Br. J. Pharmac. (1983), **79**, 863–868

The source of thromboxane and prostaglandins in experimental inflammation

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1 Although cyclo-oxygenase products have been detected at inflammatory sites the tissue of origin remains uncertain.

2 Inflammatory exudates were collected from rats 4, 6, 8, 12 or 24 h after subcutaneous implantation of carrageenin-impregnated sponges.

3 Concentrations of the cyclo-oxygenase products prostaglandin E_2 (PGE_2), 6-oxo-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) in inflammatory exudates and serum (obtained from blood clotted at 37° C) were measured by specific radioimmunoassays.

4 TXB_2 concentrations in exudates increased to about 100 ng ml^{-1} at 8 h but decreased to less than 20 ng ml^{-1} after 24 h. PGE_2 concentrations increased from 4–12 h and remained between 80 and 120 ng ml^{-1} from 12–24 h. 6-oxo-$PGF_{1\alpha}$ had the same time course as that of PGE_2 but concentrations were approximately one third of PGE_2 values.

5 TXB_2 concentrations in serum from thrombocytopaenic rats were less than 5% of control values. Thrombocytopaenia did not affect TXB_2, PGE_2 or 6-oxo-$PGF_{1\alpha}$ concentrations or total leukocyte numbers in inflammatory exudates.

6 Methotrexate-induced neutropaenia did not affect serum TXB_2 concentrations but cyclo-oxygenase products (including TXB_2) in 6 h inflammatory exudates were reduced by 60–95%.

7 Colchicine (1.0 mg kg^{-1} s.c.) prevented leukocyte accumulation in sponge exudates and this was accompanied by a reduction in TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ concentrations at 6 h.

8 These results indicate that platelets are the source of TXB_2 in clotting blood but do not contribute to cyclo-oxygenase activity in experimental inflammation. The results also suggest that migrating leukocytes are the major source of thromboxane and to a lesser degree prostaglandins in acute 6 h inflammatory exudates.

Introduction

Cyclo-oxygenase is a widely distributed enzyme which converts arachidonic acid to the cyclic-endoperoxide precursors of prostaglandins, thromboxanes and prostacyclin (for review see Samuelsson, Goldyne, Granstrom, Hamberg, Hammarstrom & Malmsten, 1978). These products have been detected in carrageenin-induced inflammation (Higgs & Salmon, 1979) and chronic joint inflammation in man (Brodie, Hensby, Parke & Gordon, 1980; Bombardieri, Cattani, Ciabattoni, Di Munno, Pasero, Patrono, Pinca & Pugliese, 1981) but it is not clear from which tissues they are derived. Thromboxane A_2 (TXA_2) is the major cyclo-oxygenase product in platelets (Hamberg, Svensson & Samuelsson, 1975) and circulating leukocytes (Morley, Bray, Jones, Nugteren & Van Dorp, 1979) whereas vascular endothelial cells produce mainly prostacyclin (Moncada, Gryglewski, Bunting & Vane, 1976). Macrophages (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977), granuloma tissues (Chang, Murota, Matsuo & Tsurufuji, 1976; Chang, Murota & Tsurufuji, 1977) and human synovial tissues (Salmon, Higgs, Vane, Bitensky, Chayen, Henderson & Cashman, 1982) produce both thromboxane and prostacyclin. Extra-vascular leukocyte accumulation is a characteristic of inflammation and these cells are a potential source of cyclo-oxygenase products in inflammatory exudates. The detection of thromboxane in inflammation may also indicate that platelets are involved. We have now investigated the tissue origin of prostaglandins and thromboxane in experimental inflammation.

Methods

Induction and collection of inflammatory exudates

Inflammatory exudates were collected from male rats (200–250 g) 4–24 h after the subcutaneous implantation of polyester sponges soaked in carrageenin (2% w/v in 0.9% sterile saline) (Higgs, Harvey, Ferreira & Vane, 1976). Sponges were implanted in groups of 5 animals and exudates were squeezed into 5 ml heparinized saline (5–10 units ml^{-1}). Total leukocyte numbers in exudates were estimated on a randomised blind basis using 'improved Neubauer' counting chambers and phase contrast microscopy. Differential leukocyte counts were performed on smears of exudates stained with haematoxylin and eosin. Drugs were administered orally, intraperitoneally or subcutaneously.

Blood samples

At the time of sponge removal, blood samples were taken from the abdominal aorta. Samples of blood (1 ml) were collected into glass test tubes and allowed to clot at 37°C for 40 min. Serum was removed from clotted blood following centrifugation at 1500 *g* for 15 min and cyclo-oxygenase products were assayed by radio-immunoassay (Patrono, Ciabattoni, Pinca, Pugliese, Castrucci, De Salvo, Satta & Peskar, 1980). Blood was also collected into tubes containing disodium edetate (EDTA) for determination of circulating blood cell numbers by conventional methods.

Radio-immunoassay

TXB_2, prostaglandin E_2 (PGE_2) and 6-oxo-$PGF_{1\alpha}$ concentrations in inflammatory exudates and serum were measured by specific radio-immunoassays (RIA) (Salmon, 1978; Higgs & Salmon, 1979) after dilution of exudate in buffer (1:10–1:100) but without prior extraction or chromatographic purification. In order to reduce the cross reaction of PGE_2 in the RIA for 6-oxo-$PGF_{1\alpha}$, exudates were treated with 1N NaOH for 1 h at 20°C, which converts E type prostaglandins to the corresponding PGB; the latter compounds exhibit minor cross-reactivity with the anti-6-keto-$PGF_{1\alpha}$ sera: consequently, this procedure makes the assay more specific. Prior to RIA for 6-keto-$PGF_{1\alpha}$ the base-treated samples were neut-

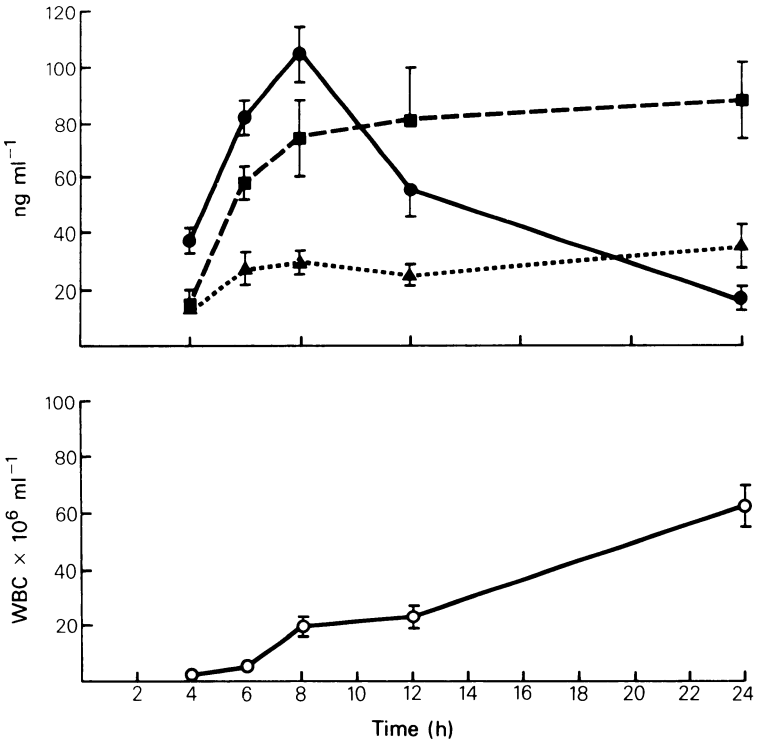

Figure 1 (a) Concentrations of prostaglandin E_2 (■), thromboxane B_2 (●) and 6-oxo-$PGF_{1\alpha}$ (▲) and (b) total leukocyte numbers (○) in inflammatory exudates collected from 4–24 h. Each point is the mean of 11–54 experiments and the bars represent s.e. mean.

ralized by addition of an equivalent amount of 1N HCl and then diluted 1:10 in assay buffer.

Anti-platelet serum

Suspensions of platelets were prepared following exsanguination of rats (350 g) from the abdominal aorta under sodium pentobarbitone anaesthesia (60 mg kg^{-1} i.p.). Platelet rich plasma was obtained from citrated whole blood (0.315% sodium citrate, final concentration) after high speed centrifugation for 2 min. Contaminating red cells were removed by centrifugation at 50 *g* for 5 min. During the preparation of platelet suspensions, platelets were washed using the method of Vargas, Radomski & Moncada (1982), with the following modifications. Platelet rich plasma was centrifuged at 100 *g* for 10 min and the plasma was removed. The platelets were then washed three times in sterile Tyrode solution containing 0.5% heparin and prostacyclin 200 ng ml^{-1}. Final suspensions were adjusted to contain approximately 5×10^9 platelets ml^{-1}.

In order to raise antibodies to rat platelets, suspensions of platelets were mixed with equal volumes of Freund's complete adjuvant and given by divided injections into a foot pad and several sites on the back of New Zealand white rabbits (2.5 kg) (Ubatuba & Ferreira, 1976). After 20–30 days, booster doses of platelets mixed with incomplete adjuvant were given into the back at 14 day intervals. After the third booster dose, rabbits were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.v.) and exsanguinated from the carotid artery. The blood was allowed to clot at room temperature for 24 h after which it was centrifuged and the serum collected. Serum was decomplemented by heating at 56°C for 30 min. The effectiveness of the rabbit anti-rat platelet serum (APS) was tested by estimating circulating platelet numbers 5 min–24 h after the injection of 300 µl APS into the tail veins of groups of 10 rats. Similar groups were treated with normal rabbit serum.

Neutropaenia

Neutropaenia was induced in groups of rats following intraperitoneal injections of methotrexate (Lederle; 2.5 mg kg^{-1}) on three consecutive days (Spector & Willoughby, 1968). The numbers of circulating neutrophils were monitored from 6 to 10 days after the first injection of methotrexate.

Results

Concentrations of TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ in inflammatory exudates were measurable 4 h after sponge implantation. TXB_2 concentrations increased to a maximum at about 8 h but then subsided to relatively low levels at 24 h (Figure 1). PGE_2 and 6-oxo $PGF_{1\alpha}$ concentrations increased up to 24 h and remained high from 12–24 h (Figure 1). From 12 h, PGE_2 was the major cyclo-oxygenase product present. Total leukocyte numbers increased from 4–24 h and at all times the predominant cell present was polymorphonuclear (> 98%). The effects of drug treatment on cyclo-oxygenase products in exudates were measured at 6 h as this was a convenient time when the three products were at near maximal concentrations (Figure 1).

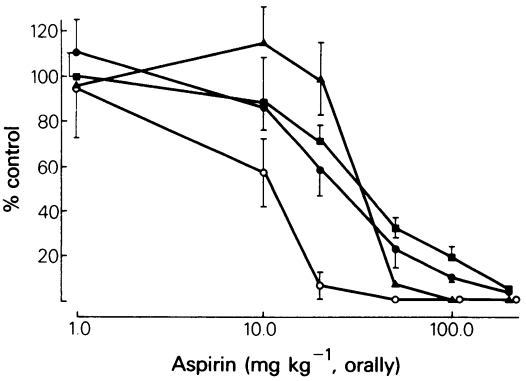

Figure 2 The effect of aspirin on the concentration of prostaglandin E_2 (■), thromboxane B_2 (●) and 6-oxo-$PGF_{1\alpha}$ (▲) in inflammatory exudates collected at 6 h, and on the concentrations of thromboxane B_2 (○) in serum samples collected from the same animals. Results are expressed as a percentages of control values obtained from animals receiving vehicle alone. Each point is the mean of 5 experiments and the bars represent s.e. mean; *indicates $P < 0.05$ compared to controls.

Serum obtained from blood clotted at 37°C contained 174.8 ± 8.6 ng TXB_2 ml^{-1} (mean ± s.e. mean; $n = 119$) and 16.7 ± 1.8 ng PGE_2 ml^{-1} ($n = 40$). 6-oxo-$PGF_{1\alpha}$ was not detectable (< 5.0 ng ml^{-1}) in serum.

Aspirin (Sigma; 1–200 mg kg^{-1}, orally), given at the time of sponge implantation, caused a dose-dependent reduction in TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ concentrations in inflammatory exudates collected at 6 h. Aspirin also inhibited TXB_2 production in clotting blood and was more potent in reducing the concentration of cyclo-oxygenase products in serum than in inflammatory exudates (Figure 2).

Thrombocytopaenia

Injection of anti-platelet serum caused an immediate and selective thrombocytopaenia in which circulating platelet numbers were reduced to less than 5% of control values 5 min–24 h after injection. Anti-

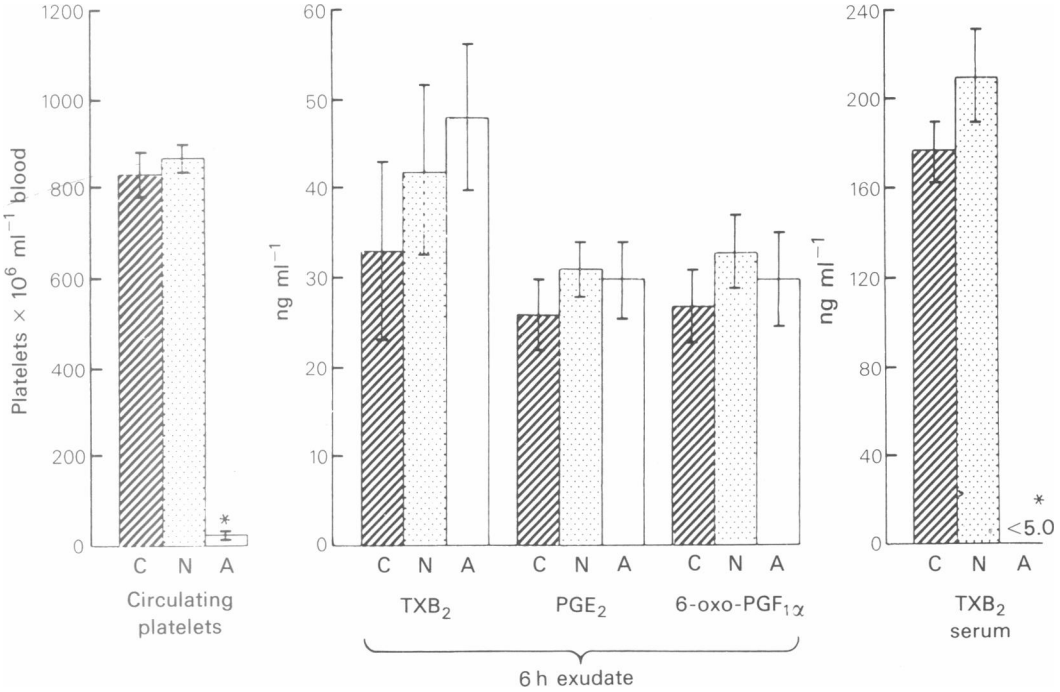

Figure 3 The effects of thrombocytopaenia on the concentrations of cyclo-oxygenase products in serum and 6 h inflammatory exudates. The histograms show the numbers of circulating platelets, serum thromboxane (TXB_2) concentrations and concentrations of TXB_2, prostaglandin E_2 (PGE_2) and 6-oxo-$PGF_{1\alpha}$ in exudates from control animals (C), animals injected with normal rabbit serum (N) and animals injected with anti-platelet serum (A). The results are expressed as mean values from five animals in each group in the same experiment and the bars represent s.e. mean; *indicates $P < 0.05$ compared to controls.

platelet serum had no effect on the numbers of circulating blood cells. Injection of normal rabbit serum did not cause thrombocytopaenia or any other haematological changes.

Serum from thrombocytopaenic rats did not contain any detectable TXB_2($< 5.0\,ng\,ml^{-1}$) whereas TXB_2 concentrations in serum from animals receiving normal rabbit serum did not differ from control values (Figure 3). Inflammatory exudates collected from thrombocytopaenic rats at 6 h contained the same concentrations of TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ as control exudates (Figure 3). Thrombocytopaenia had no effect on the numbers of leukocytes in inflammatory exudates.

Neutropaenia

Methotrexate caused a selective neutropaenia 7–9 days after the first intraperitoneal injection. Other circulating blood cells were not significantly affected. The total numbers of leukocytes in inflammatory exudates collected at 6 h from neutropaenic animals were reduced to less than 5% of control values. Methotrexate treatment did not reduce TXB_2 concentrations in serum but in 6 h inflammatory exudates from neutropaenic animals the concentration of cyclo-oxygenase products was 60–95% lower than controls (Figure 4).

Effects of colchicine

Colchicine (BDH; $1.0\,mg\,kg^{-1}$ s.c.) prevented leukocyte accumulation in 6–24 h sponge exudates without causing neutropaenia. Colchicine did not reduce TXB_2 concentrations in serum but in 6 h sponge exudates TXB_2 concentrations were less than 5% of control values. PGE_2 concentrations were reduced to $36 \pm 5\%$ ($n = 10$) of controls and 6-oxo-$PGF_{1\alpha}$ concentrations were reduced to $57 \pm 7\%$ of control values. Colchicine ($0.05–1.0\,mg\,kg^{-1}$, s.c.) caused a dose-dependent decrease in both TXB_2 concentrations and total leukocyte numbers in 6 h exudates (Figure 5).

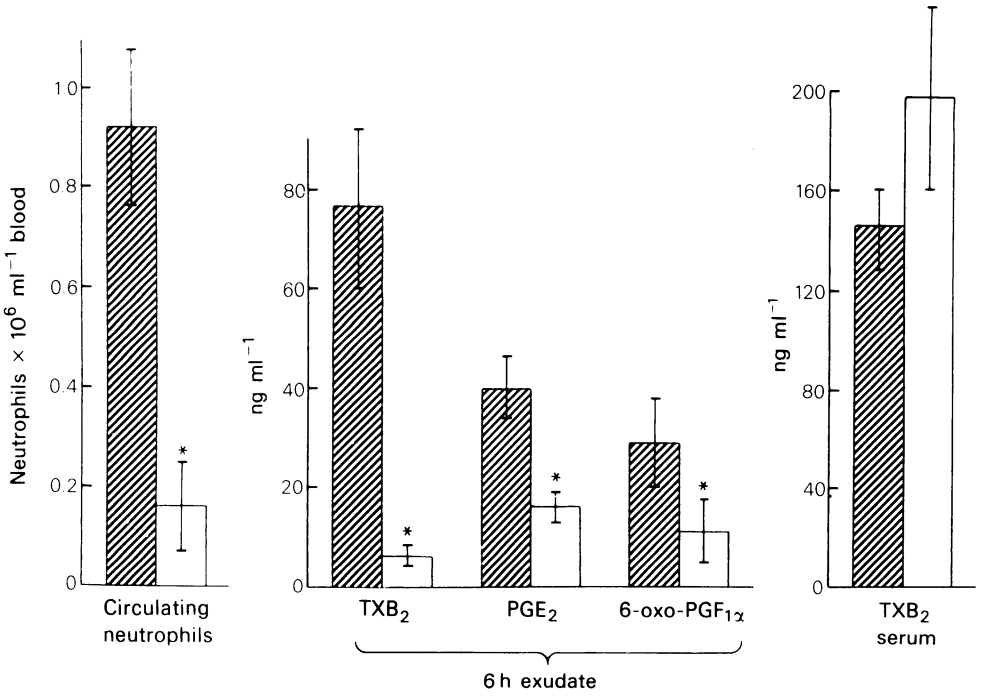

Figure 4 The effects of methotrexate-induced neutropaenia on the concentrations of cyclo-oxygenase products in serum and 6 h inflammatory exudates. The histograms show the number of circulating neutrophils, serum thromboxane (TXB_2) concentrations and concentrations of TXB_2, prostaglandin E_2 (PGE_2) and 6-oxo-$PGF_{1\alpha}$ in exudates from control animals (hatched columns) or neutropaenic animals (open columns). Each histogram is the mean of 5–30 experiments and the bars represent s.e. mean; *indicates $P < 0.05$ compared with control values.

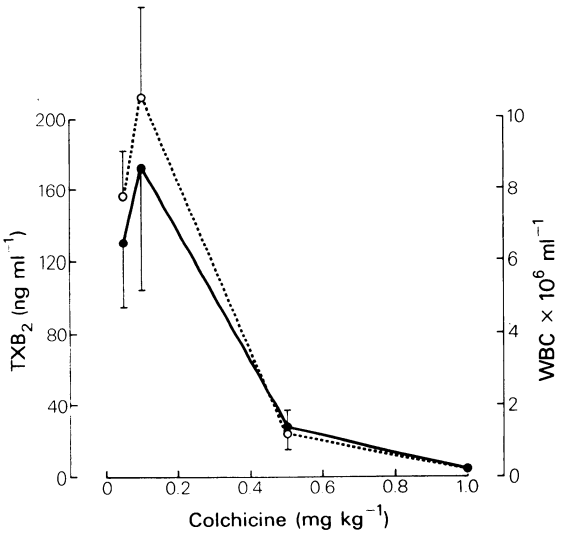

Figure 5 The effects of colchicine on thromboxane B_2 (TXB_2; ●) concentrations and total leukocyte numbers (WBC; ○) in 6 h inflammatory exudates. Each point is the mean of five experiments and the bars represent s.e. mean.

Discussion

The results presented in this paper confirm that PGE_2, TXB_2 and 6-oxo-$PGF_{1\alpha}$ are produced in carrageenin-induced inflammation and that thromboxane production follows a different time course from the other cyclo-oxygenase products (Higgs & Salmon, 1979). The oral administration of aspirin results in a significantly greater reduction of thromboxane concentration in serum than in inflammatory exudates (Figure 2). This suggests that thromboxane in serum is not derived from the same source as thromboxane in inflammatory exudates.

It has been proposed that platelets play a role in the development of inflammation by generating oxygenation products of arachidonic acid (Silver, Bills & Smith, 1978) although there is evidence that the inflammatory response is not diminished in thrombocytopaenic animals (Ubatuba, Harvey & Ferreira, 1975). The detection of thromboxane in inflammatory exudates has been offered as support for the theory that platelets are an important source of cyclo-oxygenase in inflammation (Silver *et al.*, 1978). However, the present experiments clearly indicate that thrombocytopaenia does not affect cyclo-

oxygenase activity in inflammation but leads to a complete inhibition of thromboxane synthesis in serum. It can be concluded therefore, that platelets are the source of thromboxane in clotting blood but not in carrageenin-induced inflammatory exudates. It is also clear that the presence of platelets does not influence migration in this model.

Polymorphonuclear (PMN) leukocytes produce prostaglandins (Higgs, McCall & Youlten, 1975) and TXA_2 (Higgs, Bunting, Moncada & Vane, 1976) and there is evidence that thromboxane is the predominant cyclo-oxygenase product synthesized by all types of circulating leukocyte (Morley *et al.*, 1979). A characteristic of acute inflammation is the emigration of PMN leukocytes into inflamed tissues. When leukocyte migration in this model of inflammation is prevented by inducing neutropaenia or by treating with colchicine the concentration of cyclo-oxygenase products in sponge exudates is reduced. This cannot be explained by a direct inhibition of the enzyme as thromboxane concentrations in serum from the same animals are not affected by methotrexate or colchicine. Thromboxane concentrations in exudates correspond closely to total leukocyte numbers (Figure 5) suggesting that these cells are the major source of thromboxane in this response. The lesser effects on PGE_2 and 6-oxo-$PGF_{1\alpha}$ concentrations suggest that other cells or tissues also contribute to the synthesis of these products.

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(Received November 16, 1982
Revised February 23, 1983)