Enhanced Platelet Accumulation onto Injured Carotid Arteries in Rabbits after Aspirin Treatment

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 A B S T R A C T Prostacyclin (PGI₂) is a powerful inhibitor of platelet aggregation, but its role in the pathogenesis of arterial thrombosis is uncertain. We have studied the thrombogenic effect of inhibiting $PGI₂$ production by aspirin (ASA) in carotid arteries of rabbits given 0, 3, 10, or 100 mg ASA/kg either 1, 3, 6, or 20 h beforehand. Platelet accumulation onto injured carotid arteries was enhanced with ASA in ^a dose of 10 mg/kg. A higher dose of ASA (100 mg/kg) had no further effect. The enhanced thrombogenic effect of ASA persisted for at least 20 h and was associated with a decrease in vessel wall PGI₂ production. There was a strong inverse correlation ($r = -0.55$, $P < 0.01$) between PGI₂ production and platelet accumulation. The findings suggest that the margin of safety in obtaining an antithrombotic effect of ASA and producing ^a potential thrombotic effect in arteries may not be as large as predicted by studies using cultured endothelial cells or experimentally induced thrombosis in veins.

INTRODUCTION

The production of thromboxane A_2 (TxA₂)¹ by platelets and of prostacyclin $(PGI₂)$ by vessel-wall cells is thought to be important in the regulation of hemostasis and in the pathogenesis of thrombosis $(1, 2)$. TxA₂ causes vasoconstriction and enhances platelet aggregation, whereas $PGI₂$ causes vasodilatation and inhibits platelet aggregation. Aspirin (ASA) inhibits TxA2 formation by acetylating platelet cyclooxygenase (3). This effect lasts for the life span of the platelet and is produced by low concentrations of ASA, which have recently been shown to be effective in preventing

thrombosis (4). Aspirin also inhibits $PGI₂$ formation by vascular wall cells by the same mechanism, but in contrast to platelets, its effect on cultured vessel wall cells has been reported to be relatively shortlived, presumably because the vascular wall cells have the capacity to resynthesize cyclooxygenase (5, 6).

Recently, we reported that the inhibitory effect of ASA on $PGI₂$ formation persists for up to 20 h in whole vessel wall preparations of carotid arteries, but is lost within $3-6$ h in jugular veins in rabbits $(7-9)$. To determine whether this prolonged effect of ASA on PGI₂ production in arteries is associated with a prolonged thrombogenic effect, we examined the relationship between the inhibitory effect of ASA on $PGI₂$ production and its enhancement of platelet accumulation onto injured carotid arteries in rabbits.

METHODS

Carotid injury model. Rabbits were injected with homologous 51Cr-labeled platelets. 20 h later, each animal was anesthetized with sodium pentobarbital (MTC Pharmaceuticals, Hamilton, Ontario) and both carotid arteries were isolated. Two clamps were applied ¹ cm apart onto each artery for 10 min, during which time blood remained within the isolated segment. The clamps were then removed and blood flow restored. No macroscope thrombi were ever seen to form in the segment during stasis. ¹ h later, a 5-ml citrated blood sample was collected, and the rabbit was then heparinized (200 U/kg) and killed with an overdose of sodium pentobarbital. A standard length of each vessel, encompassing both clamp injury sites, was removed, rinsed in saline, and placed in a glass tube containing 1.5 ml saline. The vessel wall radioactivity was then determined in a gamma counter (model 1085, Nuclear-Chicago Corp., Des Plaines, Ill.). The vessel was then removed from the tube, slit longitudinally, laid flat, endothelial side down, on a transparent film of acetate, and photocopied. The area of the vessel wall was determined by cutting out the photocopy and weighing it. The weight was compared with the weight of an adjacent ¹ cm square cut from the same piece of paper and was expressed as weight per unit area.

Platelet-rich plasma was prepared from the citrated blood sample collected before removing the injured vessels. Platelet counts and platelet radioactivity were determined, and

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¹ Abbreviations used in this paper: ASA, aspirin; PGI_2 , prostacyclin; TxA_2 , thromboxane A_2 .

platelet specific activity calculated. The radioactivity of the vessel wall was then expressed as platelets/10 mm2 of vessel wall.

Some animals were injected with both 51Cr-platelets and 125I-fibrinogen, and their carotid arteries were then injured as described above. 5'Cr-Platelet accumulation and 1251-fibrin accretion on the injured vessels were determined using the method described elsewhere (9). In other animals, the injured vessels were removed and prepared for electron microscope examination.

Preparation of tissues for scanning electron microscopy. Segments of injured carotid artery were fixed in 2% glutaraldehyde (0.1 M sodium cacodylate, 0.2 mM calcium chloride, pH 7.35) for 2 h at 4°C. The vessels were then postfixed in 1% aqueous osmium tetroxide for 1 h at 4° C, followed by dehydration in a series of graded ethanols. After dehydration, the vessels were critical-point dried from $CO₂$ in a Bomar critical point dryer (model SPC-900/EX, Tacoma, Wash.) and coated with gold (200 A) using a Polaron sputter coater (Polaron Equipment Ltd., Watford, Herts, England). The vessels were viewed in a Phillips 501 scanning electron microscope (Phillips Electronic Instruments, Inc., Mahwah, N. J.). All chemicals for this procedure were obtained from Polysciences Inc., Warrington, Pa.

Measurement of $PGI₂$ -like activity of carotid arteries. The effect of ASA on PGI₂ synthesis by the vessel wall was quantitated in two ways, using a radioimmune assay for 6 keto-PGF₁^a as described by Czervionke et al. (10) and by using a bioassay as described below. The antibody for the radioimmune assay was kindly supplied by Dr. J. B. Smith, Temple University, Philadelphia, Pa. The sensitivity of the bioassay was 3 pmol, and the sensitivity of the radioimmune assay was 30 pmol. The activity of the bioassay is referred to as "PGI₂-like" activity.

The basic method used to quantitate PGI₂-like activity of vessel walls is described in detail elsewhere (8, 9). In brief, rabbits were heparinized with 200 U/kg of heparin (Hepalean, Harris Laboratories, Brantford, Ontario), and the carotid arteries were isolated and removed from the animal. The heparin was used to prevent the generation of thrombin during the isolation of arteries. We have previously demonstrated that the heparin was effectively removed from the vessel wall so that it does not interfere with the PGI2-like activity or the $PGI₂$ assay procedure (11). Each vessel was flushed with calcium-free Tyrodes solution (containing 0.35% albumin, bovine fraction V; Sigma Chemical Corp., St. Louis, Mo .) and then slit longitudinally to stimulate $PGI₂$ synthesis. It was immediately placed in 300 μ l of Eagles' medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), containing 4% albumin, ⁵ mM Hepes buffer, pH 7.45 (Eagles'), and incubated at 37°C for 5 min. The vessel was then removed, and 7μ l of 1.0 M sodium carbonate was immediately added to the medium to raise the pH to 8.6 and so stabilize the PGI₂-like activity. The sample was stored at -70° C until testing. The area of the vessel wall was quantitated in the same manner as described above.

To test for the PGI₂-like activity of each test sample, the sample was thawed and 10 μ l of 0.5 N hydrochloric acid was added to the sample to bring the pH back to 7.6. Immediately, the sample (300 μ l) was added to 0.7 ml of ¹⁴Cserotonin-labeled platelet suspension which was being stirred in an aggregation module (Payton Associates, Scarboro, Ontario). 15 s later, imipramine (2 μ M) was added to block reuptake of the 14C-serotonin and 15 ^s after that, a standard amount of thrombin (0.015-0.025 U, Parke, Davis & Co., Detroit, Mich.) was added to the sample. 60 and 120 s later, $100-\mu$ l aliquots were collected from the sample and added to 100μ l of 0.25% ice-cold paraformaldehyde to terminate the release

reaction. The aliquots were then centrifuged for 60 ^s at 12,000 g and the amount of ¹⁴C-serotonin released into the supemate was determined. The percent inhibitoin of 14C-serotonin released by platelets was measured and the amount of PGI₂like activity calculated from a dose-response curve that was standardized for each experiment, using PGI₂ obtained from Upjohn Co., Kalamazoo, Mich. There was no loss of PGI₂ activity when standards containing known amounts of $PGI₂$ were treated in an identical manner.

The recovery of PGI₂-like activity in the bioassay was measured by adding known amounts of PGI₂ to either Eagles' medium or Eagles' containing a vessel wall segment. In six experiments, the level of $PGI₂$ -like activity for the vessel wall alone was 137 ± 37 pg/100 μ l Eagles' (mean \pm SEM); for the vessel wall plus the PGI₂, 723 \pm 50 pg/100 μ l Eagles'; and for the PGI₂ alone, 666 ± 70 pg/100 μ l Eagles', giving a recovery rate of 87±3%.

The PGI₂-like activity had the following characteristics. Its activity was abolished at acid pH or after boiling, it rapidly decayed at neutral pH over time, it was totally inhibited by exposure of the vessel to ASA in vitro, it was inhibited by at least 90% after the animal was treated with ASA, and there was a similar pattern of inhibition of 6-keto- $PGF_{1\alpha}$ formation in animals treated with ASA. However, in these in vivo experiments there was no measurable 6-keto- $PGF_{1\alpha}$ for up to 6 h after ASA treatment even though there were measurable (although markedly reduced levels of PGI₂-like activity by the bioassay. Because of its increased sensitivity, the bioassay was used throughout these experiments. Because of its stability and recovery characteristics, its activity is referred to as PGI₂.

Experimental design. Experiments were performed on rabbits either 1, 3, 6, or 20 h after treatment with 0, 3, 10, or 100 mg/kg of ASA given intravenously. The ASA (acetylsalicyclic acid, Sigma Chemical Co., St. Louis, Mo.) was solubilized by first adding the crystals to water and then slowly adding sodium carbonate until the crystals dissolved. During this procedure, the pH remained below 7.0, so hydrolysis of the ASA was minimized. The ASA solutions were made up in concentrations of 3, 10, and 100 mg/ml (16.5, 55, and 550 mM, respectively).

The data were analysed using analysis of variance and multiple comparison procedures. Preliminary analysis of the data obtained in the animals treated with increasing doses of ASA showed that the standard deviation increased as the mean platelet accumulation increased, and hence a logarithmic transformation was performed on all data, to meet standard statistical requirements.

RESULTS

Electron microscopic examination of injured carotid arteries. Examination of the carotid artery by scanning electron microscopy ¹ h after the stasis/injury showed that the endothelial surface was covered by a fine mesh of fibrin to which platelets adhered (Fig. 1). A control vessel is shown for comparison. The quantity of fibrin accreted onto the injured vessels did not differ between control animals and those given 100 mg/kg ASA 1, 6, or 20 h beforehand (Table I). In contrast, the number of platelets accumulated onto the injured carotid arteries in the ASA-treated rabbits was significantly increased, as is indicated by the measurement of 51Cr-platelet accumulation (see Tables ^I and II; Figs. 2 and 3).

FIGURE ¹ A scanning electron micrograph of ^a carotid injury ¹ h after restoration of flow. The endothelial surface is covered with a mesh of fibrin to which numerous platelets and platelet aggregates have adhered (right side). A control vessel is shown for comparison (left side).

Effect ofASA on platelet accumulation onto injured carotid arteries. The observed platelet accumulation onto the injured arteries in each of the four treatment groups is shown in Fig. 2. An analysis of variance of the log platelet accumulations in the three ASA-treated groups revealed no significant changes with time. Therefore, the results over time within each ASAtreated group were averaged. These mean values, as well as that for the control group, are shown in Table II.

The observed differences among the means for the four groups were statistically significant $(P < 0.001)$. Using an appropriate multiple comparison procedure

TABLE ^I Mean 125I-Fibrin Accretion and Mean 51Cr-Platelet Accumulation onto Injured Carotid Arteries in Rabbits Given 0 or 100 mg/kg ASA i.v. either 1, 6, or 20 h Beforehand

		ASA treatment		
	Control	1 h	6 h	20 _h
¹²⁵ I-Fibrin accretion, μ g/10 mm ² vessel wall	$56 + 6$	$52 + 6$	$66 + 7$	$65 + 7$
⁵¹ Cr-Platelet accumulation, $\times 10^6$ /mm ² vessel wall	1.00 ± 0.1	2.10 ± 0.25	2.51 ± 0.21	1.92 ± 0.23

There was no significant difference in fibrin accretion between control and ASA-treated animals. In contrast, platelet accumulation was significantly enhanced $(P < 0.002)$ in all ASA-treated groups.

TABLE II Mean Platelet Accumulation onto Injured Carotid Arteries of ASA-treated Rabbits

ASA treatment	Mean platelet accumulation	
mg/kg	$\times 10^{6}$ /10 mm ²	
0	0.84	
3	0.79	
10	1.24	
100	1.32	

There was no effect of ASA on platelet accumulation with time; therefore, all of the data obtained at each time interval for each treatment group were pooled and analyzed as four treatment groups. The data were transformed to log values for data handling. The table represents the antilogs of the mean.

(Newman-Keuls), it was shown that there was no significant difference between 0 (control) and 3 mg/kg ASA, or between 10 and 100 mg/kg ASA. However, there was a highly significant difference between 10 and 100 mg/kg ASA, compared with the control and 3 mg/kg groups $(P < 0.01)$.

Correlation between inhibition of $PGI₂$ production and enhanced platelet accumulation. To determine whether there was a correlation between inhibition of $PGI₂$ production and enhanced thrombogenesis, $PGI₂$ production was measured in one carotid artery, and platelet accumulation onto an injured vessel was

measured in the other carotid artery of the same animal. The measurements were determined from carotid arteries obtained from rabbits either 3 or 20 h after treatment with 0, 5, or ¹⁰ mg/kg of ASA. There was ^a significant negative correlation between $PGI₂$ production by the normal vessel wall and the log platelet accumulation onto the injured vessel wall on the opposite side. The correlations between PGI₂ production and log platelet accumulation in the 0, 5, and 10 mg/kg ASA treatment groups were $r = -0.66$, $n = 30$, $P < 0.01$; $r = -0.61$, $n = 27$, $P < 0.01$; and $r = -0.39$, $n = 32$, $P < 0.05$, respectively. There was no evidence that this relationship differed among these three groups. Therefore, all the data were combined and are plotted in Fig. 3. The overall correlation coefficient was $r = -0.55$, $n = 89, P < 0.01.$

DISCUSSION

ASA has been reported to be an effective antithrombotic agent in a number of clinical trials (4, 12-14). It has been suggested that the effectiveness of this drug might be reduced if the dosage regimen used also inhibits PGI₂ synthesis by vascular wall cells (15-16). Differences have been reported in the inhibitory effect of ASA on prostaglandin synthesis in platelets and vascular wall cells. Baenzinger et al. (17) reported that the concentration of ASA required in vitro to inhibit platelet cyclooxygenase is at least 1/25 of the concentration required to inhibit prostaglandin

ASPIRIN DOSE (mg/kg) AND TIME AFTER TREATMENT

FIGURE 2 Log platelet accumulation (mean \pm SEM) onto injured carotid arteries in rabbits that were given 3, 10, or 100 mg ASA/kg i.v. either 0, 1, 3,6, or 20 h beforehand. Control animals injected with an equivolume of suspending vehicle were included in each time-frame. Since there was no difference between control groups, these data were pooled and are shown as one control group in this figure. Each solid dot represents the mean value ofboth carotid arteries in any given animal.

FIGURE 3 Log platelet accumulation onto injured vessel wall plotted against PGI₂ production by vessel wall in the same material. Platelet accumulation was measured on one injured carotid artery and PGI2 production was measured in the other uninjured carotid artery in the same animal. Vessels were obtained from control animals (\bullet) and from animals given 5 (O) or 10 mg/kg ASA (\Box) either 3 or 20 h beforehand. There was a significant negative correlation between PGI₂ production and log platelet accumulation in the control and 5 or 10 mg/kg ASA-treated animals $(r = -0.66, P < 0.01; r = -0.61, P < 0.01,$ and $r = -0.39, P < 0.05$, respectively).

synthesis in human fibroblasts and arterial smooth muscle cells. These and other investigators have also demonstrated that the effect of ASA on prostaglandin synthesis lasts for the life span of the platelet (3). In contrast, Jaffe and Weksler (6) reported that prostaglandin synthesis by cultured endothelial cells is inhibited by concentrations of ASA similar to those that inhibit platelet prostaglandin synthesis, but that the effect on vascular wall cells is rapidly reversed after ASA is removed. Kelton et al. (9) reported that very high doses of ASA are required to enhance 1251-fibrin accretion onto injured rabbit jugular veins and that this thrombogenic effect of ASA is lost within 21/2 h of ASA treatment (9), supporting the suggestion that, compared with its effect on platelets, the inhibitory effect of ASA on vessel wall PGI₂ synthesis is short lived.

We have previously reported that the inhibitory effect of ASA on $PGI₂$ synthesis is more prolonged in arteries than in veins (7). We now report that ASA, in a dose of 10 mg/kg i.v., inhibits $PGI₂$ production in carotid arteries and that this is associated with an increased accumulation of platelets onto injured rabbit carotid arteries, an effect that lasts for at least 20 h. A significant correlation was found between $PGI₂$ production and platelet accumulation in not only the ASA-treated animals but also in the control animals. The results with control animals suggest that there may be an association between $PGI₂$ production and platelet accumulation onto the injured vessel wall, but do not provide conclusive proof of a causal relationship.

A number of investigators have reported that $PGI₂$ does not influence platelet adhesion onto vascular wall cells (18-20). Curwen et al. (19) reported that the adhesion of platelets to cultured endothelial cells in vitro was not increased when $PGI₂$ production was inhibited by ASA (19). Dejana et al. (20) reported that ASA treatment did not influence the adherence of 5'Cr-platelets to ballooned or undamaged aortae in rabbits. In contrast, Czervionke et al. (5) found that the exposure of cultured endothelial cells to ASA increased the adhesion of thrombin-induced platelet aggregates to the cells. In our experiments, ASA increased the adhesion of platelets to damaged vessel walls. Electron microscope examination revealed that the underlying endothelial surface was intact and that the platelets adhered to a fibrin mesh that covered the endothelial cells. It is likely, therefore, that thrombin was generated during stasis, which resulted in both fibrin formation (Fig. 1) and $PGI₂$ production (21), and that treatment with ASA enhanced platelet adhesion to the fibrin mesh by inhibiting $PGI₂$ production. These findings suggest that $PGI₂$ may be particularly important in contributing to the thromboresistance of the intact vessel wall when it is covered with fibrin and that ASA may enhance thrombosis under these conditions by inhibiting PGI₂ synthesis.

Although recommendations about the therapeutic

use of ASA can only be derived from results of clinical trials, our results raise the possibility that the margin of safety between obtaining an antithrombotic effect of ASA and producing ^a potential thrombogenic effect may not be as large as indicated by studies using cultured endothelial cells or injured jugular veins. The findings therefore support the suggestion that, when administered as an antithrombotic agent, ASA should be used in the lowest daily dose required to inhibit platelet TxA_2 production $(4, 17)$.

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REFERENCES

- 1. Gryglewski, R. J., S. Bunting, S. Moncada, R. J. Flower, and J. R. Vane. 1976. Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins. 12: 685-709.
- 2. Marcus, A. J. 1978. The role of lipid in platelet function with particular reference to the arachidonic acid pathway. J. Lipid Res. 19: 793-826.
- 3. Roth, C. J., and P. W. Majerus. 1975. The mechanism of the effect of aspirin on human platelets. Acetylation of a particulate fraction protein. J. Clin. Invest. 56: 624-632.
- 4. Harter, H. R., J. W. Burch, P. W. Majerus, N. Stanford, J. A. Delmez, C. B. Anderson, and C. A. Weerts. 1979. Prevention of thrombosis in patients on hemodialysis by low-dose aspirin. N. Engl. J. Med. 301: 577-579.
- 5. Czervionke, R. L., J. C. Hoak, and G. L. Fry. 1978. Effect of aspirin on thrombin-induced adherence of platelets to cultured cells from the blood vessel wall. J. Clin. Invest. 62: 847-856.
- Jaffe, E. A. and B. B. Weksler. 1979. Recovery of endothelial cell prostacyclin production after inhibition by low doses of aspirin. J. Clin. Invest. 63: 532-535.
- 7. Buchanan, M. R., E. Dejana, J.-P. Cazenave, J. F. Mustard, and J. Hirsh. 1979. $PGI₂$ production and effect of aspirin on endothelial cells and non-endothelial cells of the vessel wall. Thromb. Haemostasis. 42: 156.
- 8. Buchanan, M. R., M. A. Blajchman, E. Dejana, J. F. Mustard, A. F. Senyi, and J. Hirsh. 1979. Shortening of

the bleeding time in thrombocytopenic rabbits after exposure of jugular vein to high aspirin concentrations. Prostaglandin Med. 3: 333-342.

- 9. Kelton, J. G., J. Hirsh, C. J. Carter, and M. R. Buchanan. 1978. Thrombogenic effect ofhigh-dose aspirin in rabbits. Relationship to inhibition of vessel wall prostaglandin I_2 -like activity. J. Clin. Invest. 62: 892-895.
- 10. Czervionke, R. L., J. B. Smith, J. C. Hoak, G. L. Fry, and D. L. Haycroft. 1979. Use of a radioimmunoassay to study thrombin-induced release of PGI₂ from cultured endothelium. Thromb. Res. 14: 781-786.
- 11. Buchanan, M. R., E. Dejana, J.-P. Cazenave, J. F. Mustard, and J. Hirsh. 1979. Uncontrolled PGI₂ production by whole vessel wall segments due to thrombin generation in vivo and its prevention by heparin. Thromb. Res. 16: 551-555.
- 12. Fields, W. S., N. A. Lemak, R. F. Frankowski, and R. J. Hardy. 1977. Controlled trial of aspirin in cerebral ischemia. Stroke. 8: 301-316.
- 13. Harris, W. H., E. W. Salzman, C. A. Athanasoulis, A. C. Waltman, and R. S. DeSanctis. 1977. Aspirin prophylaxis of venous thromboembolism after total hip replacement. N. Engl. J. Med. 297: 1246-1249.
- 14. Canadian Cooperative Study Group. 1978. A randomized trial of aspirin and sulfinpyrazone in threatened stroke. N. Engl. J. Med. 299: 53-59.
- 15. O'Grady, J. and S. Moncada. 1978. Aspirin: a paradoxical effect on bleeding time. Lancet. II: 780-781.
- 16. Marcus, A. J. 1977. Aspirin and thromboembolism. A possible dilemma. N. Engl. J. Med. 297: 1284-1285.
- 17. Baenziger, N. L., M. J. Dillender, and P. W. Majerus. 1977. Cultured human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. Biochem. Biophys. Res. Commun. 78: 294-301.
- 18. Cazenave, J.-P., E. Dejana, R. L. Kinlough-Rathbone, M. A. Packham, and J. F. Mustard. 1979. Platelet interactions with the endothelium and the subendothelium: the role of thrombin and prostacyclin. Haemostasis. 8: 183-192.
- 19. Curwen, K. D., M. A. Gimbrone, and R. I. Hardin. 1980. In vitro studies of thromboresistance: the role of prostacyclin (PGI2) in platelet adhesion to cultured normal and virally transformed human vascular endothelial cells. Lab. Invest. 42: 366-374.
- 20. Dejana, E., J-P. Cazenave, H. M. Groves, R. L. Kinlough-Rathbone, M. Richardson, M. A. Packham, and J. F. Mustard. 1980. The effect of aspirin inhibition of $PGI₂$ production on platelet adherence to normal and damaged rabbit aortea. Thromb. Res. 17: 453-464.
- 21. Weksler, B. B., C. W. Ley, and E. A. Jaffe. 1978. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A 23187. J. Clin. Invest. 62: 923-930.