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Effects of the selective inhibition of platelet thromboxane synthesis on the platelet-subendothelium interaction

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1 Drugs that inhibit TxA_2 synthesis are used to reduce platelet aggregation. The aim of this study was to compare the effects of a cyclo-oxygenase (COX) inhibitor (acetylsalicylic acid, ASA), a thromboxane synthetase (TxS) inhibitor (dazoxiben) and a dual TxS inhibitor and TxA_2 receptor blocker (DT-TX 30) on platelet aggregation and the platelet-subendothelium interaction in flow conditions.

2 The techniques used in this *in vitro* study were platelet aggregometry in whole blood, and measurement of platelet thromboxane B_2 and prostaglandin E_2 production and leucocyte production of 6-keto-$PGF_{1\alpha}$. The platelet-subendothelium interaction was evaluated in rabbit aorta subendothelium preparations exposed to flowing blood at a shear stress of 800 s^{-1}. Morphometric methods were used to calculate the percentage of subendothelium occupied by platelets.

3 The 50% inhibitory concentration (IC_{50}) of DT-TX 30 in whole blood was in the range of 10^{-7} μM (induced with collagen or arachidonic acid) to 10^{-5} μM (induced with thrombin) or 10^{-4} (induced with ADP). IC_{50} values under all experimental conditions were lower with DT–TX 30 than with ASA. For thromboxane B_2 the IC_{50} were: ASA 0.84 ± 0.05 μM, dazoxiben 765 ± 54 μM, DT–TX 30 8.54 ± 0.60 μM. Prostaglandin E_2 was inhibited only by ASA (IC_{50} 1.21 ± 0.08 μM). Leucocyte 6-keto-$PGF_{1\alpha}$ was inhibited by ASA (IC_{50} 6.58 ± 0.76 μM) and increased by dazoxiben and DT–TX 30. The greatest reduction in percentage subendothelial surface occupied by platelets after blood perfusion was seen after treatment with DT–TX 30 in the range of concentrations that inhibited collagen-induced platelet aggregation (control group: 31.20 ± 3.8%, DT-TX 30 at 0.1 μM: 10.71 ± 0.55%, at 1.0 μM: 6.53 ± 0.44%, at 5.0 μM; 1.48 ± 0.07%). All three drugs reduced thrombus formation, although ASA (unlike dazoxiben or DT–TX 30) increased the percentage surface occupied by adhesions.

4 In conclusion, the effect of specific blockage of TxS together with blockage of membrane receptors for TxA_2 can surpass the effect of ASA in inhibiting the platelet-subendothelium interaction in flow conditions.

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Keywords: Thromboxane A_2; acetylsalicylic acid; dazoxiben; thromboxane synthetase inhibitors; thromboxane receptor antagonists; subendothelium

Abbreviations: A, adhesion; ADP, adenosine diphosphate; ASA, acetylsalicylic acid; C, contact; COX, cyclo-oxygenase; IC_{50}, inhibitory concentration 50%; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PCS, prostacyclin synthatase; PGE_2, prostaglandin E_2; PRP, platelet-rich plasma; T, thrombi; TxA_2, thromboxane A_2; TxS, thromboxane synthetase; WB, whole blood

Introduction

The arachidonic acid metabolic pathway is fundamental in the process of arterial thrombus formation, particularly in those products derived from the action of the enzyme cyclo-oxygenase (COX) on arachidonic acid. Among these products are thromboxane A_2 (TxA_2) – produced by the enzyme thromboxane synthetase (TxS) – which favours vasoconstriction and platelet aggregation (Needleman *et al*., 1976), and prostacyclin – which favours vasodilation and inhibits platelet aggregation (Bunting *et al*., 1976). These two substances are related *via* cyclic endoperoxide precursors which can translocate from platelets to endothelial cells, where they can serve as a substrate for PCS (Maguire & Wallis, 1983; Mayeux *et al*., 1989).

Pharmacological studies have used inhibitors of TxA_2 synthesis to diminish platelet activation as one way to prevent arterial thrombosis (Jones *et al*., 1983; Tayler *et al*., 1981). Cyclo-oxygenase inhibitors decrease TxA_2 and prostacyclin synthesis. On the other hand, TxS inhibitors selectively decrease TxA_2 synthesis and lead to the accumulation of cyclic endoperoxides that can enter the endothelium to form prostacyclin, but which can also bind to membrane receptors for other platelets, which thus become activated (Mayeux *et al*., 1988). Dual TxS inhibitors and TxA_2 receptor blockers represent an attempt to avoid this latter problem (Gresele *et*

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al., 1991). However, there is no consensus as to which of the pharmacological mechanisms summarized above is the most suitable for curtailing interactions between platelets and the wall of the damaged artery.

The aim of the present study was to compare the effects of three drugs that inhibit TxA_2 synthesis: acetylsalicylic acid (ASA) (a COX inhibitor), dazoxiben (a TxS inhibitor) and DT-TX 30 (a dual TxS inhibitor and TxA_2 receptor blocker). The experiments were done with a well characterized and recognized model for the study of platelet-vascular subendothelium interactions in flow conditions.

Methods

Whole blood for this *in vitro* study was obtained from healthy men (mean age 37.6 ± 1.5 years, range 19–47 years) who had not taken any medication for at least 15 days previously. Each subject gave his informed consent to participate in the study. Platelet-rich plasma was obtained by centrifugation of whole blood at $180 \times g$ for 10 min at 20°C. Leukocytes were obtained by centrifugation of whole blood on a Ficoll gradient and washing in phosphate-buffer saline (pH 7.4), followed by centrifugation at $1000 \times g$ for 15 min at 20°C.

Acetylsalicylic acid (Sigma Chemical Corp, St Louis, IL, U.S.A.), dazoxiben (Ferrer Internacional, Barcelona, Spain) and DT-TX 30 (E-6(4-(2-(4-chlorobenzosulfonyl-amine)-ethyl)fenyl)-6-(3-pyridil)-5-hexanoic acid) (Karl Thomae Institut, Biberach an der Riss, Germany), were incubated at different concentrations. Eight to 10 different samples were run in each of the experiments detailed below.

Platelet aggregometry

Platelet aggregation was measured both in platelet-rich plasma and in whole blood, with the electronic impedance method described by Cardinal & Flower (1980). We used a Chrono-Log 540 aggregometer (Chrono-Log Corp., Haverton, PA, U.S.A.) with ADP (2.5 μM), collagen (1 μg ml^{-1}), arachidonic acid (400 μM) and thrombin (0.5 IU ml^{-1}) (Menarini Diagnostica, Barcelona, Spain) to induce aggregation. Drugs were incubated at 37°C for 10 min before the aggregation inducer was added, and aggregation was recorded for 10 min. Maximum intensity of aggregation was quantified as the maximum change in electronic impedance in samples without the drug or a given concentration of each drug.

The aggregating agent concentrations were chosen according to previous experiments in which EC_{50} values were as follows: 2.10 ± 0.37 μM for ADP ($n = 10$), 1.10 ± 0.14 μg ml^{-1} for collagen ($n = 10$), 360 ± 50 μM for arachidonic acid ($n = 10$) and 0.43 ± 0.05 IU ml^{-1} for thrombin ($n = 10$).

Platelet production of thromboxane B_2 and prostaglandin E_2

Samples of platelet-rich plasma ($285 \pm 16 \times 10^9$ platelets l^{-1}) were stimulated with 1 μM calcium ionophore A 23187 for 3 min at 37°C, then 100 μM indomethacin was added to stop the reaction. The sample was centrifuged at $10{,}000 \times g$ and the amount of thromboxane B_2 (TxB_2) and prostaglandin E_2 in the supernatant was determined with an enzymoimmunoassay (Biotrak® RPN 220, Amersham International plc, Little Chalfont, Buckinghamshire, U.K.). The sensitivity of these methods was 3.6 pg ml^{-1} for thromboxane B_2 and 3.1 pg ml^{-1} for prostaglandin E_2; the within-assay variability for duplicate determinations was 2.8% and the between-assay variability was 9.7%.

Leukocyte production of 6-keto-$PGF_{1\alpha}$

Samples of platelets plus leukocytes ($6.5 \pm 0.3 \times 10^9$ leukocytes l^{-1}) were stimulated with 1 μM calcium ionophore A 23187 for 3 min at 37°C, then 100 μM indomethacin was added to stop the reaction. The sample was centrifuged at $10{,}000 \times g$ and the amount of 6-keto-$PGF_{1\alpha}$ (stable metabolite of prostacyclin) in the supernatant was determined with an enzymoimmunoassay (Biotrak® RPN 220, Amersham). The sensitivity of this method was 3.4 pg ml^{-1}, the within-assay variability for duplicate determinations was 2.0%, and the between-assay variability was 8.7%.

Platelet-subendothelium interaction

Blood perfusion studies were carried out in an annular chamber (Labotron S.A., Barcelona, Spain) according to a modification of the method described by Baumgartner & Haudenschild (1972) and Baumgartner & Muggli (1976). Briefly, vessel wall segments were obtained from New Zealand white male rabbits weighing 2–2.5 kg. Animals were anaesthetized with sodium pentobarbital (100 mg kg^{-1}) and their thoracic and abdominal aorta was removed, washed with cold phosphate-buffered saline (pH 7.4), dissected from adjacent fatty tissue, and cut into segments 1 cm in length, which were maintained in phosphate-buffered saline (pH 7.4). Artery segments were then turned inside-out, so that the endothelial surface was on the outside. Samples were incubated in a solution of α-chymotrypsin (0.4 mg ml^{-1} in a calcium-Tris buffer 0.1 mM at 37°C for 12 h, with continuous shaking, in order to completely remove the endothelial cell layer. The absence of endothelium was confirmed by scanning electron microscopic examination of three randomly selected artery samples from each animal. The remaining arterial segments were kept at −80°C before the perfusion studies, which were always carried out within 7 days after removal of the endothelium.

Everted aortic segments were mounted on the rod of the annular perfusion chamber and were exposed to 20 ml flowing human blood (previously treated with sodium citrate) for 10 min at 37°C (wall shear rate 800 s^{-1}). Drugs were previously incubated in blood samples for 5 min at 37°C. After exposure to blood, the vessel segments were rinsed in phosphate-buffered saline (pH 7.4), fixed in 2% glutaraldehyde for 15 min at room temperature, dehydrated in a graded series of alcohol and embedded in methacrylate inclusion medium JB4 (Agar Scientific Ltd., Stansted, Essex, U.K.). Blocks from each arterial segments were divided into six parts and 20-serial nonconsecutive axial slices 2 μm thick were cut (Ultramicrotome, C. Reichert, Jung, Austria) (total of 120 sections per arterial segment). The slices were stained with toluidine blue and examined with a Nikon Labophot-2 microscope ($\times 1250$ magnification) through which section images were projected and drawn onto a millimeter scale. A

semiautomatic image analysis system was used to evaluate platelet-subendothelium interactions. The following structures were distinguished, according to Baumgartner & Muggli (1976): *contact* (C), platelets that were attached to but not spread over the subendothelium; *adhesion* (A), platelets spread on and firmly bound to the subendothelium, forming layers less than 5 μm high; *thrombi* (T), platelet aggregates 5 μm high or more. The total covered surface was expressed as the percentage of subendothelium with C + A + T.

Just before and after the 10-min perfusion, blood samples were drawn for platelet counts and tromboxane B_2 measurement (the sample was centrifuged at $10{,}000 \times g$).

All evaluations were done by investigators who were blind to the origin of each sample.

Statistical analysis

All data in the text, tables and figures are the mean ± s.e. of the mean of all values for each experiments. The results were tested with one-way analysis of variance followed by the Minimal Significance test. All analyses were done with version 10.0 of the SPSS program (SPSS Co, Chicago, IL, U.S.A.). The minimum value used to establish statistical significance was $P < 0.05$.

Results

All three drugs inhibited platelet aggregation in a concentration-dependent manner, both in whole blood and platelet-rich plasma, and regardless of whether ADP, collagen or arachidonic acid was used as the inducer. When thrombin was the inducer, only DT-TX 30 inhibited the formation of aggregates. Figure 1 shows, by way of example, the inhibition curves for aggregates in whole blood induced with arachidonic acid. Table 1 summarizes the IC_{50} values or concentrations of each drug that inhibited 50% of the maximal aggregation obtained in samples incubated without drugs.

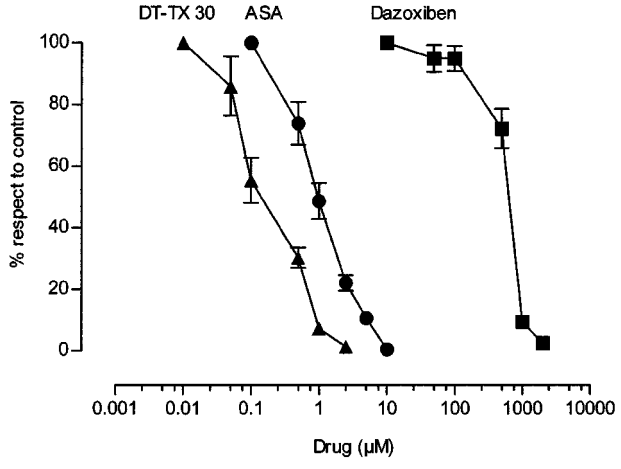

Figure 1 Concentration-effect curves of the inhibition of platelet aggregation induced in whole blood with 400 μM arachidonic acid, after treatment with acetylsalicylic acid (ASA), dazoxiben and DT-TX 30 ($n = 10$ samples in each experiment). Maximum aggregation intensity without drugs was 11.90 ± 1.13 ohms.

Table 1 Concentrations of acetylsalcylic acid (ASA), dazoxiben and DT-TX 30 (μM) that inhibited 50% (IC_{50}) maximum intensity of platelet aggregation in platelet-rich plasma (PRP) and whole blood (WB)

	ASA	*Dazoxiben*	*DT-TX 30*
ADP (2.5 μM)			
PRP	1884 ± 18	1280 ± 126	352 ± 24
WB	794 ± 19*	544 ± 51*	268 ± 41
Collagen (1 μg ml^{-1})			
PRP	339 ± 11	231 ± 9.2	49.24 ± 3.75
WB	8.42 ± 0.58**	267 ± 36	0.54 ± 0.02**
Arachidonic acid (400 μM)			
PRP	5.14 ± 0.22	745 ± 54	2.84 ± 0.13
WB	0.85 ± 0.04**	753 ± 59	0.30 ± 0.02**
Thrombin (0.5 IU ml^{-1})			
PRP	> 2000	> 2000	45.30 ± 3.25
WB	> 2000	> 2000	49.00 ± 5.13

*$P < 0.01$, **$P < 0.0001$ with respect to the value in PRP.

Prostanoid production differed in experiments with different drugs (Figure 2). Acetylsalicylic acid inhibited the production of thromboxane B_2 (IC_{50} 0.84 ± 0.05 μM), PGE_2 (IC_{50} 1.21 ± 0.08 μM) and 6-keto-$PGF_{1\alpha}$ (IC_{50} 6.58 ± 0.76 μM). Dazoxiben inhibited the platelet production of thromboxane B_2 (IC_{50} 765 ± 54 μM), did not reduce PGE_2 production ($IC_{50} > 2000$ μM), and increased the production of 6-keto-$PGF_{1\alpha}$. In comparison, DT-TX 30 inhibited thromboxane B_2 production (IC_{50} 8.54 ± 0.60 μM), did not reduce PGE_2 production ($IC_{50} > 2000$ μM), and increased the production of 6-keto-$PGF_{1\alpha}$.

In blood perfusion experiments in the annular chamber, all three drugs reduced the retention of platelets on the subendothelium and curtailed the increase in thromboxane after 10 min (Table 2). The percentage of subendothelial surface occupied by platelets was reduced after incubation with each of the drugs (Figure 3), a result that reflected mainly the decrease in the formation of thrombi (Figure 4). The formation of adhesions increased after incubation with ASA and decreased after incubation with dazoxiben and DT-TX 30 (Figure 4). Contacts were more numerous after incubation with dazoxiben and DT-TX 30 (Figure 4). The IC_{50} values for the percentage of subendothelial surface occupied by platelets were calculated graphically from the mean data for each group, and were 0.042 μM for DT-TX 30, 349 μM for dazoxiben and more than 300 μM for ASA.

The heights of platelet aggregates that accumulated on the subendothelium are shown in Table 3. Acetylsalicylic acid reduced the heights of adhesions and thrombi, and DT-TX 30 reduced all the three types of platelet structures that interacted with the subendothelial matrix.

Figure 5 shows representative examples of different types of platelet-subendothelial interactions seen in different experiments. These examples show that all three drugs led to an absolute reduction in the subendothelial surface occupied by platelets. However, in qualitative terms ASA reduced mainly thrombi, whereas DT-TX 30 reduced mainly adhesions and thrombi, such that contacts accounted for most of the subendothelial surface occupied by platelets. In these experiments the effect of dazoxiben was weaker than that of either ASAS or DT-TX 30.

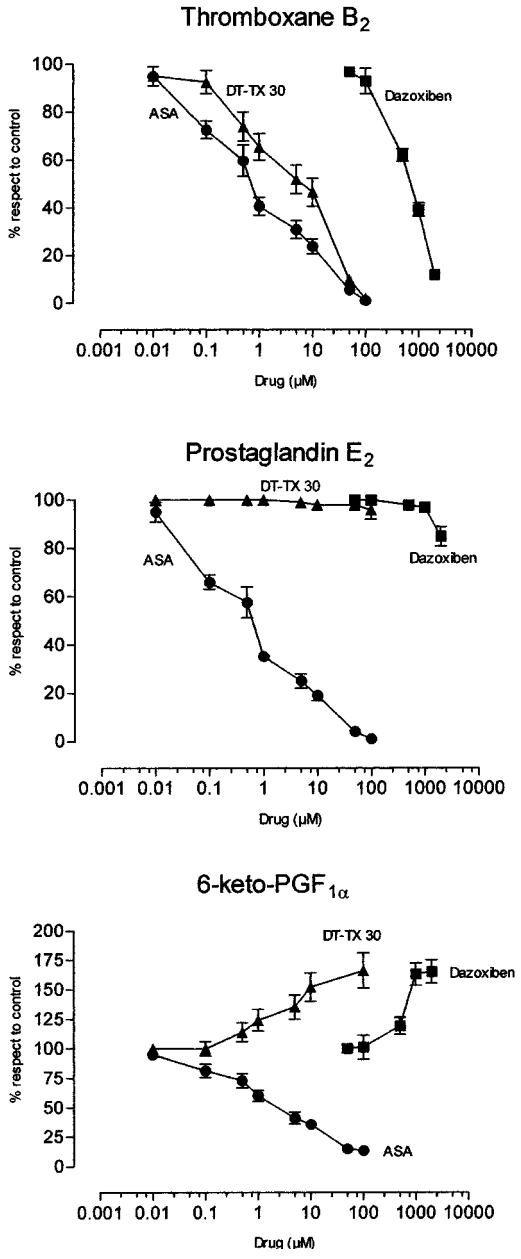

Figure 2 Production of platelet thromboxane B_2, platelet prostaglandin E_2 and leucocyte 6-keto-$PGF_{1\alpha}$ induced with 1 M calcium ionophore A23187 after incubation with acetylsalicylic acid (ASA), dazoxiben or DT-TX 30 ($n=10$ samples in each experiment). Basal values for incubation without drugs were thromboxane B_2 20.01 ± 3.31 nmol 10^{-9} platelets, prostaglandin E_2 10.01 ± 0.96 nmol 10^{-0} platelets, 6-keto-$PGF_{1\alpha}$ 108.0 ± 6.59 nmol 10^{-2} leucocytes.

Discussion

Our results show that the pharmacological inhibition of platelet TxA_2 synthesis is accompanied by inhibition of the platelet-subendothelium interaction in a perfusion system. However, there were differences in this effect depending on which enzyme was blocked, consequently the effects in our experimental model of blood flow in contact with the subendothelial matrix were also different.

The profile of antiplatelet aggregation activity of ASA was consistent with that predicted from earlier studies of how this drug behaves in whole blood (De La Cruz *et al*., 1986; 1987). In this connection, the antiaggregant effect of ASA has been shown to be greater in whole blood as a result of its ability to stimulate nitric oxide production in neutrophils (López-Farré *et al*., 1995; De La Cruz *et al*., 2000a). Moreover, it has been demonstrated that ASA stimulates constitutive nitric oxide production in the arterial wall (De La Cruz *et al*., 2002).

The specific inhibition of TxS by dazoxiben led to a smaller antiaggregant effect than that seen with ASA. In theory, the selective inhibition of TxS should yield a better antiplatelet profile than the nonselective inhibition of COX. In fact, experiments with ASA led to inhibition of thromboxane, PGE_2 and prostacyclin synthesis (Figure 2), whereas incubation with dazoxiben inhibited only thromboxane synthesis, but increased even prostacyclin production. The explanation of this effect of dazoxiben is based on the ability of cyclic endoperoxidases to act as a substrate for PCS and thus increase prostacyclin synthesis (Maguire & Wallis, 1983; Mayeux *et al*., 1989). However, endoperoxides are also able to bind to membrane TxA_2 receptors both on platelets and on the vascular wall (Hornby & Skidmore, 1982); the endoperoxide prostaglandin H_2, in fact, shows greater affinity for these receptors than does TxA_2 (Mayeux *et al*., 1988).

The pharmacological inhibition of TxS and simultaneous blockage of thromboxane receptors by DT-TX 30 (Guth & Muller, 1997; Meade *et al*., 1996) inhibited platelet aggregation in whole blood more strongly than did ASA. This effect probably resulted from the fact that accumulated cyclic endoperoxides cannot bind to thromboxane receptors, as these receptors are blocked by DT-TX 30. The effect of DT-TX 30 was also greater than that of other drugs in thrombin-induced aggregation, which was not inhibited by ASA or dazoxiben. This effect on thrombin-induced aggregation may support the earlier observation that DT-TX 30 prevented coronary artery thrombi in an experimental model with dogs (Guth & Muller, 1997), as in this process thrombin activation is fundamental to account for coronary thrombogenesis (Fuster *et al*., 1992). It is also consistent with previous experiments in streptozotocin-diabetic diabetic rats, in which DT-TX 30 prevented retinal ischaemic alterations in parallel with the increase in prostacyclin synthesis (De La Cruz *et al*., 2000b).

In experiments that compared the effects of drugs on the vascular subendothelium in a system of blood perfusion, platelets were retained on the subendothelium and platelet thromboxane production was stimulated as a result of the interaction of platelet with subendothelial collagen. Platelet retention (i.e., the number of platelets that interacted with the subendothelium) was reduced by all three drugs in this series of experiments. However, because we used concentrations in the range that inhibited collagen-induced platelet aggregation in whole blood (and because collagen is the main component of the subendothelial matrix), we found that the effect of DT-TX 30 was proportionally larger than that of ASA or dazoxiben. Moreover, the IC_{50} values for DT-TX 30 were lower than those that inhibited collagen-induced platelet aggregation in whole blood. The same pattern of relative effects was found in studies that investigated the stimulation of thromboxane synthesis, suggesting that the recruitment of new platelets was impaired as a result of stimulation by the

Table 2 Percentage change in blood platelet count and thromboxane B_2 (TxB_2) after blood perfusion for 10 min (shear stress 800 s^{-1}) in the Baumgartner annular chamber

	Change in platelet count after blood perfusion (percentage referred to pre−perfusion value)	*Change in TxB_2 value (percentage referred to pre−perfusion value)*
Control	−34.93±2.21	+64.00±1.75
ASA (μM)		
1	−18.57±1.94*	+10.56±1.10**
10	−12.14±1.10*	+7.19±0.65**
50	−11.22±0.96*	+6.64±0.71**
Dazoxiben (μM)		
100	−15.97±1.31*	+18.83±2.11*
250	−12.06±1.26*	+12.33±0.85**
500	−9.76±1.30**	+8.94±0.91**
DT=TX 30 (μM)		
0.1	−19.21±2.06*	+4.35±0.51**
1	−4.41±0.22**	+2.91±0.38**
5	−0.06±0.01**	+0.68±0.05**

*$P<0.01$, **$P<0.0001$ with respect to the control value.

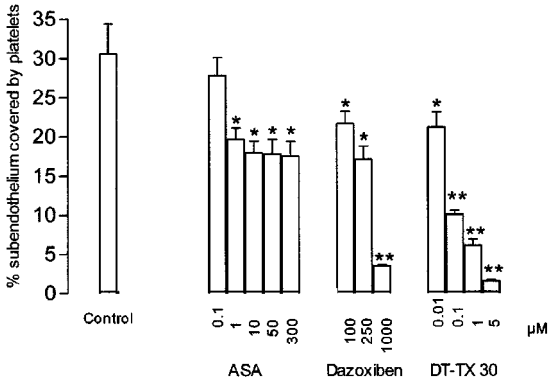

Figure 3 Percentage of the subendothelial matrix surface occupied by platelets after blood perfusion for 10 min at 37°C at a shear stress of 800 s^{-1} in the absence (control) or the presence of acetylsalicylic acid (ASA), dazoxiben or DT-TX 30. ($n=8$ to 10 samples per experiment. *$P<0.01$, **$P<0.0001$ in comparison to control assays.

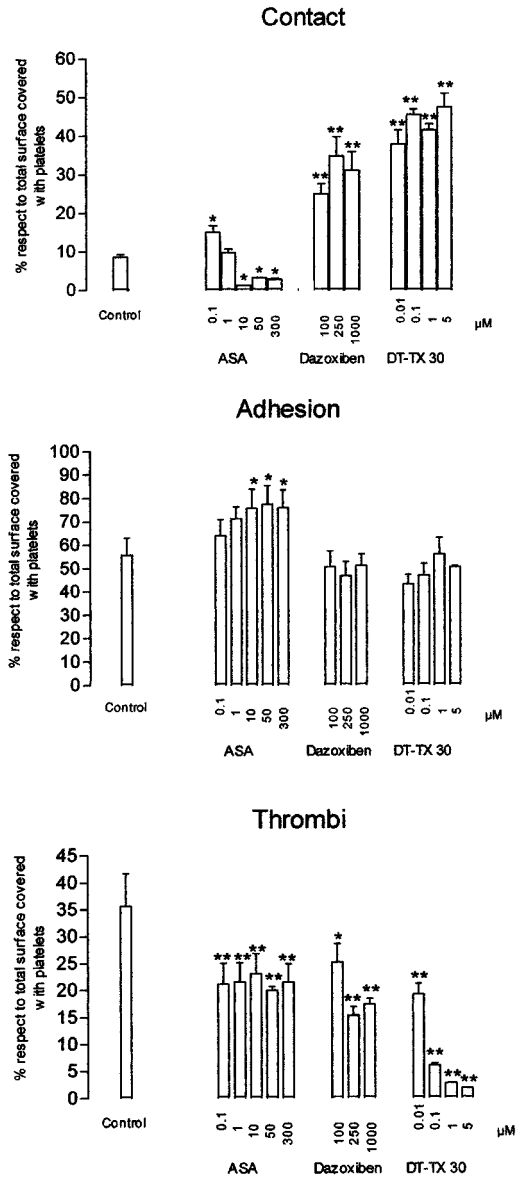

Figure 4 Percentage of the subendothelial matrix surface occupied contacts, adhesions or thrombi (referred to total surface covered with platelets) after blood perfusion for 10 min at 37°C at a shear stress of 800 s^{-1} in the absence (control) or the presence of acetylsalicylic acid (ASA), dazoxiben or DT-TX 30 ($n=8$ to 10 samples per experiment. *$P<0.01$, **$P<0.0001$ in comparison to control assays.

thromboxane released by platelets that had been previously activated by the subendothelium (Pérez-Requejo *et al*., 1985).

All three drugs reduced the subendothelial surface occupied by platelets, although in the range of concentrations that inhibited collagen-induced aggregation in whole blood, DT-TX 30 showed a greater proportional effect than ASA or dazoxiben. This finding was logical in view of the fact that DT-TX 30 had the hardest effect on platelet retention on the subendothelial matrix during blood perfusion. In experiments with the same model as we used, Escolar *et al*. (1998) found that blocking platelet thromboxane receptors impaired the platelet-subendothelium interaction to a greater extent than did ASA, an observation compatible with our present findings. However, the specific IC_{50} values in this test were lower than those obtained in platelet aggrometry experiments. Acetylsalicylic acid did not produce 50% inhibition at any of the concentrations tested, a result in line with previously published findings (Sakariassen *et al*., 2001).

The decrease in the subendothelial surface occupied by platelets reflected mainly a decrease in the formation of larger structures (thrombi); again, the effect was greatest with DT-TX 30. This effect was seen not only as a reduction in the percentage of subendothelial surface occupied by thrombi, but also as a reduction in the height of the structures that formed. Extrapolation of these observations to the blood stream of living organisms would imply less occupation of the vessel lumen by thrombi. When we compared the effects of the drugs on different structures (Figure 5), we found that ASA decreased the surface area covered by thrombi but increased the percentage surface covered by adhesions, in agreement with earlier studies (Baumgartner, 1979; De La

Table 3 Mean heights (μm) of the different types of platelet structures in relation with the subendothelium after blood perfusion for 10 min (shear stress 800 s^{-1}) in the Baumgartner annular chamber

	Total	*Contacts*	*Adhesions*	*Thrombi*
Control	3.93±0.40	1.87±0.17	3.28±0.13	7.21±0.32
ASA (μM)				
1	3.79±0.10	1.76±0.03	2.72±0.29*	6.76±0.65
10	3.36±0.29	1.73±0.16	2.77±0.09*	5.59±0.14*
50	3.32±0.25	1.75±0.11	2.73±0.16*	5.50±0.21*
Dazoxiben (μM)				
100	3.77±0.31	1.87±0.18	2.97±0.27	6.81±0.38
250	3.34±0.18	1.82±0.16	2.84±0.04*	5.72±0.16*
500	2.70±0.20*	1.50±0.10*	2.27±0.02**	5.18±0.45**
DT-TX 30 (μM)				
0.1	2.78±0.24	1.03±0.04**	2.22±0.09**	5.10±0.48*
1	2.79±0.21	1.14±0.05**	2.19±0.07**	5.08±0.54*
5	2.65±0.21*	1.00±0.20**	2.16+0.27**	5.11±0.07*

*$P<0.01$, **$P<0.0001$ with respect to the control value.

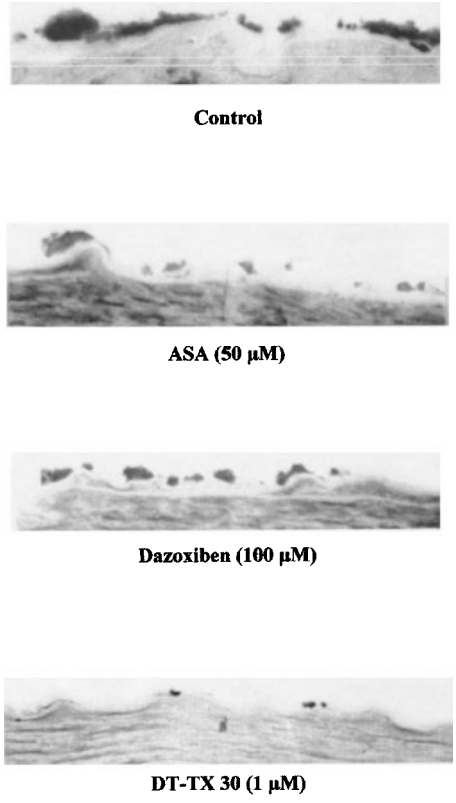

Figure 5 Representative examples of platelet-subendothelium interactions after blood perfusion for 10 min at 37°C at a shear stress of 800 s^{-1} in the absence (control) or the presence of acetylsalicylic acid (ASA) dazoxiben or DT-TX 30. ($n=8$ to 10 samples per experiment. Toluidine blue staining, ×25.

Cruz *et al*., 1995; Escolar *et al*., 1998). Several hypotheses might help explain this finding. Firstly, as a result of a physical mechanism, the formation of fewer aggregates might give rise to a rheological situation that allows platelet aggregates to accumulate and spread to form adhesions (Baumgartner, 1979). DT-TX 30 also decreased the formation of thrombi, but did not increase the formation of adhesions, possibly because its greater antiplatelet effect in comparison to ASA led to more contacts (inactivated platelets) than adhesions. This hypothesis is supported by the finding that thrombi in subendothelial preparations treated with DT-TX 30 were thinner than those in experiments with ASA (Table 3). Secondly, a biochemical mechanism might operate such that ASA does not totally annul thromboxane synthesis (Terres *et al*., 1992); as a result prostanoids might stimulate the production of smaller or weaker platelet structures such as adhesions. By blocking membrane receptors for thromboxane, DT-TX 30 might prevent thromboxane from favouring the formation of adhesions. Moreover, the thromboxane released by activated platelets in the perfusion system would not only recruit additional platelets, but would also help stabilize platelet–platelet and platelet-subendothelial collagen interactions (Wagner & Hubbell, 1992). This could also account for the greater effect of DT-TX 30 in our experimental system.

In summary, despite the clinical effectiveness of ASA in the secondary prevention of thrombotic events, the specific dual effect of inhibition of TxS together with blockage of membrane receptors for thromboxane may shed new light on pharmacological aspects of the platelet-subendothelium interaction in perfusion systems that emulate the medium-calibre human artery.

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