Effect of Aspirin and Sodium Salicylate on Thrombosis, Fibrinolysis, Prothrombin Time, and Platelet Survival in Rabbits With Indwelling Aortic Catheters

By M. Cattaneo, A. Chahil, D. Somers, R. L. Kinlough-Rathbone, M. A. Packham, and J. F. Mustard

We have studied the effect of different doses of aspirin on platelet function, PGI₂ formation, platelet survival, thrombosis, fibrinolysis, and prothrombin time in rabbits with indwelling aortic catheters. The thrombi formed around indwelling aortic catheters were found to have a large fibrin component, and their formation was inhibited by heparin administration. Thus, in these experiments we examined the effect of aspirin (a weak inhibitor of thrombin-mediated platelet aggregation) under conditions in which thrombin was a major factor in the initiation and growth of the thrombi. Only very high doses of aspirin tended to inhibit thrombus formation over the 5-day period of observation, and a statistically significant inhibition of thrombus formation was produced by equivalent concentrations of sodium salicylate. The failure of high doses of aspirin to achieve a significant inhibition of thrombosis under the conditions of these experiments (whereas an equivalent dose of sodium salicylate was inhibitory) could be due to aspirin inhibition of PGI₂ formation. Shortened platelet survival was not affected by aspirin treatment or the dose of sodium salicylate that inhibited thrombus formation. The tendency to inhibit thrombus formation appeared to be unrelated to an effect on platelets but was associated with prolongation of the one-stage prothrombin time and increased whole blood fibrinolytic activity; doses of aspirin that inhibited platelet aggregation in response to sodium arachidonate or collagen, and PGI₂ formation by the vessel wall, did not have a significant effect on the amount of thrombus present at 5 days. However, the high doses of aspirin that inhibited PGI₂ formation were associated with a tendency to increased thrombus formation during the first 3 hr after insertion of the catheter. The results of these experiments show that when thrombin is an important factor in the formation of thrombi, aspirin is a weak inhibitor of thrombosis unless doses are used that provide sufficient salicylate to interfere with blood coagulation and promote whole blood fibrinolytic activity. These results also show that thrombus formation can be inhibited without an apparent change in platelet survival.

SOME INVESTIGATORS have reported inhibition of thrombosis in experimental animals receiving aspirin, whereas in other experiments, aspirin has been found to have little inhibitory effect on thrombosis when a vessel wall is severely injured.1,9 Since several pathways may be involved in the initiation and growth of thrombi,4 aspirin may only be inhibitory if the generation of thromboxane A₂ plays a major part in thrombus formation. In circumstances in which thrombin makes a significant contribution to thrombosis, aspirin would not be expected to be inhibitory because this drug has little effect on thrombin-induced platelet aggregation and release,10 and does not influence fibrin formation unless sufficiently high doses are used to inhibit synthesis of vitamin K-dependent clotting factors. In addition, doses of aspirin that inhibit PGI₂ formation may promote thrombosis under some conditions.11,12

Shortened platelet survival is thought to be associated with vessel wall injury and thrombosis.13-15 The observation that aspirin inhibits some forms of thrombosis but is without effect on platelet survival14 indicates either that there may not be a direct relationship between thrombosis and platelet survival or that the type of thrombi that are associated with shortened platelet survival are not influenced by aspirin.

We have found that neither removal of the endothelium from a rabbit aorta with a balloon catheter (which results in a monolayer of platelets on the subendothelium) nor a single balloon-catheter-induced injury to the neointima (which is associated with the formation of platelet-fibrin thrombi) shorten platelet survival.16,17 However, continuous injury of rabbit aorta with an indwelling aortic catheter causes the formation of large platelet-fibrin thrombi, and platelet survival is reduced.18 Thus, although a single injury cannot be used to study the relation among vessel injury, thrombosis, platelet survival, and the effects of aspirin, continuous injury provides an experimental approach for such a study. We have caused continuous injury with an indwelling catheter in the rabbit aorta to examine whether there are doses of aspirin that lessen the size of thrombi that have a large fibrin component, and the effect of such doses of aspirin on platelet survival.

From the Department of Pathology, McMaster University, Hamilton, Ontario, and the Department of Biochemistry, University of Toronto, Canada.

Supported in part by Grants-in-Aid from the Medical Research Council of Canada (MT 1309 and MT 2629) and the Ontario Heart Foundation. R. L. K.-R. was in receipt of a Research Associateship of the Ontario Heart Foundation. D. S. is the recipient of a Medical Research Council of Canada Studentship.

Submitted June 11, 1982; accepted September 14, 1982.

Address reprint requests to Dr. R. L. Kinlough-Rathbone, Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

© 1983 by Grune & Stratton, Inc.

0006-4971/83/6102-0021$01.00/0
MATERIALS AND METHODS

Animals

Male New Zealand white rabbits weighing between 2.4 and 3.3 kg were used. Animals of approximately the same weight were used within an experiment.

Drugs

Aspirin (acetylsalicylic acid, Sigma Chemical Co., St. Louis, Mo., no. A-5376) was suspended in distilled water and dissolved by slow addition of a solution of Na₂CO₃. The pH of the solution was maintained below 6.8 to minimize spontaneous hydrolysis. Aspirin solutions (100 mg/ml or 10 mg/ml) were stored at −70°C and used within 15 days. Sodium salicylate (Fisher Scientific Co., Fairlawn, N.J., no. S-395) was dissolved in distilled water and stored at −20°C. Heparin (Heptalan) was purchased from Harris Laboratories, Brantford, Ontario.

Preparation of Suspensions of Washed Platelets

Suspensions of washed platelets from rabbits were prepared as previously described.²⁶ from blood collected into acid-citrate-dextrose (ACD) anticoagulant.²⁶

The platelets were labeled in the first washing solution for 60 min at room temperature with Na₂¹⁵⁶CrO₄ (Amersham/Searle, Arlington Heights, Ill., 200-500 μCi/μg of chromium; 150 μCi of ¹⁵⁶Cr were used to label platelets obtained from the blood of each rabbit). The platelets were then washed once in calcium-free Tyrode solution containing 0.35% bovine serum albumin (Pentex, Fraction V, Miles Laboratories, Elkhart, Ind.) and resuspended in platelet-poor plasma.

In the studies of PGI₂-like activity, platelets were prelabeled with ¹⁴C-serotonin as 5-hydroxytryptamine-³⁻¹⁴C-creatinine sulfate (Amersham/Searle, 50 μCi/μmol of serotonin); 1-2 μCi was used to label platelets obtained from one rabbit.

Insertion of Indwelling Aortic Catheters

The right femoral artery of rabbits anesthetized with sodium pentobarbital (30-40 mg/kg) was isolated. A polyethylene catheter (PE 90, Clay Adams; Becton, Dickinson and Company, Parsippany, N.J.) sealed at the tip with wax was inserted via the femoral artery so that it extended 12-14 cm into the aorta and reached the aortic arch. The femoral artery was ligated with the catheter in place and the incision closed with silk sutures. In sham-operated animals, the femoral artery was exposed and ligated.

Measurement of Thrombus Weight

The animals were preanesthetized with Ketamine (Ketaset, Roga/STB, Division of BTA Products, London, Ontario; 100 mg intramuscularly) and atropine (0.1 mg subcutaneously) 20 min before they were anesthetized with sodium pentobarbital (20-30 mg/kg, intravenously). The left carotid artery and the left femoral artery were cannulated with polyethylene tubing (PE 190). The rabbits were given an injection of heparin (1000 U, intravenously) and were then perfused, via the carotid cannula, with Locke's-Ringer solution (37°C) containing heparin (1 U/ml) at a pressure of 70-100 mm Hg. When the solution draining from the femoral artery was almost clear of red blood cells, the perfusion fluid was changed to 4% paraformaldehyde at 37°C. When paraformaldehyde was detected draining from the femoral artery, the vessel was clamped and the perfusion maintained at the same pressure for 10-15 min. The carotid artery was clamped and the carass stored at 4°C for 12-18 hr. The aortae were dissected free of extraneous tissue, removed, and cut into 2.5-cm lengths. Each segment of aorta was then slit longitudinally and the thrombi associated with the catheter and injured vessel wall above the aortic bifurcation (excluding thrombus in the femoral artery) were isolated, placed in preweighed plastic dishes, and dried overnight in an oven at 37°C and weighed.

The thrombi formed primarily at two sites: at the aortic bifurcation with extension into the ligated femoral artery and in the region where the tip of the catheter contacted the vessel wall. In these studies only the thrombi in the aorta were weighed and examined.

Light Microscopy

Samples of vessel wall and thrombi fixed with 4% paraformaldehyde were dehydrated through graded ethanol, embedded in Histowax, and cut into sections of 4-μ thickness, which were stained with hematoxylin-eosin or martius yellow-scarlet red-celeste blue (MSB). Sections were examined with a Zeiss photomicroscope.

Platelet Survival

The ⁵¹Cr-labeled platelets (1.0-1.5 x 10⁶ in a volume of 5 ml) were injected 2 hr before the insertion of the indwelling catheters, and samples of blood (1.5 ml) were collected from an ear vein into 0.5 ml ACD 2, 20, 26, 44, 68, and 92 hr after the injection of the ⁵¹Cr-platelets. The 2-hr sample was taken just before insertion of the catheter. The radioactivity in the 2-hr sample was assigned a value of 100% and the radioactivity in subsequent samples was expressed as a percentage of the 2-hr value. Platelet survival was calculated using Murphy's gamma function.²¹,²²

Drug Administration

Heparin. Heparin (150 U/kg) was given intravenously immediately before the insertion of the aortic catheter. Half the initial dose was repeated every 30 min for 2 hr.

Aspirin. Aspirin was given orally through a permanent pharyngeal polyethylene cannula (PE 190) introduced at least 2 days before the beginning of the experiment. Aspirin was given at the time of injection of the labeled platelet suspension, and then repeated at intervals until the end of the experiment; doses of 10 and 100 mg/kg body weight were given every 24 hr, whereas doses of 50 and 200 mg/kg body weight were given every 8 hr. None of these doses of aspirin affected the pH of the blood.

Sodium salicylate was given orally at a dose of 177.2 mg/kg body weight (calculated to be equivalent to the salicylate in 200 mg of aspirin) at the time of injection of the labeled platelet suspension and at 8-hr intervals thereafter.

Platelet Aggregation Studies

Samples of blood were taken from the central ear artery with a plastic syringe directly into trisodium citrate anticoagulant (3.8%, 9 volumes of whole blood to 1 volume of anticoagulant). The blood was centrifuged at room temperature at 190 g for 15 min, and platelet-rich plasma (PRP) obtained. The platelet count was adjusted to 5 x 10⁶/ml with platelet-poor plasma (PPP) prepared from the same blood sample. One milliliter of PRP was stirred at 37°C for 1 min in a Payton Aggregation Module (Payton Associates, Scarborough, Ontario) and the extent of platelet aggregation measured 3 min after the addition of acid-soluble collagen²³ or sodium arachidonate (grade I, Sigma).²⁴

Measurement of PGI₂-Like Activity of Thoracic Aorta

For preparation of samples of aortae for measurement of PGI₂-like activity, rabbits were anesthetized with 20-40 mg/kg sodium pentobarbital and given an injection of heparin (300 U/kg) before
they were exsanguinated through a carotid cannula. The thoracic aortae were immediately isolated and flushed in situ with Eagle's medium (GIBCO Laboratories, Grand Island Biological Co., Grand Island, N.Y.), containing 0.35% bovine serum albumin, 5 mM Hepes buffer, pH 7.45. The aortae were removed from the animals, cleared of adventitia, and cut into 0.5-cm rings. The vessel rings were stored in Eagle's medium at 37°C for 2 hr.

After 2 hr, each ring was incubated in 150 μl Eagle's medium containing 50 μM arachidonic acid at 37°C for 3 min; 100 μl of the incubation medium was then added to 1 ml of a suspension of aspirin-treated washed rabbit platelets that had been prelabeled with 3H-platelet serotonin and resuspended in a medium containing imipramine (10 μM, Ciba-Geigy, Dorval, Quebec). The sample was stirred in a Payton Aggregation module at 37°C. Sixty seconds later, thrombin (0.05 U/ml, an amount of capable of inducing 40%–50% release from a control platelet suspension) was added to the contents of the aggregation cuvette. After 120 sec, 100-μl aliquots were taken from the sample and added to ice-cold 1.2% paraformaldehyde to block further release from the platelets. The aliquots were then centrifuged at 12,000 g for 60 sec and the amount of 4C-serotonin present in the supernatant was determined. The percentage of 4C-serotonin released from platelets by thrombin in the presence of aspirin was measured and compared with the percentage of 4C-serotonin released by thrombin in the absence of this material. The inhibitory activity was considered to be aspirin, since inhibition was completely abolished by exposure of the vessel to aspirin (5 mM) in vitro or by boiling the sample for 15 sec.

Whole Blood Fibrinolytic Activity

Whole blood fibrinolytic activity was measured using the solid-phase radiometric assay described by Moroz and Gilmore.25 125I-fibrin-coated tubes were prepared using a slight modification of the method described. Human fibrinogen (grade L, AB Kabi, Stockholm, Sweden) was treated with diisopropylfluorophosphate (DFP, Sigma) to inactivate any serine proteases and purified by passage through a DEAE-cellulose column.27 Fibrinogen was labeled with 125I (Amersham/Searle) using iodine monochloride and 0.5 ml was added to each polyethylene tube (12 x 75 mm). The tubes were shaken for 3 hr at 37°C after which the 125I-fibrinogen solution was aspirated, and 3 ml of modified Tyrode solution (calcium and magnesium omitted) containing 10 mg/ml bovine serum albumin (Sigma) was added to each tube and allowed to incubate overnight at 4°C. The Tyrode-albumin solution was then discarded and the tubes washed 3 times with modified Tyrode solution. One milliliter of Tyrode solution (containing 2 mM CaCl2) and 2 U of plasmin-free human thrombin (grade 1, Sigma) were added and incubated at 37°C for 15 min. The thrombin solution was then discarded and the tubes rinsed 3 times with modified Tyrode solution before they were filled with modified Tyrode solution containing 0.1% sodium azide and stored at 4°C until required for an assay. Approximately 2 μg of fibrinogen (fibrin) became associated with the wall of the tube.

The assay for whole blood fibrinolytic activity is based on release of radioactivity from the 125I-fibrin bound to the polyethylene tubes. One milliliter of whole blood, collected in heparin (10 U/ml), was added to each tube and allowed to incubate at 37°C for 60 min, after which an excess of modified Tyrode solution (2 ml) containing 10 U/ml heparin was added, the tube contents transferred to a polyethylene tube, and the radioactivity in the solution counted in a gamma scintillation counter.

Prothrombin Time and Thrombin Time

These clotting assays were performed on plasma from blood samples collected in trisodium citrate anticoagulant (3.8%, 9 parts blood and 1 part anticoagulant) according to standard techniques.29 The thromboplastin reagent for prothrombin time determinations was purchased from the National Reference Laboratory for Anticoagulant Reagents and Control, Withington Hospital, Manchester, U.K. For thrombin time determinations, Pentex bovine thrombin was used (Miles Laboratories Inc., Elkhart, Ind.).

Plasma Salicylate Levels

Plasma salicylate levels were determined by the method described by Trinder.30

Statistical Methods

Because of the multiple comparisons used in these experiments and because the studies were designed to determine the effects of high doses of aspirin, the statistical analysis for platelet survival and thrombus weights was performed using orthogonal contrasts.19

RESULTS

In preliminary experiments to determine whether thrombin might be an important factor in the initiation and growth of the thrombi that formed on and around the catheter, we examined the effects of administering heparin during the early period following introduction of the catheter on the extent of thrombus formation. High doses of heparin (150 U/kg initially followed by 75 U/kg at half-hour intervals over a 2-hr period) produced about a 90% reduction in the amount of thrombus associated with the catheter at 2 hr (thrombus weight: heparin-treated, 0.90 ± 0.2 mg; control, 9.3 ± 4.05 mg; mean ± SEM for 3 rabbits in each group). Thus, thrombin generation contributes to thrombus formation caused by the indwelling aortic catheters.

The thrombi that formed on and around the indwelling aortic catheters reached maximum size by about 24 hr and showed little increase in size thereafter. Histologic examination of thrombi at either 3 hr or 5 or 6 days showed them to contain platelets and substantial amounts of fibrin, red blood cells, and white cells (Fig. 1, A and B). The proportion of fibrin appeared to be greater in the older thrombi.

Administration of either high or low doses of aspirin did not significantly affect thrombus formation in association with the catheter and vessel wall in the first 3 hr (Table 1), although the mean thrombus weight tended to be greater in the animals given the highest dose of aspirin. However, at 5 days, none of the doses of aspirin enhanced thrombosis; with the very high dose (200 mg/kg every 8 hr), there was a tendency for the amount of thrombus to be reduced, although under the conditions of these experiments the mean thrombus weight was not significantly different from the values for the animals given the lower doses of aspirin or from controls.

Platelet survival was significantly shortened in rabbits with indwelling aortic catheters (sham-operated...
control, 71.6 ± 3.8 hr, n = 13; indwelling aortic catheter, 42.6 ± 3.3, n = 15). None of the doses of aspirin, including the very high dose that tended to inhibit thrombus formation, significantly prolonged shortened platelet survival; there were 13 or 14 animals used for each of the 4 doses of aspirin used in Table 1. In the sham-operated animals, platelet survival was significantly longer than for the controls only in animals treated with the lowest dose of aspirin (10 mg/kg) (platelet survival 96.2 ± 5.1 hr, p < 0.005).

For all aspirin regimens, platelets in citrated plasma prepared from samples of blood taken 1 hr after the first dose on the second day (25 hr) did not aggregate in response to sodium arachidonate or acid-soluble collagen. Platelets taken just before the second dose on the second day (32 hr) from animals given aspirin three times daily, also did not respond to these aggregating agents. Platelets taken just before the third dose at 48 hr from animals given a daily dose of 10 or 100 mg/kg had partially recovered their ability to aggregate in response to sodium arachidonate or acid-soluble collagen.

### Table 1. Effect of Oral Aspirin on Thrombus Weight in Rabbits With Indwelling Aortic Catheters

<table>
<thead>
<tr>
<th>Group</th>
<th>Aspirin* (mg/kg)</th>
<th>Thrombus Weight (mg)</th>
<th>At 3 hr†</th>
<th>At 5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>8.1 ± 1.9 (9)</td>
<td>23.3 ± 4.1 (10)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>10 daily</td>
<td>6.0 ± 1.5 (8)</td>
<td>23.8 ± 5.8 (9)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>100 daily</td>
<td>10.4 ± 2.2 (8)</td>
<td>22.2 ± 4.3 (9)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>50 t.i.d.</td>
<td>—</td>
<td>22.4 ± 3.8 (8)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>200 t.i.d.</td>
<td>12.4 ± 2.5 (9)</td>
<td>12.7 ± 2.4 (8)</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SEM. The number of animals in each experiment is indicated in parenthesis.

The significance of the differences among the thrombus weights at 5 days for the 5 groups was calculated using orthogonal contrasts. Both thrombus weights and the logarithms of the thrombus weights were used in these calculations. The groups compared were: A vs B, C, D — NS; E vs B, C, D — NS; B vs C, D — NS; C vs D — NS.

*Aspirin was administered orally by pharyngeal tube 2 hr before the introduction of the aortic catheter and thereafter at the times indicated. †Thrombus weight following a single dose of aspirin.

### Table 2. Effect of Oral Aspirin on PGJ₂-Like Activity Produced From Added Sodium Arachidonate by Rabbit Aorta Samples

<table>
<thead>
<tr>
<th>Time After First Dose of Aspirin</th>
<th>Aspirin (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 hr</td>
<td>93.8 ± 5.4</td>
</tr>
<tr>
<td>32 hr</td>
<td>—</td>
</tr>
<tr>
<td>48 hr</td>
<td>93.5 ± 3.6</td>
</tr>
</tbody>
</table>

Mean values ± SEM of 6 samples of aorta from each of 3 animals in each group.

*See Materials and Methods.
daily (50 mg/kg or 200 mg/kg), the inhibitory effect after the second dose of aspirin (25
reduced PGI2-like activity to 26% of the control 1 hr
was not inhibited by a dose of 10 mg/kg aspirin daily
one-half of the control values by 32 hr (Table 2).
66% of the control. When aspirin was given 3 times
the PGI2-like activity of the aorta had recovered to
(introduction by the aorta and found that these vessels
recovered their ability to produce PGI2 after 24-48
hr (Table 2). Aspirin, at a dose of 100 mg/kg daily,
reduction of PGI2-like activity observed at 25 hr (1 hr after the
third dose of aspirin) had returned to approximately
one-half of the control values by 32 hr (Table 2). Several groups have also examined the effect of aspirin
treatment of rabbits, rats, and humans on PGI2 pro-
duction. This dose of sodium salicylate significantly
increased whole blood fibrinolytic activity, and there was no
significant difference between the extent of the
increase induced by these drugs. Fibrinolytic activity
was also increased in the blood of rabbits given aspirin
or sodium salicylate over 3 days (placebo, 9.3% before
and 8.5% after 3 days; aspirin, 200 mg t.i.d., 8.8% before
and 14.6% after 3 days; sodium salicylate, 177.2
mg t.i.d., 8.08% before and 11.7% after 3 days). The
correlation coefficient between plasma salicylate levels
and whole blood fibrinolytic activity of rabbits given
different oral doses of aspirin or sodium salicylate was
r = 0.55 (p < 0.001, n = 36).

The histologic appearance of 5-day-old thrombi
from animals given high doses of aspirin or sodium
salicylate showed a loosening of fibrin in the thrombus
with clear areas around the leukocytes (Fig. 2, A, B,
and C).

High doses of aspirin or sodium salicylate can
inhibit the synthesis of the vitamin K-dependent clot-
ing factors.13 In the present experiments, the admin-
istration of high doses of aspirin or sodium salicylate
for three days significantly prolonged the prothrombin
time (Table 5). The thrombin time was not prolonged.

DISCUSSION

In the experiments reported in this article, only the
very high dose of aspirin tended to inhibit thrombosis
induced by continuous vessel injury; lower concen-
trations of aspirin that effectively inhibited the response

Table 3. Effect of Oral Sodium Salicylate on Platelet Survival and
Thrombus Weight in Rabbits With Indwelling Aortic Catheters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Platelet Survival (hr)</th>
<th>Thrombus Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>58.7 ± 3.7</td>
<td>25.6 ± 4.7</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>56.7 ± 4.9</td>
<td>7.2 ± 1.6*</td>
</tr>
</tbody>
</table>

(177.2 mg/kg t.i.d.)

Mean values ± SEM. There were 9 animals in each experiment.

Sodium salicylate was given orally by pharyngeal tube 2 hr before the
introduction of the aortic catheter and 3 times daily for 5 days.

*p < 0.005 calculated using Student’s t test.

The formation of PGI2-like activity by rabbit aortae
was not inhibited by a dose of 10 mg/kg aspirin daily
(Table 2). Aspirin, at a dose of 100 mg/kg daily,
reduced PGI2-like activity to 26% of the control 1 hr
after the second dose of aspirin (25 hr), but at 48 hr,
the PGI2-like activity of the aorta had recovered to
66% of the control. When aspirin was given 3 times
daily (50 mg/kg or 200 mg/kg), the inhibitory effect
on PGI2-like activity observed at 25 hr (1 hr after the
third dose of aspirin) had returned to approximately
one-half of the control values by 32 hr (Table 2). Several groups have also examined the effect of aspirin
treatment of rabbits, rats, and humans on PGI2 pro-
duction by the aorta and found that these vessels
regained their ability to produce PGI2 after 24-48
hr.12,34 37

We next examined whether sodium salicylate at a
molar concentration equal to that of the 200 mg/kg
aspirin would influence the extent of thrombus for-
mation. This dose of sodium salicylate significantly
reduced thrombus weight but had no effect on platelet
survival in rabbits with indwelling aortic catheters
(Table 3). Sodium salicylate at this dose had no effect
on thrombus weight at 3 hr; sodium salicylate, 8.8 ±
2.5 mg; placebo, 8.1 ± 1.9 mg.

One of the reported effects of high concentrations of
sodium salicylate is an increase of whole blood fibrino-
lytic activity.31 High concentrations of sodium salicy-
late or aspirin (1.55-4.1 mM) added to heparinized rabbit
blood increased whole blood fibrinolytic activity by
30%-40%. The effect of intravenous administration
of sodium salicylate or aspirin on whole blood fibrino-
lytic activity over a 2-hr period is shown in Table 4.
Aspirin or sodium salicylate significantly increased
whole blood fibrinolytic activity, and there was no
significant difference between the extent of the
increase induced by these drugs. Fibrinolytic activity
was also increased in the blood of rabbits given aspirin
or sodium salicylate over 3 days (placebo, 9.3% before
and 8.5% after 3 days; aspirin, 200 mg t.i.d., 8.8% before
and 14.6% after 3 days; sodium salicylate, 177.2
mg t.i.d., 8.08% before and 11.7% after 3 days). The
correlation coefficient between plasma salicylate levels
and whole blood fibrinolytic activity of rabbits given
different oral doses of aspirin or sodium salicylate was
r = 0.55 (p < 0.001, n = 36).

The histologic appearance of 5-day-old thrombi
from animals given high doses of aspirin or sodium
salicylate showed a loosening of fibrin in the thrombus
with clear areas around the leukocytes (Fig. 2, A, B,
and C).

High doses of aspirin or sodium salicylate can
inhibit the synthesis of the vitamin K-dependent clot-
ing factors.13 In the present experiments, the admin-
istration of high doses of aspirin or sodium salicylate
for three days significantly prolonged the prothrombin
time (Table 5). The thrombin time was not prolonged.

DISCUSSION

In the experiments reported in this article, only the
very high dose of aspirin tended to inhibit thrombosis
induced by continuous vessel injury; lower concen-
trations of aspirin that effectively inhibited the response

Table 4. Effect of Intravenous Injection of Aspirin or Sodium Salicylate on Rabbit Whole Blood Fibrinolytic Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Percent Radioactivity Released From (^{125})F-Fibrin-Coated Tubes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>Aspirin</td>
<td>6</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>6</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>6</td>
<td>8.4 ± 0.9†</td>
</tr>
<tr>
<td>177.2 mg/kg</td>
<td>6</td>
<td>8.2 ± 0.5</td>
</tr>
</tbody>
</table>

Mean ± SEM. The data were analyzed using Student’s t test; the values for blood from animals that received the drugs were compared with the corresponding values for blood from animals that received placebo.

*See Materials and Methods

Significance of difference from corresponding placebo:
†p < 0.001
‡p = 0.01
§p = 0.05
Table 5. Prothrombin Time of Rabbit Plasma After 3 Days of Treatment With Aspirin or Sodium Salicylate

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Animals</th>
<th>Prothrombin Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4</td>
<td>11.0 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 10 mg/kg/daily</td>
<td>4</td>
<td>10.9 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 100 mg/kg/daily</td>
<td>4</td>
<td>10.9 ± 0.09</td>
</tr>
<tr>
<td>Aspirin 50 mg/kg/t.i.d.</td>
<td>4</td>
<td>11.2 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 200 mg/kg/t.i.d.</td>
<td>4</td>
<td>100.2 ± 10.95*</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>4</td>
<td>82.4 ± 24.6†</td>
</tr>
<tr>
<td>177.2 mg/kg/t.i.d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM

The values for blood from the animals that received the drugs were compared with the corresponding values for blood from animals that received placebo.

*Significance of difference from placebo $p < 0.001$.
†Significance of difference from placebo $p < 0.025$.

Fig. 2. Light micrographs of thrombi obtained after 5 days from the aortae of rabbits with indwelling catheters. (A) Histologic appearance of thrombus obtained from a rabbit given placebo. There are some white blood cells (wbc) and the platelet-fibrin mass is closely packed. (B) Thrombus from a rabbit given aspirin (200 mg/kg, t.i.d.). The thrombus is loosely packed with numerous clear areas, particularly in regions around the white blood cells. (C) Platelet-fibrin thrombus from a rabbit given sodium salicylate (177.2 mg/kg, t.i.d.). Again, there are numerous clear areas, particularly in regions with white blood cells. (Hematoxylin-eosin; A: x400, B and C: x750.)

Aspirin is a weak inhibitor of thrombin-induced platelet aggregation and release reaction.\textsuperscript{10} The failure of doses of aspirin that inhibited platelet function to prevent thrombosis could be attributable to the observation that thrombin seems to play a large part in the initiation and growth of thrombi under these experimental conditions, since the amount of thrombus that formed around an indwelling aortic catheter in the first
few hours could be greatly reduced by treating the animals with heparin. In addition, the doses of aspirin or sodium salicylate that inhibited thrombus formation over a 5-day period increased the one-stage prothrombin time. Although the high doses of aspirin did not produce a significant inhibition of thrombus formation, the lower mean value in this group is compatible with the reduction in the amount of thrombus material caused by the administration of sodium salicylate.

Since PG\(_1\) is a potent inhibitor of platelet aggregation, there has been some concern that drugs that inhibit cyclo-oxygenase, such as indomethacin or aspirin, and thus block PG\(_1\) production by vessel walls may be thrombogenic.\(^4\) In the first few hours following insertion of the catheter, the high doses of aspirin that inhibited PG\(_1\) formation tended to promote thrombus formation, although not to a statistically significant degree. This is similar to the thrombogenic effect of high doses of aspirin that was observed by Kelton et al.\(^1\) and Buchanan et al.\(^2\) in experiments with injured jugular veins and carotid arteries in rabbits. In both these types of experiments, thrombin generation would have played a major part in the formation of the thrombi. Both thrombin and mechanical injury stimulate PG\(_1\) production by the endothelium.\(^3\) PG\(_1\) can inhibit the enhancing effect of activated platelets on thrombin generation and inhibit platelet aggregation.\(^4\) Thus, in circumstances in which platelet aggregates may be important in enhancing thrombin generation, such as during the early stages of thrombus formation, inhibition of PG\(_1\) production may be thrombogenic. However, inhibition of PG\(_1\) formation did not enhance the size of the thrombus that was present after 5 days of continuous injury. The failure of high doses of aspirin to produce a significant inhibition of thrombosis (whereas an equivalent dose of sodium salicylate was inhibitory) could be due to aspirin inhibition of PG\(_1\) formation.

The effectiveness of aspirin as an inhibitor of experimental thrombosis is controversial, since there is wide variation in the results obtained when it is used in different experimental circumstances; sometimes it is inhibitory and sometimes it is not.\(^1\) It may be that much of the variation in the ability of aspirin to inhibit experimental arterial thrombosis is related to whether or not the response to vessel injury involves a largely thrombin-dependent reaction.

In the present experiments, the tendency for high doses of aspirin or sodium salicylate to inhibit thrombus formation secondary to chronic vessel injury appeared to be related to a prolongation of the one-stage prothrombin time as well as to activation of whole blood fibrinolytic activity, which of these pathways was most important in reducing the amount of thrombus present at 5 days is not known. It is probable that these mechanisms were complementary, and the morphological observation of an apparent increase in the digestion of fibrin around the white cells in the thrombi indicates that there was increased fibrinolytic activity in the thrombi of animals treated with the high doses of aspirin or sodium salicylate. This is in keeping with Moroz' observation that high doses of aspirin and sodium salicylate increases whole blood fibrinolytic activity through an effect of the protease action of white blood cells.\(^3\) There are reports that doses of aspirin as low as 2–3 g/day in man can prolong the prothrombin time;\(^3\) these doses of aspirin are also reported to increase whole blood fibrinolytic activity.\(^3\) It may be that the beneficial effect of high doses of aspirin (3 g/day) in preventing venous thrombosis and pulmonary embolism\(^3\) is not just due to the effect of aspirin on platelets, but could also be related to the effect of aspirin on blood coagulation and fibrinolysis. In man, the combination of aspirin and oral anticoagu- lants has been reported to be more effective in inhibiting thromboembolic complications than oral anticoagu- lants alone.\(^4\)

The results from these experiments show that aspirin and sodium salicylate did not prolong the shortened platelet survival caused by the indwelling aortic catheter even at concentrations that reduced thrombus formation. Thus, under these experimental conditions, changes in the extent of thrombosis are not related to changes in platelet survival. Salicylate-containing drugs can decrease thrombosis without affecting platelet survival. The lack of effect of aspirin on platelet survival has also been reported by other investigators.\(^4\)

Although it is difficult to apply results obtained from experiments in animals directly to human problems, it is clear from these studies that aspirin in doses that only inhibit platelet function is not a satisfactory inhibitor of arterial thrombosis when there is a significant fibrin component. Since it is likely that multiple pathways are involved in thrombus formation in diseased human arteries, it is not surprising that aspirin has not been found to be a strongly effective inhibitor of the thromboembolic clinical complications of arterial disease in man. In very high doses, aspirin may inhibit thrombosis when thrombin makes a large contribution, but this effect is not mediated through inhibition of platelet function, since sodium salicylate, which has little effect on platelet function, is more effective than aspirin. It may be that in studies in which aspirin has been reported to reduce the incidence of venous thrombosis and pulmonary embolism in man, the dosage has been high enough, and the drug has been given over a sufficiently long period of time to affect the coagulation system and whole blood fibrinolytic activity.\(^3,38,46\)
REFERENCES


tissues generate prostacyclin (prostaglandin X), a potent inhibitor of platelet aggregation. Lancet 1:18, 1977


Effect of aspirin and sodium salicylate on thrombosis, fibrinolysis, prothrombin time, and platelet survival in rabbits with indwelling aortic catheters

M Cattaneo, A Chahil, D Somers, RL Kinlough-Rathbone, MA Packham and JF Mustard