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MANIPULATION OF THE LOCAL THROMBOXANE AND PROSTACYCLIN BALANCE *IN VIVO* BY THE ANTITHROMBOTIC COMPOUNDS DAZOXIBEN, ACETYLSALICYLIC ACID AND NAFAZATROM

HANS DECKMYN, ELISABETH VAN HOUTTE, MARC VERSTRAETE and JOS VERMYLEN
Centre for Thrombosis and Vascular Research, Department of Medical Research, University of Leuven, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

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Abstract—We have developed an experimental model for the study of local prostaglandin production by platelets and the vessel wall following stimulation '*in vivo*'. A nylon thread was inserted into the external jugular vein of rabbits; its presence did not induce an occluding thrombus. Thromboxane (TXB_2) values in the blood, sampled through the facial vein, immediately distal to the stimulus, rose and remained high for at least 4 hr, while 6-keto prostaglandin (PG) $F_{1\alpha}$ levels, after a first increase, gradually returned to normal ('exhaustion' of the endothelial cells?). No changes were observed in the contralateral jugular vein without thread.

After infusion via the femoral vein of 10 mg/kg dazoxiben, a thromboxane synthetase inhibitor, local TXB_2 production was completely abolished, whereas 6-keto $PGF_{1\alpha}$ formation no longer returned to basal values, but tended to increase. This leads to the conclusion that upon inhibition of TXB_2 formation endoperoxide metabolism is reoriented '*in vivo*' towards prostacyclin, and this mainly at the site where platelets are activated.

Injection of 100 mg/kg lysine acetylsalicylic acid resulted in complete inhibition of TXB_2 and 6-keto $PGF_{1\alpha}$ formation, the latter, however, slowly recovering with time.

The administration of nafazatrom to the animals did not influence the local TXB_2 changes, but partially prevented the decline of 6-keto $PGF_{1\alpha}$ with time. The antithrombotic properties of this drug thus could be related to protection of the endothelial cells from 'exhaustion'.

During the last years many pharmacological attempts have been undertaken to shift the balance between thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) towards a more antithrombotic situation by inhibiting thromboxane synthetase, by selectively inhibiting platelet cyclo-oxygenase or by stimulating the synthesis of PGI_2.

In vitro work has shown that dazoxiben specifically inhibits thromboxane synthesis [1]; in addition, it reorients the cyclic endoperoxide metabolism in platelets towards PGE_2, $PGF_{2\alpha}$ and presumably towards PGD_2 [2], and furthermore, in whole blood [3] or in a combined platelet–endothelial cell system [2], towards PGI_2. Especially this latter effect opens interesting perspectives, since a thromboxane synthetase inhibitor would thus not only block the formation of pro-aggregatory and vasoconstricting TXA_2, but also enhance the production of anti-aggregatory and vasodilating PGI_2.

Several studies have been performed to find a dose of acetylsalicylic acid that would selectively block platelet cyclo-oxygenase. These attempts were based on the findings that endothelial cell cyclo-oxygenase is less sensitive and recovers more quickly from aspirin treatment than platelet cyclo-oxygenase [4–6]. This has led to regimens for low-dose aspirin which could discriminate the two enzymes [7, 8].

On the other hand, nafazatrom (Bay g 6575) stimulates the release of PGI_2 from 'exhausted' rat aorta rings [9, 10], from diabetic rat aorta [11] and from ram seminal vesicles [12]. Although the mechanism of action is not completely clear, it could be related to inhibition of the formation of lipoxygenase products [13] or to its capacity to act as a reducing cofactor for the hydroperoxidase component of cyclo-oxygenase [12]. Another possibility could be the inhibition of the PGI_2-degrading enzyme: 15-hydroxyprostaglandin-dehydrogenase [14].

The present '*in vivo*' study not only confirms the rapid recovery of endothelial cyclo-oxygenase following acetylsalicylic acid treatment, but also shows that the reorientation of the endoperoxide metabolism by dazoxiben and the enhanced release of PGI_2 by nafazatrom also occur '*in vivo*'; these effects are most obvious at the site where platelets and endothelial cells are activated, in other words where the action is most useful.

MATERIALS AND METHODS

Five groups of male White New Zealand rabbits weighing about 2.5 kg were studied. The rabbits were anaesthetized with ±0.3 ml/kg intramuscular injection of Hypnorm (Duphar, Amsterdam, The Netherlands); if necessary, during the course of the experiments the anaesthesia was maintained by additional 0.2 ml injections. The left and right external jugular and facial veins were carefully dissected, whereafter a catheter was inserted into both facial veins, with the tip near to the bifurcation (Fig. 1).

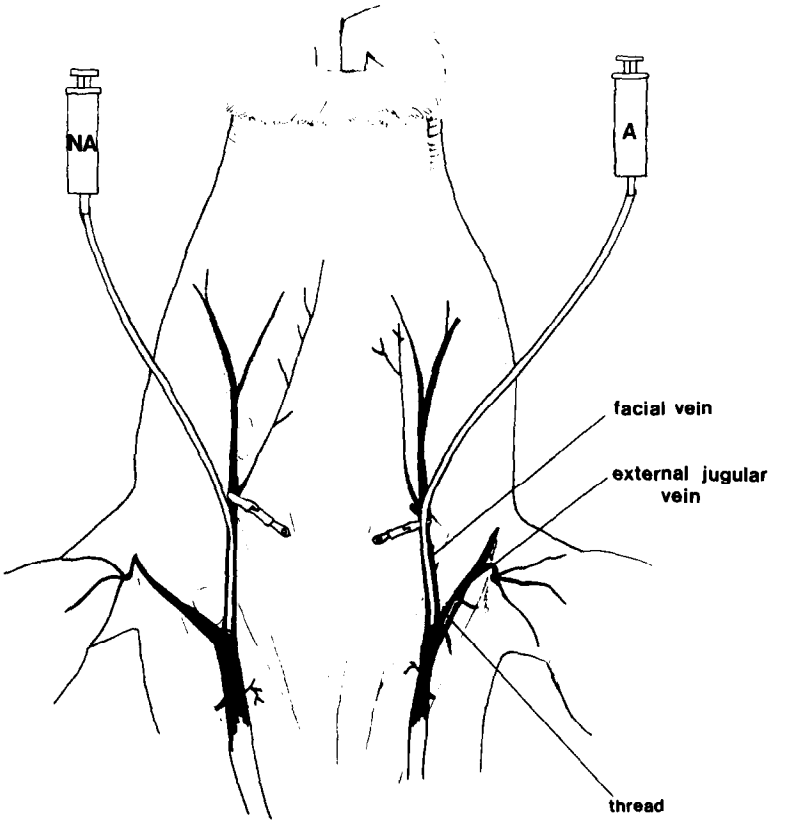

Fig. 1. Representation of the surgical procedure: a thread was inserted into the external jugular vein to cause local activation (A). Blood was sampled immediately distal to this stimulus via the facial vein. The contralateral side served as a control (NA).

The first 1 ml sample of blood was withdrawn from both sides, anticoagulated with one-tenth volume 4% trisodium citrate, and immediately centrifuged for 2 min at 12,000 *g*, whereafter plasma was kept.

A nylon thread (Ethicon, No. 3) was inserted with a needle into one of the jugular veins, so that 1 cm of the thread was in the bloodstream. Further blood samples were obtained from both sides 30 and 60 min later and processed as described.

Immediately after the 60 min sample, an infusion (5 ml over 30 min) was started via the femoral vein. The dazoxiben-treated group ($n = 5$) received 10 mg/kg of the drug (Pfizer Central Research, U.K.), whereas isotonic saline was infused into the other groups. To one of these ($n = 5$) 100 mg/kg lysine acetylsalicylic acid (Aspégic, Laboratoire Egic, France) had been administered intravenously and to another group ($n = 5$) 10 mg/kg nafazatrom (Bayer, F.R.G.) had been given via a gastric tube, both 1 hr before the start of the experiment. The fifth group ($n = 6$) was treated with a combination of dazoxiben and nafazatrom, both given as described above. Further blood samples were taken at 30 min intervals.

The plasma samples were stored at −20° until assayed for 6-keto $PGF_{1\alpha}$ and TXB_2 by specific radio-immunoassays as previously described [3], with the exception that rabbit prostaglandin-free plasma was used for constructing the standard curve.

Statistical differences were calculated using the Student's *t*-test for small numbers.

RESULTS

TXB_2 generation

TXB_2 values increased drastically, immediately distal to the inserted thread (A), as compared to the non-activated control side (NA) (A vs NA at 30 min: $P < 0.0025$). After NaCl infusion (Fig. 2A), the values remained high in A, indicating a continuous activation, which, however, was moderate, since no occluding thrombi were formed. When the thread was removed at the end of an experiment, only a minimal amount of material was seen to stick to it. No changes were observed in the TXB_2 levels in NA.

After dazoxiben infusion (Fig. 2B) TXB_2 values dropped to below basal levels both in A and in NA. At 2.30 hr, the differences between animals treated with NaCl and dazoxiben were significant, both in A ($P < 0.0005$) and NA ($P < 0.025$). A 100 mg/kg i.v. administration of lysine acetylsalicylic acid blocked all TXB_2 production (Fig. 2C), whereas nafazatrom did not significantly change the TXB_2 levels found in A and NA (Fig. 2D). The results after the combined dazoxiben–nafazatrom treatment were virtually the same as those obtained after dazoxiben alone (not shown).

6-Keto $PGF_{1\alpha}$ formation

Values of 6-keto $PGF_{1\alpha}$ also rose after the thread was introduced into the vein (A vs NA at 30 min: $P < 0.0025$), but later on they gradually decreased to basal levels (Fig. 3A). This was in sharp contrast

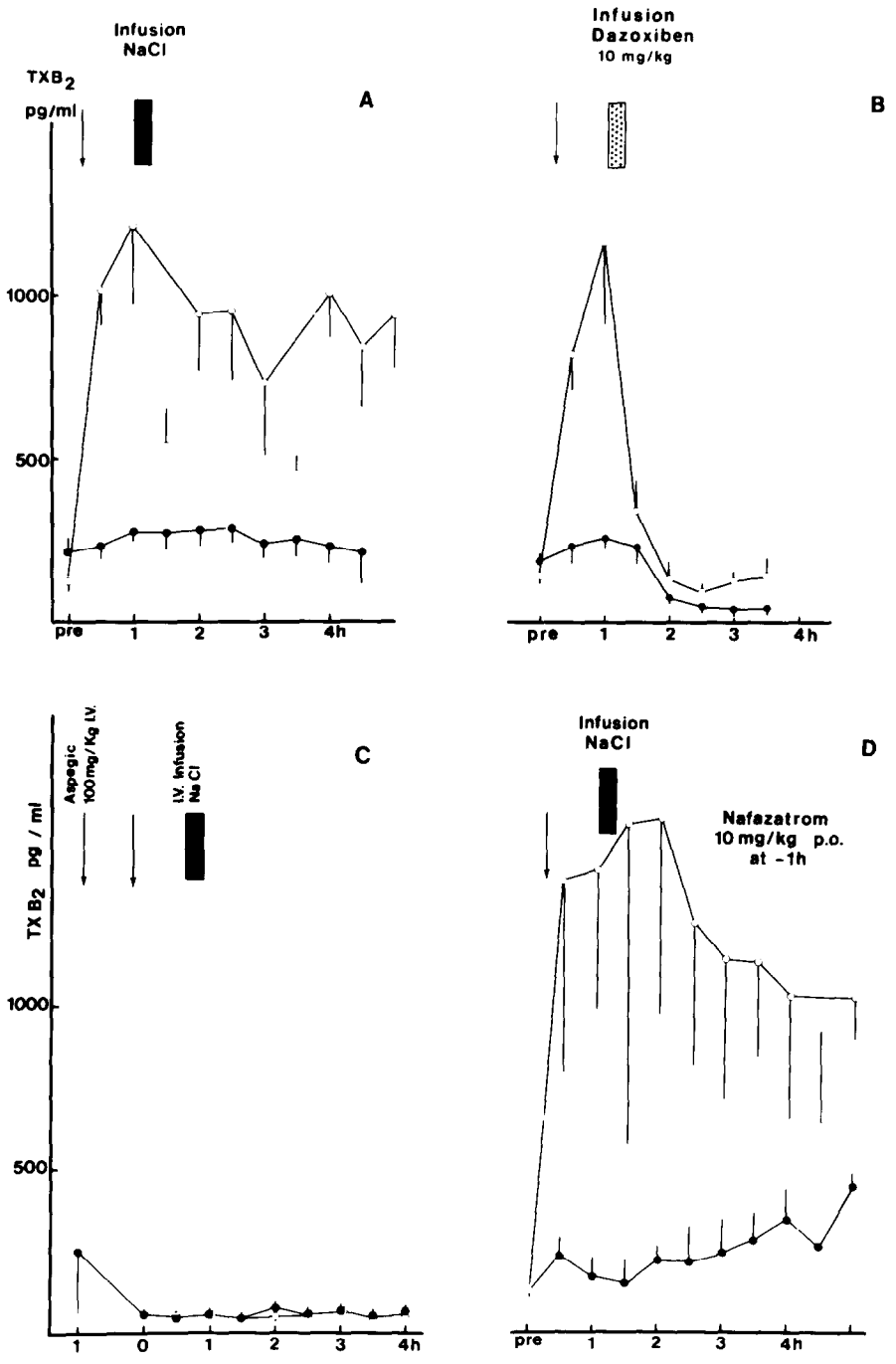

Fig. 2. TXB_2 formation at the thread-site (○) and the contralateral side (●) (mean ± S.E.M.). The arrow indicates the moment the thread was inserted. (A) Control animals with isotonic saline infusion ($n = 10$); (B) infusion of 10 mg/kg dazoxiben via the femoral vein ($n = 5$); (C) injection of 100 mg/kg lysine-acetylsalicylic acid ($n = 5$); (D) peroral administration of 10 mg/kg nafazatrom 1 hr before the start of the experiment ($n = 5$).

to what was seen in dazoxiben treatment: 6-keto $PGF_{1\alpha}$ formation in A did not decline, but even increased (NaCl vs dazoxiben in A at 2.30 hr: $P < 0.05$). On the other hand, an increase in 6-keto $PGF_{1\alpha}$ levels was also observed in NA (NaCl vs dazoxiben in NA at 2.30 hr: $P < 0.05$) (Fig. 3B).

When lysine acetylsalicylic acid was infused 1 hr before the insertion of the thread, no immediate increase occurred in A, but 6-keto $PGF_{1\alpha}$ formation recovered slowly, at least in A (A vs NA at 3 hr: $P < 0.05$) (Fig. 3C).

The nafazatrom group initially showed a pattern in A parallel to the controls: an immediate rise, followed by a gradual decease; thereafter the 6-keto $PGF_{1\alpha}$ formation, however, stabilized at a higher level (NaCl vs nafazatrom in A at 3 hr: $P < 0.025$). In NA only a slight, non-significant increase was observed (Fig. 3D).

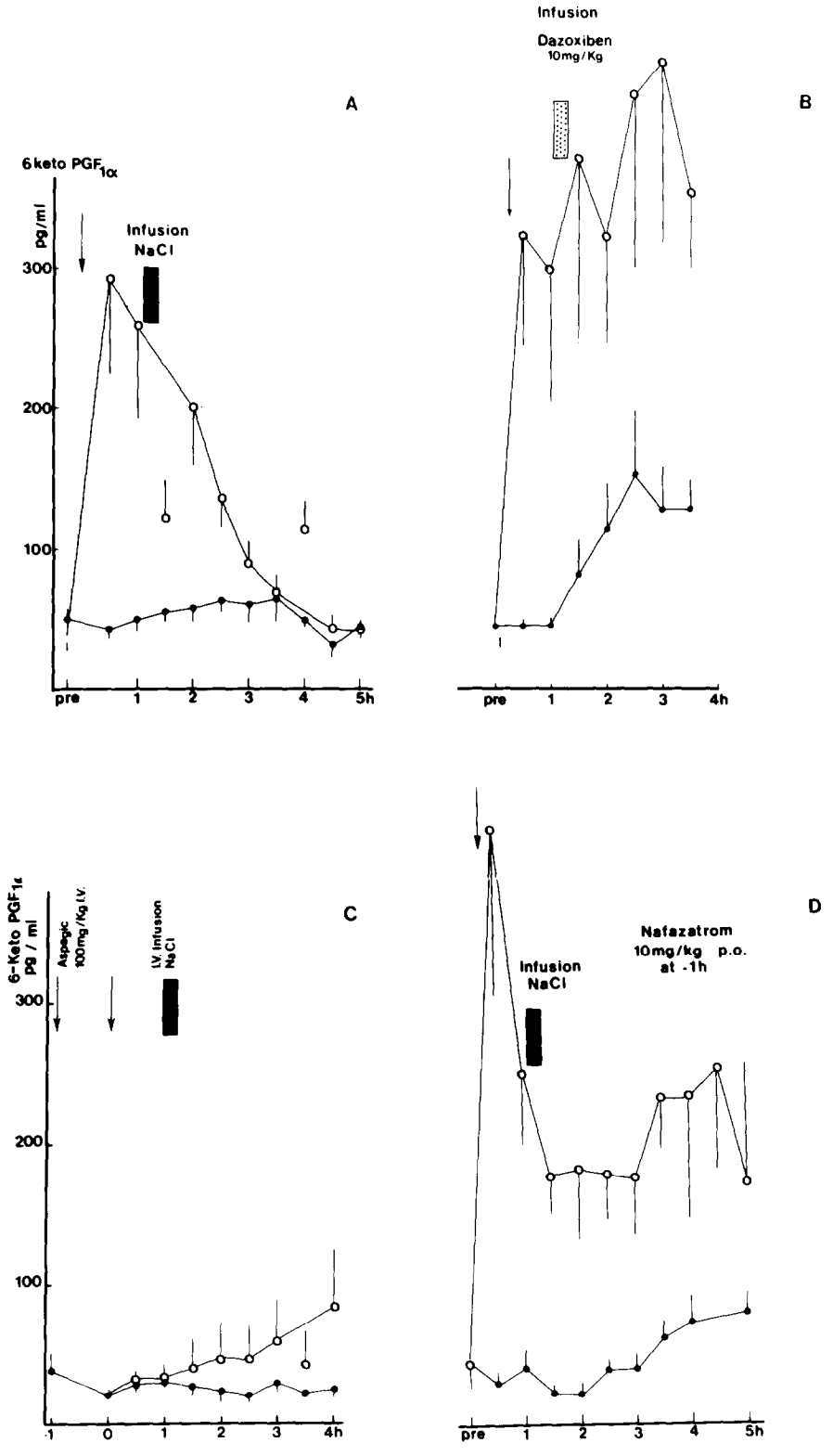

Fig. 3. 6-Keto $PGF_{1\alpha}$ formation at the thread-site (○) and the contralateral side (●) (mean ± S.E.M.). Indications are the same as in Fig. 2.

The 6-keto $PGF_{1\alpha}$ formation in the group treated with both nafazatrom and dazoxiben was not significantly different from that in the dazoxiben group (not shown).

DISCUSSION

Recent data [15, 16] indicate that prostacyclin is not a circulating hormone, and therefore should exert its activity only as a local defence mechanism. With the experimental model described here, it is possible to study local changes in prostacyclin formation: the nylon thread induces a localized and continuous stimulation and the catheter enables us to take samples, immediately distal to the stimulus, without interrupting the blood flow. In addition, the animal serves as its own control since blood can also be obtained from the contralateral side.

*Control group (*NaCl *infusion)*

In the control group, TXB_2 levels were at a sustained high level in A, which indicates that platelets indeed were continuously activated. Whether the lower values at 1.30 and 3.30 hr might be indicative of a phasic TXB_2 formation, possibly correlated with periodic embolization of adhering platelets, is unclear. The pattern of 6-keto $PGF_{1\alpha}$ formation is interesting. The rapid initial increase could be due to several stimuli: the moderate puncture damage, mechanical stimulation by the thread, platelet-derived products such as escaping cyclic endoperoxides [17] or platelet-derived growth factor [18]. Thrombin stimulation, on the other hand, is less likely to be involved since rabbit endothelium is rather insensitive to it [19]. As a function of time, the 6-keto $PGF_{1\alpha}$ formation gradually decreased to basal levels. Exhaustion of the prostacyclin production by vessel rings is observed *in vitro* [20] and may be due to the inactivation of the peroxidase component of the cyclo-oxygenase by own products [21] or by lipid peroxides formed by lipoxygenase [22]. Our present findings indicate that such exhaustion may also occur in irritated vessels *in vivo*.

Dazoxiben group

With dazoxiben a clear inhibition of TXB_2 formation was achieved. The levels after inhibition were lower than in the controls. The higher values in the control animals can be explained by activation of the platelets during the sampling resulting in some TXB_2 production. The increase in 6-keto $PGF_{1\alpha}$ values in the control vein, which corresponds to what was observed in the human volunteer study [2], could also be partly due to activation of the blood [3] during sampling.

The much higher levels at the thread-site, however, would be due to a local escape of platelet cyclic endoperoxides when thromboxane synthetase is blocked, and their conversion into prostacyclin by endothelial cells. Thus, this finding would furthermore indicate intact endothelial prostacyclin synthetase. A number of studies have already shown the antithrombotic potential of thromboxane synthetase inhibitors [23, 24], and one has indicated that inhibition of TXA_2 formation may be accompanied by increased PGI_2 synthesis; the present study gives a direct demonstration of the latter hypothesis '*in vivo*'.

Acetylsalicylic acid group

Acetylsalicylic acid inhibits cyclo-oxygenase both in platelets and endothelial cells. With the described rabbit model it was possible to confirm '*in vivo*' the recovery of endothelial cell cyclo-oxygenase with time, in good agreement with previous observations '*ex vivo*' [25]. TXB_2 formation was completely abolished throughout the experiment and the levels were lower than in the controls, again suggesting some platelet activation during blood sampling.

Nafazatrom group

The antithrombotic compound nafazatrom allows the endothelial cells to produce PGI_2 over a longer period of time. The difference between the non-significant increase of 6-keto $PGF_{1\alpha}$ at the control side and the sustained high production in the neighbourhood of the thread is indicative of a protective action of nafazatrom against endothelial cell exhaustion. Since this exhaustion can be due to inactivation of the cyclo-oxygenase by lipid peroxides [21], the findings that nafazatrom can act as a reducing cofactor for the peroxidase component of cyclo-oxygenase [12] could explain the observed phenomena. This hypothesis is compatible with the apparent lack of effect of the drug on normal blood vessel PGI_2 formation in acute experiments [26], as also indicated here by the normal rise of 6-keto $PGF_{1\alpha}$ immediately after insertion of the thread, and with its significant antithrombotic properties when a cannula is placed for a long period in rabbit aortae [26].

Dazoxiben–nafazatrom group

Upon combined administration of dazoxiben and nafazatrom, the local changes in TXB_2 and 6-keto $PGF_{1\alpha}$ were identical to those with dazoxiben alone. This could indicate that prevention of self-inactivation of cyclo-oxygenase is of minor importance when prostacyclin is mainly generated from cyclic endoperoxides derived from platelets.

In conclusion, we can state that although endothelial cyclo-oxygenase indeed recovers more quickly from acetylsalicylic acid inhibition than the platelet enzyme, acetylsalicylic acid should never stimulate local PGI_2 formation. A thromboxane synthetase inhibitor, on the other hand, not only blocks thromboxane formation selectively, but also reorients the cyclic endoperoxides partly towards PGI_2, resulting in a clearly higher local PGI_2 formation than in controls. Nafazatrom finally allows activated endothelial cells to defend themselves for much more prolonged periods by producing high amounts of PGI_2, but it does not change TXA_2 formation. In contrast to systemic prostacyclin infusion, which at most will give a low concentration at the site of vessel injury, drugs such as dazoxiben and nafazatrom would induce a higher 'local' formation of PGI_2 exactly where this is most useful, namely where platelets are activated and/or endothelial cells risk getting exhausted.

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