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Regulation of prostaglandin generation in carrageenan-induced pleurisy by inducible nitric oxide synthase in knockout mice

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Abstract

In the present study, by comparing the responses in wild-type mice (iNOSWT) and mice lacking (iNOSKO) the inducible (or type 2) nitric oxide synthase (iNOS), we investigated the correlation between endogenous nitric oxide (NO) and prostaglandin (PG) generation in carrageenan-induced pleurisy. The inflammatory response in iNOSKO mice was significantly reduced in respect to iNOSWT animals, as demonstrated by the exudate volume (-63%) and numbers of infiltrating cells (-62%). The levels of NOx in the pleural exudate from carrageenan-treated mice were significantly ($p < 0.01$) decreased in iNOSKO mice (16 ± 7.6 nmoles/mice) compared to iNOSWT animals (133 ± 9 nmoles/mice). Similarly, the amounts of PGE_2 in the pleural exudates of carrageenan-treated animals were significantly ($p < 0.01$) lower in iNOSKO compared to iNOSWT mice (120 ± 20 pg/mice vs. 308 ± 51 pg/mice). Also the amounts of 6-keto-$PGF_{1\alpha}$ produced by lungs from carrageenan-treated iNOSKO mice (1.01 ± 0.10 ng/tissue mg) were significantly ($p < 0.01$) reduced compared to iNOSWT carrageenan-treated mice (2.1 ± 0.09 ng/tissue mg). In conclusion our results confirm, by the use of iNOSKO mice that in carrageenan-induced pleurisy NO positively modulates PG biosynthesis.

Keywords: Carrageenan; Cyclooxygenase; Inflammation; Nitric oxide; Prostaglandins

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Introduction

Nitric oxide (NO) is a pleiotropic mediator formed from L-arginine by nitric oxide synthase (NOS). Several major isoforms of NOS have been identified. Two Ca^{2+}/calmodulin-dependent isoforms are constitutively expressed in the endothelium of blood vessel (eNOS or type 3 NOS) and in the neurons of the brain (nNOS or type 1 NOS) and release NO under physiological conditions. The Ca^{2+}/calmodulin independent isoform (iNOS or type 2 NOS) is expressed following its transcriptional induction by pro-inflammatory cytokines and bacterial wall components in different cell types such as macrophages, neutrophils, endothelial and smooth muscle cells [1,2]. Enhanced formation of NO following the induction of iNOS has been implicated in the pathogenesis of shock and inflammation [2].

Enhanced arachidonic acid metabolism generally accompanies inflammation and tissue injury. Biochemical and pharmacological data support the existence of two isoforms of cyclooxygenase (COX), the enzyme which converts arachidonic acid to prostaglandins (PGs). The constitutive isoform (COX-1), which is present in most cells and tissues, regulates many physiological functions through the release of PGs, while the inducible isoform (COX-2), which is induced at the inflammation site by inflammatory and/or immunological stimuli, seems to be responsible of the elevated PG generation which characterizes the inflammatory process [3–5].

The exposure to inflammatory mediators such as cytokines and endotoxin (LPS) leads to the induction of both COX-2 and iNOS [6,7]. This co-induction has encouraged researchers to look for a possible cross talk between the NOS and COX pathways. It has been reported from in vitro and ex vivo experiments that the production of large amounts of NO decreased PG generation [6,8–11]. Conversely, using similar experimental models in vitro and ex vivo, we and others have found that NO increases PG biosynthesis [12–17]. In support of these latter studies, experiments in LPS-treated rats and in rats subjected to acute inflammation (peritonitis and paw oedema) have led to the suggestion that also NO produced by iNOS in vivo positively regulates PG generation [18–21]. Unfortunately these previous investigations were limited by the lack of highly selective iNOS inhibitors. This is particularly relevant for the in vivo studies, where all the three isoforms of NOS are present [22], and where NO produced by eNOS is central to the regulation of regional blood flow and systemic blood pressure [22]. In this study, we have investigated the cross talk between the NOS and COX systems in carrageenan-induced pleurisy, using knockout mice for iNOS (iNOSKO). It has been previously demonstrated that, in this model of acute inflammation, COX-2 and iNOS are the predominant isoforms of their respective enzymes, with a similar profile of activity [23]. Moreover in the same experimental model it has been shown that either the potentiation or the inhibition of NO generation produced a corresponding increase or decrease in PGE_2 biosynthesis [24]. In this study we have determined, after pleurisy induction by carrageenan, the following endpoints of the inflammatory response in iNOSKO mice and in corresponding wild-type (iNOSWT) mice: (1) exudate formation, (2) polymorphonuclear (PMN) infiltration in the pleural cavity, (3) NOx and PGE_2 levels in the exudate and 6-keto-$PGF_{1\alpha}$ amount in lung tissue.

Methods

Animals

Male iNOSKO and iNOSWT mice (20–25 g, kindly supplied by Fons A.J. Van de Loo, Department of Rheumatology, University Hospital Nijmegen, Nijmegen, The Netherlands) were used. All animals

were allowed access to food and water ad libitum. 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).

Carrageenan-induced pleurisy

Mice were anaesthetized with isoflurane and submitted to a skin incision at the level of the left sixth intercostals space. The underlying muscle was dissected and 0.1 ml saline (sham group) or 0.1 ml saline containing 2% λ-carrageenan (carrageenan group) were injected into the pleural cavity. At 4 h after the injection of saline or carrageenan, the animals were killed by inhalation of CO_2. The chest was carefully opened and the pleural cavity rinsed with 1 ml of saline solution containing heparin (5 U/ml) and indomethacin (10 μg/ml). The exudate and washing solution were removed by aspiration and the total volume measured. Any exudate, which was contaminated with blood was discarded. The amount of exudate was calculated by subtracting the volume of the saline solution (1 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.

Measurement of nitrite + nitrate (NOx) in pleural exudate

Nitrite + nitrate (NOx) production, an indicator of NO synthesis, was measured in the pleural exudate as previously described [21]. Briefly, the nitrate in the samples was first reduced to nitrite by incubation with nitrate reductase (670 mU/ml) and NADPH (160 μM) at room temperature for 3 h. The nitrite concentration in the samples was then measured by the Griess reaction, by adding 100 μl of Griess reagent (0.1% naphthylethylenediamine dihydrochloride in H_2O and 1% sulphanilamide in 5% concentrated H_2PO_4; vol. 1:1) to 100 μl samples. The optical density at 550 nm (OD_{550}) was measured using ELISA microplate reader (SLT-Labinstruments Salzburg, Austria). Nitrite concentrations were calculated by comparison with OD_{550} of standard saline.

Measurement of prostaglandin E_2 in pleural exudate

The amount of prostaglandin E_2 (PGE_2) present in the pleural fluid was measured by radioimmunoassay (RIA) without prior extraction or purification [25] and expressed as pg/mice.

Measurement of 6-keto-$PGF_{1\alpha}$ in lung tissue

Lungs obtained at 4 h after the induction of pleurisy by carrageenan injection were immediately homogenised and processed as described by Westcott et al. [26]. The amount of 6-keto-$PGF_{1\alpha}$ present in the lung homogenate was measured by RIA as previously described [25] and expressed as ng/tissue mg.

Measurement of COX-2 mRNA expression using RT-PCR

RT-PCR was carried out on lung tissues collected 4 h after carrageenan administration. Following careful washing, total RNA was purified from approximately 200 mg of each tissue and reverse transcribed into cDNA as previously reported [27]. PCR amplification of cDNA product was carried out

using COX-2 specific primers. The mRNA for the constitutive GAPDH enzyme was examined as the reference transcript. GAPDH mRNA amplification products (195 bp) were present at equivalent levels in all tissue lysates.

Estimates of the relative COX-2 mRNA amounts were obtained dividing the area of the COX-2 band by the area of the GAPDH band (Bio-Rad Multi-Analyst/PC Version 1.1).

Data analysis

All values in the figures and text are expressed as mean ± SEM of the mean of *n* observations, where *n* represents the number of animals studied. Data sets were examined by one- and two-way analysis of variance. Individual group means were then compared with Student's unpaired t-test. A *p*-value less than 0.05 were considered significant.

Materials

[^{3}H]-PGE_2 (specific activity 170 Ci/mmol) and [^{3}H]-6-keto-$PGF_{1\alpha}$ (specific activity 130 Ci/mmol) were from Du Pont de Nemours Italiana (Cologno Monzese, Italy). Antisera for both compounds were a kind gift of Prof. G. Ciabattoni (Catholic University, Rome, Italy). All other reagents and compounds used were obtained from Sigma Chemical Company (Sigma, Milan, Italy).

Results

Exudate volume and cell count

Injection of 0.1 ml of 2% λ-carrageenan into the pleural cavity of iNOSWT mice caused a significant accumulation of the inflammatory exudate in respect to iNOSWT sham animals. As shown in Table 1 the average volume of exudate for iNOSWT mice was 1.1 ± 0.13 and 0.12 ± 0.06 ml/mice ($p < 0.01$) in carrageenan-treated and sham animals, respectively. Total leucocytes number migrated into the pleural cavity was 66 ± 1.3 × 10^6 in carrageenan-treated iNOSWT mice and 1.2 ± 0.6 × 10^6 in sham iNOSWT animals ($p < 0.01$). Carrageenan-treated iNOSKO mice showed a significant ($p < 0.01$)

Table 1
Exudate volume and number of emigrated cells in pleural cavity at 4 h after carrageenan injection

	Volume (ml)		Polymorphonuclear (million cells/mice)	
	Sham	Carrageenan	Sham	Carrageenan
iNOSWT	0.12 ± 0.06	1.1 ± 0.13*	1.2 ± 0.6	66 ± 1.3*
iNOSKO	0.11 ± 0.08	0.41 ± 0.11$^{\#,\circ}$	1 ± 0.8	25 ± 1.1$^{\#\#,\circ}$

Data are means ± SEM of 10 mice for each group.
* $p < 0.01$ vs iNOSWT sham.
$^{\#}$ $p < 0.05$ vs iNOSKO sham.
$^{\#\#}$ $p < 0.01$ vs iNOSKO sham.
$^{\circ}$ $p < 0.01$ vs iNOSWT carrageenan.

reduction (− 63%) of the pleural exudate as well as the number of emigrated PMNs (− 62%) in respect to iNOSWT carrageenan-treated mice, although a significant increase of both pleural exudate and infiltrated cells was observed in respect to iNOSKO sham animals (Table 1).

Nitrite + nitrate (NOx) and PGE_2 in pleural exudate

The levels of NOx (NO_2^- + NO_3^-) were significantly ($p < 0.01$) increased in the exudate from carrageenan-treated iNOSWT mice when compared to iNOSWT sham mice (Fig. 1A). The NOx levels found in the exudate of carrageenan-treated iNOSKO animals were significantly ($p < 0.01$) decreased

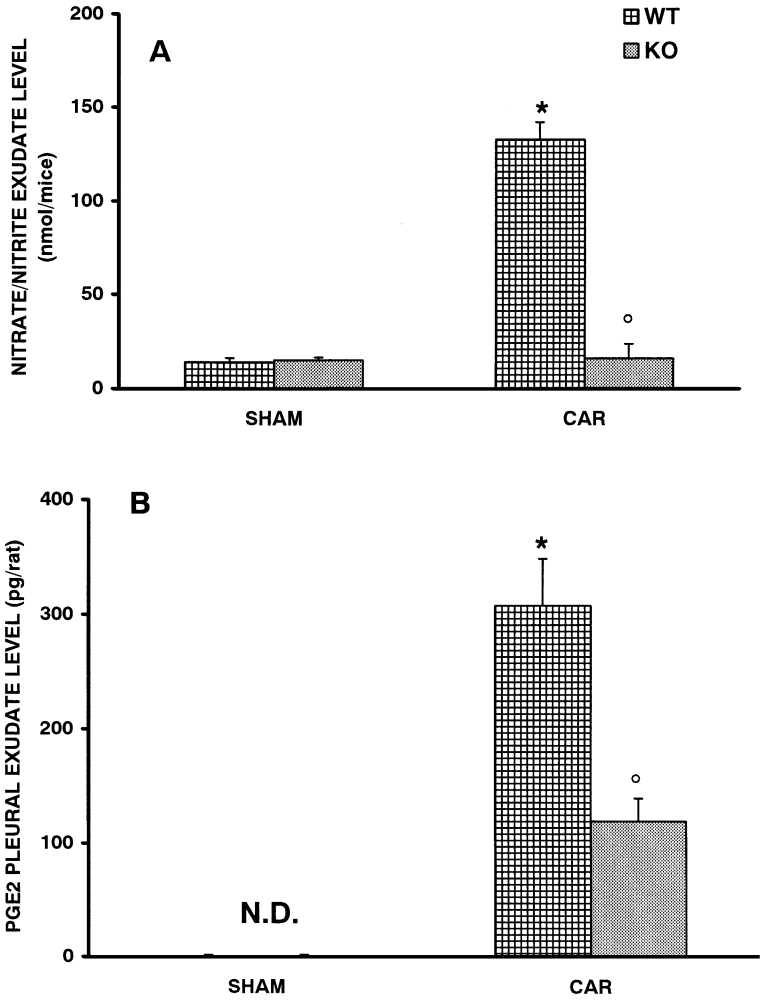

Fig. 1. Nitrite + nitrate (NOx) (**A**) and PGE_2 (**B**) levels in pleural exudate at 4 h after carrageenan administration. Data are expressed as nmoles/mice for NOx and pg/mice for PGE_2 and represent the means ± SEM of 10 mice for each group. *$p < 0.01$ vs iNOSWT sham. ◦$p < 0.01$ vs iNOSWT carrageenan.

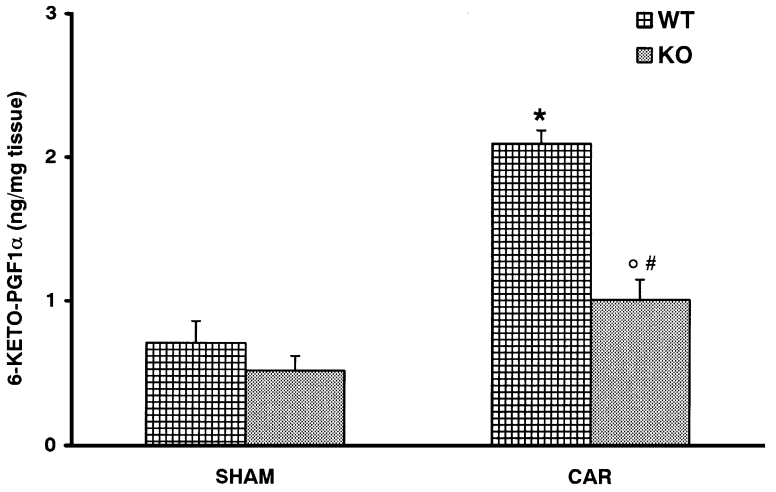

Fig. 2. Amount of 6-keto-$PGF_{1\alpha}$ in the lung at 4 h after carrageenan injection. Data are expressed as ng/tissue mg and represent the means ± SEM of 10 mice for each group. *$p < 0.01$ vs iNOSWT sham. $^{\#}p < 0.05$ vs iNOSKO sham. ◦$p < 0.01$ vs iNOSWT carrageenan.

by about 88% compared to carrageenan-treated iNOSWT mice and similar to what observed in iNOSKO sham animals (Fig. 1A).

The amount of PGE_2 found in the pleural exudate from either iNOSWT or iNOSKO sham animals were under the least detectable concentration of the RIA (6 pg/ml). In carrageenan-treated iNOSKO mice the levels of PGE_2 were significantly ($p < 0.01$) lower, by about 61%, compared to carrageenan-treated iNOSWT mice (Fig. 1B).

Amount of 6-keto-$PGF_{1\alpha}$ in lung tissue

A significant increase was observed in both iNOSWT and iNOSKO lungs from carrageenan-treated mice compared to the respective sham group. When the amount of 6-keto-$PGF_{1\alpha}$ detected in the lungs from carrageenan-treated iNOSWT mice was compared to that observed in the tissues from carrageenan-treated iNOSKO animals a significant ($p < 0.01$) reduction, by about 52%, was observed. However the amount of the prostanoid detected in lung from iNOSWT and iNOSKO sham animals was not statistically different (Fig. 2).

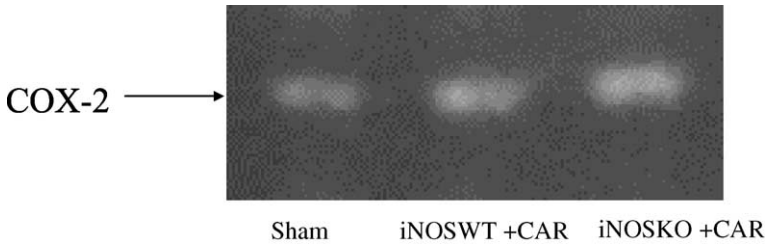

Fig. 3. Gene expression of COX-2 in the mice lung after vehicle or carrageenan administration.

COX-2 mRNA expression

Although the gene expression of COX-2 was negligible in the normal lung collected from iNOSWT mice, the expression of COX-2 mRNA was found to be up-regulated in the lung from carrageenan-treated iNOSWT mice, the expression was clearly detected in the lung as early as 4 h after the carrageenan administration (Fig. 3). The up-regulation of COX-2 was similarly observed in the lung collected from carrageenan-treated iNOSKO mice (Fig. 3).

Discussion

COX is the rate-limiting enzyme involved in the biosynthesis of prostaglandins, thromboxane A_2 and prostacyclin. In addition to the well characterized constitutive form of COX (COX-1) [28] an inducible isoform (COX-2) is present in endothelial cells [29], fibroblasts [30], and macrophages [3,4] after treatment with pro-inflammatory agents including LPS and IL-1β. Regulation of this enzyme shares similarities with the regulation of iNOS [17] and is also under the regulation of nuclear factor kappa B (NFkB) and MAP kinase [31,32]. A cross talk between NOS and COX pathways has been suggested by many studies. Thus NO has been reported to either negatively or positively modulate PG generation (for review see Refs. [33,34]).

In this study, we have shown that in carrageenan-induced pleurisy, almost in the absence of NO production, as in iNOSKO mice, PGE_2 generation in the pleural exudate was significantly decreased, by about 61%, compared to what was observed in iNOSWT mice. Thus PG generation at the inflammation site seems to be, at least in part, under the control of NO. The lack of NO generation in iNOSKO mice is demonstrated by the levels of NOx found in the pleural exudates of carrageenan-treated mice, which were similar to what was observed in sham animals. In fact in carrageenan-induced pleurisy NO is strongly generated by the iNOS, which is the predominant isoform [23]. Thus our results suggest that the action of NO on PG biosynthesis seems to be dependent on iNOS induction. These results are in agreement with previous data, obtained in rat carrageenan pleurisy [24] and in other models of acute inflammation [14,16,19–21] showing that the modulation of NO pathway, either by inhibiting, with NOS inhibitors, or increasing, with L-arginine or NO donors, NO generation corresponds to a parallel modulation of PG biosynthesis. Moreover in the lung of carrageenan-treated iNOSKO mice we observed a 52% decrease in 6-keto-$PGF_{1\alpha}$ generation compared to iNOSWT animals. Thus the lack of NO generation, due to the absence of iNOS expression, which is present in the lung from iNOSWT animals treated with carrageenan [35], is correlated with decreased prostanoid production. No difference in PG production was observed in the lung of iNOSWT and iNOSKO sham animals, suggesting that NO does not affect the unstimulated release of the prostanoid. These results are in agreement with previous data, obtained in another model of inflammation in the rat lung [16]. The effect of null iNOS genotype on COX enzyme has been investigated in different experimental models with different results. PG formation by stimulated peritoneal macrophages from iNOS-deficient mice was decreased compared to cells from wild type animals as well as PG levels in the urine [36] suggesting a positive correlation. Similar positive correlation was found concerning the antinociceptive effect of iNOS deletion which correlated with loss of stimulation of PG biosynthesis, probably through an action on COX activity [37]. On the contrary, in a mouse model of familial adenomatous polyposis no correlation between the antitumorigenic role of iNOS and COX-2 expression and activity was found [38].

The mechanism of action of NO in the modulation of PG biosynthesis is still under debate. In the present study we demonstrate that the genetic inhibition of iNOS (iNOSKO mice) did not modify the up-regulation of the expression of COX-2 mRNA in the lung after carrageenan administration. These results demonstrate that in the carrageenan-induced acute lung inflammation NO modulates PG biosynthesis interfering with COX-2 activity and not at the expression level of the enzyme. Therefore, several mechanisms have been suggested in different experimental models. In rat mesangial cells and human lung epithelial cells NO seems to modulate, at least in part, PG generation stimulated by IL-1β, by potentiating COX-2 mRNA and protein expression [17]. Other reports (for review see Ref. [39]) have suggested that NO may act, at least in part, both at transcriptional and post-transcriptional levels. However other study [40] has been reported that ischemia-induced PGE_2 biosynthesis, in the brain, was significantly reduced in iNOS knockout mice compared with wild-type animals, although COX-2 expression was not reduced, thus suggesting a direct interaction between NO and COX. Stimulation of PG biosynthesis by a direct interaction between NO and COX has been indicated by other studies, although the type of interaction has not been clearly elucidated [39].

Conclusion

In conclusion, our results demonstrate that in the carrageenan-induced acute lung inflammation NO modulates PG biosynthesis interfering with COX-2 activity and not at the expression level of the enzyme. Therefore, we demonstrate by the use of iNOSKO mice, that in carrageenan-induced pleurisy NO positively modulates PG biosynthesis and that the pro-inflammatory action of NO seems to be also related to the cross talk with the COX pathway.

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References

[1] Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43(2):109–42.
[2] Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 1992;6(12):3051–64.
[3] Masferrer JL, Seibert K, Zweifel B, Needleman P. Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. Proc Natl Acad Sci U S A 1992;89(9):3917–21.
[4] Fu JY, Masferrer JL, Seibert K, Raz A, Needleman P. The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. J Biol Chem 1990;265(28):16737–40.
[5] Raz A, Wyche A, Fu J, Seibert K, Needleman P. Regulation of prostanoids synthesis in human fibroblasts and human blood monocytes by interleukin-1, endotoxin, and glucocorticoids. Adv Prostaglandin Thromboxane Leukot Res 1990;20:22–7.

[6] Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-Bailey D, Croxtall J, Willoughby DA. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc Natl Acad Sci U S A 1994;91(6):2046–50.
[7] Swierkosz TA, Mitchell JA, Warner TD, Botting RM, Vane JR. Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. Br J Pharmacol 1995;114(7):1335–42.
[8] Stadler J, Harbrecht BG, Di Silvio M, Curran RD, Jordan ML, Simmons RL, Billiar TR. Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. J Leukoc Biol 1993;53(2):165–72.
[9] Kanner J, Harel S, Granit R. Nitric oxide, an inhibitor of lipid oxidation by lipooxygenase, cyclooxygenase and hemoglobin. Lipids 1992;27(1):46–9.
[10] Amin AR, Attur M, Patel RN, Thakker GD, Marshall PJ, Rediske J, Stuchin SA, Patel IR, Abramson SB. Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest 1997; 99(6):1231–7.
[11] Habib A, Bernard C, Lebret M, Creminon C, Esposito B, Tedgui A, Maclouf J. Regulation of the expression of cyclooxygenase-2 by nitric oxide in rat peritoneal macrophages. J Immunol 1997;158(8):3845–51.
[12] Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci U S A 1993;90(15):7240–4.
[13] Inoue T, Fukuo K, Morimoto S, Koh E, Ogihara T. Nitric oxide mediates interleukin-1-induced prostaglandin E2 production by vascular smooth muscle cells. Biochem Biophys Res Commun 1993;194(1):420–4.
[14] Salvemini D, Seibert K, Masferrer JL, Misko TP, Currie MG, Needleman P. Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation. J Clin Invest 1994;93(5):1940–7.
[15] Franchi AM, Chaud M, Rettori V, Suburo A, McCann SM, Gimeno M. Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri. Proc Natl Acad Sci U S A 1994;91(2):539–43.
[16] Sautebin L, Di Rosa M. Nitric oxide modulates prostacyclin biosynthesis in the lung of endotoxin-treated rats. Eur J Pharmacol 1994;262(1–2):193–6.
[17] Tetsuka T, Daphna-Iken D, Miller BW, Guan Z, Baier LD, Morrison AR. Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. J Clin Invest 1996;97(9):2051–6.
[18] Salvemini D, Settle SL, Masferrer JL, Seibert K, Currie MG, Needleman P. Regulation of prostaglandin production by nitric oxide; an in vivo analysis. Br J Pharmacol 1995;114(6):1171–8.
[19] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Modulation by nitric oxide of prostaglandin biosynthesis in the rat. Br J Pharmacol 1995;114(2):323–8.
[20] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Endogenous nitric oxide increases prostaglandin biosynthesis in carrageenin rat paw oedema. Eur J Pharmacol 1995;286(2):219–22.
[21] Cuzzocrea S, Zingarelli B, Sautebin L, Rizzo A, Crisafulli C, Campo GM, Costantino G, Calapai G, Nava F, Di Rosa M, Caputi AP. Multiple organ failure following zymosan-induced peritonitis is mediated by nitric oxide. Shock 1997; 8(4):268–75.
[22] Moncada S. Nitric oxide in the vasculature: physiology and pathophysiology. Ann N Y Acad Sci 1997;811:60–7.
[23] Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoughby DA. Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. Br J Pharmacol 1994;113(3):693–8.
[24] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Relationship between nitric oxide and prostaglandins in carrageenin pleurisy. Biochem Pharmacol 1998;55(7):1113–7.
[25] Sautebin L, Ianaro A, Rombola L, Ialenti A, Sala A, Di Rosa M. Cyclooxygenase-2-dependent generation of 8-epiprostaglandin F2alpha by lipopolysaccharide-activated J774 macrophages. Inflamm Res 1999;48(9):503–8.
[26] Westcott JY, Chang S, Balazy M, Stene DO, Pradelles P, Maclouf J, Voelkel NF, Murphy RC. Analysis of 6-keto PGF1 alpha, 5-HETE, and LTC4 in rat lung: comparison ofGM/MS, RIA, and EIA. Prostaglandins 1986;32(6):857–73.
[27] Hierholzer C, Harbrecht B, Menezes JM, Kane J, Mac-Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. Anal Biochem 1987;162:156–9.
[28] DeWitt DL. Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim Biophys Acta 1991; 1083(2):121–34.
[29] Maier JA, Hla T, Maciag T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. J Biol Chem 1990;265(19):10805–8.
[30] Raz A, Wyche A, Siegel N, Needleman P. Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. J Biol Chem 1988;263(6):3022–8.
[31] Yamamoto K, Arakawa T, Ueda N, Yamamoto S. Transcriptional roles of nuclear factor kappa B and nuclear factor-

interleukin-6 in the tumor necrosis factor alpha-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. J Biol Chem 1995;270(52):31315–20.
[32] Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR. Induction of cyclooxygenase-2 by the activated MEKK1 → SEK1/MKK4 → p38 mitogen-activated protein kinase pathway. J Biol Chem 1998;273(21):12901–8.
[33] Di Rosa M, Ialenti A, Ianaro A, Sautebin L. Interaction between nitric oxide and cyclooxygenase pathways. Prostaglandins Leukot Essent Fatty Acids 1996;54(4):229–38.
[34] Salvemini D, Manning PT, Zweifel BS, Seibert K, Connor J, Currie MG, Needleman P, Masferrer JL. Dual inhibition of nitric oxide and prostaglandin production contributes to the antiinflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 1995;96(1):301–8.
[35] Cuzzocrea S, Sautebin L, De Sarro G, Costantino G, Rombola L, Mazzon E, Ialenti A, De Sarro A, Ciliberto G, Di Rosa M, Caputi AP, Thiemermann C. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. J Immunol 1999;163(9):5094–104.
[36] Marnett LJ, Wright TL, Crews BC, Tannenbaum SR, Morrow JD. Regulation of prostaglandin biosynthesis by nitric oxide is revealed by targeted deletion of inducible nitric-oxide synthase. J Biol Chem 2000;275(18):13427–30.
[37] Guhring H, Gorig M, Ates M, Coste O, Zeilhofer HU, Pahl A, Rehse K, Brune K. Suppressed injury-induced rise in spinal prostaglandin E2 production and reduced early thermal hyperalgesia in iNOS-deficient mice. J Neurosci 2000; 20(17):6714–20.
[38] Scott DJ, Hull MA, Cartwright EJ, Lam WK, Tisbury A, Poulsom R, Markham AF, Bonifer C, Coletta PL. Lack of inducible nitric oxide synthase promotes intestinal tumorigenesis in the Apc(Min/+) mouse. Gastroenterology 2001; 121(4):889–99.
[39] Goodwin DC, Landino LM, Marnett LJ. Effects of nitric oxide and nitric oxide-derived species on prostaglandin endoperoxide synthase and prostaglandin biosynthesis. FASEB J 1999;13(10):1121–36.
[40] Nogawa S, Forster C, Zhang F, Nagayama M, Ross ME, Iadecola C. Interaction between inducible nitric oxide synthase and cyclooxygenase-2 after cerebral ischemia. Proc Natl Acad Sci U S A 1998;95(18):10966–71.