Effect of Aspirin on Experimentally Induced Arterial Thrombosis During the Healing Phase

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Abstract:

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Twenty dogs were treated with either acetylsalicylic acid or a lactose placebo for 5.5 ± 2.3 days before surgical or chemical injury to the carotid and femoral arteries and for the following 34.5 days. Only the laboratory diener had knowledge of the random table used to select type of treatment until after all determinations had been completed. Following sacrifice the arteries were classified for the presence of intimal proliferation, defects in the internal elastica, presence of organized thrombi and the percentage of recanalization, and the presence of fresh thrombi and the percentage of occlusion. Thrombi were present in 8% of the arteries of dogs treated with acetylsalicylic acid and in 36% of those treated with placebo. This difference is significant (P < 0.01). The degree of intimal proliferation and defects in the internal elastica were not significantly different between the two groups. We conclude that in dogs acetylsalicylic acid therapy during the healing phase following arterial injury protects against thrombosis and does not retard the healing process.

Additional Key Words: acetylsalicylic acid, platelet aggregation inhibitors, adenine diphosphate, intimal repair, arterial injury, thrombosis, platelet-fibrin emboli, transient ischemic attacks.

Preliminary studies in humans suggest that transient ischemic attacks in the distribution of the internal carotid arteries may be decreased by antiplatelet aggregation medication.1-5 Acute studies have established that acetylsalicylic acid (aspirin) and other platelet aggregation inhibitors reduce the incidence of stenosis or occlusion in arteries of dogs following surgical or chemical injury.6-8 To the authors' knowledge all animal arterial determinations have been made four to 48 hours after injury. These studies would be clinically applicable only if the drug could be used safely over a long period of time and during this time continue to protect the artery from thrombosis and not interfere with the normal repair processes. Therefore, the present experiment was designed to compare the response to arterial injury between dogs treated with acetylsalicylic acid and those treated with placebo (lactose) over a time period of at least one month.

Methods

Mongrel dogs were selected by a semirandom table designed so that 20 dogs would be equally divided between those treated with aspirin and those treated with placebo (lactose). The weight ranged from 8 to 14.5 kilograms with a mean of 10.4 ± 1.7. The random table was assigned to the laboratory diener with bottles of identical Lilly pulvule capsules containing 325 mg of acetylsalicylic acid or 325 mg of powdered lactose. Only the diener had access to the code until after the study was completed. Following the table each dog was given a code number and one capsule was administered with a special applicator orally each day for at least three days and an average of 5.5 ± 2.3 days before the injury to the arteries.

At the time of the surgical procedure, the dogs were anesthetized with intravenous sodium pentobarbital and intubated. Both common carotid and both femoral arteries were exposed. The left carotid and femoral arteries were clamped with disposable clip clamps and a 2-cm arteriotomy made. With magnification the intima was then stripped off. The arteriotomy site was then closed with 6-0 arterial silk and oozing controlled by gel foam. On the arteries on the right a 2-cm segment was isolated by clip clamps. The blood was aspirated from the isolated segments and 2 ml of 0.1 N H₂SO₄ barbitaged intraluminally with a 25-gauge needle over a two-minute period. Following this the segment was flushed for two minutes with sterile water and the clip clamps then removed.
Following the surgical procedure a capsule was administered each day until sacrifice. During this time using a spectrophotometric method at least one plasma salicylate level was obtained on all but one dog as an additional check that proper medication had been given. No investigator involved with performing or evaluating the study was permitted access to these data. The dogs were sacrificed from 29 to 42 days following surgery. The median was 35 and the mean 34.5 days.

On the day of sacrifice the dogs were anesthetized with intravenous sodium pentobarbital and all four arteries again exposed. Each artery was transected distal to the site of injury and the absence or presence of bleeding from the proximal end was recorded. The animal was then sacrificed using sodium pentobarbital. The arteries were removed and placed in 10% formalin for fixation.

After proper fixation, blocks were taken at a right angle to the long dimension of the artery at the site of visible surgery or prior manipulation and imbedded in paraffin. Cross-sections were routinely stained with hematoxylin and eosin and microscopic descriptions given only under the code number. Before the code was broken, a second investigator rigidly classified the changes in each artery. Arteries were classified for (1) the presence of intimal proliferation (1+ = minimal, 2+ = moderate, and 3+ = severe), (2) defects in internal elastica (0 = none, 1+ = minimal, 2+ = moderate, and 3+ = severe), (3) presence of organized thrombus and the percentage of recanalization, and (4) the presence of fresh thrombus and the percentage of occlusion. After all final decisions had been made, the code was broken and comparisons were made between the two treatment groups.

After the code was broken, comparison of salicylate levels revealed that one dog assigned to the placebo group had levels of 6.4 and 1.4 mg/100 ml on two occasions. The upper limit for animals not on salicylates is 2.0 mg/100 ml. Therefore, this dog was discarded from analysis.

**Results**

After the code was broken and the one dog with an elevated salicylate level discarded, ten dogs (40 arteries) were in the treated group and nine (36 arteries) in the control. None of the control dogs had a level above 2.0 mg/100 ml and the mean of the eight with technically satisfactory studies was 0.6 ± 0.7. The ten in the treated group had a mean plasma salicylate level of 9.5 ± 3.2 mg/100 ml.

The mean time from injury to sacrifice in the treated group was 34.6 ± 4.5 days and of the controls 34.4 ± 3.7 days.

Tables 1 and 2 summarize the results. Table 1 demonstrates that only three of 38 arteries injured by 0.1 N H₂SO₄ (right carotid and femoral arteries) had thrombi, and these were all in the control group. Thirteen of the 38 arteries injured by endarterectomy (left carotid and femoral arteries) had thrombi and ten of these were in the placebo group.
Only three (8%) of the 40 arteries of dogs who had received acetylsalicylic acid had thrombosis compared to 13 (36%) of the 36 arteries from the placebo group. A Chi square analysis reveals that this difference is significant (P less than 0.01).

Intimal proliferation and defects in the internal elastica were compared between the groups for the arteries without thrombi (table 2). Intimal proliferation was present in 25 (68%) of 37 arteries in the acetylsalicylic acid treated group versus 11 (49%) of the placebo group. This is not significantly different. Defects in the internal elastica were identical (11 or 30% of the 37 treated versus seven or 30% of the 23 in the placebo group).

Discussion and Conclusions
The initial factor in arterial thrombus formation appears to be platelet adhesion to collagen exposed by damage to the intima. Adhesion of platelets associated with release of substances, including adenosine diphosphate (ADP), which causes rounding, aggregation and consequent release reaction in other platelets in a progressive chain reaction. The platelet aggregation is followed by deposition of fibrin in and around the original platelet mass with possible microembolization of platelet-fibrin emboli. Aspirin prevents the release reactions.10-11

Clinical studies suggest that aspirin does not have significant effects on prevention of postoperative venous thrombosis.12

The previous observation by others in acute studies6-8 that acetylsalicylic acid protects the injured artery from thrombosis is supported by our chronic study. We have established that long-term aspirin therapy in addition to protecting against arterial thrombosis does not adversely affect intimal repair. When arteries without thrombi were compared, the degree of intimal proliferation and defects in the internal elastica were not significantly different between the treated and the control groups.

We conclude that in dogs acetylsalicylic acid therapy during the healing phase following arterial injury protects against thrombosis and does not retard the healing process.

References
Thrombosis During the Healing Phase
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