Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy

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1 The profiles of cyclo-oxygenase (COX) and nitric oxide synthase (NOS) isoforms were determined in the rat carrageenin-induced pleurisy model of acute inflammation.

2 The enzymes were assessed in peripheral blood leucocyte (PBL) cell pellets taken from untreated animals and at 2, 6 and 24 h after injection of the irritant in pleural exudate cell pellets and lung homogenates.

3 COX activity was assessed by the generation of prostacyclin (PGI₂, measured as the stable metabolite, 6-keto prostaglandin F_{1α}) and prostaglandin E₂ (PGE₂). Western blot analysis and immunohistochemistry were also carried out.

4 NOS activity was based on the conversion of [³H]-L-arginine to [³H]-L-citrulline in the presence (total NOS activity) or absence of Ca²⁺ (inducible NOS; iNOS).

5 Peripheral blood leucocyte samples contained low levels of COX activity. In pleural exudate cell pellets, COX activity peaked at 2 to 6 h after injection of the carrageenin. At 24 h, COX activity was significantly reduced.

6 Western blot analysis demonstrated that the inducible isoform of COX (COX-2), was the predominant enzyme at all time points. Low levels of COX-2 were seen in PBLs. In pleural exudate cell pellets maximal COX-2 protein levels were seen at 2 h.

7 Immunohistochemistry confirmed the findings of Western blot studies. Approximately 10% of polymorphonuclear neutrophils (PMNs) in PBLs from untreated animals were immunopositive for COX-2. In cell pellet smears from carrageenin-induced pleurisy taken 2 h after injection of the irritant, PMNs were also the major source of COX-2 immunoreactivity. A small proportion of macrophages and mesothelial cells were also immunolabelled for COX-2.

8 Low levels of NOS activity were seen in PBLs. In pleural exudates NOS activity was maximum at 6 h and greatly reduced by 24 h. This activity was solely attributable to iNOS.

9 The present results illustrated a similar profile of COX and NOS activity in the carrageenin-induced pleurisy model of acute inflammation. It was demonstrated that COX-2 and iNOS were the predominant isoforms of their respective enzymes.

Keywords: Inducible cyclo-oxygenase; COX-2; nitric oxide synthase; inflammation; carrageenin pleurisy

Introduction

Arachidonic acid is an integral part of cell membrane phospholipids. The release of free arachidonic acid is via the rate limiting action of phospholipase A₂ on phosphatidylcholine or phospholipase C on phosphatidylinositol. Once generated, arachidonic acid is converted to prostaglandin G₂ (PGG₂) followed by PGH₂ and subsequently to prostaglandins and thromboxanes by the cyclo-oxygenase (COX) pathway (see Vane & Botting, 1987). Inhibition of COX is the mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs; Vane, 1971). Recently, two isoforms of COX have been identified, a constitutive COX (COX-1) and a mitogen-inducible COX (COX-2; see Xie *et al.*, 1992). We have shown *in vitro*, that NSAIDs have a differential inhibitory capacity on the two COX isoforms (Mitchell *et al.*, 1993a). This raises the possibility that selective inhibition of the predominant isoform of COX in inflammation, may prove more effective than conventional therapies.

Nitric oxide (NO) is a free radical involved in neurotransmission, cell mediated cytotoxicity and endothelium-dependent relaxation of vascular smooth muscle (for review see Moncada *et al.*, 1991). A number of cell types are capable of generating NO via nitric oxide synthase (NOS) from L-

arginine (for review of NOS isoforms see Forstermann *et al.*, 1991). A constitutive, calcium-dependent isoform of NOS is present in endothelial cells, neurones and platelets (Radomski *et al.*, 1990). A second, calcium-independent isoform of NOS (iNOS) can be induced in endothelial cells, macrophages, vascular smooth muscle cells and fibroblasts.

Acute inflammation is characterized by vasodilatation, plasma extravasation and polymorphonuclear neutrophil (PMN) influx. Vasodilatation induced by a number of agents including bradykinin, histamine, substance P, 5-hydroxytryptamine and thrombin, is dependent on the release of a relaxing factor derived from the endothelium (endothelium-derived relaxing factor; EDRF) which has been shown to be identical to NO (Palmer *et al.*, 1987). In contrast, the vasodilatation induced by the COX metabolites PGE₂ and PGI₂ is independent of the endothelium, acting directly on smooth muscle cells (Chand & Altura, 1981; Cherry *et al.*, 1982). The observation that the vasodilator effects of numerous agents involved in the inflammatory process are mediated by NO signifies a role for this molecule in inflammation, although at present the precise role is obscure. However, modulation of the inflammatory response has been demonstrated in a number of models using inhibitors of NOS. For example, treatment with the NOS inhibitor N^G-monomethyl-L-arginine protects against immune complex-induced inflammation (Mulligan *et al.*, 1991) and use of

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N^G -nitro-L-arginine methyl ester suppresses adjuvant-induced arthritis (Ialenti *et al.*, 1993).

Although the products of COX and NOS are implicated in inflammation, the relative contribution of the different isoforms of these enzymes has not been fully established. We have shown previously, that in a murine model of chronic granulomatous inflammation, inducible COX and NOS are the predominant isoforms of their respective enzymes (Vane *et al.*, 1994). Here we have extended these studies and characterized COX and NOS activity in the rat carrageenin-induced pleurisy model of acute inflammation.

Methods

Induction of pleurisy

Male Wistar rats (200 ± 20 g) were anaesthetized with ether and $150 \mu\text{l}$ of a 1% carrageenin solution in 0.9% NaCl injected into the pleural cavity (Velo *et al.*, 1973). Exudates were collected at 2, 6 and 24 h after injection of the irritant, by pleural lavage with 1 ml protease inhibitory buffer containing: trisodium citrate (3.15%), phenylmethylsulphonyl fluoride (1 mM), pepstatin A (1.5 mM) and leupeptin (0.2 mM) in 10 mM phosphate buffered saline (PBS) pH 7.3. Any exudates with blood contamination were rejected. Cells were counted in a Coulter counter, model Dn (Coulter Electronics Ltd., Luton). Each sample was centrifuged at 800 g for 10 min and the resultant cell pellet prepared as outlined below. At all time points lungs from experimental animals were dissected out and samples taken for subsequent procedures. Peripheral blood leucocytes (PBL) from untreated animals were used as control.

Preparation of peripheral blood leucocytes

Blood samples from untreated animals were taken by cardiac puncture and placed immediately in protease inhibitory buffer. Samples were centrifuged at 800 g for 10 min, the supernatant discarded and the red blood cells flash lysed by resuspending the cell pellet in 10 ml distilled water for 10 s, followed immediately by the addition of 10 ml, $2 \times$ concentrated protease inhibitory buffer. Cellular debris was removed by two successive centrifugation (800 g for 10 min) and resuspension steps. Leucocyte integrity was assessed morphologically by smearing and staining with haematoxylin and eosin.

Assessment of COX activity

Cell pellets from pleural exudates and PBLs were sonicated and tissues from lungs were homogenized at 4°C in protease inhibitory buffer, in a ratio of 5:1 (v/w). The protein concentration in the homogenates was measured by the Bradford assay (Bradford *et al.*, 1976), with bovine serum albumin (BSA) used as standard. Homogenates were incubated at 37°C for 30 min in the presence of excess arachidonic acid ($30 \mu\text{M}$). The samples were boiled and centrifuged at $10,000 g$ for 30 min. The concentration of 6-keto $\text{PGF}_{1\alpha}$ or PGE_2 present in the supernatant was measured by radioimmunoassay. Results are expressed as ng eicosanoid per mg protein per 30 min ($\text{ng mg}^{-1} 30 \text{ min}^{-1}$).

Western blot analysis

Cell pellets from pleural exudates and PBLs were lysed by the addition of protease inhibitory buffer containing 1% Triton X100 and boiled (10 min) with gel loading buffer (Tris, 50 mM; SDS, 10%; glycerol, 10%; 2-mercapthoethanol, 10%; bromophenol blue, 2 mg per ml) in a ratio of 1:1 and centrifuged at $10,000 g$, for 10 min. The protein concentrations of supernatants were determined as above, and total protein-equivalents ($17 \mu\text{g}$) for each sample separated on

10% sodium dodecyl sulphate-polyacrylamide mini-gels (Hoefer; Staffordshire) using the Laemmli buffer system and transferred to polyvinylidene difluoride membranes (Millipore, Hertfordshire). Nonspecific IgGs were blocked with 5% dried milk protein and incubated with specific antibody to COX-1 or COX-2 (both 1:1,000). Bands were detected with an amplified alkaline phosphatase kit and developed with nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3-indonyl phosphate (BCIP). Rainbow marker and pre-stained blue protein markers were used for molecular weight determinations. In experiments using COX-2 antibody, protein equivalents from rat seminal vesicles served as a positive control.

Immunohistochemistry

Peripheral blood and exudate cell samples were smeared onto poly-L-lysine coated slides and allowed to air dry. Prior to immunolabelling or histological staining, smears were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 1 h or acetone for 10 min. Endogenous peroxidases were quenched with 0.3% H_2O_2 in methanol and sections washed with 0.1% Triton X100 in PBS. Non-specific binding of IgG's was blocked using normal goat serum (NGS) 1:50 in 0.1% essentially globulin free BSA in PBS. The sections were incubated overnight with polyclonal rabbit anti-COX-1 (1:300) or polyclonal rabbit anti-COX-2 (1:400) at 4°C , washed and incubated for a further 30 min with biotinylated goat anti-rabbit secondary antibody. Following a further 30 min incubation with Vectastain ABC horseradish-peroxidase, the substrate (0.05% diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer pH 7.6) was added for the appropriate time period (5–10 min). This resulted in positive immunoreactivity labelling brown. Primary antiserum was replaced with NGS, as a negative control. Morphological observations were confirmed by use of the routine histological stain, haematoxylin and eosin.

Morphometric analysis

Differential cell counts and the number of immunopositive cells per unit area were determined using a 21 mm eye piece graticule (model NEII, Graticules Ltd.). Results are expressed as the percentage of total cells from an average of $n = 8$ smears, 1000 cells per smear, for peripheral blood and exudate samples at each time point.

Assessment of NOS activity

NOS activity was measured as the ability of exudate and peripheral blood samples to convert [^3H]-L-arginine to [^3H]-L-citrulline. Samples were prepared as for assessment of COX activity and incubated at room temperature for 30 min in the presence of NADPH (1 mM), calmodulin (30 nM), L-valine (50 mM), tetrahydrobiopterin ($5 \mu\text{M}$), calcium (2 mM) and a mixture of unlabelled and [^3H]-L-arginine ($10 \mu\text{M}$). In experiments to assess the contribution of the iNOS (calcium-independent) to the total NOS activity, calcium was replaced with EGTA (1 mM). In addition, L-citrulline formation was determined in the absence of NADPH as a measure of nonspecific conversion of L-arginine. Incubations were terminated by the addition of 1 ml HEPES (20 mM, pH 5.5) containing EGTA (1 mM) and EDTA (1 mM). The newly formed L-citrulline was separated from L-arginine by passing the reaction mixture over Dowex columns (1 ml) and the eluted labelled material was measured in a Beckman scintillation counter.

Materials

Radiolabelled prostanoids and Rainbow protein markers were obtained from Amersham International, Buckinghamshire. Polyclonal rabbit anti-COX-1 and polyclonal rabbit

anti-COX-2 were obtained from Cayman Chemicals, MI, U.S.A. Amplified alkaline phosphatase kit and NBT-BCIP colour development reagents were from Bio-Rad Laboratories Ltd., Hemel Hempstead. Biotinylated secondary antibody and ABC Elite kit were from Vector Laboratories, Peterborough. All other chemicals were from Sigma Chemical Co., Poole.

Statistics

Results are expressed as the mean $\pm$ s.e.mean for (*n*) experiments. Statistical analysis was determined by Student's unpaired *t* test, with a *P* value of <0.05 considered significant.

Results

Exudate volume and cell count

The COX activity in PBL cell pellets, and carrageenin-induced pleural exudate cell pellets and lung homogenates 6 h and reached a peak of 1.63 ± 0.23 ml at 24 h. The rise in exudate volume was paralleled by an increase in cell influx. At 2 h the total number of cells per ml exudate fluid was $8.9 \pm 2.4 \times 10^6$, this rose to $53.1 \pm 10.9 \times 10^6$ at 6 h and was maximal at 24 h ($111.5 \pm 16.9 \times 10^6$). This is illustrated in Figure 1.

COX activity (PGE_2)

The COX activity in PBL cell pellets, and carrageenin-induced pleural exudate cell pellets and lung homogenates was assessed by the generation of PGE_2 and 6-keto $PGF_{1\alpha}$. Peripheral blood leucocytes from untreated animals contained barely detectable levels of COX activity, as determined by PGE_2 generation. At 2 h after injection of carrageenin, 49.1 ± 10.7 ng PGE_2 per mg protein per 30 min ($ng\ mg^{-1}\ protein\ 30\ min^{-1}$) were generated by the exudate cell pellet (Figure 2a). This fell at 6 h and was significantly reduced ($P < 0.01$) by 24 h to 0.9 ± 0.1 ng $PGE_2\ mg^{-1}\ protein\ 30\ min^{-1}$. In lungs from affected animals low levels of PGE_2 were seen at 2 (5.8 ± 1 ng $mg^{-1}\ protein\ 30\ min^{-1}$), 6 (4.1 ± 0.5 ng $mg^{-1}\ protein\ 30\ min^{-1}$) and 24 h (4.06 ± 0.5 ng $mg^{-1}\ protein\ 30\ min^{-1}$). However, no significant differences between time points was observed for PGE_2 levels in lung homogenates (see Figure 2a).

Figure 1 Time course of exudate volume (□) and inflammatory cell influx (■) in the rat carrageenin-induced pleurisy. Each point represents the mean $\pm$ s.e.mean from *n* = 8 experiments.

COX activity (6-keto $PGF_{1\alpha}$)

It was not possible to detect 6-keto $PGF_{1\alpha}$ liberation in PBL samples. The profile of 6-keto $PGF_{1\alpha}$ in cell pellets from pleural exudates and lung homogenates is illustrated in Figure 2b. In the case of carrageenin-induced pleural cell pellets the production of 6-keto $PGF_{1\alpha}$ was similar, but always proportionally greater than that of PGE_2 . Maximal levels of 6-keto $PGF_{1\alpha}$ in exudate samples were seen at 6 h (128.1 ± 32.6 ng $mg^{-1}\ protein\ 30\ min^{-1}$) and were significantly ($P < 0.01$) reduced to 14.5 ± 2.5 ng $mg^{-1}\ protein\ 30\ min^{-1}$, at 24 h. The production of 6-keto $PGF_{1\alpha}$ by lung homogenates was significantly lower than in exudate cell pellets at all time points, with levels of 38.4 ± 12.3 , 26.4 ± 6.7 and 14.3 ± 2.9 ng $mg^{-1}\ protein\ 30\ min^{-1}$ at 2, 6 and 24 h respectively. No statistically significant difference was observed between time points.

Western blot analysis

At all time points, barely detectable levels of COX-1 protein were discerned (data not shown). The COX-2 protein levels in seminal vesicles, PBLs and carrageenin exudate cell pellets are shown in Figure 3. Low levels of COX-2 were seen in PBLs. The intensity of COX-2 labelling in exudate cell pellets varied, with maximal levels at 2 h in *n* = 8 experiments, whereas in 2 out of the total 10 experiments, the peak occurred at 6 h. A characteristic doublet at 72 and 74 kDa

Figure 2 Production of prostaglandin E_2 (PGE_2) (a) and 6-keto $PGF_{1\alpha}$ (b) by homogenates of cell pellets from pleural exudates (□) and lungs (■) from the rat carrageenin-induced pleural model of acute inflammation. Prostaglandins were measured at 2, 6 and 24 h after injection of the irritant. Peripheral blood leucocyte pellets from untreated rats contained little or no COX activity. Each point represents the mean $\pm$ s.e.mean from *n* = 8 experiments.

was observed in samples taken at 2 h. In all experiments COX-2 protein was present at lower levels at 24 h. At 6 and 24 h a band at approximately 60 kDa was also seen, which was always less intense than the 72 kDa band. At all time points the levels of COX-2 protein from carrageenin-induced pleural cell pellets were lower than protein equivalents from seminal vesicles.

Immunohistochemistry

In peripheral blood smears, COX-2 immunoreactivity was specifically localized to a discrete population of PMNs and monocytes. Morphometric analysis revealed that of the PMN population, $9.3 \pm 1.94\%$ were observed to label positively for COX-2. In the case of the mononuclear cell population this figure was lower with only $0.25 \pm 0.08\%$ of monocytes labelling.

At 2 h after injection of the carrageenin, the profile of cells from the exudate smears was as follows: 58% PMNs, 8% macrophages, 17% lymphocytes and 17% mesothelial cells (cells lining the pleural cavity). Of the PMN population $84 \pm 6.5\%$ were observed to label for COX-2, $82.5 \pm 5.1\%$ of macrophages and $53 \pm 9.9\%$ of mesothelial cells. No lymphocytes were seen to label for COX-2.

The cell profile at 6 h changed and consisted of 88% PMNs, 10% macrophages, 1.5% lymphocytes and 0.5% mesothelial cells; $69.5 \pm 6.5\%$ of the PMNs labelled for COX-2, $13 \pm 4\%$ macrophages and $25 \pm 2.5\%$ mesothelial cells. No lymphocytes showed COX-2 immunoreactivity.

At 24 h after injection of the carrageenin the exudate cell profile was 63% PMNs, 36% macrophages, 0.6% lymphocytes and 0.4% mesothelial cells. Of the total number of PMNs present $87 \pm 3\%$ showed COX-2 immunoreactivity and $15 \pm 5.8\%$ of macrophages. The positive immunoreactivity associated with PMNs in a 24 h carrageenin-induced pleurisy is shown in Figure 4.

NOS activity

Cell pellets of PBLs contained barely detectable levels of NOS activity. In cell pellets from carrageenin-induced pleurisy, high levels of total NOS activity were measured at 2 h reaching a maximum at 6 h (550.6 ± 107.7 pmol L-citrulline 30 min^{-1}) (Figure 5). By 24 h, the level of activity was significantly ($P < 0.01$) reduced to 15.2 ± 5.6 pmol L-citrulline. The proportion of total NOS activity due to iNOS was determined by the exclusion of calcium. The profile of iNOS paralleled that of total NOS reaching a peak at 6 h

Figure 3 Western blot of the levels of COX-2 protein from extracts of seminal vesicles (SV), peripheral blood leucocytes (PBL) and exudate cell pellets from the rat carrageenin-induced pleural model of inflammation, taken at 2, 6 and 24 h after injection of the irritant. The specific antibody to COX-2 recognised a protein which migrated at 72 kDa molecular weight when compared to protein standards. The levels of COX-2 protein in cell pellets were always lower than in seminal vesicle controls. Note the characteristic doublet at 72 and 74 kDa in the 2 h time point. Note also the presence of a second band at approximately 60 kDa in the exudate samples. Western blots primed with a selective antibody to COX-1 showed low levels of protein, which remained constant throughout the depicted time course (data not shown).

Figure 4 Immunolocalization of COX-2 in a cell smear derived from a rat carrageenin-induced pleurisy taken 24 h after injection of the irritant. The specific localization to PMNs is shown (arrows). Note however, the lack of labelling in a proportion of PMNs (large arrow) and some macrophages (M).

Figure 5 Total NOS activity in homogenates of cell pellets from pleural exudates (□) and lungs (■) from the rat carrageenin-induced pleural model of acute inflammation. NOS activity was measured at 2, 6 and 24 h after injection of the irritant. The calcium-independent activity (iNOS) of cell pellets (Δ) and lungs (▲) is also shown. Each point represents the mean $\pm$ s.e.mean from $n = 8$ experiments.

(587.3 ± 82.6 pmol L-citrulline) with a significant ($P < 0.01$) reduction in activity by 24 h (43.5 ± 15.8 pmol L-citrulline). Detectable levels of calcium-dependent NOS and iNOS activity were seen in lung homogenates, but no significant differences in activity were observed throughout the time course (see Figure 5). The generation of L-citrulline was significantly ($P < 0.001$) reduced in the absence of the essential co-factor NADPH in all samples measured (data not shown).

Discussion

The principal findings of this study are that, in the rat carrageenin-induced pleurisy the predominant isoforms of COX and NOS are COX-2 and iNOS. Furthermore, approximately 10% of circulating PMNs expressed the inducible isoform of COX, i.e. COX-2.

The activity of COX was assessed by the generation of the arachidonic metabolites PGE_2 and the stable metabolite of PGI_2 , 6-keto $\text{PGF}_{1\alpha}$. In the case of PGE_2 , the levels were maximal at 2 h after injection of the carrageenin, whereas 6-keto $\text{PGF}_{1\alpha}$ levels were greatest at 6 h. This may be due to differences in the kinetics of their respective isomerases. The peak of COX activity between 2–6 h is consistent with earlier studies measuring prostanoid levels in carrageenin-induced pleural exudates (Velo *et al.*, 1973). The results of the Western blot analysis confirm the findings of Otto *et al.* (1993) where, using murine COX-2 run on SDS-polyacrylamide gel electrophoresis, a 72 and 74 kDa doublet was observed due to N-glycosylation at Asn 580. Glycosylation of COX-2 is essential for enzymic activity, however, glycosylation at Asn 580 has no effect on activity. As the time course progressed a second protein band at approximately 60 kDa occurred, which was most intense at 24 h. This may represent COX-2 degradation products and could explain the decreased COX activity seen at 24 h. Western blot analysis demonstrated that PBLs contained COX-2 protein. The results also show correlation of the activity data for PGE_2 , with levels of COX-2 protein, the peak occurring at 2 h after injection of the carrageenin (see Figure 3). In conjunction with the results of the immunohistochemistry, which revealed that a proportion of PMNs were labelled for COX-2, this may indicate that the rapid induction of COX activity seen at 2 h is primarily due to the influx of PMNs.

Monocytes were also immunopositive for COX-2, although these formed a small proportion in relation to the number of PMNs labelled. Only a minority of peripheral blood PMNs were immunolabelled for COX-2. This may be due to the process of *ex vivo* extraction, which might result in their activation, or could be an indication that circulating PMNs have a basal level of expression of COX-2, which upon exposure to an inflammatory stimulus is rapidly utilised. Mesothelial cells of the pleural cavity and macrophages were also shown to label positively for COX-2 and could therefore in addition to PMNs contribute to the high levels of COX activity seen at 2 h.

At 24 h, COX activity and protein levels diminished. The percentage of PMNs in the exudate decreased, whereas macrophage numbers had increased. However, although the proportion of PMNs which labelled for COX-2 remained constant, the percentage of immunopositive macrophages decreased. This seems to indicate that as the inflammation progresses, the COX-2 present in macrophages is down regulated, but is unaffected in PMNs. Endothelial cells, once stimulated to produce PGI_2 become unresponsive to the effects of restimulation due to deactivation of COX (Kent *et al.*, 1983; Brotherton & Hoak, 1983). Unlike PMNs, macrophages have a longer life span. Hence, the decrease in COX-2 immunoreactivity seen in macrophages may be due to down regulation after exposure to the initial stimulus. *In vitro*, as promonocytes differentiate into monocytes/macrophages their ability to liberate prostaglandin is increased (Koehler *et al.*, 1990). The findings of the present study showed that a proportion of macrophages were immunolabelled for COX-2. Whether this represents a facet of maturity or is under the constraint of exogenous factors present in the inflammatory exudate requires further investigation. COX and NOS activity was detected in lungs from affected animals. However, the levels were always lower than in pleural exudate cell pellets, indicating that the induction of COX and NOS was localized to the site of injection i.e. the pleural cavity.

We have also shown that NOS activity was similar in profile to COX. In the pleural exudate cell pellet, maximal NOS activity occurred at 6 h after injection of the carrageenin and was significantly decreased after 24 h. Furthermore, it was found that this activity was solely attributed to iNOS, as depletion of calcium from the reaction mixture had no effect on subsequent levels of activity. Both constitutive and inducible forms of NOS are reported to be present in PMNs (Wright *et al.*, 1989; McCall *et al.*, 1989; 1991), whilst macrophages contain iNOS (Iyengar *et al.*, 1987; Marletta *et al.*, 1988). It is possible therefore, that at 6 h after injection of the irritant, the newly influxed monocytes/macrophages, although representing only 10% of the total exudate cell population, may be responsible for this activity. In macrophages, high levels of NO feedback to switch off iNOS activity, due to NO binding to the haem moiety of the enzyme (Griscavage *et al.*, 1993). Interestingly, NO may also account for the decreased COX activity seen at 24 h as NO can inhibit PGI_2 release (Mitchell *et al.*, 1993b). Hence, the decrease in NOS and COX activity seen at 24 h could be due to a self modulatory, NO-mediated, negative feedback loop.

This study has demonstrated that, in the rat carrageenin-induced pleurisy model of acute inflammation, inducible COX and NOS are the predominant isoforms of their respective enzymes. As the inflammation progressed and the cell population changed from PMN to mononuclear dominated, there was an accompanying decrease in COX and NOS activity. It is possible that in acute inflammation the use of selective inhibitors of COX-2 and iNOS may be more beneficial than existing therapies.

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