

Biochemical Selectivity of Oral Versus Intravenous Aspirin in Rats

Inhibition by Oral Aspirin of Cyclooxygenase Activity in Platelets and Presystemic but Not Systemic Vessels

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Abstract

In rats intravenous aspirin was only slightly more effective an inhibitor of platelet thromboxane B₂ (TxB₂) than of aorta 6-keto-prostaglandin (PGF)_{1 α} generation (1.9 versus 2.1 mg/kg). In contrast, oral aspirin was about five times more effective on platelet than on aorta cyclooxygenase activity. The "biochemical selectivity" of aspirin as an inhibitor of platelet and vascular cyclooxygenase was not apparent after intravenous administration of the drug. However, this could be achieved by relatively low doses of oral (or intraduodenal) aspirin, on account of "presystemic" acetylation of platelet cyclooxygenase. Even in this condition, though, aspirin selectivity was relative to "systemic" peripheral vessels but not to the vessels of the enterohepatic circulation. Indeed after an oral or intraduodenal dose of 5 mg/kg aspirin, generation of portal vein 6-keto-PGF_{1 α} was inhibited to much the same extent as platelet TxB₂, while inferior vena cava 6-keto-PGF_{1 α} formation was spared.

Introduction

It has long been known that aspirin inhibits cyclooxygenase (1), the enzyme catalyzing the aggregatory prostaglandin cyclic endoperoxides and (TxA₂)¹ in platelets (2) and the antiaggregatory prostaglandin I₂ (PGI₂) in vascular cells (3). On the basis of early studies showing different sensitivity to aspirin of cyclooxygenase in different tissues *in vitro* (4, 5), it was suggested that low doses could dissociate the drug's effect on platelet TxA₂ generation from its effect on vascular PGI₂ (6). However, the selectivity between platelet and vascular cyclooxygenase obtained by a single low dose of aspirin in man was relative, owing to the wide variability from one study to another and within subjects in the same study, especially as far as inhibition of PGI₂ synthesis was concerned (7–10). Repeated low-dose aspirin resulted invariably in cumulative inhibition of platelet TxA₂ generation, but the effect on vascular PGI₂ formation (11, 12) or its major urinary metabolites was variable (13, 14).

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1. Abbreviations used in this paper: PGI₂, prostaglandin I₂ or prostacyclin; 6-keto-PGF_{1 α} , 6-keto-prostaglandin F_{1 α} ; TxA₂, TxB₂, thromboxane A₂, B₂.

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It has been suggested more recently that the "biochemical selectivity" of aspirin could be related to its kinetics and data were presented consistent with "presystemic" inhibition of platelets after oral low-dose or enteric-coated, slow-release aspirin (15–17). This was mainly based on the significant suppression of platelet TxB₂ generation seen in the absence of measurable levels of aspirin in the peripheral circulation.

Oral aspirin is mainly absorbed by the stomach and is subject to first-pass metabolism both in the gastrointestinal tract and in the liver (18, 19). Presystemic deacetylation of aspirin after oral administration is responsible for reduced peripheral drug levels. Consequently, after oral aspirin peripheral vascular cyclooxygenase might be exposed to lower concentrations of the drug and/or its inactive metabolite, salicylate (20). In contrast, platelets passing through the gut capillaries during absorption could be effectively acetylated by the drug. However, actual protection of vascular PGI₂ generation through presystemic deacetylation of aspirin has not yet been demonstrated.

We compared the effect of aspirin given by a systemic intravenous or oral route on cyclooxygenase activity in platelets and in both presystemic and systemic vascular tissues in the rat.

Methods

Male CD-COBS rats (300–350 g body wt, Charles River, Calco, Italy) were used. They received single doses of either isotonic saline or aspirin in the form of its soluble lysine salt (Flectadol, Maggioni, Italy).

A first group of rats ($n = 25$) was anesthetized with pentothal (35 mg/kg *i.p.*) and the right femoral vein was catheterized with a heparinized polyethylene tube (PE-50) for intravenous injection. A second group of animals ($n = 30$) was used for *per os* administration. In a third group of rats ($n = 15$), 6 d before the experiment a polyethylene tube (PE-60) was implanted in the duodenum (1 cm after the gastroduodenal junction) under ketamine HCl (100 mg/kg *i.p.*) anesthesia, for intraduodenal administration, as described in detail elsewhere (21). At the time of the experiment the conscious implanted rats were given either saline or aspirin through the cannula at a rate of 50 μ l/min for 30 min using an infusion Haward pump.

The aspirin dose for each rat was dissolved in 1.5 ml of isotonic saline for intraduodenal and *per os* administration; for intravenous bolus (30 s) injection the dose was dissolved in 0.35–0.40 ml of isotonic saline. The dosages of aspirin in milligrams per kilogram used are detailed in Results.

All animals were exsanguinated by heart puncture 45 min after the beginning of the treatment. Native blood was allowed to clot in a glass tube at 37°C for 1 h; serum was separated by centrifugation and stored at –20°C until assayed for TxB₂ generation.

Immediately after exsanguination rings (~2 mm long) were cut from presystemic (portal vein) and systemic (inferior vena cava and the abdominal aorta) vessels. The rings were incubated at 37°C for 5 min in 100 μ l Tris-HCl buffer 0.15 M, pH = 8, containing 25 μ M arachidonic

acid (Na salt, >99% pure, Sigma, Milan, Italy); the clear supernatant was removed at the end of incubation and stored at -20°C until assayed for 6-keto $\text{PGF}_{1\alpha}$ production (22).

TxB_2 and 6-keto $\text{PGF}_{1\alpha}$ were quantitated by radioimmunoassay, using specific antisera kindly provided by C. Patrono (Catholic University, Rome, Italy) as described (22, 23). Radioimmunoassays for TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ were validated as described (24).

In control rats, platelet counts were $1.5 \pm 0.2 \times 10^9/\text{ml}$ of blood and TxB_2 was 346.0 ± 39.4 pmol/ml serum. The wet weight (milligrams, mean \pm SEM) of comparable vascular rings prepared from a group of 10 rats was 4.8 ± 0.5 (portal vein); 1.6 ± 0.3 (inferior vena cava) and 2.6 ± 0.2 (abdominal aorta). In the experimental conditions used in the present study the greater weight of portal vein segments provided in control rats equivalent production of 6-keto- $\text{PGF}_{1\alpha}$ as the inferior vena cava (39.4 ± 4.9 and 40.5 ± 5.8 pmol/ml of incubation medium, respectively). As the two vessels might have different amounts of perivascular tissue, the results were not expressed on a per milligram tissue basis. 6-keto- $\text{PGF}_{1\alpha}$ generation by abdominal aorta rings from control rats was 132.5 ± 12.6 pmol/ml of incubation medium.

All values are means \pm SEM. For easier comparison of the effects of aspirin on different systems the results are given as percentages of the respective control values.

Data were analyzed by one-way analysis of variance and Duncan's test.

Results

Effects of oral and intravenous aspirin on cyclooxygenase activity in platelets and abdominal aorta

The ID_{50} of oral or intravenous aspirin on cyclooxygenase activity was calculated in platelets and in abdominal aorta. From the data shown in Fig. 1, it appeared that intravenous aspirin was more effective than the oral drug in preventing both platelet TxB_2 and aorta 6-keto- $\text{PGF}_{1\alpha}$ generation. Intravenous aspirin was almost as effective on platelets and on vascular enzyme, however, while oral aspirin was about five times more potent on platelets than on the vessel wall preparation. The ratio of oral to intravenous aspirin ID_{50} was 2.7 in platelets but 11.4 in aorta (Table I). It also appeared that at the highest doses used (20 or 100 mg/kg body wt) oral aspirin tended to inhibit both platelet TxB_2 and aorta 6-keto- $\text{PGF}_{1\alpha}$ formation to a comparable degree (Fig. 1).

Effects of aspirin on cyclooxygenase activity in platelets, portal vein, and inferior vena cava

We tested the inhibitory effect of 5 mg/kg body wt oral aspirin on a systemic and a presystemic venous vessel, namely the inferior vena cava and the portal vein. This dose was selected as

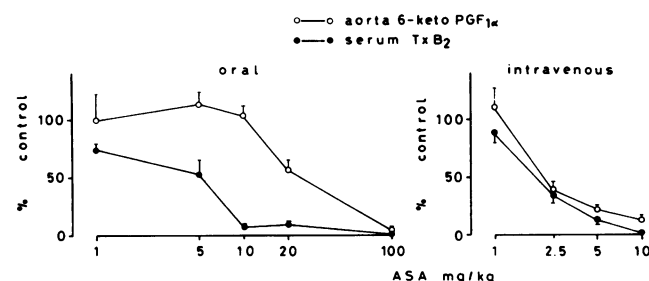


Figure 1. Dose-response curves of rat aorta 6-keto- $\text{PGF}_{1\alpha}$ and serum TxB_2 production tested 45 min after oral or intravenous aspirin. Results are expressed as a percentage of the respective control values and are reported as means and SEM for groups of 4–6 rats.

Table I. ID_{50} (mg/kg) of Intravenous and Oral Aspirin Obtained from Dose-response Curves on Serum TxB_2 and Aorta 6-keto- $\text{PGF}_{1\alpha}$ Generation in Rats

Route of administration	Serum TxB_2	Aorta 6-keto- $\text{PGF}_{1\alpha}$
Intravenous	1.9	2.1
Oral	5.2	24.0
Ratio oral/intravenous	2.7	11.4

close to the calculated ID_{50} on platelet TxB_2 generation. As shown in Fig. 2, 6-keto- $\text{PGF}_{1\alpha}$ generated by the vena cava was not affected while that produced by a portal vein was prevented to virtually the same extent as TxB_2 inhibition. Essentially similar or an even clearer dissociation of the effects of 5 mg/kg aspirin on platelets and portal vein on the one hand and vena cava on the other was observed after intraduodenal administration of the drug (Fig. 2). In contrast, 6-keto- $\text{PGF}_{1\alpha}$ generation by both venous vessels was equally and significantly inhibited after the same dose of intravenous aspirin (37.3 ± 5.9 and $40.3 \pm 5.4\%$ of control values for portal vein or vena cava, respectively) (data not shown).

Discussion

Oral aspirin is rapidly hydrolyzed to salicylate during gastrointestinal absorption before entering the peripheral systemic circulation. The area under the plasma concentrations versus time curve for unchanged aspirin after oral administration of 10 mg/kg aspirin to rats was about one-third of that obtained following intravenous administration of the same dose. As urinary excre-

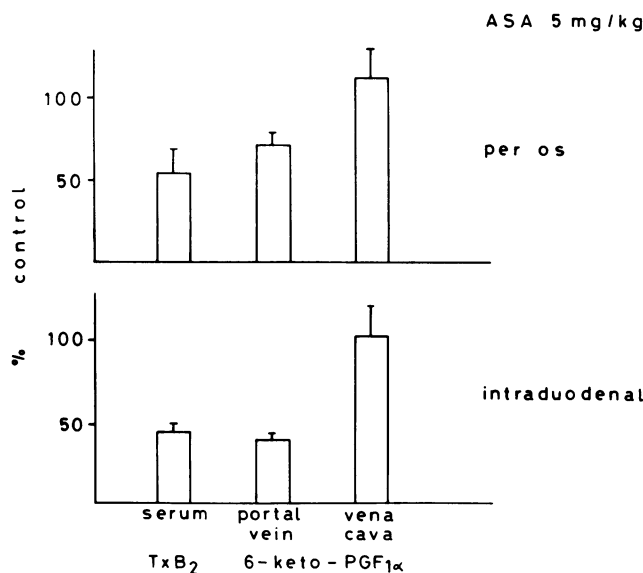


Figure 2. Serum TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ generation by rat portal vein and vena cava segments tested 45 min after oral or intraduodenal aspirin 5 mg/kg. Results are expressed as a percentage of the respective control values and are reported as means and SEM for groups of five to eight rats. Statistical analysis showed significant differences ($P < 0.01$) between serum TxB_2 and vena cava 6-keto- $\text{PGF}_{1\alpha}$ production after oral or intraduodenal aspirin and between portal vein and vena cava 6-keto- $\text{PGF}_{1\alpha}$ only after intraduodenal aspirin.

tion of salicylic acid and its conjugated forms was similar after oral or intravenous administration of aspirin, reduced systemic availability after oral administration indicated "first pass" metabolism of aspirin (18).

This study was designed to test the hypothesis that aspirin administered orally might show greater biochemical selectivity toward platelet rather than aorta cyclooxygenase after intravenous injection. Moreover, oral aspirin might affect a presystemic venous vessel such as portal vein similarly to platelets, while sparing a systemic vessel, such as vena cava. The hypothesized different effects of aspirin on portal vein and vena cava would be the consequence of the observed contribution of the liver to the first pass metabolism of aspirin in rats (18).

This hypothesis was experimentally verified by comparing the ID_{50} of intravenous and oral administration of aspirin obtained from dose-response curves on platelet and aorta cyclooxygenase activity in rats. Intravenously administered aspirin was a similar inhibitor of platelet TxB_2 and aorta 6-keto-PGF $_{1\alpha}$ generation (1.9 versus 2.1 mg/kg). In contrast, oral aspirin was about five times more effective on platelet than aorta cyclooxygenase activity. Cyclooxygenase in platelets and in a vascular tissue (such as aorta) thus appears similarly sensitive to the inhibitory effect of aspirin.

This supports a previous *in vitro* observation of similar susceptibility to aspirin inhibition of platelets and cultured endothelial cells (25). Aspirin thus is not a selective platelet cyclooxygenase inhibitor *per se*, at least in the rat. Biochemical selectivity can however be achieved in some conditions after oral administration of the drug. Indeed it has been suggested (15–17) that acetylation of platelet enzyme occurs on exposure to aspirin in the portal circulation during absorption. Following first-pass deacetylation in the enterohepatic circulation, much less or even no aspirin may be delivered to the peripheral blood, thus partially or totally sparing peripheral systemic vascular cyclooxygenase. This suggestion was based on the observation that after oral aspirin, inhibition of serum TxB_2 generation could be obtained before or even in the absence of measurable drug levels in peripheral blood (15–17, 26–28). The concomitant sparing of vascular cyclooxygenase activity was not measured in any of these studies in human volunteers. In the present study an almost complete dissociation between the effects of aspirin on platelet and aorta cyclooxygenase activity was achieved after 10 mg/kg body wt or a lower dose of aspirin (Fig. 1). After 20 or 100 mg/kg of oral aspirin, the presystemic component of drug action became less evident as enzyme inhibition approached a maximum. This supports the suggestion that in man biochemical selectivity of aspirin is more marked at relatively low doses (13, 14, 16).

If the biochemical selectivity of oral aspirin is related to presystemic acetylation of the platelet enzyme, one would expect it not to apply with respect to enterohepatic vessels. As shown in Fig. 2, this was indeed the case when the effect of aspirin was compared on portal vein and inferior vena cava. 6-keto-PGF $_{1\alpha}$ was inhibited in the former (presystemic) vessel to an extent not significantly different from platelets, while in the same conditions the latter (systemic) vessel was spared. In relation to these experimental data it may be of interest to recall that an oral dose of 40 mg aspirin resulted in inhibition of 6-keto-PGF $_{1\alpha}$ generated by mesenteric vein (29), but not by vena saphena (9). Both studies were performed in humans by the same group. Fig. 2 also shows that intraduodenal administration of the same dose of aspirin, possibly by avoiding aspirin hydrolysis, which occurs in the

stomach (19, 30), induced significantly ($P < 0.01$) more inhibition of both platelet and portal cyclooxygenase activity, but did not result in any measurable inhibition of the enzyme in vena cava.

In conclusion, this study shows that the biochemical selectivity of aspirin on inhibition of platelet and vascular cyclooxygenase is not apparent after intravenous administration of the drug. It can be achieved by relatively low doses of oral (or intraduodenal) aspirin, through presystemic acetylation of platelet cyclooxygenase. Even in this condition, however, aspirin selectivity is relative to systemic peripheral vessels but not to the vessels of the enterohepatic circulation.

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