



Induction of antioxidant mechanisms in lung during experimental pancreatitis in rats

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Lung complications are the most common cause of death in patients with acute pancreatitis. In this study, we investigated how induction of mild acute pancreatitis could modify the course of lung inflammation, eventually, induced by a severe acute pancreatitis in rats. A severe and a mild forms of an experimental acute pancreatitis were respectively established by intraductal administration of sodium taurocholate to final dose of 50 µg/kg body wt. and intra-peritoneal injection of caerulein to supramaximal dose of 40 µg/kg body wt. We observed reduced levels of thiobarbiturate acid reacting substances when severe pancreatitis was preceded by the induction of mild pancreatitis. Moreover, mRNAs expression of both HSP-70 and Mn-SOD was increased in the lung. By contrast, the level of glutathione was reduced, but no change in the infiltration of neutrophils was observed. Therefore, we conclude that during the course of pancreatitis and its related lung inflammation, the pulmonary cells response involved in the expression of different protective proteins, including HSP-70 and Mn-SOD, which possibly improves the defensive mechanisms against inflammation in lung cells.

Keywords: ARDS, Caerulein, HSP-70, Inflammation; Mn-SOD, mRNA expression

Acute pancreatitis is a severe disease, frequently associated with systemic complications such as shock, renal failure, coagulation disorders, and acute respiratory failure whose clinical manifestation is referred to as “adult respiratory distress syndrome” or ARDS¹. Pulmonary complications are the most frequent cause of death of patients by acute pancreatitis. Studies on animal models of pancreatitis have shown that the inflammatory lung injury is mediated by the sequestration of activated neutrophils in the pulmonary microvasculature^{2,3}. It is known that during pancreatitis, the generation of oxygen-derived free radicals plays an important role responsible of increasing the extension of the lesions in the pancreas^{4,5}. Depletion of neutrophils using antineutrophil antibodies abolishes the generation of superoxide anions and prevents neutrophil-dependent acute pancreatitis associated lung injury⁶. Xanthine oxidase and activated neutrophils are the main source of free radicals in the early stages of the disease^{7,8}. In addition to their direct toxic effects, free radicals promote the generation of arachidonic acid metabolites

and other inflammatory mediators that aggravate the severity of the lesions⁹.

In the pancreas, a rapid and massive rearrangement of the patterns of genes expression was observed during the course of acute pancreatitis¹⁰. A reduction of both the content and the secretion of potentially harmful pancreatic enzymes characterize this acute phase reaction¹⁰. Moreover, a strong overexpression of other proteins that restrain the development of acute pancreatitis or participate in pancreatic regeneration has been observed^{11,12}. Hence, during acute inflammatory injury, the pancreas develops cellular defence mechanisms that limit pancreatic alteration and, accordingly, may temper the adverse systemic effects of pancreatitis and improve survival. Indeed, Fiedler *et al.*¹³ had reported that induction of mild pancreatitis by caeruleine triggers an acute phase response in the pancreas of rats and, therefore, protected animals against possible severe necrotizing pancreatitis caused by intraductal administration sodium taurocholate (NaTc). Actually, the mortality of the rats was significantly reduced, demonstrating that caerulein had induced a mechanism of defence against further systemic aggression¹³.

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The lung injury is the main cause of death during pancreatitis and, therefore, it could be surmised that the pancreatic acute phase response helped to reduce the lung inflammatory response. However, whether the observed protection occurred through the reduction of the pro-inflammatory signals received by the lung or the stimulation of lung cell anti-inflammatory mechanisms is still unknown⁴. In addition, it has been reported that oxidative damage, characterized by the generation of superoxide and activation of neutrophils in the lung, was an important component of the respiratory distress associated with acute pancreatitis. Circulating xanthine oxidase was involved in the selectins expression in the lung endothelial cells, promoting the inflammatory process¹⁴. Therefore, as occurs with the pancreas, it could be suspected that inflammatory response produced by acute pancreatitis would induce a lung defensive response.

In the present work, we tried to investigate how the caerulein induction of mild acute pancreatitis in rats could induce an antioxidant response, which may modify the course of lung inflammation, eventually induced by a severe acute pancreatitis.

Material and Methods

Chemicals

NaTc, caerulein, hexadecyltrimethylammonium bromide, superoxide dismutase (SOD), nitroblue tetrazolium, xanthine, xanthine oxidase and 3,3',5,5'-tetramethylbenzidine were purchased from Sigma Chemical Co. (St. Louis, MO). The thiobutabarbitol was provided from Research Biochemicals International (Inactin®, Natick, MA). The lipase assay kit was purchased from Randox (Antrim, UK).

Animals

Male Wistar rats (Iffa Credo, France), weighing 250-300 g, were used for all studies of acute pancreatitis. The animals were maintained on a 12 h light-dark cycle for at least one-week before use. The rats were given free access to food (Panlab, France) and water. All operations were performed according to the European Union guidelines concerning the care and treatment of experimental animals.

Experimental design of acute pancreatitis models

In order to produce mild acute pancreatitis (acute interstitial edematous pancreatitis), the animals were given a first dose of caeruleine (in 200 μ L of 0.9% NaCl) at a rate of 40 μ g/kg body wt. After 60 min, the treatment was repeated under the same conditions¹⁵.

The animals in the control group were treated with 200 μ L of saline solution (0.9% NaCl). The severe acute pancreatitis model was established by anaesthetizing the animals with an intraperitoneal dose of 100 mg/kg body wt. of thiobutabarbitol. After laparotomy, the biliopancreatic duct was cannulated through the duodenum and a small bulldog clamp closed the hepatic duct. Pancreatitis was induced in rats by retrograde injection into the biliopancreatic duct of NaTc, using a Harvard '22' infusion pump (Harvard Instruments, Edenbridge, UK)¹⁶.

A total of 48 animals were randomly allocated into four groups of 12 animals each, processed by the caerulein (final dose of 40 μ g/kg body wt. in 200 μ L of 0.9% NaCl) and the four groups have been separately re-treated with the same dose of caerulein for different periods (6, 12, 24 and 48 h). Six animals from each group were treated with 100 μ L of NaTc (final dose of 50 μ g/kg body wt.), in order to induce a necrotic pancreatitis, while the remaining animals have been intra-ductally infused with 100 μ L of 0.9% NaCl (saline) and used as the control groups. Additional control groups of 12 rats were intra-peritoneally infused with saline instead of caerulein, divided into two batches of six rats each, and then separately treated with 100 μ L of NaTc (final dose of 50 μ g/kg body wt.) or saline solution. All animals were sacrificed, after 3 h of intraductal administration of saline or NaTc. Samples from plasma, lung and pancreas were immediately frozen and maintained at -80°C , until use. At the same time, additional samples of lung tissues were collected for histology.

Total proteins, lipase and amylase assays

Total protein concentration of homogenates were estimated using a commercial kit from BioRad (Munich, Germany) and bovine serum albumin as standard. Plasma activities of lipase and amylase were determined, respectively using commercial kits from Randox (Antrim, UK) and Boheringer (Mannheim, Germany), according to the supplier's specifications.

MPO assay

Myeloperoxidase (MPO) activities in both pancreas and lung were photometrically monitored, using 3,3',5,5'-tetramethylbenzidine as substrate¹⁷. Samples were macerated in 50 mM phosphate buffer pH 6.0 and centrifuged at 3000 \times g for 15 min. Supernatants were discarded and pellets were re-suspended in 50 mM phosphate buffer containing 0.5% hexadecyltrimethyl-ammonium bromide. Resuspended pellets were then disrupted for 30 s, using

a Labsonic sonicator (B. Braun, Germany) at 20% power and were subsequently snap-frozen in dry ice and thawed. The procedure was repeated twice before a final 30 s sonication. Samples were incubated at 60°C for 2 h, and then spun down at 4000×g for 12 min. Supernatants were collected for MPO assay. Enzyme activity was assessed photometrically at 630 nm. The assay mixture consisted in: 20 mL supernatant; 10 mL tetramethylbenzidine dissolved in DMSO (final concentration: 1.6 mM); 70 mL H₂O₂ (final concentration: 3 mM) diluted in 80 mM phosphate buffer pH 5.4. MPO activity was expressed as IU/mg protein and the enzyme unit was defined as the amount of the enzyme that produces an increase of one absorption unit per min.

Quantification of TBARS

Lipid peroxidation was determined by the reaction of thiobarbiturate (TBA) which involves measuring the formation of substances reacting with thiobarbituric acid (TBARS). For this purpose, 2 mL of trichloroacetic acid (20%) was added to 2 mL of lung homogenate. After mixing and centrifuging, 1.0 mL of a TBA (0.67%) was added to the supernatant. The mixture was boiled for 60 min and cooled, and then the optical density was recorded at 530 nm¹⁸.

Quantification of GSH

To determine the reduced glutathione (GSH) concentration, the lung samples were homogenized in a solution of KCl (1.15%). The proteins were precipitated with 500 µL of perchloric acid (1 N). After centrifugation, the samples were neutralized with 10% K₂CO₃. The amount of GSH was measured, using glutathione transferase and 1-chloro-2,4-dinitrobenzene¹⁹. The reaction mixture contained 215 µL of 0.1 M potassium phosphate buffer pH 7.0, 50 µL of neutralized sample and 10 µL of 10 mM 1-chloro-2,4-dinitrobenzene solution in ethanol. The reaction was started with 5 µL of glutathione transferase solution (12 IU/L) and monitored at 340–400 nm reaching the end-point, 5 min after enzyme addition.

Total RNA preparation

A part of the lung was dissected and immediately processed for RNA isolation or frozen in liquid nitrogen and stored at –80°C, until use. Total RNA was extracted with TRIzol Reagent following the manufacturer's instructions (GibcoBRL, Life Technologies) and RNA concentration was estimated using absorbance at 260 nm.

Quantitative RT-PCR analysis

The mRNA expression analysis of both manganese superoxide dismutase (Mn-SOD) and heat shock

protein 70 (HSP-70) was done by a semi-quantitative RT-PCR method, called 'One Step Life Technologies System', according to the manufacturer's protocol. About 1.0 µg of total RNA was reverse transcribed at 50°C for 30 min in 50 µL final volume, using 2 µL of One Step RT-PCR enzyme mix. For each gene, a specific primer was used to synthesize the first cDNA strand. Then, the PCR reactions were done using a couple of specific primers: for Mn-SOD, the forward primer was: 5'-CCGTGTTCTGAGGAGAGCAG-3' and the reverse primer was: 5'-CTCCTCGGTGACGTTCAGAT-3'; for HSP-70, the primers 5'-AGGAGATCTCGTCCATGGTG-3' and 5'-CAGAGAGTCGATCTCCAGGC-3' were used as forward and reverse primers, respectively. The conditions of enzymatic amplification were established for 35 cycles involving an initial denaturation step at 94°C for 2 min and, then, each cycle performed at 94°C for 15 s, 60°C for 30 s and 68°C for one minute.

As a negative control, water was used in RT-PCR instead of total RNA to exclude any contamination. Elsewhere, the integrity and the amounts homogeneity of RNA samples used in RT-PCR experiments were controlled by analysing the expression profiles of ribosomal RNAs (18S and 28S) and GAPDH. Finally, the PCR products were resolved using 1.8% (Mn-SOD, HSP-70) or 1% (GAPDH, 18S and 28S rRNAs) agarose gel electrophoresis under non-denaturing conditions and ethidium bromide coloration.

Histological study

Tissue samples of the lung were fixed in 10% neutral buffered formalin, paraplast-embedded, cut into 5 µm sections, stained with haematoxylin-eosin and observed under optical microscope (×400), according to standard procedures.

Statistical analysis

All data of concentration and activity quantifications in our experiments (Total proteins, lipase, amylase, TBARS, GSH and MPO) were expressed as mean ± SEM. Means of different groups were compared using a one-way analysis of variance. Student's *t* test was performed for evaluation of significant differences between groups. Differences were assumed to be significant when *P* < 0.05.

Results

Pancreas injury decreases during the course of severe pancreatitis

Plasma levels of digestive enzymes reflect the severity of pancreatic injury¹⁰. In our experimental conditions, intraductal saline resulted in mild

pancreatic injury. Folch *et al.*³ have already noted the presence of a significant infiltration of the neutrophils in the lung, after 3 h of induction pancreatitis by 50 µg/kg body wt. of NaTc in the rat and, therefore, it was used as the appropriate control of intraductal NaTc experiments. Administration of caerulein to these animals induced an increase of lipase and amylase concentrations in plasma. Maximum was reached, after 6 h of caerulein injections (Table 1). Compared to caeruleine, intraductal administration of NaTc induced a much greater increase in these parameters, consistent with the fact that NaTc resulted in severe pancreatitis. However, when caerulein was given before NaTc administration, the levels of these two enzymes were lower than with NaTc alone.

Pancreatic and Lung inflammation increases during the course of severe pancreatitis

The pancreatic MPO activity indicates inflammatory damage¹⁰. As shown in Table1, MPO activity increased in the pancreas, both in mild oedematous caerulein-induced pancreatitis and severe necrotizing NaTc-induced pancreatitis. Contrary to serum enzymes levels, pancreatic inflammation induced by NaTc was not reduced by previous caerulein treatment. The infiltration of neutrophils into the lung, estimated by MPO activity (Fig. 1), showed progressive increase, after caerulein-induced acute pancreatitis, achieving significant values, after 24 h of induction. By contrast, NaTc-induced severe pancreatitis evoked immediate increase of MPO activity in lung. Administration of caerulein, before NaTc treatment, did not alter the infiltration of neutrophils into the lung.

Lung oxidative stress induces Mn-SOD and HSP-70 expression during the course of severe pancreatitis

The oxidative damage measured, as generated TBARS in the lung, was found increased after caerulein administration significantly after 12 h and returned to control values later (Fig. 2). In addition,

the infusion of NaTc alone showed a significant TBARS increase. However, the oxidative damage consecutive to NaTc infusion was significantly reduced, prior injection of caerulein (6 or 12 h). As

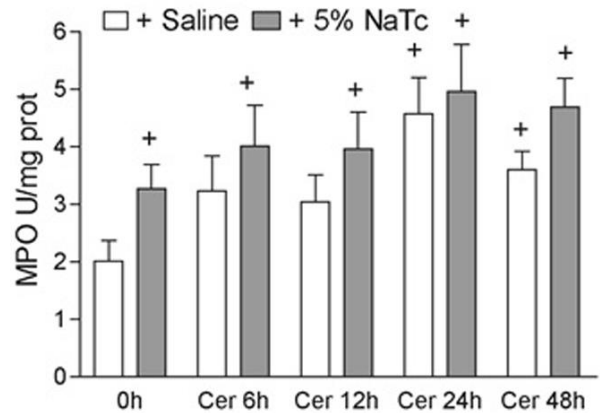


Fig. 1 — Measurement of lung MPO activity. [The lung activity of MPO *in vitro* was assessed photometrically with 3,3',5,5'-tetramethylbenzidine as substrate; +=P <0.05 vs. control (saline) without caerulein (0 h); Cer: caerulein]

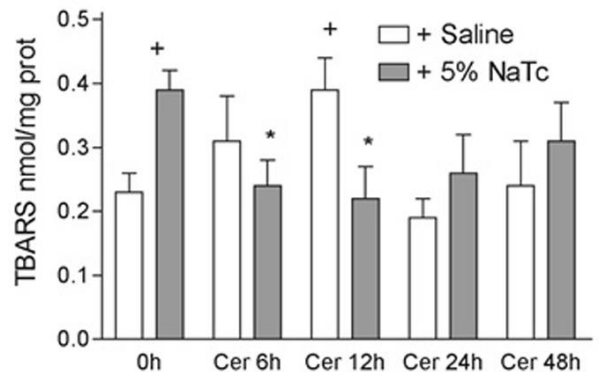


Fig. 2 — Evaluation of lung TBARS levels. [Lipid peroxidation was determined by the thiobarbiturate (TBA) reaction measuring the formation of thiobarbiturate acid reacting substances; +=P <0.05 vs. control (saline) without caerulein (0 h); *=P <0.05 vs. NaTc 5% without caerulein (0 h); Cer: caerulein]

Table 1 — Pancreatic MPO activity and plasma levels of lipase and amylase

	Control group	Caerulein-pre-treated groups				
		6 h	12 h	24 h	48 h	
Plasma Lipase (IU/mL)	Saline	778±188	3939±603*	1417±402*	62±11*	109±21*
	NaTc	7426±1309*	4484±660*	3472±750*§	2351±428*	1099±112§
Plasma Amylase (IU/mL)	Saline	17.4±0.6	38.6±5.3*	27.4±3.0*	16.9±0.8	17.0±1.3
	NaTc	42.3±9.5*	38.9±8.9*	31.6±5.1*	29.3±2.2*	20.3±1.6*§
Pancreas MPO (IU/mg protein)	Saline	0.21±0.09	0.56 ± 0.07*	2.05 ± 0.83*	1.29 ± 0.25*	0.85 ± 0.21*
	NaTc	0.90 ± 0.16*	0.98 ± 0.04*	1.39 ± 0.37*	1.42 ± 0.36*	0.86 ± 0.08*

[The activities of pancreatic MPO and both lipase and amylase in plasma were estimated in rats 3 h after intraductal administration of saline or NaTc. The effect of caerulein on these parameters was done by its administration at different times before intraductal infusions of NaTc or saline. The results are expressed as mean ±SE for 6 animals. *=P <0.05 vs. control (no caerulein, intraductal saline); §=P <0.05 vs. NaTc (no caerulein, intraductal NaTc)].

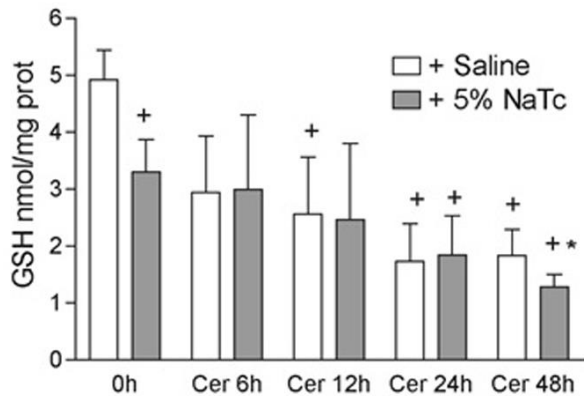


Fig. 3 — Evaluation of GSH levels in the lung. [Changes in GSH concentration into the lung were measured using glutathione transferase and 1-chloro-2, 4-dinitrobenzene; +=*P* <0.05 vs. control (saline) without caerulein (0 h); *=*P* <0.05 vs. NaTc 5% without caerulein (0 h); Cer: caerulein]

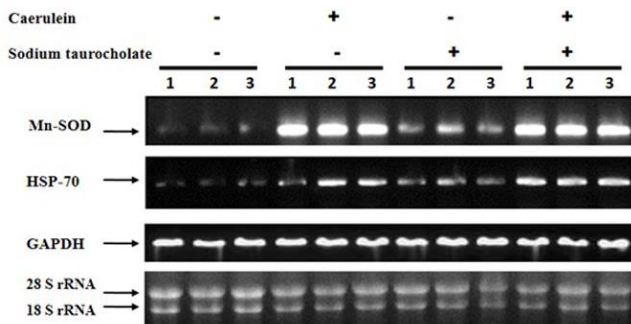


Fig. 4 — RT-PCR analysis of Mn-SOD and HSP-70 mRNAs expression. [The mRNAs expression of Mn-SOD and HSP-70 in lung was analyzed by a semi-quantitative RT-PCR method using 1 µg of total RNA and specific primers for each trial and repeated three times. Four groups of treatment (saline, caerulein, NaTc or caerulein/NaTc after 12 h of treatment), with three different animals per group were assayed. The expression profiles of ribosomal RNAs (18S, 28S) and GAPDH were used as internal controls of mRNA integrity and homogeneity. Amplified cDNA sequences were resolved in 1.8% (Mn-SOD, HSP-70 and GAPDH) or 1% (18S and 28S rRNA) agarose electrophoresis gel containing ethidium bromide under non-denaturing conditions]

shown in Fig. 3, GSH levels gradually decreased during acute caerulein-induced pancreatitis and, subsequently, administration of NaTc did not change the concentration of GSH. By contrast, the administration of NaTc (alone) resulted in moderate decrease in GSH concentration.

Analysis of Mn-SOD and HSP-70 mRNAs expression using RT-PCR approach is shown on Fig. 4. A marked increase in the expression of Mn-SOD mRNA could be observed, after 12 h of caerulein administration. The treatment with NaTc, after 12 h of

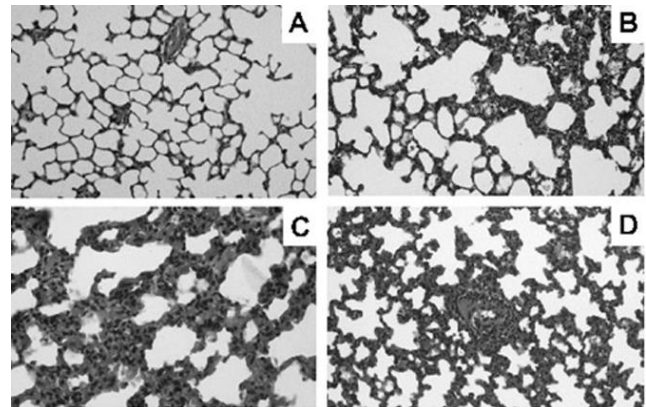


Fig. 5 — Histological study of the lung parenchyma. [Five groups of lung tissues were analyzed using haematoxylin-Eosin and optical microscopy (×400). A: animals treated with saline for 12 h; B: animals treated with 40 µg/kg body wt. of caerulein (12 h); C: animals treated with 50 µg/kg body wt. of NaTc (12 h); D: animals treated with 40 µg/kg body wt. of caerulein (12 h) + 50 µg/kg body wt. of NaTc (12 h). Control group shows normal lung parenchyma. All the other groups showed similar features: Moderate thickening of alveolar walls with PMN infiltration]

caerulein injection did not lead to further increase in the expression of the mRNA encoding the Mn-SOD, however, the effect of the NaTc (alone) was much smaller. Furthermore, both the caerulein and NaTc induced moderate increases in the expression of HSP-70, while their combination resulted in higher expression. As shown in Fig. 5, the histological aspect of the lung was normal in the control group, whereas, similar modifications, including, moderate thickening of alveolar walls and PMN infiltration were observed in other groups: caerulein (12 h); NaTc (12 h); caerulein+ NaTc (12 h).

Discussion

The acute-phase response of the pancreas in both rats and humans during the course of pancreatitis has been described earlier^{10,13}. It appears to be a powerful emergency defence process that provides protection against further pancreatic aggression. This was shown by Fiedler *et al.*¹³ who reported that induction of an acute-phase response by treatment with caerulein in rats, before triggering severe acute pancreatitis by NaTc, improved survival of the animals. To extend these observations, we used a similar design to investigate the effect of an initial challenge with caerulein on the inflammatory process, induced in the pancreas and the lung by a second aggression triggered by NaTc.

Amylase and lipase are good enzymatic markers for the diagnosis of acute pancreatitis²⁰. The evolution of these pancreatic enzymes in plasma was noteworthy (Table 1). The increase in plasma concentrations of lipase and amylase following NaTc administration was much smaller in the animals pre-treated with caerulein, suggesting lesser severity of pancreatitis. Yet, it is known that treatment of animals by caerulein strongly inhibits pancreatic synthesis of both amylase and lipase¹⁰. These results are consolidated by a recent work, which indicated that cordycepin pre-treatment of the caerulein-induced acute pancreatitis model in mice down regulated serum levels of both amylase and lipase and modulated neutrophil infiltration (MPO staining) in AP mice²¹. Hence, under this context of pancreatic damage, plasma levels of these two enzymes are indeed expected to be lower, after caerulein treatment. In fact, failure of caerulein to reduce the infiltration of neutrophils (measured as MPO activity) into the pancreas, after infusion of NaTc, might suggest that pre-treatment with caerulein does not mitigate NaTc-induced pancreatic inflammation (Table 1).

Yet, Fiedler *et al.*¹³ have reported a better outcome of the animals, after severe pancreatitis, if they had been previously exposed to mild pancreatitis. On this basis, we investigated whether, contrary to the pancreas, the inflammation of the lung would be attenuated by that treatment. Three hours after NaTc administration, a strong inflammation reflected by thickening of the alveolar wall and infiltration of neutrophils and macrophages could be observed in the lung (Fig. 5). Caerulein treatment and its combination with NaTc had the same effects. In addition, the measurement of the infiltration degree of neutrophils by determination of MPO activity demonstrated²² that the increase in the NaTc-induced infiltration of neutrophils was not modified by caerulein pre-treatment at any of the time-periods assessed (Fig. 1). This increase in MPO levels during acute pancreatitis lung injury seems to be consistent with previous findings²³. Therefore, like in pancreas, pre-treatment with caerulein does not protect the lung against pancreatitis-induced inflammation; it could be concluded that the reduced mortality associated with this pre-treatment may not be due to reduced lung inflammation.

Free radicals participate in the aggravation of oxidative stress and may play important roles in oxidative lung injury during acute pancreatitis⁵. Thus,

the biochemical parameters of oxidative stress were evaluated to predict the disease severity. Nevertheless, the increase in lung lipid peroxidation induced by NaTc treatment, measured as TBARS, was suppressed when animals were previously challenged with caerulein (Fig. 2). This fact could be clearly observed at 6 and 12 h after caerulein treatment suggesting that although the intensity of inflammation was not modified, its consequences in lung cells were attenuated. The protective effect evidenced by decreased lipid peroxidation in the lung, might be explained by an increase in the cellular antioxidant mechanisms. This was monitored by measuring the concentration of GSH and the changes in the expression of Mn-SOD and HSP-70 in lung cells. NaTc-induced pancreatitis was associated with a decrease in the GSH concentrations in the lung, as a probable consequence of free radicals generation by infiltrating neutrophils (Fig. 3). A similar decrease was observed during the course of caerulein-induced pancreatitis. Yet, combination of both challenges did not induce further decrease, suggesting that GSH was not involved in the increased antioxidant activity observed in the lung of animals treated with caerulein, plus NaTc.

The superoxide dismutases (SOD) could also take part in increased antioxidant activities. They are the first and most important line of antioxidant enzymes defence systems against ROS²⁴. These enzymes catalyze the reaction of the superoxide radical to hydrogen peroxide, which in turn, will be detoxified to water by the action of catalase. While it is known that lung cells show higher levels of both the activity and the content of SOD, due to increased expression of manganese superoxide dismutase (Mn-SOD) in response to an acute oxidant stress, the expression of the copper/zinc superoxide dismutase (Cu/Zn-SOD) remained constant²⁵. We analyzed the expression of Mn-SOD mRNA in the lung (Fig. 4). It was induced by caerulein pre-treatment, without further effect of NaTc. By contrast, infusion of NaTc alone induced a moderate increase in Mn-SOD mRNA levels. Hence, Mn-SOD mRNA induction caused by mild caerulein-induced pancreatitis could participate in the protective mechanism that lung cells develop in response to inflammation. This protective process may include the induction of Mn-SOD expression by NF- κ B and/or TNF- α , since, it has been demonstrated that these two proteins are required for increase of Mn-SOD expression during oxidative stress²⁶. Moreover, the

Mn-SOD action against oxidant injury may be mediated by phosphorylation of STAT 3, a transcription factor, indispensable for PAPI expression, as supported by previous studies²⁷. Elsewhere, the alprostadil, a typical vasodilator with high activity, has been shown to play a main role in reducing acute pancreatitis²⁸. This exogenous prostaglandin is able to reduce damage and apoptosis in the pancreas, and possesses significant anti-inflammatory and antioxidative stress effects, which associated with inhibition of the JAK2/STAT3 signalling pathway²⁹. Therefore, we suggest that the action of pulmonary Mn-SOD against oxidative stress and inflammation during severe acute pancreatitis could be mediated by the JAK2/STAT3 pathway.

Another factor, that could play a role in lung protection, is a highly conserved family of heat shock proteins (HSP), their expression increases in response to stress. The HSP-70 constitutes the best-known stress-inducible cytoplasmic chaperone^{30,31}. This acute phase protein is synthesized under cellular stress, and conferred cells protection by different mechanisms³². In particular, Hsp-70 has an essential role in substrate degradation through the ubiquitin-proteasome system, as well as through different autophagy pathways³³. Indeed, the oxidative damage can induce misfolding or degradation of proteins and it represents a cellular stress signal responsible for triggering the synthesis of HSP, which help to stabilize the macromolecular structure of denatured proteins and promote their reactivation³⁴. In addition, overexpression of HSP-72 has been involved in the protection against lipid peroxidation by inhibition of the NADPH oxidase, a main source of free radicals generated by neutrophils³⁵.

The analysis of HSP-70 mRNA showed a moderate increase of its synthesis, after caerulein administration and, to a lesser degree, after NaTc challenge (Fig. 4). The increase in HSP-70 mRNA levels was more pronounced when NaTc-induced pancreatitis was induced, after caerulein pre-treatment, suggesting the involvement of HSP-70 in the lung protective response. Indeed, the association between Hsp70 expression and oxidative stress is relatively well established³⁶. In particular, HSP-70 overexpression was shown to accelerate the recovery from caerulein-induced pancreatitis and to confer protection in multiple different stress situations^{37,38}. Moreover, the expression of HSP-70 and its effect on the regulation of inflammatory response during acute pancreatitis involves p38 MAPK signaling pathway³⁹.

Elsewhere, it has been reported that cordycepin markedly decreased the levels of pro-inflammatory factors (IL-6, IL-1 β and TNF- α), while inhibiting the activation of the nuclear factor κ B (NF- κ B) and protein 3 containing the NLR family pyrin domain (NLRP3) inflammasome in AP mice²¹. Besides, the accelerated recovery of lung cells during caerulein-induced pancreatitis has been associated, at least in part, to a delay in activation of NF- κ B signaling by HSP-70³¹. Given that overexpression of HSP-70 suppresses the paraquat-induced neuro-degeneration by reducing oxygen species, which in turn inhibits JNK and caspase-3 activation³⁴, we suggest that expression of HSP-70 may protect lung cells against inflammation by inhibition of JNK signalling pathway. Furthermore, it has been shown that Mn-SOD activity is significantly decreased in HSP-70 KO mice than in the wild type⁴⁰. Therefore, we suggest that HSP-70 might be involved in the regulation of Mn-SOD expression.

The stimulation of lung antioxidant mechanisms by mediators released during the pancreatic acute phase could explain, at least partially, the increased survival rate reported when a pancreatic acute phase reaction is induced prior to severe acute pancreatitis. Altogether, our results indicate that during the course of pancreatitis and consecutive lung inflammation, pulmonary cell response involves the synthesis of different protective proteins, including, HSP-70 and Mn-SOD. The possibility that HSP-70 and Mn-SOD work together to cause the defence mechanism against lung injury during acute pancreatitis is still intriguing, given that previous studies have yielded conflicting results regarding the relationship between NF- κ B and the antioxidant activities of these two proteins.

Conclusion

The mechanism involved in the induction of the protective response of the lung during acute pancreatitis remains unknown. A local lung response to neutrophils infiltration is possible. In contrast, the pancreas exhibits an intense acute phase response, after induction of pancreatitis. This process is characterized by significant changes in the phenotype of pancreatic cells, including, the overexpression of proteins, such as HSP-70 and Mn-SOD, which are poorly expressed in the normal pancreas. It is possible that some of these proteins are released into the blood and may mediate systemic defence mechanisms. The characterization of these acute phase pancreatic mediators responsible for

the induction of pulmonary protection could help to develop new therapeutic strategies against severe acute pancreatitis. However, more research is essential to determine the precise mechanism by which these antioxidant proteins protect lung tissue from ROS.

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Conflicts of interest

Authors have declared no conflict of interests.

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