 μl of plasma used/ELT (min).

Discussion

Generally, the pathogenesis of thrombosis is thought to involve factors such as changes in the blood vessel, changes in blood flow and changes in the properties of the blood components, especially platelets. The present study revealed that AAP has potent anti-thrombotic action in various thrombosis models in which platelets are mainly concerned.

In the extracorporeal shunt model, white thrombus was formed by platelet adhesion to a silken thread and subsequent aggregation under inhibition of blood coagulation by continuous injection of heparin.[11] AAP inhibited thrombus formation in this model when given *i.v.*, *i.p.* or *p.o.* before blood circulation for 1 h. The peptide given *i.v.* or *i.p.* did not affect blood flow, a decrease of which inhibited thrombus formation in this model.[11] Therefore, the action of AAP seemed to be mainly due to the effect on the platelet properties.

Jorgensen *et al.*[12] noted that intermittent infusions of ADP into the coronary circulation of pigs caused circulatory collapse, electrocardiographic evidence of myocardial ischemia, ventricle arrhythmias and formation of platelet aggregates in the microcirculation. Dejana *et al.*[7] also reported that slow intravenous infusion of ADP was accompanied by a decrease of platelet count cardiovascular collapse and ECG alterations typical of myocardial ischemia, and that all these effects were inhibited in rats given antiplatelet aggregating compound or made thrombocytopenic. In the present experiment, a model of coronary thromboembolism was prepared in rats according to the procedure reported by Dejana *et al.*,[7] except for the infusion dose (1 mg/kg/10 min) of ADP, because about one-third of rats given the reported dose (6 mg/kg/10 min) of ADP died during the infusion. AAP clearly diminished the platelet decrease, ECG alterations typical of myocardial ischemia and ectopic beats induced by the ADP infusion. On the other hand, although ticlopidine and aspirin diminished the platelet decrease, they were ineffective as regards ECG alterations and ectopic beats, suggesting that myocardial ischemia in this model can not be reversed only by antiplatelet aggregating action. Previously, we reported that AAP (10 mg/kg, *i.v.*) significantly shortened the duration of arrhythmias induced by rapid intravenous injection of ADP, which caused cardiovascular alterations due to the direct effect of ADP rather than to the formation of platelet aggregates,[7] but aspirin (50 mg/kg, *s.c.*) did not affect the duration of arrhythmias and quinidine (10 mg/kg, *i.v.*) prolonged it.[4,6b] Therefore, the action of AAP on the ECG alterations might be related to its essential and direct action on the heart in addition to its inhibitory effect on the thrombocytopenia induced by the ADP infusion.

Experimental pulmonary thrombosis was induced by lactic acidosis. As lactic acidosis progressed, the platelet count decreased and microthrombi were formed in the lung, accompanied by disseminated intravascular coagulation.[8] This thrombosis was shown to be prevented by treatment with anti-platelet-aggregating drugs, anticoagulant and fibrinolytic agents.[5] AAP inhibited thrombus formation and platelet decrease in this model probably through its inhibitory action on platelet aggregation, because this peptide clearly inhibited collagen-induced thromboembolic death, which is generally considered to be a result of *in vivo* platelet aggregation, and it did not affect blood coagulation or fibrinolysis.

The possibility that AAP inhibited the thromboses mainly through its anti-platelet-aggregating effect, was supported by the ineffectiveness of AAP in the venous thrombosis model, which appears to be not associated to a large extent with platelet aggregation and adhesion.

In the rats given laurate into the femoral artery, the early vascular lesions were degeneration and denudation of the endothelium and edematous swelling of the intimal and medial layers, and there was secondary formation of occlusive thrombi due to platelet adherence and aggregation with some participation of the coagulation system, followed by fibrosis and organization with cellular infiltration.[13] AAP inhibited the progression of disease

in this model in the same way as ticlopidine, although there was a difference in the time course of inhibitory action between the two samples. The effect of AAP is thought to be at least in part due to its anti-platelet-aggregating action *in vivo*, like that of ticlopidine, as mentioned above. AAP was effective in inhibiting the later progression of disease, however, unlike ticlopidine. This peptide was found to increase the spreading phenomenon, to increase macromolecular synthesis and to prolong the survival of rat myocardial cells in serum-free or low-serum culture.[14] The restorative effect of AAP might, therefore, contribute to the inhibition of the progression of the vascular lesions. Aspirin was ineffective in this model.[6b]

Although it is difficult to compare directly the antithrombotic action of AAP demonstrated in the present study, with those of ticlopidine and aspirin because of the differences in doses, routes and times, it is probable that there are differences in antithrombotic spectrum among the three drugs. AAP was effective in various thrombosis models including white thrombus formation in the extracorporeal shunt, coronary thromboembolism, pulmonary thrombosis and peripheral arterial thrombosis, in which platelets are mainly concerned. Ticlopidine also possessed a wide antithrombotic spectrum, but it lacked the action against myocardial ischemia. Aspirin possessed the narrowest spectrum among the three samples in the thrombosis models used in the present study. Thus, AAP is a unique peptide possessing antithrombotic action together with antiarrhythmic action. The results of a study on the mode of action on platelet function will be presented shortly.

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