Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury

ALBERT M. SONG, LAKSHMI BHAGAT, VIJAY P. SINGH, GIJS G. D. VAN ACKER, MICHAEL L. STEER,* AND ASHOK K. SALUJA*

Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215

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Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury. Am J Physiol Gastrointest Liver Physiol 283: G1166–G1174, 2002.—Cyclooxygenase-2 (COX-2), a widely distributed enzyme, plays an important role in inflammation. We have studied the role of COX-2 in acute pancreatitis and pancreatitis-associated lung injury using both the pharmacological inhibition of COX-2 and genetic deletion of COX-2. Pancreatitis was induced in mice by 12 hourly injections of cerulein. The severity of pancreatitis was assessed by measuring serum amylase, pancreatic trypsin activity, intrapancreatic sequestration of neutrophils, and acinar cell necrosis. The severity of lung injury was evaluated by measuring lactate dehydrogenase levels in the bronchoalveolar lavage fluid and by quantitating neutrophil sequestration in the lung. In both the pharmacologically inhibited and genetically altered mice, the severity of pancreatitis and pancreatitis-associated lung injury was reduced compared with the noninhibited strains of COX-2-sufficient mice. This reduction in injury indicates that COX-2 plays an important proinflammatory role in pancreatitis and its associated lung injury. Our findings support the concept that COX-2 inhibitors may play a beneficial role in the prevention of acute pancreatitis or in the reduction of its severity.

prostaglandin; proinflammatory; cerulein; neutrophil; heat shock protein 70; inducible nitric oxide synthase; trypsin; myeloperoxidase

CLINICAL ACUTE PANCREATITIS can present with varying degrees of severity. It is frequently associated with lung injury, manifesting as the adult respiratory distress syndrome. Approximately 80% of patients experience a mild attack with minimal sequelae; however, the other 20% of patients develop severe disease with a mortality rate that can reach 40% (37). The factors that determine the development and ultimate severity of pancreatitis are still not fully understood.

The initiation of prostanooid synthesis from arachidonic acid involves the enzyme cyclooxygenase (COX), an enzyme that is also referred to as PGH synthase or PG endoperoxide synthase, because it is also the rate-limiting enzyme for PGE2 synthesis. Two COX isoforms have been identified: a constitutive form (COX-1), which is thought to have an important housekeeping function, and an inducible form (COX-2), which has been implicated as an important proinflammatory mediator (21). COX-2 is upregulated in response to a variety of proinflammatory stimuli including IL-1, TNF-α, and bacterial lipopolysaccharide (1, 9, 23). COX-2 mRNA and protein levels are increased during experimental pancreatitis, but the role of COX-2 in pancreatitis has not been well defined (13).

In this communication, we report the result of studies dealing with the role of COX-2 in acute pancreatitis and pancreatitis-associated lung injury. In our studies, some mice were pretreated with COX-2 inhibitors (15, 33), whereas other mice were bred with genetic deletion of COX-2. Pancreatitis was induced via supramaximal secretagogue stimulation. Both the genetic deletion of COX-2 and the pharmacological inhibition of COX-2 were found to reduce the severity of secretagogue-induced pancreatitis as well as its associated lung injury. Our observations indicate that COX-2 plays an important proinflammatory role in regulating the severity of acute pancreatitis and its associated lung injury.

MATERIALS AND METHODS

All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center. Breeding pairs of COX-2-deficient mice, back-crossed to a C57/Bl6 background, were purchased from Taconic laboratories (Germantown, NY). The identity of COX-2-deficient (−/−) homozygotes was confirmed by PCR analysis. Wild-type C57/Bl6 mice, purchased from Taconic laboratories, were used as COX-2-sufficient (+/+ ) controls. All animals were bred and housed in standard shoebox cages in a climate-controlled room with an ambient temperature of 23 ± 2°C and a 12:12-h light-dark cycle. Animals were fed standard laboratory chow, given water ad libitum, randomly assigned to control or experimental groups, and fasted overnight before each experiment. Cerulein, the decapeptide analog of the potent pan-
creatic secretagogue CCK, was obtained from Research Plus (Bayonne, NJ). N-(2-cyclohexylxoy-4-nitrophenyl)methane sulfonamide (NS-398) was purchased from Sigma (St. Louis, MO). Celecoxib was a kind gift from the Beth Israel Deaconess Medical Center Pharmacy. RNA Later, 18s primer, and the Total RNA kit were purchased from Ambion (Austin, TX). Antibody to inducible heat shock protein 70 (HSP70; SPA-810) was purchased from Stressgen (Victoria, Canada). Antibodies to COX-2 (rabbit polyclonal) and inducible nitric oxide synthase (iNOS), and anti-rabbit and anti-mouse IgG peroxidase conjugates were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Other reagents were purchased from sources previously reported (5, 6) and were of the highest purity available.

**Semiquantitative PCR analysis for COX-2.** Pancreas samples were stored in RNA Later at 4°C until further processing. Total RNA was extracted using the Totally RNA kit. Primer for 18s was used as an internal standard. Five micromolars of total RNA were reverse transcribed in a 50-μl reaction mixture containing 60 ng of random hexamer as a primer, together with 200 U Superscript II RT, according to the manufacturer’s instructions (Catalyzed Reporter Decay; MOB-RLI). For the PCR, 2 μl CDNA mixture were added to 50 μl master mix containing 200 μM of each dNTP, 100 μM of each specific primer, as well as 1.5 mM MgCl2 and 25 U Taq DNA polymerase. DNA oligonucleotide primers for COX-2 were selected from NIH GenBank (accession no. NM-01198). The sense primer was based on the sequence number 357–382 5′-GAC-CCA-CTT-CAA-AGG-GGT-CTG-GAA-C-3′ and the antisense number 857–881 5′-CTG-CTG-TGG-GGC-TGA-ACC-CCA-CAA-CCT-C-3′. The optimal reaction conditions chosen were an annealing temperature of 40°C, cycle number 35, and Mg2+ concentration of 1.5 mM. The PCR products were separated on 1.5% agarose gel and stained with ethidium bromide. The stained gels were photographed under ultraviolet illumination using Polaroid 667 film (Polaroid, Cambridge, MA).

**Western blotting for COX-2, HSP70, and iNOS.** Pancreas samples were homogenized in suspension buffer, pH 7.4, containing 100 mM NaCl, 10 mM Tris·HCl, 1 mM EDTA, 1 μg/ml aprotinin, and 100 μg/ml PMSF. The homogenates were centrifuged (14,000 g for 15 min at 4°C), the supernatants were collected, and the protein concentration in the supernatant was measured by the method of Bradford (8). The samples (10 μg/lane) were subjected to gel electrophoresis as described by Laemmli (29) using 10% Ready gels and transferred onto nitrocellulose membranes for Western blot analysis by the method of Towbin et al. (39). COX-2, HSP70, and iNOS levels were monitored using antibodies to COX-2 (1:300), HSP70 (1:1,000), and iNOS (1:1,000), respectively. Labeled proteins were visualized by the enhanced chemiluminescence method using horseradish peroxidase-coupled secondary antibodies (1:5,000) and quantitated using a Scion Image Analysis program (Scion, Frederick, MD).

**Blood and tissue preparation.** Animals were given hourly intraperitoneal injections of saline (control) or saline containing a supramaximally stimulating concentration of cerulein (50 μg/kg) for 12 h. The animals involved in the pharmacological study were also given either a dose of NS-398 (20 mg/kg), celecoxib (20 mg/kg), or vehicle (10% DMSO) just before the first cerulein injection. One hour after the last injection, mice were killed in a CO2 gas chamber. Blood and tissue samples of the pancreas and lungs were then prepared for study. Harvested blood was allowed to clot and was then centrifuged. Serum was harvested and used for measurement of amylase activity. For morphological studies, the pancreas was removed rapidly, and complete random cross-sections of the head, body, and tail of the pancreas were fixed in 4% neutral phosphate-buffered formalin for histological study. After the pancreas was removed, the trachea was cannulated and the lungs were lavaged in situ three times with saline (1 ml per lavage). The bronchoalveolar lavage (BAL) fluid was collected and combined with an overall fluid recovery between 85 and 90%. Other samples of pancreas and lung were prepared for the measurement of tissue MPO activity as described in Other assays.

**Morphological examination.** Paraflin-embedded pancreas samples were sectioned (5 μm), stained with hematoxylin-eosin, and examined by an experienced morphologist who was not aware of the sample identity. Acanin cell injury necrosis was quantitated by morphometry as previously described (6). For these studies, 10 randomly chosen microscopic fields (×125) were examined for each tissue sample, and the extent of acinar cell injury necrosis was expressed as the percentage of the total acinar tissue that was occupied by areas meeting the criteria for injury/necrosis. Those criteria were defined as either 1) the presence of acinar-cell ghosts or 2) vacuolization and swelling of acinar cells and the destruction of the histoarchitecture of whole or parts of the acini, both of which had to be associated with an inflammatory reaction.

**Other assays.** Serum amylase activity was measured using 4,6-ethylidene(G1)-p-nitrophenyl(G2)k1,β-D-maltolheptoside(Sigma) as the substrate (34). BAL lactate dehydrogenase (LDH) activity was measured using pyruvate and NADH as the substrates (3). Neutrophil sequestration in pancreas and lung was quantitated by measuring tissue MPO activity. For these measurements, tissue samples harvested at the time of death were stored at −70°C. The samples were thawed and homogenized in 1 ml of 20 mM phosphate buffer (pH 7.4) and centrifuged (10,000 g for 10 min at 4°C), and the resulting pellet was reconstituted in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecylmethylammonium bromide. The suspension was further disrupted by sonication (40 s). The sample was then centrifuged (10,000 g for 5 min at 4°C), and the supernatant was used for MPO assay. The MPO assay is a modification of the bromide-dependent chemiluminescence technique as described by Hafqani et al. (18).

PGF2α levels in pancreatic homogenates were measured using an ELISA kit (Caymon Chemicals, Ann Arbor, MI). TNF-α levels were measured in homogenates of pancreatic tissue and in BAL fluid using an ELISA kit (BD Pharmingen, Franklin Lakes, NJ). Values for pancreas were expressed per microgram of DNA in each sample, whereas those for BAL fluid were expressed per 3 ml BAL fluid.

For measurement of intrapancreatic trypsin activity, pancreas samples were homogenized in cold (4°C) MOPS buffer, pH 7.0 (250 mM sucrose, 5 mM MOPS, and 1 mM MgSO4), using a motorized glass-Teflon homogenizer. The resulting homogenate was centrifuged (50 g, 5 min), and the supernatant was taken for assay. Trypsin activity was measured fluorometrically using Boc-Glu-Ala-Arg-methyl-coumaryl-7- amide as the substrate according to the method of Kawabata et al. (26) and described in detail in our earlier publication (22), whereas DNA was measured using Hoechst 33258 dye, according to the method of Labarca and Paigen (28). Our results were expressed as trypsin activity (slope of fluorescence emission) per microgram of DNA in the homogenate.

**EMSA for NF-κB.** Nuclear extracts were prepared as described by Dyer and Herzog (12). Reaction mixtures (25 μl, pH 7.5) contained 7.5–10 μg of nuclear protein, 5 mM Tris, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 4% (vol/vol) glycerol, and 0.08 mg/ml salmon sperm DNA. The oligonucleotide probe [5′-AGT TGA GGG GAC TTC CCC AGG C-3′ (Promega, Madison, WI)] containing the κB binding motif

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was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase and purified over two successive 1-ml G-50 columns (Amersham Pharmacia Biotech). The probe (1 × 10^6 counts/min) was added to the reaction mixture, and the binding reaction was allowed to proceed for 20 min at room temperature. DNA-protein complexes were resolved in a 6% non-denaturing polyacrylamide gel in buffer containing 22.5 mM Tris, 22.5 mM boric acid, and 0.5 mM EDTA, pH 8.3, at 140 V for 2–3 h. Gels were dried and exposed to Kodak Bio Max MR films at −70°C. NF-κB bands from films were quantitated by using a Hewlett-Packard Scanjet 4100 scanner and a Scion image-analysis program.

Data presentation. The results reported represent means ± SE for multiple determinations from at least four or more animals. In the figures, vertical bars denote SE. The Student’s t-test or ANOVA using Tukey’s post hoc test was used to evaluate the significance of the changes. A P value <0.05 was considered to be statistically significant.

RESULTS

Pancreatitis-induced changes in COX-2 mRNA and protein levels. COX-2 mRNA levels in the pancreas of control animals were low, but within 15 min of the start of cerulein administration, COX-2 mRNA levels were markedly increased. They continued to be elevated during the initial 3 h following the start of cerulein administration (Fig. 1). COX-2 protein levels, measured by Western blotting, were also low in control animals and were unchanged for the first 2 h of cerulein administration but increased in the pancreas within 4 h of the start of cerulein administration, and the COX-2 levels remained elevated over the subsequent 8 h (Fig. 2). Similar changes have been noted by others (13). PGE2 (the COX-2 product) levels were significantly increased in the pancreatic homogenates of the mice given cerulein compared with saline-injected controls. Pretreatment of the animals with NS-398 (a COX-2 inhibitor) attenuated the cerulein-induced rise in PGE2 levels (data not shown).

Effects of COX-2 deletion on cerulein-induced pancreatitis. Mice given 12 hourly injections of a supramaximally stimulating dose of cerulein develop acute necrotizing pancreatitis and lung injury. As shown in Fig. 3, this is manifested by a rise in pancreatic MPO activity, which reflects neutrophil sequestration in the pancreas, and by extensive acinar cell necrosis. Morphological changes of pancreatitis (Fig. 4) include acinar cell vacuolization, intralobular edema, an inflammatory infiltrate, and acinar cell necrosis. This model of pancreatitis is also associated with lung injury, and that lung injury is characterized by a rise in lung MPO activity as well as a rise in the LDH activity in BAL fluid (Fig. 5). The former change reflects neutrophil sequestration within the lung, whereas the latter reflects injury to type II pneumocytes. Secretagogue-induced pancreatitis is also associated with a rise in pancreatic and BAL fluid levels of TNF-α (Fig. 6).

Genetic deletion of COX-2 was associated with a reduction in the severity of both pancreatitis and lung injury. As shown in Fig. 3, pancreatic MPO activity and acinar cell necrosis after supramaximal secretagogue stimulation were decreased in the COX-2-deficient (−/−) animals compared with the COX-2-sufficient (+/+ ) controls. Similarly, the morphological changes of pancreatitis were less severe in the COX-2 −/− group than in the COX-2+/+ group (Fig. 4). Serum amylase and pancreatic water content (an indicator of edema) were elevated in both groups, and the
magnitude of those elevations was similar in both groups (data not shown). Lung MPO activity and BAL-LDH levels during pancreatitis were significantly reduced in the COX-2 −/− group such that MPO and LDH levels in COX-2 −/− mice given cerulein were no greater than the control levels found in COX-2 +/+ mice not given cerulein (Fig. 5). Pancreatic and BAL fluid levels of TNF-α were also reduced in the COX-2 −/− animals given cerulein (Fig. 6).

**Effects Of COX-2 deletion on trypsinogen and NFkB activation.** Previous studies have indicated that intrapancreatic activation of trypsinogen and NF-κB occurs within 30 min of the start of cerulein administration (17, 20, 22). As shown in Figs. 7 and 8, neither activation of trypsinogen nor early activation of NF-κB are reduced by genetic deletion of COX-2. However, later activation of NF-κB (i.e., 6 h after the start of cerulein administration) was much less marked in COX-2 −/− mice compared with the COX-2 +/+ animals.

![Graph showing effects of COX-2 deletion on trypsinogen and NFkB activation](image1)

**Fig. 3.** Effects of COX-2 deletion on cerulein (Cer)-induced pancreatitis. Mice were given 12 hourly injections of Cer (50 μg/kg ip) or saline (control). One hour after the last Cer injection, mice were killed and pancreatic MPO activity and acinar cell necrosis were measured as described in the text. Values are expressed as a percentage of the values obtained for wild-type animals given Cer. Control (saline administered) values were similar in COX-2 +/+ and −/− animals. Results shown are means ± SE for 10 or more animals in each group. *P < 0.05 when COX-2 −/− animals given Cer were compared with COX-2 +/+ animals given Cer.

![Graph showing effects of COX-2 deletion on pancreatitis](image2)

**Fig. 4.** Morphological effects of COX-2 deletion. Representative micrographs are shown from control mouse pancreas (A). Pancreas from a COX-2 +/+ animal injected with Cer shows extensive acinar cell vacuolization, edema, and inflammation (B). Pancreas from a COX-2-deficient animal injected with Cer shows marked reduction in acinar cell vacuolization and inflammation but minimal-to-no change in edema (C).

![Graph showing effects of COX-2 deletion on HSP70 and iNOS expression during pancreatitis](image3)

**Fig. 5.** Effects of COX-2 deletion on pancreatitis-associated lung injury. Mice were given 12 hourly injections of Cer (50 μg/kg ip) or saline (control). One hour after the last injection, mice were killed and lung MPO and bronchoalveolar lavage (BAL) lactate dehydrogenase (LDH) were measured as described in the text. Values are expressed as a percentage of the value obtained for wild-type animals given Cer. Control (saline administered) values were similar in COX-2 +/+ and −/− animals. Results shown are means ± SE for 6 or more animals in each group. *P < 0.05 when COX-2 −/− animals given Cer were compared with COX-2 +/+ animals given Cer.

**Effect of COX-2 deletion on HSP70 and iNOS expression during pancreatitis.** Pancreas HSP70 and iNOS levels, quantitated by Western blotting, were low in nonstimulated wild-type and COX-2-deficient animals (Figs. 9 and 10). After 12 h of supramaximal cerulein stimulation, a rise in both HSP70 and iNOS could be detected in both groups of animals. The rise in HSP70 was significantly greater in the COX-2 −/− group than in the wild-type COX-2 +/+ group, whereas the rise in iNOS noted in the COX-2 −/− group was less than that in the COX-2 +/+ controls.

**Effects of COX-2 inhibition on the severity of secretagogue-induced pancreatitis.** We used NS-398 and Celecoxib, two well-characterized COX-2-specific inhibitors, to evaluate the effects of COX-2 inhibition on the severity of pancreatitis. As shown in Figs. 11 and 12, the COX-2 inhibitors reduced the severity of secretagogue-induced pancreatitis and its associated lung injury, and that reduction was similar in magnitude to that associated with genetic deletion of COX-2. Pancreatic rises in MPO activity and acinar cell necrosis were reduced by both inhibitors, and both inhibitors reduced the rise in lung MPO that was noted during secretagogue-induced pancreatitis. Serum amylase as well as
DISCUSSION

pancreatic water content were increased, and NF-κB was activated by cerulein administration, but those changes were not altered by administration of the COX-2 inhibitors (data not shown).

We noted that pancreatic COX-2 mRNA levels rose within 15 min of the start of cerulein administration and that pancreatic COX-2 protein levels were increased within 4 h of cerulein administration (Figs. 1 and 2). These observations suggested to us that COX-2 might play an important role in regulating the severity of pancreatitis and, possibly, of pancreatitis-associated lung injury as well.

Two groups of studies were performed to evaluate the role of COX-2 in pancreatitis. The first involved use of genetically altered mice that do not express COX-2 (11, 31), whereas the second involved administration, to wild-type mice, of agents known to inhibit COX-2 (15, 25, 33, 41). Both groups of studies yielded similar results. Either genetic deletion or pharmacological inhibition of COX-2 resulted in a marked reduction in the severity of secretagogue-induced pancreatitis. Both approaches also resulted in a marked reduction in the severity of pancreatitis-associated lung injury. This reduction in pancreatitis and lung-injury severity, brought about by interfering with COX-2, leads us to conclude that COX-2 plays an important proinflammatory role in both pancreatitis and its associated lung injury.

The mechanisms by which COX-2 might promote inflammation in pancreatitis are not immediately obvious. We considered the possibility that it might modulate the early intra-acinar cell events that characterize this model of pancreatitis. These include activation of trypsinogen and NF-κB (17, 22, 38), two temporally and mechanistically parallel events that occur within 30 min of the start of cerulein administration (20). We found, however, that neither cerulein-induced trypsinogen activation nor cerulein-induced early NF-κB activation were altered by COX-2 deletion after 30 min of cerulein administration (Figs. 7 and 8). Thus the...
protective effects of COX-2 deletion must involve events that occur downstream to these early acinar cell changes during pancreatitis. However, it is interesting to note that COX-2 deletion substantially reduces the NF-κB activation that is observed to occur 6 h after the start of cerulein administration (Fig. 8). As previously reported by Gukovsky et al. (17), cerulein-induced NF-κB activation during pancreatitis occurs in two phases, an early phase that peaks at 30 min and appears to reflect activation of acinar cell NF-κB and a later phase that peaks at 6 h. This later phase can be reduced by neutrophil depletion, and it is not observed when acinar cells are exposed to a supramaximally stimulatory concentration of cerulein in vitro. These observations have suggested that the later phase of NF-κB activation reflects changes that occur within or involve inflammatory cells. Our finding that NF-κB activation at 6 h is markedly reduced by COX-2 deletion is, therefore, consistent with the possibility that the proinflammatory effects of COX-2 in pancreatitis involve neutrophils.

Furthermore, Ethridge et al. (14) recently showed that HSP70 expression is reduced in intestinal cells that overexpress COX-2. That reduction was believed to reflect diminished heat shock factor binding, and it could be reversed by inhibiting COX-2 with NS-398. They suggested that COX-2 activity and HSP70 expression were inversely related phenomena. Bhagat et al. (4) and Kruger et al. (27) recently showed that hyperthermia and resulting HSP70 expression can reduce the severity of secretagogue-induced pancreatitis and that the upregulated expression of HSP70 that occurs during pancreatitis may act to dampen the

Fig. 8. Effects of COX-2 deficiency on NF-κB activation. COX-2+/+ and −/− mice were given Cer (50 μg/kg ip) or saline (control) as described in the text. After 30 min or 6 h, the mice were killed and pancreas tissue samples were removed. Nuclear extracts were prepared from the pancreas tissue samples, and NF-κB activation was monitored by EMSA as described in the text. Results are representative of 3 such experiments.

Fig. 9. Effect of COX-2 deficiency on heat shock protein 70 (HSP70). Mice were given 12 hourly injections of Cer (50 μg/kg ip) or saline (control). One hour after the last Cer injection, the mice were killed and pancreas samples were removed. Protein lysates (10 μg/lane) extracted from pancreas samples were subjected to gel electrophoresis and transferred onto nitrocellulose membranes. HSP70 levels were monitored using anti-HSP70 antibodies. Results shown are means ± SE for 4 or more animals in each group. *P < 0.05 when COX-2 −/− animals given Cer were compared with COX-2 +/+ animals given Cer.

Fig. 10. Effect of COX-2 deficiency on inducible nitric oxide synthase (iNOS) expression. Mice were given 12 hourly injections of Cer (50 μg/kg ip) or saline (control). One hour after the last Cer injection, the mice were killed and pancreas samples were removed. Protein lysates (10 μg/lane) extracted from pancreas samples were subjected to gel electrophoresis and transferred onto nitrocellulose membranes. iNOS levels were monitored using antibodies to iNOS. Results shown are means ± SE for 4 or more animals in each group. *P < 0.05 when COX-2 −/− animals given Cer were compared with COX-2 +/+ animals given Cer.
severity of pancreatitis itself. In the currently reported studies, we have found that upregulated HSP70 expression is even more robust in COX-2-deficient animals (Fig. 9). Thus it is tempting to speculate that COX-2 might play a proinflammatory role by dampening the rise in HSP70 that occurs during the evolution of pancreatitis and that, itself, acts to lessen the severity of pancreatitis.

It is likely, however, that the proinflammatory effects of COX-2 in pancreatitis and lung injury also involve other, non-HSP70-related, events. For example, the proinflammatory effects of COX-2 may reflect events involving neutrophils. Studies by others have suggested that COX-2 plays a proinflammatory role during the early, neutrophil-dominated phases of inflammation but that it may play a more anti-inflammatory role during the later phases of inflammation, which do not appear to involve neutrophils (16). Our own studies (Fig. 3) have shown that the cerulein-induced rise in pancreatic MPO activity, an index of neutrophil activation and sequestration in the pancreas, is partially reduced by either COX-2 deletion or COX-2 inhibition. This phenomenon is even more profoundly manifest in the lung, where genetic deletion of COX-2 was observed to completely prevent the rise in MPO content during cerulein-induced pancreatitis (Fig. 5). Thus the proinflammatory role of COX-2 in both pancreatitis and pancreatitis-associated lung injury may reflect the effect of COX-2 in regulating neutrophil activation and/or sequestration at sites of inflammation.

Recent studies have suggested that iNOS may play an important proinflammatory role in pancreatitis (2, 40). We, therefore, considered the possibility that COX-2 regulates the severity of pancreatitis and lung injury indirectly by modulating iNOS expression. Indeed, our observation (Fig. 10) that COX-2 deletion...
reduces the rise in iNOS expression during pancreatitis that would support that conclusion. Further studies will be needed to establish the significance of these observations, because it is also possible that the reduction in iNOS noted in COX-2-deficient animals during pancreatitis reflects the fact that pancreatitis is less severe in those animals and that it is not causally related to the reduced pancreatitis severity.

In summary, the studies reported in this communication indicate that COX-2 plays an important proinflammatory role in pancreatitis and pancreatitis-associated lung injury. COX-2 appears to regulate the severity of pancreatitis via mechanisms that are downstream to the early intra-acinar cell events. The proinflammatory effects of COX-2 in this model of pancreatitis may be multifactorial and involve, among other things, alterations in HSP70 expression, iNOS expression, and neutrophil function. Further studies will clearly be needed to elucidate those mechanisms, but regardless of the results of those studies, the observations reported in this communication indicate that pharmacological interventions that inhibit COX-2 may prove beneficial in the prevention and/or treatment of acute pancreatitis.

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