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Protective effects of Celecoxib on lung injury and red blood cells modification induced by carrageenan in the rat

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Abstract

In the present study, we evaluated the effect of Celecoxib, a selective COX-2 inhibitor, in an acute model of lung injury induced by carrageenan administration in the rats. Injection of carrageenan into the pleural cavity of rats elicited an acute inflammatory response characterized by: fluid accumulation in the pleural cavity which contained a large number of polymorphonuclear neutrophils (PMNs) as well as an infiltration of PMNs in lung tissues and subsequent lipid peroxidation, and increased production of prostaglandin E_2 (PGE_2), tumor necrosis factor α (TNFα), and interleukin-1β. All parameters of inflammation were attenuated by Celecoxib. Furthermore, carrageenan induced an upregulation of the adhesion molecules ICAM-1 and P-selectin, as well as nitrotyrosine and poly(ADP-ribose) synthetase (PARS) as determined by immunohistochemical analysis of lung tissues. The degree of staining for the ICAM-1, P-selectin, nitrotyrosine and PARS was reduced by Celecoxib. These results clearly confirmed that COX-2 plays a critical role in the development of the inflammatory response by altering key components of the inflammatory cascade. Therefore, selective inhibitor of COX-2 such as Celecoxib, offers a therapeutic approach for the management of various inflammatory diseases.

Keywords: Polymorphonuclear neutrophils; Celecoxib; Carrageenan-induced pleurisy

1. Introduction

The inflammatory process is invariably characterized by a production of histamine, bradykinin, platelet-activating factor (PAF) and interleukin-1 (IL-1) and by a release of chemicals from tissues and migrating cells [1]. Furthermore, there is a large amount of evidence that reactive oxygen species (ROS) play an important role in the tissue destruction associated with the inflammatory process (see Section 4). Pro-inflammatory cytokines including TNFα and IL-1 and interleukin-6 (IL-6) contribute to the extension of the inflammatory process. There is substantial evidence that inflammation is associated with an increase in arachidonic acid (AA) metabolites in blood and tissues [2]. This increase was associated with the *de novo* synthesis of a new cyclooxygenase (COX) protein, termed COX-2, which is encoded by a different gene (located on chromosome 1) from that which encodes for the constitutive enzyme (COX-1), which is located on chromosome 9 [3–5]. The expression of COX-2 afforded by inflammatory stimuli in many different cell types is secondary to the activation of protein tyrosine kinases [6] and of the transcription factor NF-κB [5,7].

Nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, are effective anti-inflammatory and analgesic agents commonly used in the treatment of acute and chronic inflammation. NSAIDs inhibit PG formation through inhibition of both the COX-1 and COX-2 enzymes [8,9]. Long-term NSAID treatment is often limited, however, by gastrointestinal ulcerogenicity that may result from the suppression of physiological PG production in these tissues. It has been recently demonstrated the ability

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Abbreviations: PARS, poly(ADP-ribose) synthetase; PMNs, polymorphonuclear neutrophils; COX, cyclooxygenase; ROS, reactive oxygen species; RBC, red blood cell; PG, prostaglandin.

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of a selective COX-2 inhibitor to block PG production [10] and acute tissue inflammation [11] *in vivo* at dosages that do not affect stomach PG production, suggesting that COX-2 inhibitors may provide a safer therapeutic alternative to NSAIDs. In this study, the effects of the selective COX-2 inhibitor Celecoxib on the inflammatory response (pleurisy) caused by injection of carrageenan in the rat. In addition, we have investigated the effects of Celecoxib on cytokines production, lung injury (histology), and red blood cells damage as well as the increases in nitrotyrosine (immunohistochemistry) and PARS activity caused by carrageenan in the lung.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (300–350 g; Charles River; Milan; Italy) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986)

2.2. Carrageenan-induced pleurisy

Rats were anaesthetized with isoflurane and submitted to a skin incision at the level of the left sixth intercostal space. The underlying muscle was dissected and saline (0.2 mL) or saline containing 1% λ-carrageenan (0.2 mL), injected into the pleural cavity. The skin incision was closed with a suture and the animals allowed to recover. Celecoxib (5–20 mg/kg), or an equivalent volume (0.3 mL) of vehicle, was administered orally (o.s.) 6, 12 hr after carrageenan. At 24 hr after the injection of carrageenan, the animals were killed by inhalation of CO_2. The chest was carefully opened and the pleural cavity rinsed with 2 mL of saline solution containing heparin (5 unit/mL) and indomethacin (10 μg/mL). The exudate and washing solution were removed by aspiration and the total volume measured. Any exudate, that was contaminated with blood was discarded. The amount of exudate was calculated by subtracting the volume injected (2 mL) from the total volume recovered. The leukocytes in the exudate were suspended in phosphate-buffer saline (PBS) and counted with an optical microscope in a Burker's chamber after vital Trypan Blue staining.

2.3. Measurement of lung-tissue myeloperoxidase activity and malondialdehyde

Myeloperoxidase (MPO) activity, a hemoprotein located in azurophil granules of neutrophils, has been used as a biochemical marker for neutrophil infiltration into tissues [12]. In the present study, MPO was measured photometrically by a method similar to that described previously [13]. At 24 hr following the intrapleural injection of carrageenan, lung tissues were obtained and weighed. Each piece of tissue was homogenized in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 *g* at 4°. An aliquot of the supernatant was then allowed to react with a solution of tetramethylbenzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide/min at 37° and was expressed in milliunits per 100 mg weight of wet tissue. Malondialdehyde (MDA) levels in the lung tissue were determined as an indicator of lipid peroxidation [14]. Lung tissue, collected at the specified time, were homogenized in 1.15% KCl solution. An aliquot (100 μL) of the homogenate was added to a reaction mixture containing 200 μL of 8.1% SDS, 1500 μL of 20% acetic acid (pH 3.5), 1500 μL of 0.8% thiobarbituric acid and 700 μL distilled water. Samples were then boiled for 1 hr at 95° and centrifuged at 3000 *g* for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 650 nm.

2.4. Immunofluorescence localization of ICAM-1, P-selectin, nitrotyrosine and PARS

Indirect immunofluorescence staining was performed on 7 μm thick sections of unfixed rat lung. Sections were cut in with a Slee and London cryostat at −30°, transferred onto clean glass slides and dried overnight at RT. Sections were permeabilized with acetone at –20° for 10 min and rehydrated in PBS, 150 mM NaCl, 20 mM sodium phosphate pH 7.2) at RT for 45 min. Sections were incubated overnight with (1) rabbit anti-human polyclonal antibody directed at P-selectin (CD62P) which react with rat and with mouse anti-rat antibody directed at ICAM-1 (CD54) (1:500 in PBS, v/v) (DBA, Milan, Italy) or (2) with anti-nitrotyrosine rabbit polyclonal antibody (1:500 in PBS, v/v) or with anti-poly(ADP-ribose) goat polyclonal antibody rat (1:500 in PBS, v/v). Sections were washed with PBS, and incubated with secondary antibody (TRITC-conjugated anti-rabbit and with FITC-conjugated anti-mouse (Jackson, west Grove, PA) or with FITC-conjugated anti-goat antibody (1:80 in PBS, v/v) for 2 hr at RT. Sections were washed as before, mounted with 90% glycerol in PBS, and observed with a Nikon RCM8000 confocal microscope equipped with a 40× oil objective.

2.5. Histological examination

Lung biopsies were taken at 24 hr after injection of carrageenan. The biopsies were fixed for 1 week in buffered

formaldehyde solution (10% in PBS) at room temperature, dehydrated by graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, NJ). Tissue sections (thickness 7 μm) were deparaffinized with xylene, stained with trichromic Van Gieson and studied using light microscopy (Dialux 22 Leitz). Blood were passed on the slide, fixed at 37°, stained with May Grunward-Giemsa and studied using light microscopy.

2.6. Measurement of cytokines

TNFα and interleukin-1β (IL-1β) levels were evaluated in the exudate at 24 hr after the induction of pleurisy by carrageenan injection. The assay was carried out by using a colorimetric, commercial kit (Calbaiochem-Novabiochem Corporation, USA). The ELISA has a lower detection limit of 10 pg/mL.

2.7. Measurement of prostaglandin E_2 in the pleural exudate

The amount of PGE_2 present in the pleural fluid was measured by radioimmunoassay without prior extraction or purification [15].

2.8. Assessment of COX activity

Lungs obtained at 24 hr after the induction of pleurisy by carrageenan injection. The material was homogenized at >4° in a buffer containing the following protease inhibitors: in ration of 5:1 (v/w). The protein concentration in the homogenates was measured by the Bradford assay [16], with bovine serum albumin (BSA) used as standard. Homogenates were incubated at 37° for 30 min in the presence of excess arachidonic acid (30 μM). The samples were boiled and centrifuged at 10,000 *g* for a minute. The concentration of 6-keto-$PGF_{1\alpha}$ present in the supernatant was measured by radioimmunoassay as previously described [17].

2.9. Scanning electron microscopy (SEM)

Blood samples (taken from femoral vein) for RBCs evaluation were taken at 24 hr after the carrageenan administration. The morphological alteration of RBCs were followed by scanning electron microscopy (SEM). Blood samples were fixed (at >4°) in modified Karnovsky fixative (1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M cacodylate buffer). The blood were then transferred to ice-cold 0.1 M phosphate buffer and postfixed in 1% OSO_4 in 0.1 M cacodylate buffer for 1 hr. After thorough rinsing in 0.1 M phosphate buffer, blood samples were dehydrated in a graded series of ethanols and transferred into liquid CO_2 in a critical point dryer. The dried specimens were mounted, sputtercoated with gold, and examined in a scanning electron microscope at 20 W.

2.9.1. Materials

Cell culture medium, heparin and fetal calf serum were obtained from Sigma (Milan, Italy). Perchloric acid was obtained from Aldrich (Milan, Italy). Primary anti-nitrotyrosine antibody was from Upstate Biotech (DBA, Milan, Italy). All other reagents and compounds used were obtained from Sigma (Milan, Italy).

2.10. Data analysis

All values in the figures and text are expressed as mean ± SE of the mean (SEM) for *n* observations. For the *in vitro* studies, data represent the number of wells studied (6–9-well from two to three independent experiments). For the *in vivo* studies, *n* represents the number of animals studied. The results were analyzed by one-way ANOVA followed by a Bonferroni *post-hoc* test for multiple comparisons. A *P*-value less than 0.05 was considered significant. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days.

3. Results

3.1. Effects of Celecoxib in carrageenan-induced pleurisy

Histological examination of lung sections revealed significant tissue damage (Fig. 1B). Thus, when compared to lung sections taken from saline-treated animals (Fig. 1A), histological examination of lung sections of rats treated with carrageenan showed edema, tissue injury as well as infiltration of the tissue with PMNs (Fig. 1B). Celecoxib at the highest dose tested (20 mg/kg, intraperitoneally, i.p.), significantly reduced the degree of injury as well as the infiltration of PMNs (Fig. 1C). Furthermore, the injection of carrageenan into the pleural cavity of rats elicited an acute inflammatory response characterized by the accumulation of fluid (edema) that contained a large amounts of PMNs (Fig. 2A and B). Neutrophils also infiltrated in the lung tissues (Fig. 3A) and this was associated with lipid peroxidation of lung tissues as evidenced by an increase in the levels of malonyldialdehyde (Fig. 3B). Edema, neutrophil infiltration in lung tissue, and lipid peroxidation were attenuated in a dose-dependent fashion by the oral administration of Celecoxib (5–20 mg/kg, $n = 10$) (Figs. 2 and 3).

3.2. Effects of Celecoxib on the expression of adhesion molecules (ICAM-1, P-selectin)

Staining of lung tissue sections obtained from saline-treated rats with anti-ICAM-1 antibody showed a specific staining along bronchial epithelium, demonstrating that

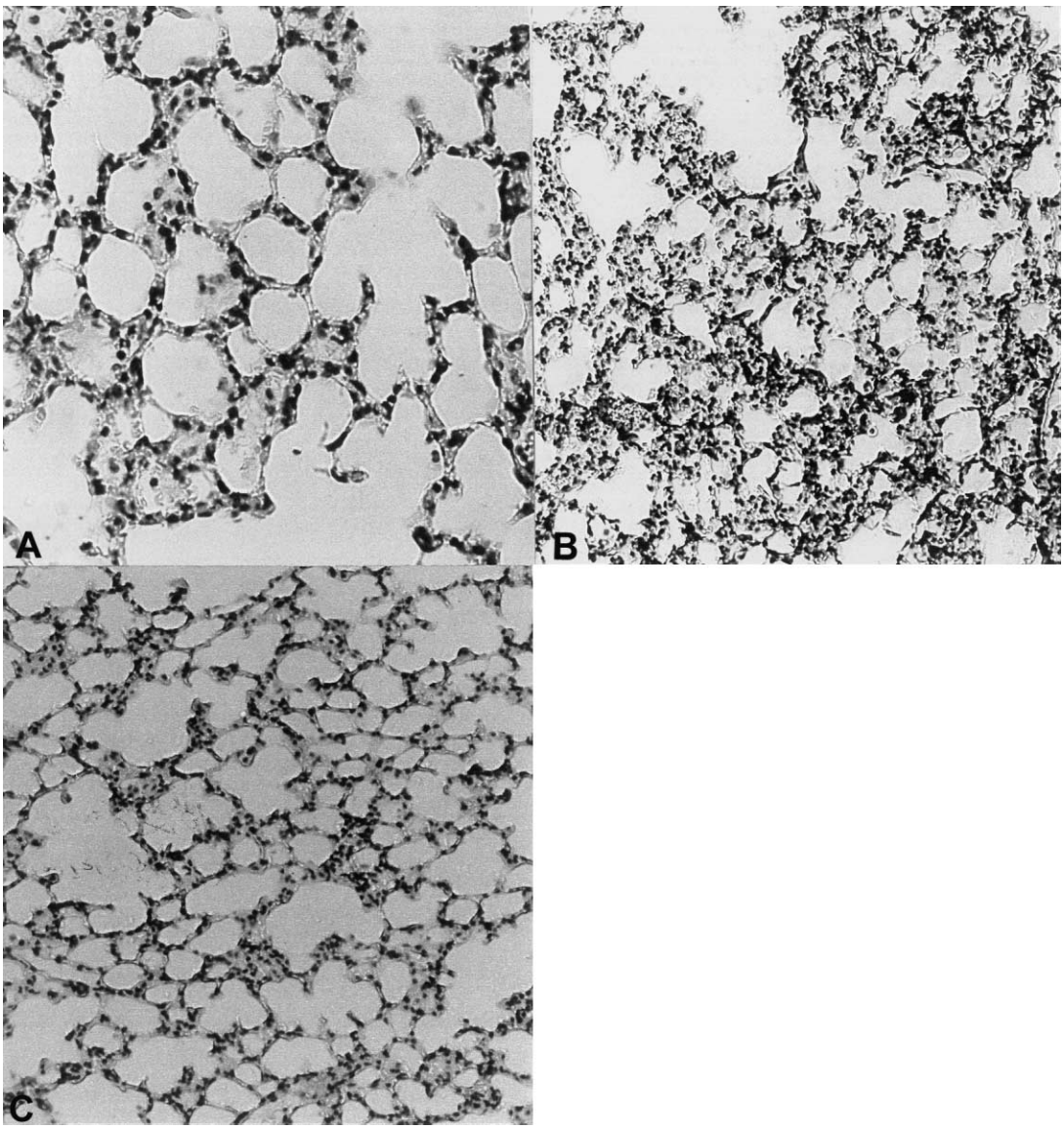

Fig. 1. Effect of Celecoxib, on lung injury: when compared to lung sections taken from control animals (A) lung sections from carrageenan-treated rats (B) demonstrates interstitial hemorrhage and polymorphonuclear leukocyte accumulation. Lung sections from a carrageenan-treated rat that had received Celecoxib (20 mg/kg) (C) exhibit reduced interstitial hemorrhage and a lesser cellular infiltration. Original magnification: 62.5×. Results are representative of at least three experiments performed on different experimental days.

ICAM-1 is constitutively expressed (data not shown). At 24 hr after carrageenan injection, the staining intensity substantially increased along the bronchial epithelium (Fig. 4A). Lung tissue section obtained from carrageenan-treated rats showed positive staining for P-selectin localized in the bronchial epithelium (Fig. 4B). No positive staining for ICAM-1 or P-selectin was found in the lungs of carrageenan-treated rats that received intraperitoneal injection of Celecoxib (20 mg/kg) (Fig. 4D and E). To verify the binding specificity for ICAM-1 or P-selectin, some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). In these situations, no positive staining was found in the sections indicating that the immunoreaction was positive in all the experiments carried out.

3.3. Effects of Celecoxib on nitrotyrosine and PARS

At 24 hr after carrageenan injection, lung sections were taken in order to determine the immunohistological staining for nitrotyrosine or PARS. Sections of lung from saline-treated rats did not reveal any immunoreactivity for nitrotyrosine (data not shown) and for PARS (data not shown) within the normal architecture. A positive staining for nitrotyrosine (Fig. 5A) and for PARS (Fig. 5B) was found primarily localized in the vessels and in the bronchial epithelium. Celecoxib (20 mg/kg, i.p.) reduced the staining for both nitrotyrosine and PARS (Fig. 5D and E). In order to confirm that the immunoreaction for the nitrotyrosine was specific some sections were also incubated with the primary antibody (anti-nitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding speci-

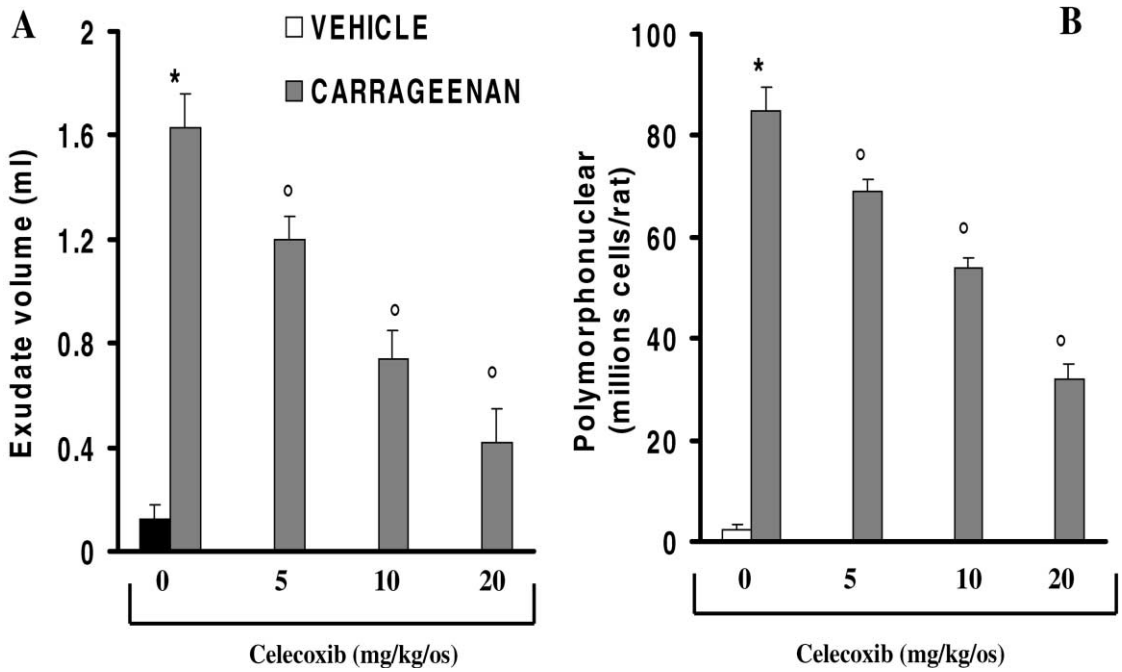

Fig. 2. Effect of Celecoxib, on carrageenan-induced inflammation: the increase in volume exudate (A) and accumulation of PMNs (B) in pleural cavity at 4 hr after carrageenan injection was inhibited in a dose-dependent manner by Celecoxib (5–20 mg/kg). Each value is the mean ± SEM for $n = 10$ experiments; (✱) $P < 0.01$ vs. sham, (○) $P < 0.01$ vs. carrageenan.

ficity. To verify the binding specificity for PARS, some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). In these situations, no positive staining was found in the sections indicating that the immunoreaction was positive in all the experiments carried out.

3.4. Effects of Celecoxib on the release of cytokine

When compared to controls at 24 hr after the injection of carrageenan, an increase in the levels of TNFα and IL-1β was observed in pleural exudates (Fig. 6A and B). Citokines production was attenuated in a dose-dependent

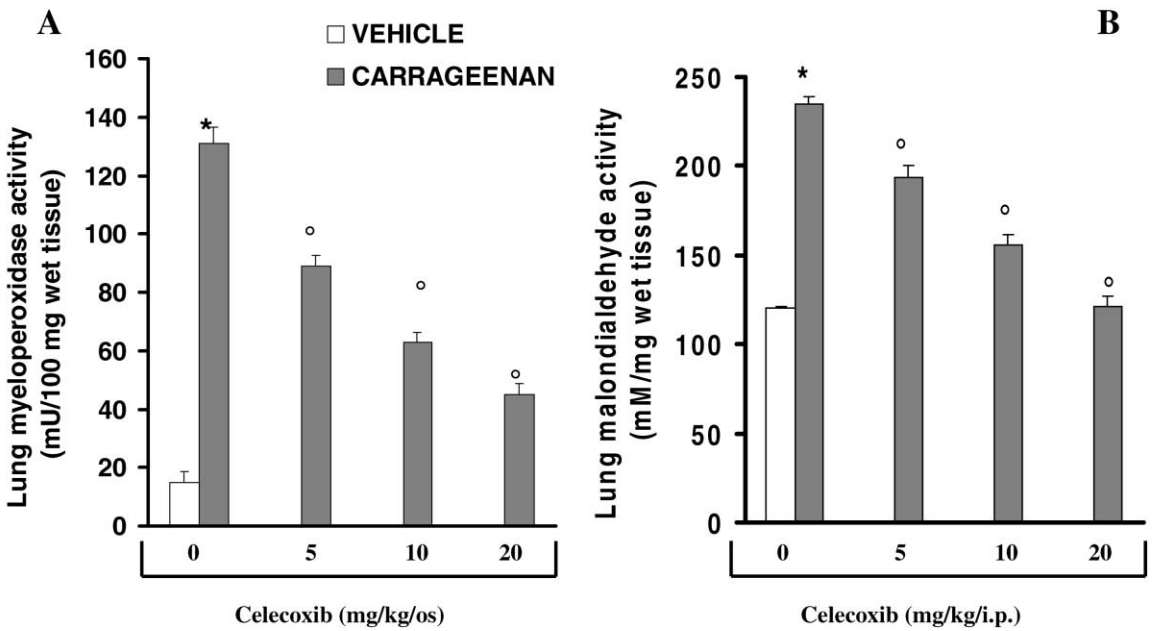

Fig. 3. Effect of Celecoxib, on MPO activity and MDA levels in the lung. Within 24 hr, pleural injection of carrageenan led to an increase in neutrophil accumulation in the lung (as measured by MPO activity, A) an effect that was associated with increased lipid peroxidation of lung tissue (as measured by MDA, B). Celecoxib inhibited in a dose-dependent (5–20 mg/kg) fashion neutrophil infiltration and lipid peroxidation. Each value is the mean ± SEM for $n = 10$ experiments; (✱)$P < 0.01$ vs. sham, (○) $P < 0.01$ vs. carrageenan.

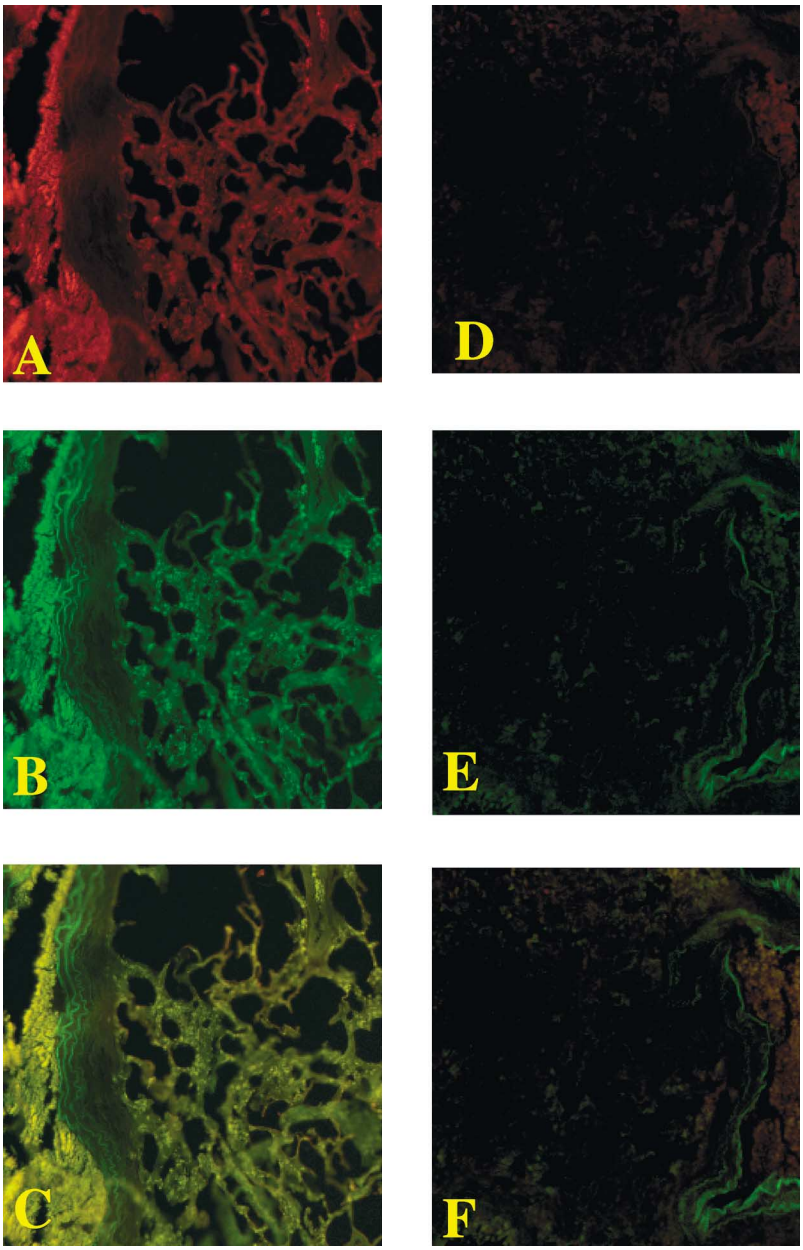

Fig. 4. Immunohistochemical localization of ICAM-1 and P-selectin in the lung. Section obtained from carrageenan-treated rats showed intense positive staining for ICAM-1 (A) and for P-selectin (B) on bronchial epithelium. The degree of bronchial epithelium staining for ICAM-1 (D) and for P-selectin (E) was markedly reduced in tissue section obtained from Celecoxib-treated rats (20 mg/kg). C and F represent the staining combination of panel A and B, and D and E, respectively. Original magnification: 100×. Results are representative of at least three experiments performed on different experimental days.

fashion by the oral administration of Celecoxib (5–20 mg/kg, $n = 10$).

3.5. Effects of Celecoxib on the release of prostaglandin

The COX activity in carrageenan-induced pleural exudate and lung homogenates was assessed by measuring the increase in the formation of PGE_2 in the exudate. The amounts of PGE_2 found in the pleural exudate of carrageenan-treated rats was 289 ± 13 pg per rat ($n = 10$) (Fig. 7A). The amounts of PGE_2 were significantly lower in the exudate obtained from carrageenan-treated rats, which had been treated with Celecoxib. In lungs from carrageenan-treated rats, the amount of 6-keto-$PGF_{1\alpha}$ was 142 ± 20 pg/mg per tissue (Fig. 7B). The amount of 6-keto-$PGF_{1\alpha}$ was significantly reduced in the lungs from carrageenan-treated rats, which had been treated with Celecoxib (Fig. 7B).

3.6. Effects of Celecoxib on RBCs alteration

At 24 hr after the injection of carrageenan, a significant alteration in the RBCs morphology was observed (Fig. 8B). Celecoxib (20 mg/kg, $n = 10$) significantly prevent RBCs alteration (Fig. 8C).

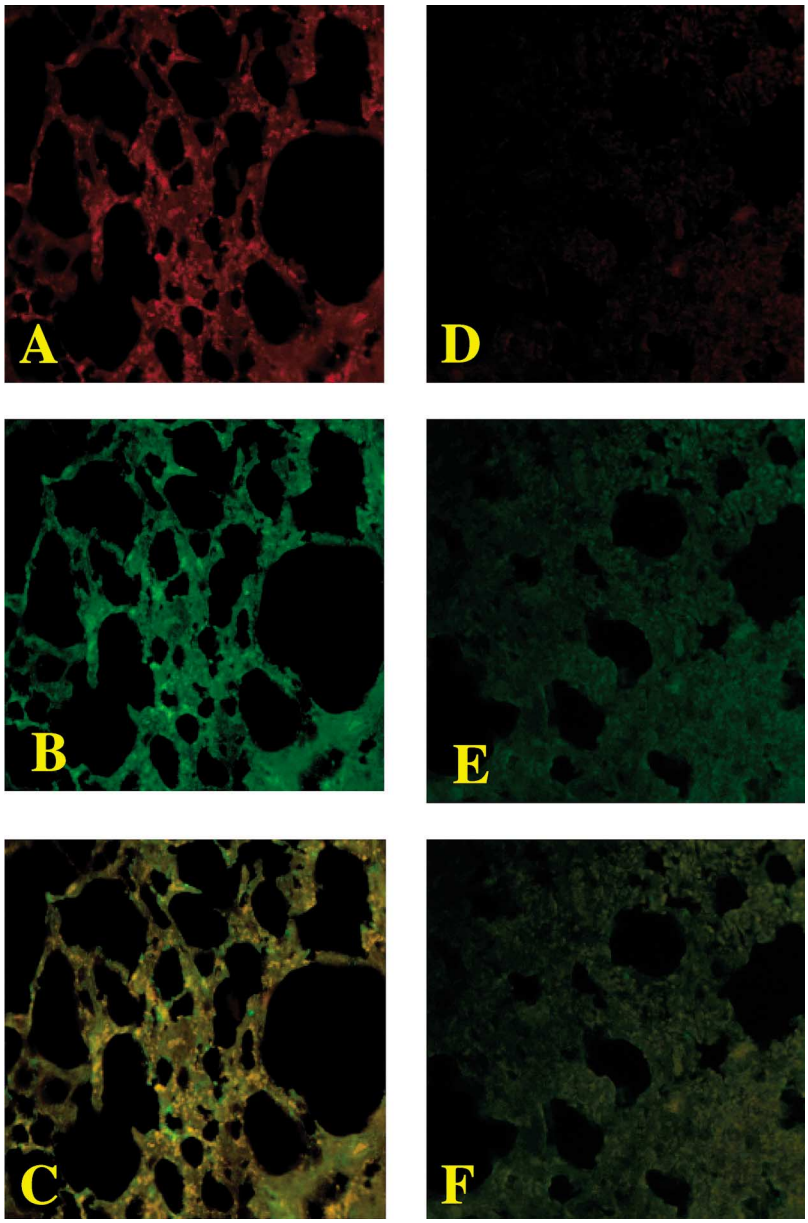

Fig. 5. Immunohistochemical localization for nitrotyrosine and for PARS in the lung. Immunohistochemistry for nitrotyrosine (A) and for PARS (B) show positive staining along the vessels and in the bronchial epithelium from a carrageenan-treated rats. The intensity of the positive staining for nitrotyrosine (D) and for PARS (E) was significantly reduced in the lung from Celecoxib-treated rats (20 mg/kg). C and F represent the staining combination of panel A and B, and D and E, respectively. Original magnification: 100×. Results are representative of at least three experiments performed on different experimental days.

4. Discussion

The results of this study indicate that the development of lung inflammation in experimentally induced pleurisy is associated with PG production in affected tissues. This result extends the findings reported by Harada *et al.* [18] by identifying COX-2 as the induced COX isoform. Our result also confirms the observation reported by Crofford *et al.* [19] indicating that proinflammatory stimuli upregulate cell production of functional COX-2 enzyme without modulating the production of COX-1. While our data do not directly demonstrate the induction of COX-2 transcription in the affected lungs, Harada *et al.* [18] found high level COX protein expression in the lungs and in infiltrating inflammatory cells in this model, suggesting that *de novo* synthesis of COX-2 is upregulated in the inflamed lung. The regulation of COX-2 gene expression involves both transcriptional and translational mechanisms [20], but details of the mechanisms remain elusive. Ristimki *et al.* [21] recently showed that IL-1β induces a rapid but transient activation of COX-2 transcription in inflammatory cells and stabilizes the COX-2 mRNA in the absence of transcription. It is likely that carrageenan induces the production of IL-1β as well as TNFα in the exudate and that IL-1β

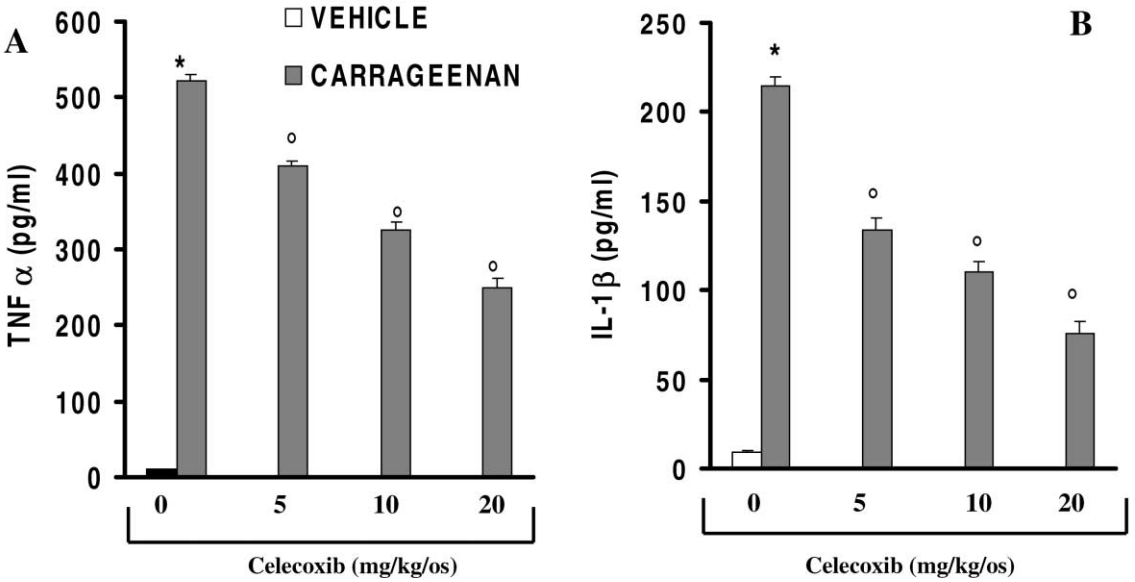

Fig. 6. Pleural injection of carrageenan caused by 24 hr an increase in the release of the cytokines TNFα (A), IL-1β (B). Celecoxib (5–20 mg/kg) significantly inhibited TNFα and IL-1β. Each value is the mean ± SEM for $n = 10$ experiments; (✱) $P < 0.01$ vs. sham, (○) $P < 0.01$ vs. carrageenan.

as well as TNFα plays an important role in the regulation of sustained COX-2 polypeptide synthesis in inflammatory responses.

This study also provides insight into the proinflammatory activities of COX-2-derived PGs in established acute inflammation. Administration of the selective COX-2 inhibitor Celecoxib to carrageenan-treated animals rapidly reversed exudate formation, reduced lung inflammation and returned PGE_2 levels to normal. Furthermore, inhibition of COX-2 activity diminished the level of COX-2 activity in lung tissue, suggesting that PGs enhance the local expression of COX-2 itself in inflamed lung tissue. Inhibition of COX-2 activity also modulated cytokine production in carrageenan-treated rats. The development of lung injury was associated with increased levels of TNFα and IL-1β in the exudate. Treatment of carrageenan-treated rats with Celecoxib markedly reduced the level of TNFα and IL-1β in the exudate. Partial inhibition of systemic cytokines production by treatment with indomethacin has been documented previously [22]. Both enhancement and suppression of TNFα production have been demonstrated to be dose dependently regulated by PGE_2 and cAMP in rat peritoneal macrophages [23]. In addition, treatment with Celecoxib markedly reduced (1) the cellular infiltrate in the pleural cavity as well as in the lung, and (2) inflammation of lung tissue. The ability of COX inhibitors to partially reduce the inflammatory cells infiltrate in the lung could in part explain the observed reduction in the levels of COX-2 and cytokines production. In addition, we have observed that Celecoxib significantly reduced P-selectin and ICAM-1 in the lung from carrageenan-treated rats. Lung inflammation is usually characterized by extensive infiltration of pulmonary tissue by polymorphonuclear leukocytes, which is more marked

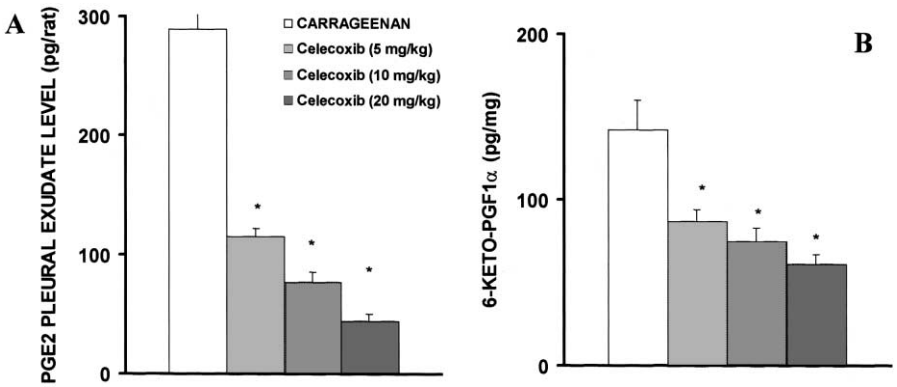

Fig. 7. PGE_2 levels in the pleural exudate (A) and 6-keto-$PGF_{1\alpha}$ in the lungs (B) from carrageenan-treated rats. The amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ was significantly reduced in a dose-dependent manner in rats treated with Celecoxib (5–20 mg/kg). Data are mean ± SEM of 10 rats for each group; (✱) $P < 0.01$ vs. carrageenan.

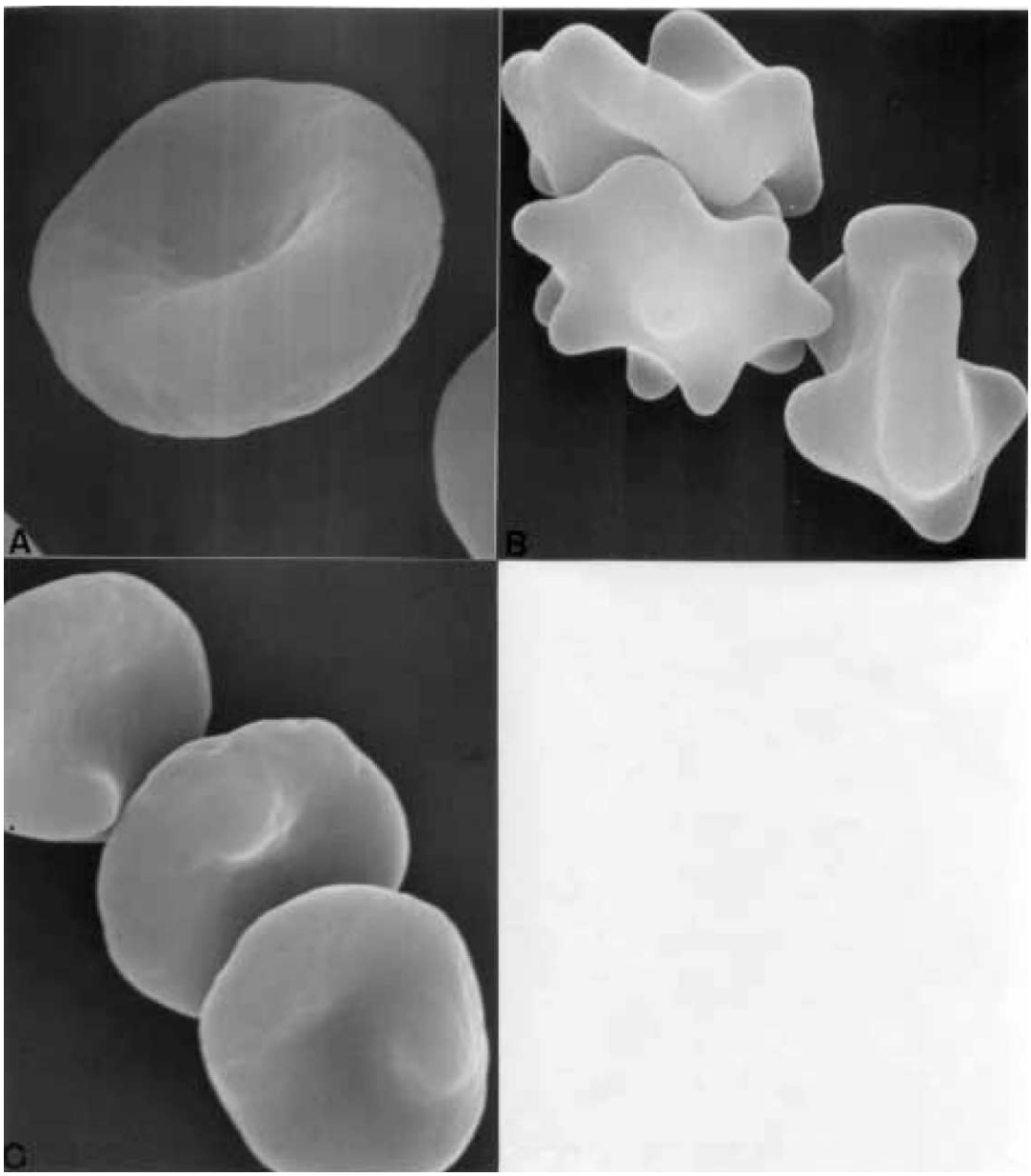

Fig. 8. Effect of Celecoxib, on RBCs modification (*SEM observation*): when compared to RBCs taken from control animals (A) RBCs from carrageenan-treated rats (B) demonstrates significant alteration. RBCs from a carrageenan-treated rat that had received Celecoxib (20 mg/kg) (C) exhibit a significant morphology protection. Original magnification: 7500×. Results are representative of at least three experiments performed on different experimental days.

in bronchioalveolar lavage fluid during acute, infectious exacerbations. Neutrophil activation represents an important source of ROS [24].

Furthermore, there is much evidence that the production of ROS such as hydrogen peroxide, superoxide and hydroxyl radicals at the site of inflammation contributes to tissue damage [25–31]. ROS can also cause DNA single-strand damage which is the obligatory trigger for PARS activation [32,33] resulting in the depletion of its substrate NAD^+ *in vitro* and a reduction in the rate of glycolysis. Since NAD^+ functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD^+ depletion leads to a rapid fall in intracellular ATP and, ultimately, cell injury [34]. Furthermore, substantial evidence exists to support the fact that PARS activation is important in inflammation [35]. Using the experimental model described here, previous work has demonstrated the anti-inflammatory potential of various therapeutic approaches aimed at the inhibition of NO synthesis, peroxynitrite formation and PARS activation [25,29,30].

In the present study, we have found that Celecoxib reduced nitrotyrosine and PARS staining. This effect in the Celecoxib-treated animals is more likely to be related to the reduced mononuclear cell infiltration.

As with most pharmacological inhibitors, we cannot exclude that additional, prostaglandin-independent effects which may contribute to the anti-inflammatory effects observed with Celecoxib in the current study.

In addition in this study, we show that Celecoxib can block RBCs modification *in vivo*. RBCs modification is by the lung injury that in turn is correlated with less oxygen exchange.

Taken together, our findings support a model in which carrageenan induces local TNFα, COX-2, and IL-1β production in pleurisy. COX-2-derived PGs appear to mediate a variety of pro-inflammatory effects in this model of lung injury. These results support the view that the overproduction of COX-2-derived PG contributes to acute inflammation.

Acknowledgments

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References

[1] Vane J, Botting R. Inflammation V and the mechanism of action of anti-inflammatory drugs. FASEB 1987;1:89.
[2] Feuerstein G, Hallenbeck JM. Prostaglandins, leukotrienes, and platelet-activating factor in shock. Ann Rev Pharmacol Toxicol 1987;27:301–13.
[3] Yokoyama C, Tanabe T. Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of enzyme. Biochem Biophys Res Commun 1989;1665:888–94.
[4] Kosaka T, Miyata A, Ihara H, Hara S, Sugimoto T, Takeda O, Takahashi E, Tanabe T. Characterization of human gene encoding prostaglandin endoperoxide synthase-2. Eur J Biochem 1994;221: 889–97.
[5] Mitchell JA, Larkin S, Williams TJ. Cyclooxygenase-2: regulation and relevance in inflammation. Biochem Pharmacol 1995;50:1535–42.
[6] Akarasereenont P, Bakhle YS, Thiemermann C, Vane JR. Cytokine-mediated induction of cyclooxygenase-2 by activation of tyrosine kinase in bovine endothelial cells stimulated by bacterial lipopolysccaride. Br J Pharmacol 1995;115:401–8.
[7] Xie W, Robertson DL, Simmons DL. Mitogen-induced prostaglandin G/H synthase: a new target for non-steroidal anti-inflammatory drugs. Drug Dev Res 1992;25:249–65.
[8] Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other nonsteroidal anti-inflammatory drugs. J Biol Chem 1993;28:6610–4.
[9] O'Neill GP, Mancini JA, Krgman S, Yergey J, Kwan MY, Falgueyrat MP, Abramovitz M, Kennedy BP, Ouellet M, Cromlish W. Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. Mol Pharmacol 1994;45:245–54.
[10] Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K. Selective inhibition of inducible cyclooxygenase-2 *in vivo* is anti-inflammatory and non-ulcerogenic. Proc Natl Acad Sci USA 1994;91:2046–50.
[11] Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, Isakson PC. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation and pain. Proc Natl Acad Sci USA 1994;91:12013–7.
[12] Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Clin Invest Dermatol 1982;78:206–9.
[13] Laight DW, Lad N, Woodward B, Waterfall JF. Assessment of myeloperoxidase activity in renal tissue after ischemia/reperfusion. Eur J Pharmacol 1994;292:81–8.
[14] Ohkaw H, Ohishi H, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
[15] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Modulation by nitric oxide of prostaglandin biosynthesis in the rat. Br J Pharmacol 1995;114:323.
[16] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248.
[17] Tomlinson A, Appleton I, Mooregilroy AR, Willis D, Mitchell JA, Willoughby A. Cyclooxygenase and nitric oxide isoforms in rat carrageenan-induced pleurisy. Br J Pharmacol 1994;113:693–8.
[18] Harada Y, Hatanaka K, Michiko K, Saito M, Ogino M, Majima M, Ohno T, Yamamoto K, Taketani Y, Yamamoto S, Katori M. Role of prostaglandin H synthase-2 in prostaglandin E_2 formation in rat carrageenan-induced pleurisy. Prostaglndins 1996;51:19–33.
[19] Crofford LJ, Wilder RL, Ristimake AP, Sano H, Remmers EF, Epps HR, Hla T. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. J Clin Invest 1994;93:391–6.
[20] Raz A, Wyche A, Needleman P. Temporal and pharmacological division of fibroblast cyclooxygenase expression into trascriptional and traslational phases. Proc Natl Acad Sci USA 1989;86:1657–61.
[21] Ristimki A, Garfinkel S, Wessendorf J, Maciag T, Hla T. Induction of cyclooxygenase-2 by interleukin-1a. Clin Exp Immunol 1994;73: 449–55.
[22] Theisen-Popp P, Pape H, Muller-Peddinghaus R. Interleukin 6 (IL-6) in adjuvant arthritis of rats and its pharmacological modulation. Int J Immunopharmacol 1992;14:565–71.
[23] Renz H, Gong JH, Schmidt A, Nain M, Gemsa D. Release of tumor necrosis factor-α from macrophages: enhancement and suppression are dose-dependently regulated by prostaglandin E2 and cyclic nucleotides. J Immunol 1988;141:2388–93.
[24] Dawson J, Sedgwick AD, Edwards JC, Lees PA. comparative study of the cellular, exudative and histological responses to carrageenan, dextran and zymosan in the mouse. Int J Tissue React 1991;13:171–85.
[25] Cuzzocrea S, Caputi AP, Zingarelli B. Peroxynitrite-mediated DNA strand breakage activates poly(ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy. Immunology 1998;93:96.
[26] Peskar BM, Trautmann M, Nowak P, Peskar BA. Release of 15-hydroxy-5,8,11,13-eicosatetraenoic acid and cysteinyl-leukotrienes in carrageenan-induced inflammation: effect of non-steroidal anti-inflammatory drugs. Agents Actions 1991;33:240–6.
[27] Da Motta JI, Cunha FQ, Vargaftig BB, Ferreira SH. Drug modulation of antigen-induced paw oedema in guinea-pigs: effects of lipopolysaccharide, tumour necrosis factor and leucocyte depletion. Br J Pharmacol 1994;112:111–6.
[28] Salvemini D, Wang ZQ, Wyatt P, Bourdon DM, Marino MH, Manning PT, Currie MG. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 1996;118:829–38.
[29] Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C. Protective effect of melatonin in carrageenan-induced models of local inflammation. J Pineal Res 1997;23:106–16.
[30] Tracey WR, Nakane M, Kuk J, Budzik G, Klinghofer V, Harris R, Carter G. The nitric oxide synthase inhibitor, L-N^G-monomethylarginine, reduces carrageenan-induced pleurisy in the rat. J Pharmacol Exp Ther 1995;273:1295–9.
[31] Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, Huang FP, Xu D, Muller W, Moncada S, Liew FY. Altered immune responses in

mice lacking inducible nitric oxide synthase. Nature 1995;375: 408–11.

[32] Inoue S, Kawanishi S. Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. FEBS Lett 1995;371:86–8.

[33] Salgo MG, Bermudez S, Squadrito G, Pryor W. DNA damage and oxidation of thiols peroxynitrite causes in rat thymocytes. Arch Biochem Biophys 1998;322:500–5.

[34] Szabó C, Dawson VL. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia–reperfusion. Trends Pharmacol Sci 1998;19:287–98.

[35] Szabó C. Nitric oxide, peroxynitrite and poly(ADP-ribose) synthetase: biochemistry and pathophysiological implications. In: Rubanyi GM, editors. Pathophysiology and clinical applications of nitric oxide. 1999, p. 69–98.