Salicylate-Aspirin Interaction in the Rat

EVIDENCE THAT SALICYLATE ACCUMULATING DURING ASPIRIN ADMINISTRATION MAY PROTECT VASCULAR PROSTACYCLIN FROM ASPIRIN-INDUCED INHIBITION

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ABSTRACT Aspirin inhibits cyclooxygenase, thus preventing thromboxane A_2 production in blood platelets and prostacyclin in vascular cells. Aspirin is rapidly hydrolyzed to salicylate in the circulation. The objectives of this study were (*a*) to evaluate whether administration of salicylate, though ineffective by itself, prevents the inhibitory effect of aspirin on platelet and/or vascular cyclooxygenase activity; (*b*) to verify whether salicylate accumulating in blood after aspirin administration interferes with the pharmacological activity of further doses of aspirin. Pretreatment of rats with sodium salicylate (25–100 mg/kg i.p.) resulted in dose-related prevention of the effect of a subsequent dose of aspirin (2.5–10 mg/kg i.v.) on both platelet and vascular cells. Sodium salicylate appeared to amplify the greater response of platelets to aspirin compared with vessel wall.

Pretreatment of rats with repeated high doses of aspirin (200 mg/kg) resulted after 24 h in blood salicylate levels (150–200 μg/ml) that significantly prevented the inhibitory effect of a subsequent dose of aspirin on newly synthesized vascular prostacyclin. Blood salicylate levels obtained after 36 or 48 h (<50 μg/ml) were too low to blunt aspirin's effect. The interference with aspirin of its major endogenous metabolite should be borne in mind when interpreting results obtained with high dose aspirin or during repeated administration of this drug.

INTRODUCTION

Aspirin (acetylsalicylic acid) irreversibly inhibits cyclooxygenase, the enzyme that catalyzes the conversion of arachidonic acid into cyclic-prostaglandin endoperoxides (1). These unstable compounds are in turn metabolized preferentially to thromboxane A_2 (TxA_2)[1] in blood platelets (2) and to prostacyclin (PGI_2) in vascular cells (3). TxA_2 can induce platelet aggregation and vasoconstriction, and PGI_2 is a powerful inhibitor of platelet aggregation and a vasodilator (4). If TxA_2 and PGI_2 production are both suppressed by aspirin, then the antithrombotic activity of this drug might be reduced (5). Much effort has been devoted during the last few years to finding the most appropriate treatment schedule with aspirin to dissociate the drug's effects at platelet and vascular level. However studies in laboratory animals and in man have all been inconclusive so far (6–14). The available results are based on studies with single doses of aspirin, but any regimen using aspirin as a potential antithrombotic drug should be applied for long periods.

Aspirin is rapidly hydrolyzed in the body to salicylate (SA), which is eliminated slowly and can therefore accumulate in the circulation following repeated aspirin treatment (15). Since SA by itself does not modify prostaglandin synthesis (4) no attention has been paid to the disposition of this substance in the body when discussing the potential antithrombotic effect of aspirin. Several investigators reported recently that in vitro SA could prevent the inhibitory effect of aspirin on platelet and vascular cyclooxygenase activity (16–18).

The purpose of the present work was dual: (*a*) To investigate whether SA-aspirin interaction could occur at platelet and/or vascular level in rats given both compounds. (*b*) To verify whether SA accumulating in blood from previous aspirin treatment could interfere with the pharmacological activity of further aspirin.

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[1] *Abbreviations used in this paper*: AA, arachidonic acid; MDA, malondialdehyde; PG, prostaglandin; PGI_2, prostacyclin; SA, salicylate; Tx, thromboxane.

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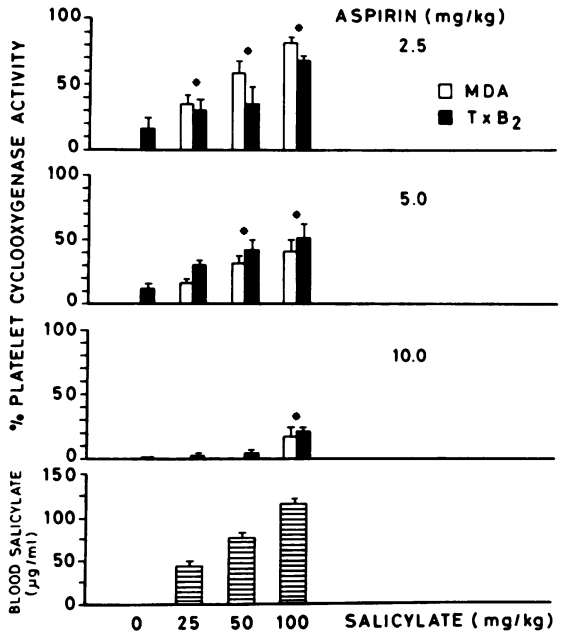

FIGURE 1 Inhibition of platelet cyclooxygenase activity by aspirin and its prevention by previous administration of Na-SA. Doses of aspirin and Na-SA are indicated. Cyclooxygenase activity was measured as MDA and TxB_2 produced by 0.4 mM AA. For each series of experiments the results are expressed as a percentage (means±SEM, $n = 4$) of the mean values measured in animals given saline (control groups). The absolute values in the control groups were 0.77±0.15, 0.85±0.10 nmol/10^9 platelets for MDA and TxB_2, respectively. The lower panel of the figure reports blood levels of SA measured just before aspirin administration. *$P < 0.01$ compared with treatment with aspirin alone.

METHODS

Experiment A. This experiment was devised to assess the effects of previous Na-SA administration on aspirin-induced inhibition of platelet and vascular cyclooxygenase activity. Male CD-COBS rats (Charles River Breeding Laboratories, Italy) weighing 250–300 g were randomly divided into groups given the following treatments: isotonic saline, aspirin (2.5, 5, or 10 mg/kg i.v.) in the form of its soluble lysine salt (Flectadol, Maggioni, Italy) or Na-SA (100 mg/kg i.p.) (Farmitalia-Carlo Erba, Milan, Italy). Other groups of animals received different combined treatments with Na-SA (25, 50, or 100 mg/kg i.p.) followed by aspirin (2.5, 5, or 10 mg/kg i.v.) 30 min later. All animals were exsanguinated by heart puncture under ether anesthesia 30 min after the last drug dose.

Experiment B. This experiment was devised to assess the effects of SA accumulating after aspirin administration on further aspirin-induced inhibition of vascular cyclooxygenase. Rats were randomly divided into groups that were given three injections of isotonic saline or aspirin (200 mg/kg each, i.p.) 4 h apart. 30 min before scheduled death, 24, 36, or 48 h after the last of these aspirin injections, all animals received an additional challenge dose of 5 mg/kg aspirin i.v.

Malondialdehyde (MDA) and TxB_2 production by platelets was measured respectively by a spectrophotometric method and by radioimmunoassay after incubation of platelet-rich plasma with arachidonic acid (AA) (>99% pure, Nuchek Prep, Elysian, Minn.) (19).

PGI_2 synthesis by rat thoracic aorta. Immediately after exsanguination the thoracic aorta was isolated, cleaned of adventitia, and flushed *in situ* with 5 ml of calcium-free tyrode solution. The vessel was then removed and a ring (~7 mg wet wt, 0.5 cm length) was cut in the middle part. The rings were immediately incubated at 37°C for 5 min in 100 μl of Tris-HCl buffer 0.15 M, pH 9.2 containing 25 μM AA. The supernatant buffer was then removed, rapidly frozen, and stored at −70°C until use. A standard curve of synthetic PGI_2 (Upjohn Co., Kalamazoo, Mich.) was constructed in Tris HCl buffer for each experiment and stored in an identical manner. PGI_2 synthesis by the vessel wall was quantitated in two ways, using a bioassay of PGI_2 (20) and a radioimmunoassay for 6-keto-$PGF_{1\alpha}$ (21).

Measurement of blood SA levels. SA levels were measured by a spectrophotofluorimetric method (22) on blood sampled from the animal's tail vein on Na_2 EDTA solution (0.4% wt/vol, final concentration).

Statistical analysis. For each experiment data were expressed as a percentage of the mean value of controls and statistically analyzed by ANOVA and Duncan's multiple range test.

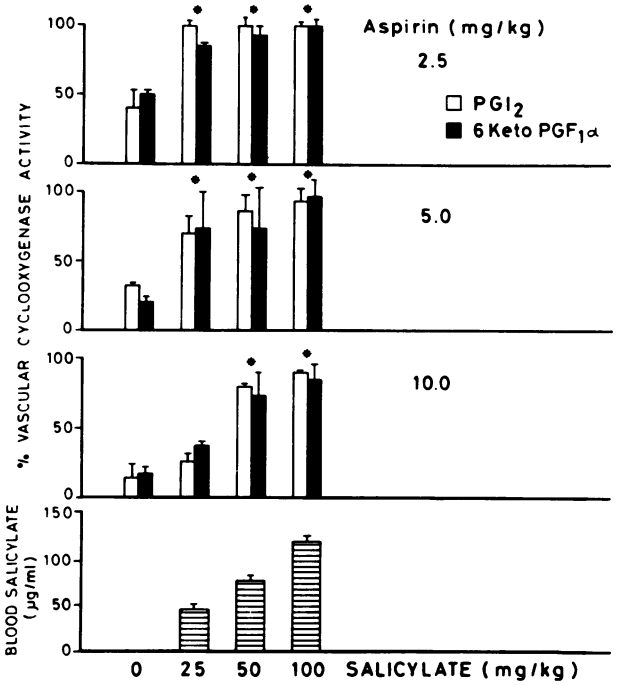

FIGURE 2 Inhibition of vascular cyclooxygenase activity by aspirin and its prevention by previous administration of Na-SA. Doses of aspirin and Na-SA are indicated. Cyclooxygenase activity was measured as platelet aggregation inhibitory effect of PGI_2 and as the amount of 6-keto-$PGF_{1\alpha}$. For each series of experiments the results are expressed as a percentage (means±SEM, $n = 4$) of the mean values measured in animals given saline (control groups). The absolute values in these groups were 4.3±0.4 and 3.4±0.5 pmol/mg wet wt, respectively, for PGI_2 activity and 6-keto-$PGF_{1\alpha}$. The lower panel of the figure reports blood levels of SA measured just before aspirin administration. *$P < 0.01$ compared to treatment with aspirin alone.

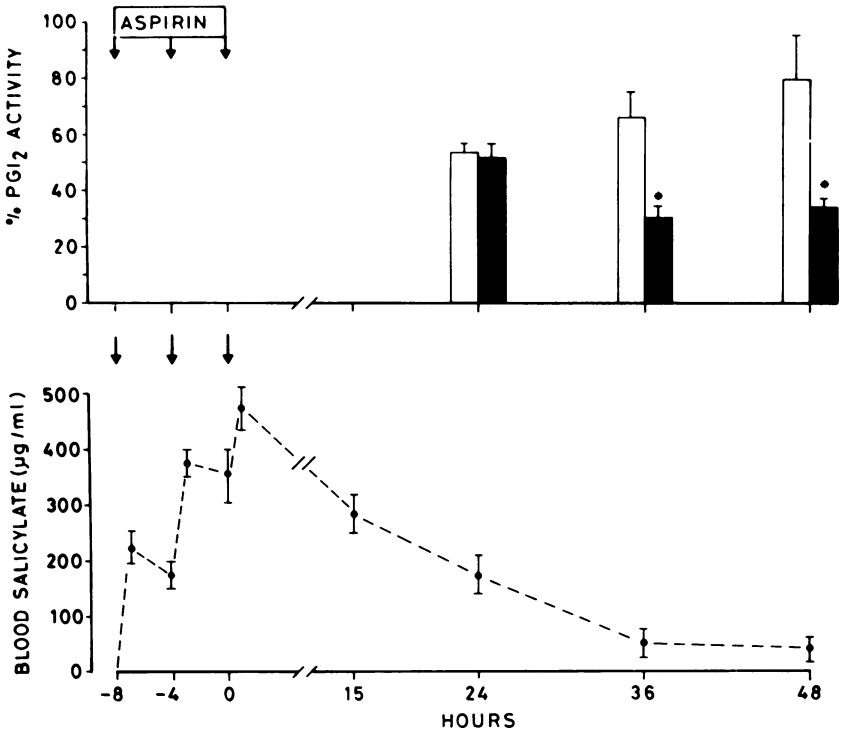

FIGURE 3 Vascular PGI_2 activity (upper panel, white columns) and blood SA levels (lower panel) after three consecutive doses of aspirin 200 mg/kg i.p., 4 h apart. Black columns represent PGI_2 activity 30 min after a fourth dose of aspirin (5 mg/kg i.v.) given at the intervals indicated. In aspirin-pretreated rats, this last dose of aspirin further reduced PGI_2 activity at 36 and 48 h ($P < 0.01$), but not at 24 h. For each series of experiments the results are expressed as a percentage (means±SEM, $n = 10$) of the mean values, measured in animals given no aspirin. The absolute value of PGI_2 activity in control animals was 9.3±0.9 pmol/mg wet wt.

RESULTS

Prevention by previous Na-SA administration of aspirin-induced inhibition of platelet and vascular cyclooxygenase activity (experiment A). Platelet cyclooxygenase activity (measured by the amount of MDA and TxB_2 produced in platelet-rich plasma by 0.4 mM AA) was not modified by Na-SA (100 mg/kg i.p.) administered 1 h before testing (data not shown). In contrast, single doses of aspirin (2.5–10 mg/kg i.v.) resulted in complete inhibition of MDA production and a marked dose-related reduction of TxB_2. When Na-SA was administered before aspirin, the inhibitory effect of the latter was significantly reduced as shown in Fig. 1. The interaction was dependent on the doses of both drugs, being maximal when 100 mg/kg of Na-SA was followed by 2.5 mg/kg of aspirin and barely detectable when the same Na-SA dose was followed by 10 mg/kg of aspirin. Fig. 1 also reports the blood levels of SA measured just before aspirin administration.

Cyclooxygenase activity of thoracic aortic rings (measured by the amount of PGI_2 activity and 6-keto-$PGF_{1\alpha}$ released upon incubation with 25 μM AA) was not modified by Na-SA at any dose (data not shown). In contrast, aspirin induced a dose-related inhibition of cyclooxygenase activity. When Na-SA was administered before aspirin, the inhibitory effect of the latter was significantly reduced as shown in Fig. 2. Both PGI_2 activity and 6-keto-$PGF_{1\alpha}$ levels were between 70 and 100% of control values at all drug combinations except when the lowest dose of Na-SA (25 mg/kg) was given before the highest dose of aspirin (10 mg/kg).

Prevention by SA accumulating after aspirin administration of aspirin-induced inhibition of vascular cyclooxygenase activity (experiment B). As reported in Fig. 3 three doses of aspirin (200 mg/kg i.p.) were administered 4 h apart. 24 h after the last dose, PGI_2 activity had recovered by ~50% and SA levels were ~175 μg/ml. At that moment the administration of a test dose of aspirin (5 mg/kg i.v.) did not modify PGI_2 activity. In contrast, it significantly inhibited PGI_2 activity when given after 36 or 48 h, when SA levels had dropped to 50 μg/ml or less.

It can be excluded that the lack of inhibition of PGI_2 activity by aspirin (after 24 h) was due to refractoriness

of the newly synthesized vascular enzyme to the drug. Administration of a single dose of aspirin (200 mg/kg i.p.) to rats resulted after 24 h in ~35 μg/ml of blood SA and 50% recovery of PGI_2 activity. In this condition the test dose of aspirin significantly reduced PGI_2 activity (data not shown).

DISCUSSION

We report here that SA may effectively prevent the inhibitory action of aspirin on both platelet and vascular cyclooxygenase activity when administered as such and when accumulated as a result of aspirin hydrolysis in vivo. Pretreatment of rats with increasing single doses of Na-SA (from 25 to 100 mg/kg i.p.) resulted in dose-related prevention of the effect of subsequent single doses of aspirin (from 2.5 to 10 mg/kg i.v.) on both platelet and vascular cells. Although platelet cyclooxygenase showed higher sensitivity to aspirin than vascular cyclooxygenase, complete dissociation between these two effects could not be obtained. This was achieved however when rats were pretreated with Na-SA. As an example, both platelet and vascular cyclooxygenase were almost completely blocked by 10 mg/kg aspirin, whereas they were inhibited by 95 and 20%, respectively, when the same dose of aspirin was given after Na-SA (50 mg/kg). Thus, SA appears to amplify the difference in the response to aspirin of platelet and vascular cells (9). Whether this newly discovered property of SA derives from a different sensitivity to and/or access of the drug at various cellular levels remains to be established. Nevertheless, our observation may constitute the experimental basis for an original approach aimed at resolving the "aspirin dilemma" in clinical practice (5).

In the second part of this study the SA levels reached after administration of Na-SA were comparable to those after administration of aspirin, its parent molecule. It was thus possible to demonstrate a hitherto unrecognized pharmacological interaction between the endogenously formed metabolite and its parent molecule. Indeed, pretreatment of rats with high doses of aspirin resulted in blood SA levels high enough to block the inhibitory effect of a subsequent dose of aspirin on vascular PGI_2. The interaction between endogenous SA and aspirin could only be studied at vascular level since recovery of PGI_2 activity from aspirin inhibition was sufficient when relatively high blood levels of SA were still present. In contrast, the inhibitory effect of aspirin on platelets was still complete 24 h after administration (7, 19), so that an experiment similar to that described for vascular cells could not be performed.

The interference with aspirin of its metabolite SA was obtained in rather critical experimental conditions. In fact, the relation of these high doses to those used in man remains to be established. Therefore, any extrapolation to clinical situations would be hazardous. However, the findings should be taken into account when interpreting results obtained with high dose aspirin or during repeated administration of this drug.

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