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Slow Intravenous Administration of Low Dose Aspirin Inhibits Both Vascular and Platelet Cyclooxygenase Activity: An Experimental Study in the Rat (42340)

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Abstract. Aspirin irreversibly inhibits cyclooxygenase, thus preventing thromboxane (Tx)A_2 production in platelets and prostacyclin in vascular cells. While it is generally accepted that the inhibitory effect of low dose aspirin is cumulative on platelet cyclooxygenase, it is still a matter of debate whether a similar phenomenon also occurs on vascular cyclooxygenase. We have measured in anesthetized rats the inhibitory effect of two doses of aspirin (2.5 and 5.0 mg/kg), given intravenously either as a bolus or as a continuous infusion (for 30 min), on platelet TxB_2 and 6-ketoprostaglandin $F_{1\alpha}$ generation by different vascular segments. Aspirin significantly inhibited both platelet and vascular cyclooxygenase independently of the rate of drug administration. The aspirin peak plasma levels at the end of bolus injection was about 170 times higher than the average level measured during the slow infusion (1.21 ± 0.15 μg/ml). At this concentration aspirin did not affect *in vitro* either platelet or vascular cyclooxygenase activity. Thus the inhibitory effect of aspirin on both platelet and vascular cyclooxygenase seems to be related to total exposure of the enzyme to the drug rather than to the maximal drug concentration attainable in the systemic circulation. These findings may be relevant to the current debate on optimal conditions for the biochemical selectivity of aspirin as an antithrombotic drug. © 1986 Society for Experimental Biology and Medicine.

The irreversible acetylation of cyclooxygenase exerted by aspirin (1) induces inhibition of thromboxane (Tx)A_2 in blood platelets (2) and of prostacyclin (PGI_2) in vascular cells (3–4). Since these two compounds may have antagonistic effects on platelet aggregation and thrombus formation (5) it was suggested that the limited efficacy of aspirin as an antithrombotic agent, particularly at high doses, might be related to this opposite effect (6–8). Therefore a considerable interest has developed to find out schedules, doses, and routes of administration to maximize the effect of aspirin in inhibiting platelet TxA_2 in respect to vascular PGI_2 (9–15); advantage has been taken of the fact that the inhibition of cyclooxygenase by single doses of aspirin lasts longer on platelets than on vascular cells due to the lack of protein synthesis in the former cell in respect to the latter (16). In this study we compared the effect of the same doses of aspirin, given either as a single, rapid bolus or as a slow continuous infusion, on both platelet TxB_2 and vascular PGI_2 generation. The results obtained were correlated to aspirin plasma levels to evaluate whether the aspirin peak levels or the total exposure are important to determine the inhibition of cyclooxygenase activity in platelets and vascular cells.

Materials and Methods. Male CD-COBS rats (300–350 g body wt, Charles River, Italy) were anesthetized with pentothal (35 mg/kg ip). The right femoral vein was catheterized with a heparinized polyethylene tube (PE-50) for intravenous injection. Isotonic saline or aspirin (2.5 or 5.0 mg/kg) in the form of its soluble lysine salt (Flectadol, Maggioni, Italy) was given through the cannula for 30 min (slow infusion) or for 30 sec (bolus). The dose of aspirin for each rat was dissolved in 0.3–0.35 ml isotonic saline for the bolus injection or in 1.5 ml saline for the slow infusion (50 μl/min) using an infusion pump (Haward).

All animals were exsanguinated by heart puncture 45 min after the beginning of the treatment. Native blood was incubated in a glass tube at 37°C for 1 hr; serum was separated by centrifugation and stored at −20°C until assayed for TxB_2 generation.

Immediately after exsanguination the portal vein, the inferior vena cava, and the abdominal aorta were isolated and a ring of each vessel (approximately 2 mm long) was cut. The rings were incubated at 37°C for 5 min in 100 μl Tris–HCl buffer (0.15 *M*, pH 8) containing 25 μ*M* arachidonic acid (Na salt, >99% pure; Sigma, Italy). The ring was removed and the clear supernatant stored at −20°C until as-

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sayed for 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) production.

The wet weight (mg, mean ± SEM, $n = 10$) of the vascular rings was 4.8 ± 0.5 (portal vein), 1.6 ± 0.3 (inferior vena cava), and 2.6 ± 0.2 (abdominal aorta).

TxB_2 and 6-keto-$PGF_{1\alpha}$ were quantitated by radioimmunoassay, using specific antisera kindly provided by Professor C. Patrono (Catholic University, Rome, Italy) as described (17, 18). TxB_2 and 6-keto-$PGF_{1\alpha}$ values are reported respectively as picomoles per milliliter of serum and of incubation medium.

Another group of rats was given similar treatment to determine the plasma concentrations of aspirin and salicylate. Besides cannulation of the femoral vein to infuse aspirin (5.0 mg/kg), the carotid or the femoral artery was cannulated for blood collection in anesthetized rats receiving respectively aspirin bolus or slow infusion. Blood was collected right at the end of injection (30 sec) and 45 min after the bolus, and at the end (30 min) and 15 min after the slow infusion. Blood samples were collected in cold Heparin with 0.3% KF (50/50 w/v) to inhibit hydrolysis of aspirin by plasma esterases. Measurements were made on plasma obtained by rapid centrifugation at 4°C and frozen at −80°C, by a selective high-performance liquid chromatography method described elsewhere (14).

In vitro studies were made on citrated (3.8% sodium citrate, 1/10 v/v) blood and on aortic rings, incubated in 100 μl Tris–HCl buffer (0.15 *M*, pH 8) at 37°C for 30 min with 1.2 μg/ml aspirin. At the end of the incubation the vascular rings were treated with 25 μ*M* arachidonic acid as in *ex vivo* studies, whereas 25 μ*M* $CaCl_2$ and 5 NIH U thrombin/ml were added to citrated blood to induce clotting and obtain serum.

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

Results. Figure 1 shows serum TxB_2 generation in rats receiving 2.5 or 5.0 mg/kg iv aspirin as a slow infusion or as a bolus. Aspirin significantly ($P < 0.01$) inhibited TxB_2 generation at both doses but no significant difference was found between the two administration procedures.

Figure 2 shows 6-keto-$PGF_{1\alpha}$ production by different vascular segments. Aspirin significantly ($P < 0.01$) inhibited 6-keto-$PGF_{1\alpha}$ in the portal vein and in the abdominal aorta but not in the inferior vena cava segments at 2.5 mg/kg while a significant inhibition was found in all three vascular segments at 5.0 mg/kg. However, the inhibition induced by the two administration procedures was not significantly different.

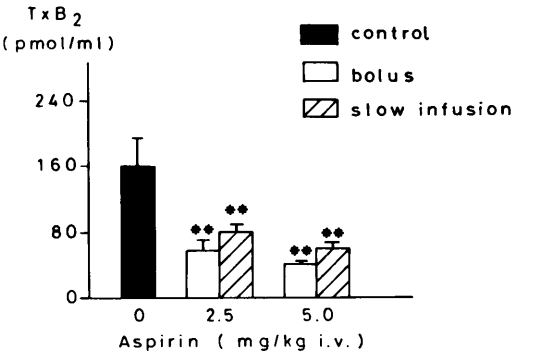

FIG. 1. Effect of aspirin given to rats as a slow intravenous infusion or as a bolus injection on serum TxB_2 production. No significant difference was found in TxB_2 generation between rats infused with isotonic saline either as a bolus or as slow infusion. Control animals were therefore pooled (black column). No significant differences were found between TxB_2 production in rats treated with aspirin as a bolus or by slow infusion. In contrast, both administration routes and aspirin doses resulted in a significant (**$P < 0.01$) reduction of TxB_2 generation compared to controls. Each experimental group consisted of five rats.

Aspirin plasma concentrations were measured after iv administration of 5.0 mg/kg aspirin. At the end of the slow infusion (30 min) aspirin plasma concentrations averaged 1.21 ± 0.15 μg/ml and had fallen to 0.14 ± 0.01 μg/ml 15 min later when the animals were killed; at the end of bolus injection (30 sec) they averaged 207.1 ± 16.4 μg/ml while they were undetectable (<0.05 μg/ml) at killing (45 min later).

The average concentration of aspirin (1.2 μg/ml) measured after slow iv infusion did not affect either TxB_2 generation or 6-keto-$PGF_{1\alpha}$ synthesis by aortic rings when it was incubated *in vitro* for 30 min with total blood or vascular rings, respectively (data not shown).

Discussion. There is general agreement on the cumulative inhibitory effect of aspirin given under repeated treatment on platelet cyclooxygenase as measured by serum TxB_2 generation (17); in contrast, it is still a matter

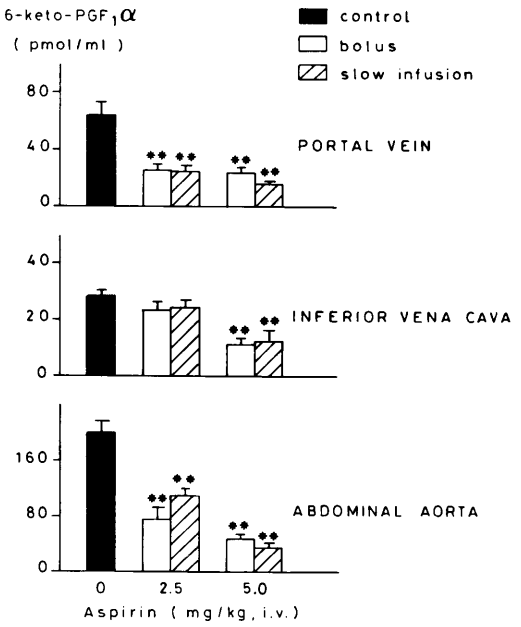

FIG. 2. Effect of aspirin given to rats as a slow intravenous infusion or as a bolus injection on 6-keto-PGF$_{1\alpha}$ production by different vascular segments. No significant difference was found in vascular 6-keto-PGF$_{1\alpha}$ between rats infused with isotonic saline either as a bolus or as slow infusion. Control animals were therefore pooled (black column). Both administration routes and aspirin doses resulted in a significant (**$P < 0.01$) reduction of 6-keto-PGF$_{1\alpha}$ generation compared to controls, except for the inferior vena cava at the dose of 2.5 mg/kg (bolus or slow infusion). In contrast, no significant differences were found between 6-keto-PGF$_{1\alpha}$ production in rats treated as a bolus or by slow infusion. Each experimental group consisted of five animals.

of debate whether a similar phenomenon also occurs on vascular cyclooxygenase. In fact several investigators (10, 11, 15, 19) found a cumulative inhibition by aspirin on vascular 6-keto-PGF$_{1\alpha}$ whereas others (12, 20) were unable to find a decrease of the urinary excretion of PGI$_2$ metabolites. This study shows that a slow iv infusion of aspirin (5.0 mg/kg) in rats induced a significant inhibition of both platelet TxB$_2$ and vascular 6-keto-PGF$_{1\alpha}$ formation. The fact that 1.2 μg/ml aspirin *in vitro* did not affect either platelet or vascular cyclooxygenase activity would indicate that the drug concentration measured at the end of the slow infusion was a very low one for the rat. Extrapolation of *in vitro* data obtained in a closed experimental system to *in vivo* effects of aspirin should, however, be made with great caution. In particular it is still unclear whether the *in vitro* conditions used in this and previous studies (13) approximate *in vivo* conditions for cumulative acetylation of cyclooxygenase.

The inhibitory effects of aspirin given as a slow iv infusion were very similar to those obtained by a rapid iv bolus injection. A significant inhibitory effect was also observed when half the dose was given (2.5 mg/kg), except in the inferior vena cava. This failure could be related to the lower absolute levels of 6-keto-PGF$_{1\alpha}$ produced in controls by this vessel than by the others. However, the vena cava and portal vein produced similar amounts of 6-keto-PGF$_{1\alpha}$ when these were expressed in terms of picomoles per milligram wet tissue (average 17.53 and 13.31 pmole/mg, respectively).

The average aspirin level in plasma during the slow infusion was about 170 times lower than at the end of bolus injection. Thus the inhibitory effect of aspirin on platelet and vascular cyclooxygenase is clearly not directly related to the peak plasma levels of the drug. It was previously shown that the same dose of aspirin administered iv as a bolus or as a constant rate infusion in the rat resulted in no significant difference between the areas under the curve (drug plasma levels versus time) (21). We can therefore conclude that inhibition by aspirin of both platelet and vascular cyclooxygenase activity is related to the area under the curve (total exposure of the enzyme to the drug) rather than to the maximal concentration of the drug attainable in the circulation. This finding indicates that at least at short time intervals after iv administration of aspirin, significant inhibition of platelet cyclooxygenase activity cannot be achieved without also affecting somewhat vascular PGI$_2$ synthesis.

An implication of our findings is that the possibility exists of an inhibitory effect of even low doses of aspirin on vascular cyclooxygenase in the systemic circulation; to avoid this and obtain a biochemical selectivity of the drug, one should therefore minimize the entry of intact aspirin into the systemic circulation. It has recently been proposed (13, 14) that this might be obtained if substantial first-pass deacetylation of aspirin occurs in the enterohepatic circulation. Presystemic acetylation of

platelets would occur within gastrointestinal vessels, while peripheral vascular PGI_2 generation might be spared. The present study suggests that systemic effects of aspirin would be difficult to preclude if even low levels of aspirin come in contact with vascular tissues for a sufficient time. In view of the wide variation in aspirin kinetics between individuals (14), the clinical relevance of the first-pass deacetylation of aspirin requires careful evaluation.

Finally it is of interest to consider that under some particular conditions, as occurred in the present study after iv administration of 2.5 mg/kg aspirin, cumulative inhibition by aspirin can be observed in one vessel (e.g., portal vein), but not in another one from the same animal (e.g., inferior vena cava). This observation should be taken into account when discussing the vascular effect of aspirin based on PGI_2 measurements carried out in urine or in single vessels.

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