The role of xanthine oxidase in platelet activating factor induced intestinal injury in the rat

X W Qu, R A Rozenfeld, W Huang, G B Bulkley, W Hsueh

Abstract

***Background*—Xanthine oxidase (XO) is an important source of reactive oxygen species in the small intestine.**

***Aims*—To examine the interaction of platelet activating factor (PAF), XO, and neutrophils in mediating intestinal injury in rats.**

***Methods*—Two doses of PAF were used to induce either reversible hypotension, or irreversible shock with intestinal necrosis. The activities of XO, and its precursor xanthine dehydrogenase (XD), in both the whole intestinal tissue and epithelial cells, were measured. XO was localised by histochemical staining.**

***Results*—PAF dose dependently induced an increase in XO activity, predominantly in the ileal epithelium, without altering the total activity of XD+XO. Most of the XD to XO conversion was via proteolysis. PAF induced XO activation and intestinal injury were prevented by prior neutrophil depletion. PAF induced XO activation is probably not due to reperfusion, as XO activation preceded the recovery of mesenteric flow. Allopurinol pretreatment substantially inhibited intestinal neutrophil sequestration induced by high dose (but not low dose) PAF.**

***Conclusions*—PAF rapidly activates intestinal XO through proteolytic XD-XO conversion, predominantly in the ileal epithelium. This effect is mediated by neutrophils. XO activation promotes PAF induced polymorphonuclear leucocyte sequestration in the intestine.**

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Keywords: xanthine dehydrogenase; reactive oxygen species; leucocyte adhesion; neutrophils; shock

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There is a large body of evidence linking the production of reactive oxygen species (ROS) to the pathophysiology of tissue damage associated with the inflammatory response. One of the major in vivo sources of ROS is xanthine oxidase (XO).[1] Xanthine dehydrogenase (XD), the precursor of XO, is constitutively expressed as a 150 kDa protein[2] which catalyses the conversion of hypoxanthine to xanthine and subsequently to uric acid, coupled with the reduction of NAD^+ to NADPH. XD can be transformed into XO either reversibly by oxidation of free thiol groups, or irreversibly by limited proteolysis.[3] It has been shown that XD to XO conversion occurs during ischaemia.[1] As XO preferentially uses molecular oxygen to NAD^+ as the electron receptor and thereby generates superoxide, XD to XO conversion appears to play a role in reperfusion injury.[1] This hypothesis is substantiated by the observation that ischaemia/reperfusion (I/R) induced damage in many organs is ameliorated by the administration of superoxide dismutase (SOD), or allopurinol, a specific inhibitor of XO.[4 5] An important site of this conversion is hypothesised to be the microvasculature, where the generation of superoxide and other ROS results in leucocyte-endothelial adhesion and consequent tissue injury.[1]

Despite the well established role of XO in initial I/R injury, questions remain as to whether this enzyme is involved in other inflammatory states. XD/XO is especially abundant in the small intestine, where it is expressed predominantly in the villus epithelium.[6] Using a model of acute inflammatory intestinal injury induced by platelet activating factor (PAF), an endogenous phospholipid mediator, we found that the injury could be significantly attenuated by administration of allopurinol.[7] This observation implies a role for XO in PAF induced intestinal injury. However, the mechanism by which PAF activates XO is unclear. The present study was designed to examine: (1) the impact of PAF administration on the XD-XO system in different segments of the intestine; (2) the cellular location of the activated XO in the small intestine; (3) the mechanism of XO activation; and (4) its relation to polymorphonuclear leucocytes (PMN).

Materials and methods

MATERIALS

All reagents were purchased from Sigma Chemical Co. (St Louis, Missouri, USA), except polyclonal antirat PMN antibody which was purchased from Accurate Chemical & Scientific Co. (Westbury, New York, USA).

ANIMAL EXPERIMENTS

Male Sprague-Dawley rats (80–120 g) were anaesthetised with Nembutal (65 mg/kg, intraperitoneally) and tracheotomised. The carotid artery and jugular vein were cannulated for continuous blood pressure monitoring, blood sampling, and drug administration. The animals were divided into five groups to receive one of

Abbreviations used in this paper: MPO, myeloperoxidase; PAF, platelet activating factor; PMN, polymorphonuclear leucocyte; ROS, reactive oxygen species; XD, xanthine dehydrogenase; XO, xanthine oxidase; DMSO, dimethylsulphoxide; WBC, white blood cell; DTT, dithiothreitol; SMA, superior mesenteric artery; PMSF, phenylmethylsulphonyl fluroide; I/R, ischaemia/reperfusion.

the following treatment regimens: (*a*) sham operated, sterile saline (vehicle) only; (*b*) PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) 2.8 or 1.5 μg/kg, intravenously; (*c*) allopurinol (10 mg/kg, 20 mg/ml in dimethylsulphoxide (DMSO), intravenously, 20 minutes before PAF) + PAF (2.8 or 1.5 μg/kg); (*d*) vehicle (DMSO, 0.5 ml/kg, intravenously, 20 minutes before PAF) + PAF (2.8 μg/kg); or (*e*) anti-PMN antibody pretreatment (5 ml/kg/day, intraperitoneally, for two days), followed by PAF (2.8 μg/kg).

Mean arterial blood pressure was monitored continuously throughout the experimental period. Packed cell volume and peripheral blood leucocyte (white blood cell, WBC) counts were measured before and 60 minutes after PAF injection. At the end of the experiment (15, 30, or 60 minutes after PAF injection), the animals were euthanised. (A few animals died before the end of the experiment, and were excluded from the study.) The small and large intestines were removed, and washed with ice cold phosphate buffered saline (PBS) containing dithiothreitol (DTT) 10 mM. Tissue sections were taken from grossly abnormal segments (multiple random sections were taken when no gross injury was found), fixed in formalin, processed, sectioned, and stained for assessment of histological injury by a blinded examiner. The histological severity score is defined as follows: no injury (0); minimal (0.5), with separation of surface epithelial cells from villi; mild (1.0), with necrosis limited to the tip of villi; moderate (2.0), with necrosis involving more than the top half of the length of the villi; severe (3.0), transmucosal necrosis. The injury score was calculated by multiplying the histological severity score of the segment with the percentage of involvement (length of injured segment divided by the total length of the small bowel).[8] The small intestine (jejunum and ileum) was separated from the large intestine (including ascending, transverse, and descending colon), and tissues were minced, snap frozen in liquid nitrogen, and stored at −70°C for enzyme assays. Handling of animals was in accordance with the guidelines of the Institutional Animal Care and Use Committee.

MEASUREMENT OF SUPERIOR MESENTERIC ARTERY BLOOD FLOW

Superior mesenteric artery (SMA) flow was measured in some animals in groups (*a*) and (*d*) using a T-101 blood flow meter (Transonic System Inc.), as previously described.[9]

ISOLATION OF INTESTINAL EPITHELIAL CELLS

Epithelial cells were isolated from the small intestine following a previously described procedure.[10] Briefly, the freshly collected small intestine was everted, cut into 1–2 cm segments, and incubated in a chelating buffer (Tris-sodium citrate 27 mM, Na_2HPO_4 5 mM, NaCl 96 mM, KH_2PO_4 8 mM, KCl 1.5 mM, D-sorbitol 55 mM, sucrose 44 mM, and DTT 0.5 mM, pH 7.3) at 4°C while stirring for one hour. The suspension was passed through a piece of cheese cloth, and centrifuged at 1000 *g* at 4°C for five minutes. The cell pellet was immediately frozen in liquid nitrogen and stored at −70°C.

XD/XO ACTIVITY ASSAY

Frozen intestinal tissue or epithelial cells were ground into fine powder with a pestle and mortar cooled with liquid nitrogen. The tissue/cell powder was suspended (0.1 g/ml) in a potassium phosphate buffer (100 mM, containing EDTA 1 mM, phenylmethylsulphonyl fluoride (PMSF) 3 mM, pH 7.8), and centrifuged at 40 000 *g* at 4°C for 30 minutes. An aliquot of the supernatant was mixed with a xanthine containing substrate (100 mM pyrophosphate buffer, containing 0.1 mM xanthine, 0.2 mM EDTA, pH 8.5) with or without 0.4 mM NAD^+,[11] and the rate of formation of uric acid over time was monitored by reading Ab_{295}. (In the absence of NAD^+, the production of uric acid reflects XO activity only. As XD relies on NAD^+ as the electron receptor for the reaction, the formation of uric acid in an NAD^+ containing system denotes the combined activity of XD and XO.) The assay was performed in duplicate or triplicate. XO activity was converted into units by referring to a standard curve constructed with serially diluted bovine xanthine oxidoreductase (from buttermilk), and normalised against either the weight of the tissue used, or the protein content in the sample determined using a Sigma total protein kit.

HISTOCHEMICAL LOCALISATION OF XO

A separate set of animals was used for histochemical studies. In these experiments the entire ileum was removed, rolled in a spiral, and frozen in liquid N_2 for sectioning and staining. This method allows histological examination of the entire ileum. The histochemical localisation of XO was carried out as previously described.[12] Briefly, 8 μm cryostat sections were incubated in incubation medium (100 mM Tris-maleate buffer, pH 8.0, containing 10 mM cerium chloride, 100 mM sodium azide, 0.5 mM hypoxanthine, and 10% polyvinyl alcohol) for 30 minutes at 37°C. The sections were then rinsed with hot distilled water (60°C) and further incubated in visualisation buffer (50 mM acetate buffer, pH 5.3, containing 42 mM cobalt chloride, 100 mM sodium azide, 1.4 mM diaminobenzidine, and 0.6 mM hydrogen peroxide) for 30 minutes at room temperature before embedding.

MYELOPEROXIDASE ASSAY

Myeloperoxidase (MPO) activity was assayed in ileal samples, as previously described.[8] Briefly, tissues were homogenised in 0.05 M potassium phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide and EDTA (5 mM), and sonicated. An aliquot was mixed with substrate (*O*-dianisidine HCl + H_2O_2 in potassium phosphate buffer) and the optical density read at 460 nm. The assay was done in duplicate or triplicate. A standard curve was constructed with serial dilutions of human MPO (Sigma).

PREPARATION AND CHARACTERISATION OF ANTI-XO ANTIBODY

Bovine xanthine oxidase (a kind gift from Professor Ian Mather, College Park, Maryland, USA) was purified from milk fat globule membrane, as previously described.[13] Briefly, extraction of fresh bovine milk with butan-1-ol was followed by precipitation with ammonium sulphate. This material was then subjected to preparative electrofocusing and chromatography on concanavalin A/agarose for protein separation and purification.[13] New Zealand White rabbits were immunised with a total of 2 mg of this purified XO in three injections, 10 days apart, at multiple sites near the axillary lymph nodes. The resulting immunoglobulin was affinity purified from the polyclonal serum on a Sepharose 4B column (Pharmacia, Piscataway, New Jersey, USA) derivatised with the purified XO described above.

The binding sensitivity of the polyclonal anti-XO has been confirmed in previous studies,[13] with the ability to detect XO at ng/ml and pg/ml concentrations. Analysis of the antibody by immunodiffusion (Ouchterlony method) gave rise to a single precipitation line when reacted against purified XO. This preparation produced a specific, high avidity polyclonal antibody which, at a 1/2000 dilution, still bound strongly to human, bovine, or rat XO. The Ouchterlony diffusion assay performed in a previous study using this same polyclonal antibody (immunodiffusion studies) confirmed the purity of this polyclonal antibody preparation, showing no evidence of IgG contamination.[13] Electrophoresis of protein precipitated by the XO antibody from human liver homogenate produced a gel pattern identical to that which has been reported for purified human XO by Krenitsky *et al*.[14] In this study the gel electrophoresis produced five discrete bands at 150, 135, 95, 55, and 38 kDa respectively. While most of the protein (representing intact XO) travelled with the 150 kDa band, the smaller fragments were thought to represent post-translational and subsequent preparative proteolysis. We observed an identical pattern.

WESTERN BLOTTING OF XO

Ileal epithelial cells were lysed with ice cold lysing buffer (2.0 mM Tris-HCl, pH 7.6, 30 mM NaCl, 1 mM EDTA, 0.2 mM benzamidine, 1 mM DTT, 1 mM PMSF, 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 1% Nonidet P-40) at 4°C with shaking. The cell lysate was centrifuged at 10 000 *g* for 10 minutes at 4°C after sonication for two minutes. The protein concentration of the supernatant was determined, and made homogenous by appropriate dilution with the lysing buffer. The protein in the sample was then resolved by electrophoresis on 7.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel.

Western blotting was performed by following the conventional procedure, with the above described anti-XO antibody (1/1000 diluted) and HRP labelled goat antirabbit IgG antibody (1/2000 diluted) at room temperature. The blot was detected with an ECL system (Amersham, Arlington Heights, Illinois, USA).

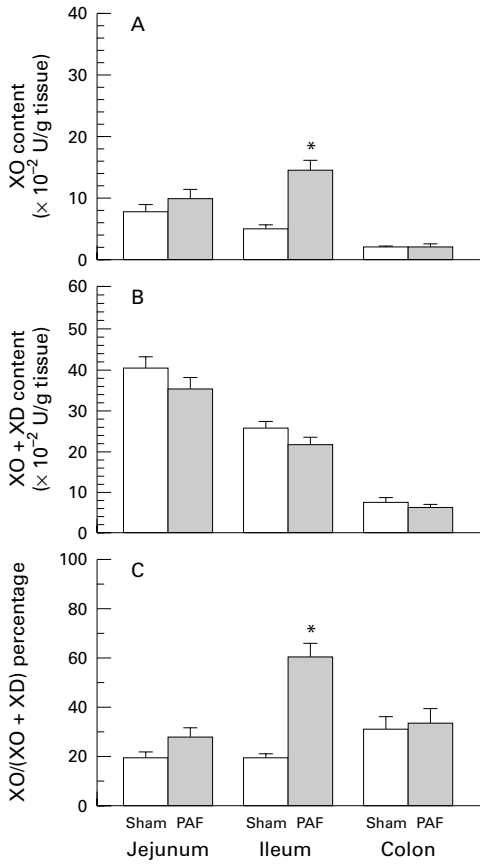

*Figure 1 The activity of XO (A), total xanthine oxidoreductase (XO+XD) (B), and the percentage of XO/(XD+XO) (C) in the jejunum, ileum, and colon in sham operated (n=5) and PAF (2.8 µg/kg) treated animals (n=7) at 60 minutes. *Significantly different (p<0.05) from sham operated group.*

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was used for the analysis of all data of multiple groups (Bonferroni method was used for the post-tests), and two sided Student's *t* test for the comparison of data of any two single groups. Data are presented as mean (SEM). The difference between groups was considered significant if the p value was less than 0.05.

Results

PAF INDUCED XD TO XO CONVERSION IN THE ILEUM

Figure 1 shows the activities of XO, XD+XO, and the percentage of XO in the total activity of tissue xanthine oxidoreductase (XD+XO). Xanthine oxidoreductase was found to be present constitutively within the small intestine, most abundantly in the jejunum, followed by the ileum, and was low in the large intestine (fig 1B). PAF administration (2.8 µg/kg) caused no significant change in the total xanthine oxidoreductase (XD+XO) activity in any of the intestinal segments (fig 1B), but induced a more than twofold increase in XO activity in the ileum (due to XD to XO conversion), within 60 minutes.

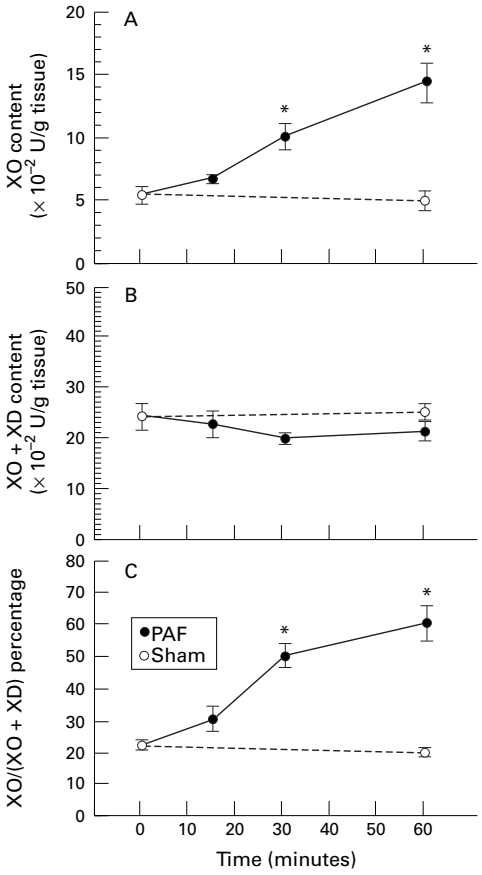

*Figure 2 The time course of PAF induced XO activation in the ileum. (A) XO activity; (B) total XO+XD activity; (C) percentage XO/(XD+XO). Open circles, sham operated animals (n=5); closed circles, PAF (2.8 µg/kg, injected at time 0) treated animals (n=5). *Significantly different from sham operated (p<0.05).*

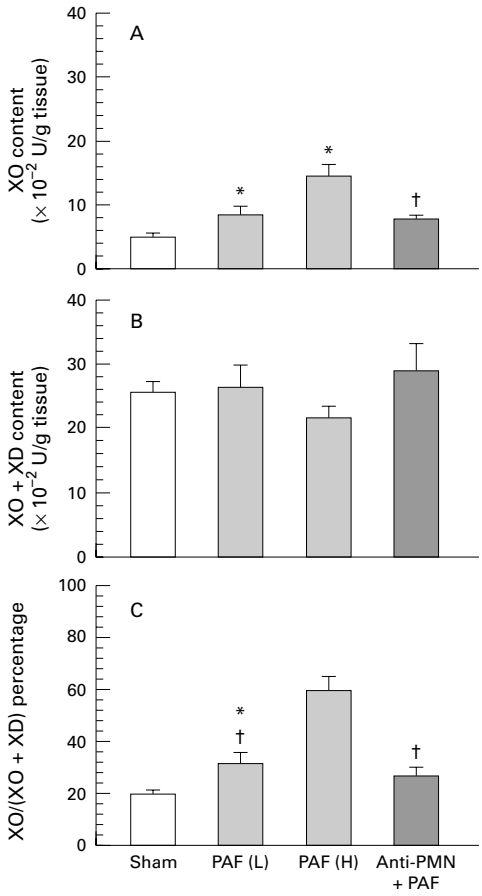

*Figure 3 PAF induced XD to XO conversion in the ileum and the preventive effect of PMN depletion. (A) XO activity; (B) total XO+XD activity; (C) percentage XO/(XD+XO). Sham operated group (n=5); PAF(H), 2.8 µg/kg (n=7); PAF(L), 1.5 µg/kg (n=5); PMN depleted rats injected with PAF (n=5). *Significantly different from sham operated group (p<0.05); †significantly different (p<0.05) from PAF (2.8 µg/kg) treated group.*

PAF INDUCED XO ACTIVATION IS TIME AND DOSE DEPENDENT

Figure 2 shows the time course of XO activation within the ileum after PAF injection. There was no significant change in either XO or total XD+XO activity in the sham operated group over the entire experimental period. PAF rapidly induced XO activation (fig 2A). An increase in XO activity (and of the percentage of the total xanthine oxidoreductase activity) became detectable at 15 minutes, and reached significance at 30 minutes (fig 2A,C). At 60 minutes, XO constituted over 60% of the total xanthine oxidoreductase activity in PAF treated animals, in contrast to approximately 20% in the control group (fig 2C). This effect of PAF appeared to be dose dependent (fig 3). PAF at the low (1.5 µg/kg) dose significantly elevated the XO content and XO/(XD+XO)% at 60 minutes (fig 3C); this effect almost doubled after administration of the high dose (2.8 µg/kg) (fig 3A,C).

LOCALISATION OF PAF INDUCED XO ACTIVATION

Histochemical studies indicated that the XO activity was localised predominantly in the villus epithelia in the normal ileum, indicated by faint staining (fig 4A). In contrast, crypt cells were unstained, indicating that XO was below detectable concentrations in the crypts. The intensity of staining (XO activity) did not notably change in sham operated rats throughout the entire experimental period (fig 4A). PAF (2.8 µg/kg) induced a mild but noticeable increase in XO staining intensity in the ileum at 15 minutes. However, the change was focal, observed only in some villi (fig 4B). At 30 minutes the epithelial cells of all villi were diffusely stained (fig 4C), and the staining became very intense at 60 minutes (fig 4D). The crypt cells remained negative throughout the experimental period. The specificity of the staining was confirmed by negative control sections which failed to stain in the presence of allopurinol in the reaction solution (fig 4E).

The histochemical localisation of XO activity in the epithelia was corroborated by the finding that XO activity was predominantly in the epithelial cell preparation, as shown in fig 5. The activity of XO and total XD+XO in ileal epithelial cells in control rats was approximately threefold higher than that of the whole tissue homogenate. The pattern of XO activation following PAF injection was almost identical in the whole tissue and the epithelial cells, suggesting that the main source of XO was the epithelial cells.

PAF INDUCED XO ACTIVATION RESULTED MAINLY FROM PROTEOLYSIS

In order to elucidate the mechanism of the PAF induced XO activation, we compared the XO activity from two assay systems, the standard procedure as stated earlier, and another containing 50 mM DTT to reverse the disulphide bond oxidation which leads to reversible, oxidative XD-XO conversion. Only a small difference (9.6 (6)%, n=4) was observed between the XO activity yielded by the two assay systems, suggesting that mechanisms other than thiol oxidation are involved in XD to XO conversion. Therefore, western hybridisation of ileal epithelial cells was performed to examine the role of the proteolytic pathway in XO activation (fig 6). As shown in the figure, PAF treatment resulted in an increase in two bands that reacted with anti-XO antibody: one at 150 kDa representing XO produced by thiol oxidisation, and another larger one at 40 kDa. The molecular weight (40 kDa) of the latter fits one of the proteolytic products of XD,[3] suggesting that the major pathway of XD to XO conversion is via proteolysis.

EFFECT OF PAF ON THE SYSTEMIC INFLAMMATORY RESPONSE AND INTESTINAL INJURY, AND THE PROTECTIVE EFFECT OF ALLOPURINOL AND PMN DEPLETION

Figures 7 and 8 show the effects of PAF, at low and high doses, on mean arterial pressure, superior mesenteric artery (SMA) blood flow, WBC count, packed cell volume, and intestinal injury. PAF at low dose (1.5 μg/kg) induced an immediate, severe hypotensive response (30–40 mm Hg), which partially reversed at 15 minutes and returned to near normal values at 30 minutes. PAF at this dose also caused haemoconcentration, mild leucocytosis, and minimal intestinal injury. At the high dose (2.8 μg/kg), PAF caused irreversible shock (fig 7). The initial hypotension was severe (around 30 mm Hg), and the recovery was slow and minimal: the blood pressure only reached an average of 56 mm Hg at 60 minutes. At the high dose used, PAF also caused a drastic reduction in SMA flow, which remained low throughout the experimental period without significant recovery (fig 7C). No change in SMA flow was observed in sham operated animals.

At the high dose (2.8 μg/kg), PAF induced notable haemoconcentration (fig 8B), leucope-

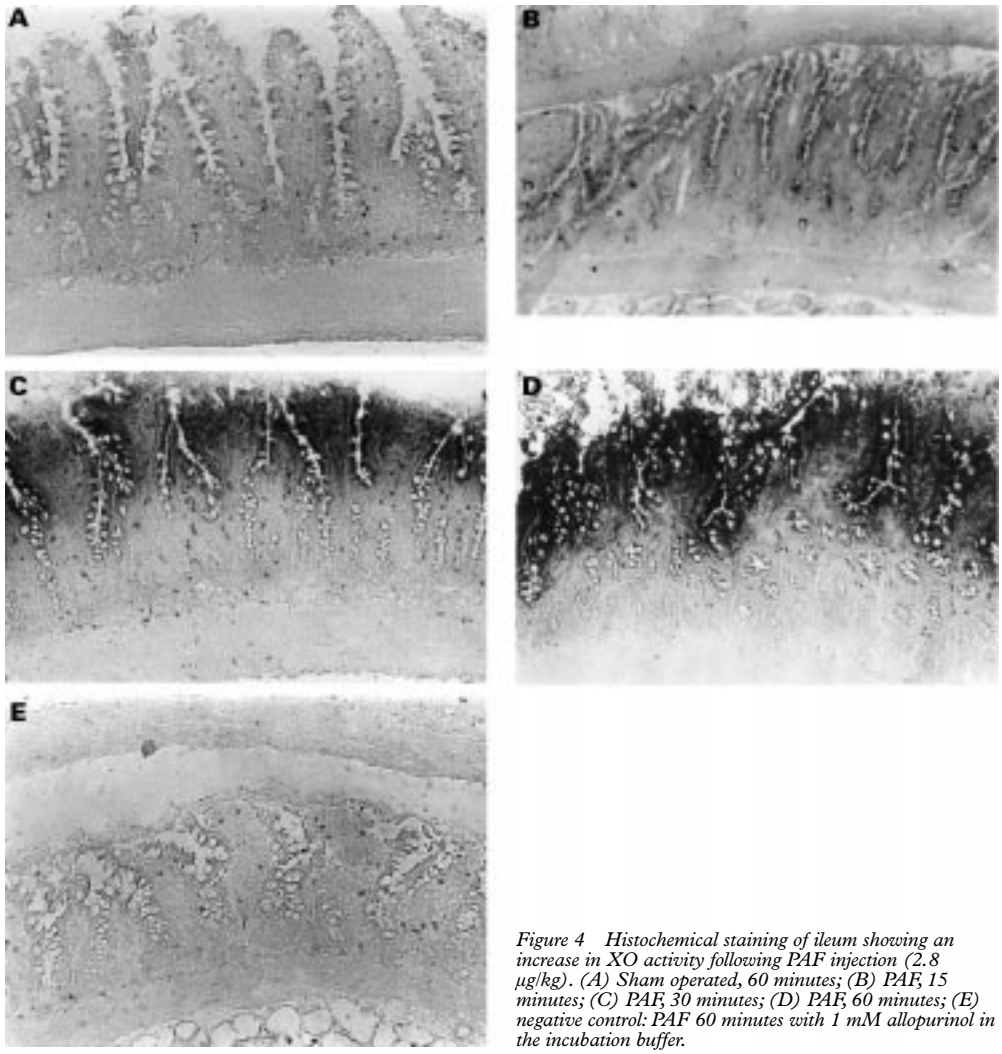

Figure 4 Histochemical staining of ileum showing an increase in XO activity following PAF injection (2.8 μg/kg). (A) Sham operated, 60 minutes; (B) PAF, 15 minutes; (C) PAF, 30 minutes; (D) PAF, 60 minutes; (E) negative control: PAF 60 minutes with 1 mM allopurinol in the incubation buffer.

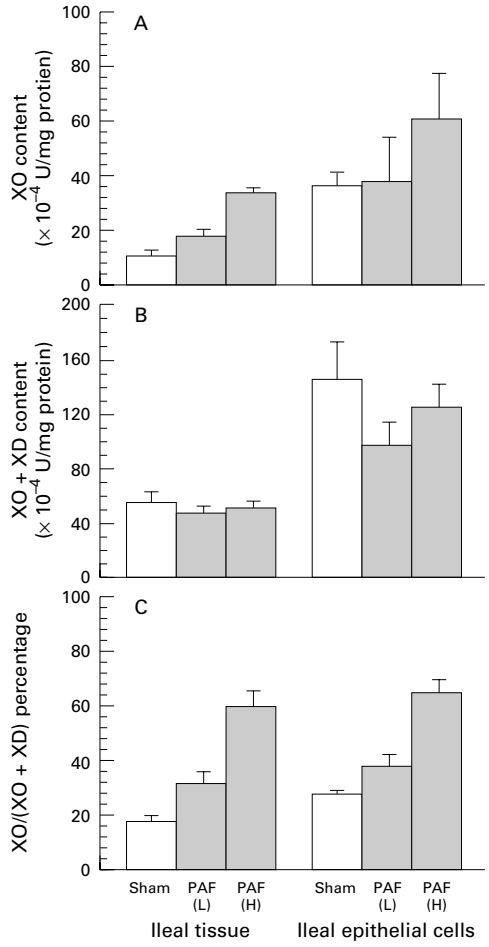

Figure 5 Comparison of activity of XO (A), total XO+XD (B), and percentage XO/(XO+XD) of ileal tissue homogenates and isolated ileal epithelial cells (C) in sham operated and PAF treated rats. For tissue homogenates: n=5 in sham operated; n=7 in PAF(H) (2.8 μg/kg); n=5 in PAF(L) (1.5 μg/kg). For epithelial cells: n=4 in all groups. In (A) and (B), there is a significant difference (p<0.05) between the values of tissue homogenates and epithelial cells in each group. No statistical difference was found between the values of tissue homogenates and epithelial cells in any of the three groups in (C).

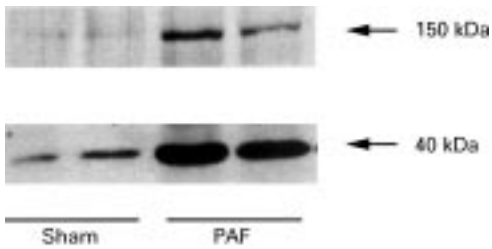

Figure 6 Western blot of XO in the epithelial cells isolated from the ileum of sham and PAF (2.8 μg/kg) treated animals.

nia (fig 8A), and severe intestinal injury (fig 8C). Interestingly, the injury was usually first noted at the ileum, often in the distal portion, where XD to XO conversion was most pronounced. With time the injury might expand to involve more areas or multiple segments. At the end of one hour, most animals had injury in the ileum, some animals developed necrosis in both the ileum and jejunum, but only rare animals had injury confined to the jejunum.

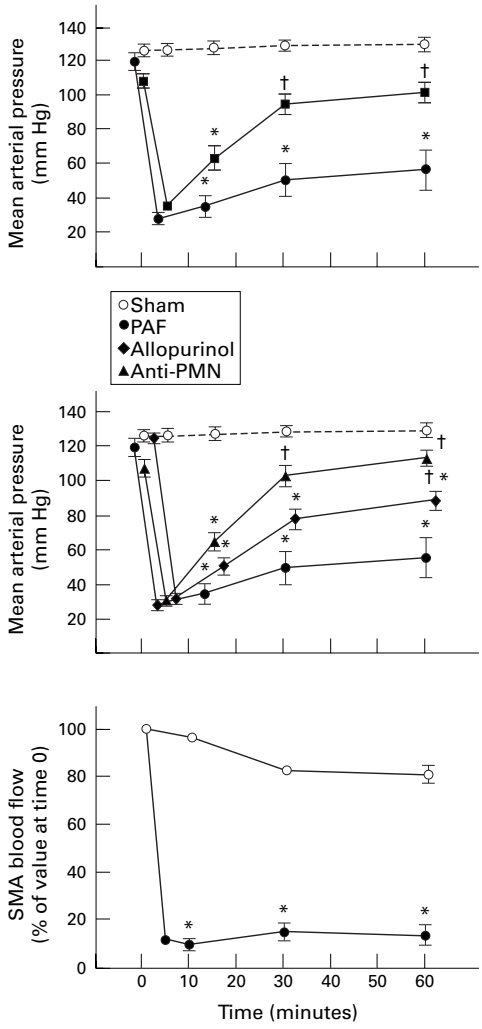

*Figure 7 (A) PAF induced systemic hypotension. Open circles, sham operated (n=5); closed circles, PAF (2.8 μg/kg, n=7); closed squares, PAF (1.5 μg/kg, n=5). PAF was injected at time 0. (B) Effect of allopurinol pretreatment and PMN depletion. Open circles, sham operated (n=5); closed circles, PAF (2.8 μg/kg, n=7); closed diamonds, allopurinol + PAF (2.8 μg/kg, n=5); closed triangles, anti-PMN + PAF (2.8 μg/kg, n=5). As the group receiving DMSO (vehicle) + PAF shows no difference from the group receiving PAF alone in all the parameters measured, only data from the PAF group are presented. (C) PAF induced reduction of SMA flow. Open circles, sham operated (n=4); closed circles, PAF (2.8 μg/kg, n=4). PAF was injected at time 0. *Significantly different (p<0.05) from sham control; †significantly different (p<0.05) from PAF (2.8 μg/kg) treated animals.*

Allopurinol (10 mg/kg) pretreatment and PMN depletion almost completely prevented PAF induced bowel necrosis (fig 8C). PMN depletion and allopurinol also completely or partially reversed PAF induced shock (fig 7B). Interestingly, both pretreatment regimens reversed high dose PAF induced leucopenia to leucocytosis.

EFFECT OF PMN DEPLETION ON PAF INDUCED XO ACTIVATION

Figure 3 illustrates the role of PMNs in PAF induced XO activation. PMN depletion largely abolished PAF induced XD to XO conversion in the ileum, suggesting that PAF induced XO activation was via PMN activation.

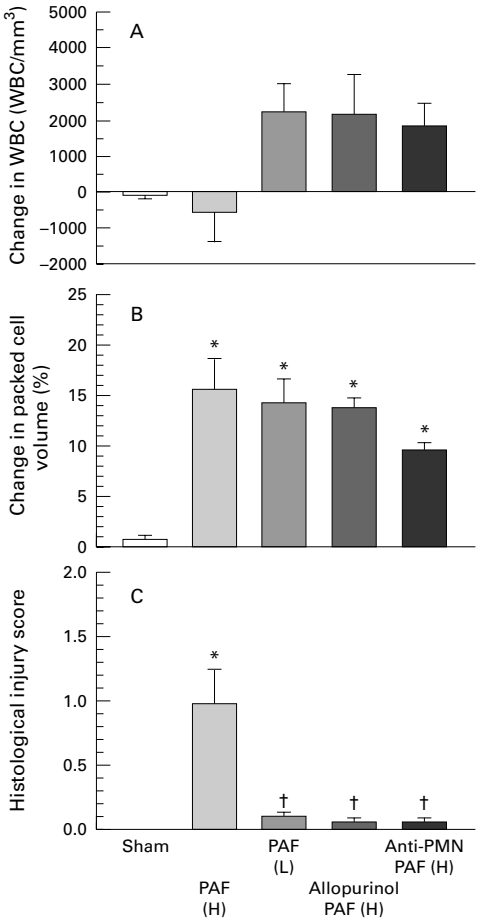

*Figure 8 PAF induced changes in peripheral WBC count (A), packed cell volume (B), and intestinal injury (C), and the preventive effect of allopurinol pretreatment and PMN depletion. n=7 in PAF(H); n=5 in all other groups. Values were taken at time 0, and at 60 minutes after PAF injection. *Significantly different (p<0.05) from sham control; †significantly different (p<0.05) from PAF (2.8 μg/kg) treated animals.*

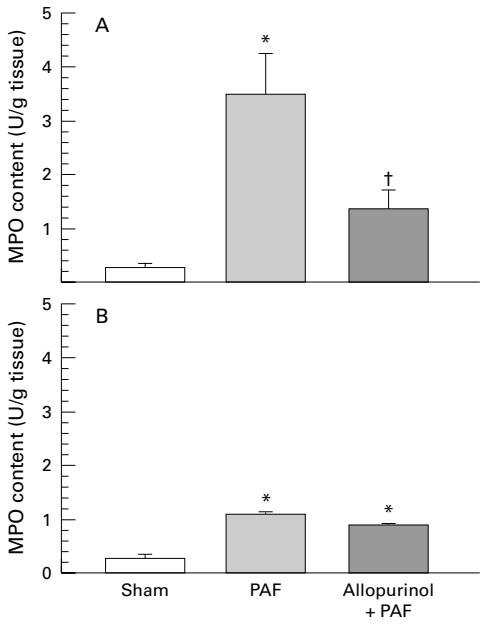

*Figure 9 Effect of allopurinol on PAF induced PMN sequestration in the intestine (reflected by increased MPO activity). Tissue samples were collected 60 minutes after PAF injection. (A) PAF 2.8 μg/kg; (B) PAF 1.5 μg/kg. n=5 in all groups. *Significantly different (p<0.05) from sham control; †significantly different (p<0.05) from PAF treated animals.*

EFFECT OF ALLOPURINOL ON PAF INDUCED PMN SEQUESTRATION IN THE INTESTINE

Only the ileal MPO data are presented because four preliminary experiments showed no statistical difference between values obtained from the jejunum and the ileum. In sham operated rats, there is a baseline activity of MPO in the jejunum and ileum (0.55 (0.13) and 0.34 (0.13) U/g, respectively). Following PAF (2.8 μg/kg) injection, the MPO concentrations in both the jejunum and ileum were notably elevated. Although the injury was more frequently observed in the ileum, ileal MPO values (3.75 (0.48) U/g) are not statistically different from those of the jejunum (4.25 (0.71) U/g).

PAF, at the low dose, caused a significant increase in PMN sequestration in the ileum, as indicated by the elevation of MPO activity (fig 9B), which was not affected by allopurinol pretreatment. Injection of a high dose of PAF resulted in notable PMN sequestration (as evidenced by a more than ninefold increase in MPO activity), which was partially but significantly reduced by allopurinol pretreatment (fig 9A).

Discussion

Reactive oxygen species play an important role in the pathogenesis of a variety of inflammatory processes, and XO may be an important source of these oxygen radicals. As the small intestine contains high xanthine oxidoreductase activity,[6] it is not unlikely that this enzyme is involved in intestinal inflammation and injury. However, the regulation of XO activation is incompletely understood. Ischaemia/reperfusion (I/R) has been found to induce XD to XO conversion, and consequent reperfusion injury in the intestine.[15] Furthermore, lipopolysaccharide has been implicated in the induction of XO activation, as an increase in mucosal permeability in the rat ileum during endotoxaemia could be blocked by allopurinol.[16] This increased intestinal XO activity may lead to bacterial translocation, resulting in further injury.[16]

PAF is an endogenous phospholipid mediator that has many proinflammatory actions.[17 18] PAF is probably a pivotal mediator in endotoxic shock,[17] and intestinal injury in endotoxaemia[19]; when injected systemically, it induces intestinal necrosis in rats[20] and mice.[21] Our previous study indicated the involvement of ROS, particularly those generated by xanthine oxidase, in the pathogenesis of PAF induced intestinal necrosis,[7] as much of the injurious action of PAF was abolished by pretreatment with allopurinol.[7] The present study provides us with the following insights into the interaction of PAF and the XD/XO system in the small intestine: PAF induces, in a time and dose dependent fashion, an increase in XO activity in the rat ileum through XD to XO conversion. This activation could be detected as early as 15 minutes after PAF

administration. The XD to XO conversion takes place predominantly in the ileal epithelium. While the two known mechanisms for XD-XO conversion, namely the thiol group oxidation and limited proteolysis, are both involved in PAF induced XO activation, the latter is predominant. This XD-XO conversion is triggered by certain signal(s) or mediator(s) from PMNs, and the activation of XO and PMN sequestration in the small intestine are related to each other in a positive feedback relation.

The exact mechanism of PAF induced XD to XO conversion, and its relation to the PAF induced intestinal injury is still not entirely clear. The current study suggests that PAF modulates XO activity via neutrophil activation. PAF is a potent agonist for PMNs as well as for endothelial cells and promotes the expression of adhesion molecules on both cell types, leading to leucocyte-endothelial adhesion.[22 23] As a consequence, many proinflammatory signals and mediators such as ROS[24] and proteases[25] may be released. Proteases from PMNs, especially elastase, have been shown to induce XD to XO conversion in vitro.[26] It is thus conceivable that some of these mediators or enzymes may trigger XD proteolysis to form XO. However, previous reports using histochemistry[27] and in situ hybridisation[28] showed the predominant localisation of xanthine oxidoreductase in the villus epithelium, and the present study showed that the villus epithelium is also the site where PAF induced XO activation occurs. It thus remains to be clarified how neutrophil-endothelial adhesion affects the XD/XO system in the epithelial cells. One possibility is the local diffusion of neutrophil derived mediators (for example, proteases, elastase, and ROS) into the intestinal villi. Alternatively, epithelial cells may effect the conversion by their own proteases: hepatocyte homogenates have been found to convert XD to XO in vitro.[29]

Once activated, XO could assume active roles in the now unfolding inflammatory cascades, by further promoting PMN-endothelial adhesion, and producing ROS.[30] A positive feedback relation is thereby established: further PMN activation/adhesion could thus lead to more XO activation, which in turn could lead to higher ROS production. Such a relation may be especially operative at high dose PAF. Allopurinol had little effect on PMN sequestration when low dose (1.5 μg/kg) PAF was used, indicating that the initial PMN-endothelial adhesion was largely independent of XO activation. However, when high dose PAF was used, allopurinol clearly inhibited PMN sequestration, suggesting that a large part of the PMN-endothelial interaction was due to XO activation, probably via generated ROS. It remains unclear which XO, endothelial or epithelial, is responsible for promoting PMN-endothelial adhesion in this model. Although XO in the epithelial cell is at a distance from the site of initial leucocyte adherence, the enzyme in the epithelium is far more abundant overall than in the endothelium. It is possible that ROS produced in the intestinal epithelium could diffuse into the microvasculature and stimulate leucocyte adhesion.

ROS may have injurious effects other than those described. The XO generated superoxide could also elicit epithelial damage by the formation of hydroxyl radicals.[31] It is well known that iron chelators, such as deferoxamine, protect against I/R induced injury.[32] It has also been reported that XO can liberate ferrous iron (which catalyses hydroxy radical formation) from ferritin.[33] Furthermore, superoxide could cause tissue damage through the formation of peroxynitrite by interacting with the nitric oxide (NO)[34] generated in the intestinal epithelia. (We have detected the expression of inducible nitric oxide synthase, the major source of NO, during inflammatory conditions, in rat ileal epithelia.[35]) The selective localisation of XO in the villus epithelia in the ileum may partly account for the preferential site of damage in PAF induced necrosis seen in the ileal villi.

Mesenteric ischaemia/reperfusion (I/R) occurs after PAF injection.[7] Both PAF and PMNs have been shown to play a role in I/R,[36] suggesting an overlapping of the mechanisms involved in PAF and I/R induced bowel injury. However, the effect of PAF is much more rapid than I/R, probably because PAF potently and rapidly activates PMNs and endothelial cells.[17 18] The systemic changes in blood pressure, packed cell volume, and WBC are detectable within five minutes, and the early histological changes (separation of the epithelia from the lamina propria at the villus tips) can be observed as early as 15 minutes. In contrast, mesenteric reperfusion is a relatively late event in this model. In an earlier study, we observed a partial recovery of the SMA vascular tone 30 minutes after PAF injection,[7] suggesting that I/R may contribute to PAF induced injury after 30 minutes. However, when a high dose of PAF was used, as in the present study, no recovery of SMA blood flow was observed up to 90 minutes after PAF injection. This observation does not support a role of I/R in the pathogenesis of bowel injury in this model.

That mechanisms other than simple splanchnic ischaemia are involved in PAF induced bowel injury is supported by the following evidence. (1) Our previous studies have shown that reversal of PAF induced hypotension and mesenteric flow by vasodilators did not prevent the intestinal injury.[9] (2) Allopurinol completely prevented PAF induced bowel necrosis but only partially reversed the hypotension. (3) Preliminary experiments in a model of haemorrhagic shock showed that bleeding rats in order to drop the blood pressure to approximately 30 mm Hg for 60 minutes did not cause any gross intestinal necrosis (unpublished observation). Nevertheless, it is likely that an interaction exists between the PAF and I/R induced inflammatory responses, and that the complex networking of these responses eventually leads to tissue damage.

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