Effect of Aspirin and Salicylate on Platelet-Vessel Wall Interactions in Rabbits

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We performed studies to examine the mechanism responsible for the antithrombogenic effect of aspirin in experimental animals. We measured the effect of 10 and 100 mg/kg doses of aspirin, salicylate, or a combination of both on the accumulation of radio labeled platelets onto injured carotid arteries in rabbits. The effects of these agents on thrombogenicity measured as platelet accumulation onto injured carotid arteries, were correlated with the ability to inhibit platelet thromboxane A$_2$ and vessel wall prostacyclin synthesis. We found that a low dose of aspirin significantly inhibited platelet accumulation onto the injured vessels, while a high dose reversed this effect. Thromboxane A$_2$ and prostacyclin production, however, were maximally inhibited in rabbits given either aspirin dose. The reversal of the antithrombotic effect of the low dose of aspirin by a high dose of aspirin was simulated by administering a combination dose of high-dose salicylate and low-dose aspirin. We concluded that the reversal of the antithrombotic effect of a 10 mg/kg dose of aspirin by a higher dose of aspirin could not be explained solely by the inhibition of PGI$_2$ synthesis and was affected by the salicylate moiety of aspirin. (Arteriosclerosis 4:403-406, July/August 1984)

We reported that aspirin in doses of 1 to 10 mg/kg inhibits thrombus formation, whereas higher doses enhance thrombus formation and accelerate hemostasis in a number of experimental animal models. The thrombogenic effect of aspirin was attributed to the inhibition of PGI$_2$ synthesis. However, we noted that aspirin retained its antithrombotic effect even at doses that inhibited PGI$_2$ synthesis, and that much higher doses were required to produce a thrombogenic effect. This disparity between the antithrombotic effect of aspirin and the dose required to inhibit PGI$_2$ synthesis prompted us to investigate again the mechanisms by which aspirin is antithrombotic, and in particular, to test the possibility that the loss of this effect is through mechanisms other than the inhibition of PGI$_2$ production.

Methods

Arterial Injury Model

Accumulation of $^{51}$Cr-labeled platelets onto injured rabbit carotid arteries was determined using a modification of our injury model previously described. Briefly, rabbits were injected with homologous $^{15}$Cr-labeled platelets. After 24 hours, each animal was anesthetized with sodium pentobarbital, and both carotid arteries were isolated. The blood was emptied from the vessel, and two clamps were applied 2 cm apart onto each artery. After 10 minutes, the clamps were removed, and blood flow was restored. Then 1 hour later, a 5-ml citrated blood sample was collected, and each rabbit was 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 the glass tube containing 1.5 ml of saline. The vessel wall radioactivity was determined in a gamma counter. The vessel was removed from the tube, slit longitudinally, and laid flat with the endothelial side down on a transparent film of acetate, and was photocopied. The surface area of the vessel wall was determined by cutting out the photocopy, weighing it, and comparing it with the weight of an adjacent 1 cm$^2$ cut from the same piece of paper.

In the interim, platelet-rich plasma was prepared from the citrated blood collected before removing the injured vessel walls and both platelet count and platelet specific activity were determined. The radioactivity of the vessel wall was then expressed as platelets/mm$^2$ of vessel wall surface.

In other experiments, both uninjured and injured carotid artery segments were prepared from the same animals for scanning electron microscopic examination with standard techniques. Each vessel segment was fixed in 2% glutaraldehyde, postfixed in 1% aqueous osmium tetroxide, and dehydrated in a series of graded ethanol (50% to 95%). After dehydration, the vessels were critical-point dried.
with CO₂, coated with gold/platinum (200A), and viewed in a Philips scanning electron microscope (PSEM Model 501B).

**Experimental Design**

We injected 42 rabbits (male and female New Zealand White, 2.1 kg to 3.6 kg) via the marginal ear vein with 0 (suspending vehicle), 10 or 100 mg/kg of aspirin, 10 mg/kg of aspirin followed 1 hour later with 100 mg/kg sodium salicylate (salicylate), or 100 mg/kg of salicylate alone. All treatments were administered in 1 ml/kg equivalents. After 2 hours, both carotid arteries were injured as described above. Then, 1 hour later, the injured carotid artery segments were removed and the number of platelets adherent to the injured surface was determined. At the same time, citrated blood samples were collected to determine thromboxane A₂ (TxA₂) production.

Another group of rabbits were given similar treatments; 2 hours later the carotid arteries were removed and their PGI₂ activity was determined.

**Determination of 6-Keto-PGF₁α**

PGI₂ activity was determined by measuring a stable endproduct, 6-keto-PGF₁α. Purified 6-keto-PGF₁α was purchased from Upjohn Company (Kalamazoo, Michigan), and the specific antibody to 6-keto-PGF₁α was a gift from Dr. Edward Rorsman (Upjohn Company, Kalamazoo, Michigan). The antibody was purchased from Seragen Company, Boston, Massachusetts. The assay was done by means of a double-antibody technique.

**Scanning Electron Microscopy**

The luminal surface of injured carotid arteries was markedly abnormal 2 hours after the stasis- and anoxia-induced injury. The surface was partially denuded, and in several places the endothelial cells were lifted off the basement membrane (Figure 1 A). Platelets and platelet aggregates adhered both to the partially detached endothelial cells and to the underlying basement membrane. Figure 1 B shows a control vessel for comparison.

**Effect of Aspirin and Salicylate on Platelet Accumulation**

Approximately 2.44 x 10⁶ platelets adhered to a 10-mm² area of the vessel wall of the injured carotid arteries in untreated animals. This represented the thrombogenicity of the injured vessels (Figure 2 A). A dose of 10 mg/kg of aspirin significantly inhibited platelet accumulation (p < 0.01), whereas the higher aspirin dose of 100 mg/kg reversed this effect. Similarly, treatment with a combination of salicylate and low-dose aspirin reversed the inhibitory effect on platelet accumulation achieved with the low dose of aspirin alone. Salicylate alone had no effect.
Vessel wall 6-keto-PGF₁₀ production was also maximally inhibited in all three aspirin-treated groups of rabbits (Figure 2 B). In contrast, the 6-keto-PGF₁₀ production was elevated 40% in rabbits given salicylate alone \( (p < 0.05) \).

**Discussion**

We demonstrated that aspirin in a dose that inhibits both TxB₂ and PGI₂ synthesis is antithrombotic in an experimentally injured vessel wall model and that this antithrombotic effect is reversed either by increasing the dose of aspirin or by adding a high dose of salicylate to the antithrombotic aspirin dose. These observations suggest that the reversal of the antithrombotic effect of a low dose of aspirin by increasing the aspirin dose is not solely due to the inhibition of PGI₂ and that this reversal is contributed to by an effect of the salicylate moiety on platelet-vessel wall interactions.

![Figure 1](image1.png)

**Figure 1.** Scanning electron micrograph of the luminal surface of an injured (A) and an uninjured (B) carotid artery. BM = basement membrane; P = platelets; EC = endothelial cells. \( \times 1000 \).

![Figure 2](image2.png)

**Figure 2.** Effect of aspirin and salicylate on (A) \(^{51}\)Cr-platelet accumulation onto injured carotid arteries and (B) 6-keto-PGF₁₀ production in rabbits given 0 (none), 10 mg/kg aspirin (LO aspirin), 100 mg/kg aspirin (HI aspirin), 10 mg/kg aspirin plus 100 mg/kg salicylate (ASA/SAL), or 100 mg/kg salicylate alone (HI salicylate). Data are expressed as means ± SEM; \( n = 8 \).
The mechanism by which salicylate influences the effect of aspirin on platelet-vessel wall interactions is unknown, but there are a number of possibilities. Cerletti et al.9 and Dejana et al.9 have reported that salicylate inhibits the acetylation of platelet cyclooxygenase by aspirin and therefore interferes with the inhibition of TxA2 production. However, this does not explain our observations since salicylate was given 1 hour after the aspirin treatment and both TxA2 and PGI2 production were maximally inhibited even in the group of animals given aspirin plus salicylate. Salicylate reportedly10 affects platelet function, but this effect is to inhibit platelet aggregation, so this would not explain the loss of antithrombotic effect in the rabbits treated with a low dose of aspirin and a high dose of salicylate. Therefore, it is more likely that salicylate in high doses acts on the vessel wall through an unknown mechanism independent of the cyclooxygenase pathway and the inhibition of PGI2 synthesis. Lipoxygenase metabolites have reportedly4 been produced by rabbit vessel wall segments, and salicylate in high concentrations has been shown12 to inhibit the production of lipoxygenase metabolites in platelets.

More recently, we reported13 that human endothelial cells in culture produce a lipoxygenase metabolite that inhibits platelet-endothelial cell interactions; salicylate inhibits the production of this metabolite by endothelial cells and reverses its effect on platelet-endothelial cell interactions. It is therefore possible that the observed effect of salicylate on reversing the antithrombotic effect of aspirin is caused by this mechanism. Our observations of an increase in 6-keto-PGF1α levels in animals treated with salicylate alone is consistent with an inhibitory effect of salicylate on the lipoxygenase pathway and the shunting of excess arachidonic acid to the cyclooxygenase pathway. A similar shunting mechanism from the lipoxigenase to the cyclooxygenase pathway has been reported in platelets.14,15 These observations do not, however, exclude the possibility that salicylate has other effects independent of an effect on arachidonic acid metabolism, although this possibility seems unlikely.

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