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Role of oxidative stress in the pathogenesis of caerulein-induced acute pancreatitis

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Abstract

In the last decade, the role of oxidative stress has been extensively evaluated in different experimental models of acute pancreatitis. This review shows that there is strong evidence that this stress occurs as an early phenomenon in pancreatic tissue in the course of caerulein-induced acute pancreatitis. Oxidative stress was documented in pancreatic tissue by means of methods showing generation of reactive oxygen species (e.g., chemiluminescence) and accumulation of products of reactive oxygen species-mediated lipid peroxidation, with concomitant depletion of enzymatic and low molecular weight antioxidants. Features of acinar cell injury and inflammation, especially pancreatic edema, show a marked improvement following treatment with a broad spectrum of antioxidants, platelet activating factor antagonists, or donors of nitric oxide (NO). Unfortunately, in most cases these beneficial effects are temporary and generally restricted to an early phase of the disease. However, results of well-designed clinical trials should finally evaluate the importance of oxidative stress-oriented treatment in acute pancreatitis in humans.

Keywords: Pancreatitis; Caerulein; Oxygen reactive species; Antioxidant; Nitric oxide (NO)

1. Introduction

Progress continues to be made in the management of acute pancreatitis in different experimental models, but the exact pathomechanisms leading to this disease are not well understood. In the last decade, a large body of experimental data has accumulated, suggesting that reactive oxygen species may play a critical role in the pathogenesis of acute pancreatitis. As far as we know, it has been the topic of four reviews (Schoenberg et al., 1992, 1994, 1995; Sweiry and Mann, 1996).

Under normal conditions, approximately 95% of the molecular oxygen in biological systems undergoes controlled reduction through the addition of four electrons (tetravalent) in the mitochondrial cytochrome oxidase system to form water. The remaining molecular oxygen undergoes sequential, univalent reduction to produce the partially reduced intermediates, known as reactive oxygen species, such as superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$). Besides the mitochondria, there are other important biological sources of reactive oxygen species, including xanthine oxidase, activated leukocytes, prostaglandin synthetase, and catecholamine auto-oxidation, but xanthine oxidase and leukocytes appear to be the major sources in clinical disease states (Forman and Thomas, 1986; Parks, 1989). A mutilayer system of defense has been evolved by nature to counter cytotoxicity (Freeman and Crapo, 1982; Halliwel, 1991). The primary defense is provided by the enzymes, superoxide dismutase, catalase and GSH peroxidase. The second line of defense against oxidant-induced cellular injury is provided by low molecular weight scavengers such as α-tocopherol, carotenoids, thiols, especially reduced GSH peroxidase, cysteine, ascorbate, melatonin, flavonoids, uric acid, methionine, etc. (Fig. 1).

The third line of defense against reactive oxygen species includes the repair of DNA and proteins, reduction of protein SH groups, restoration of mucosal ATP production and decrease of intracellular Ca^{2+}.

Endogenous nitric oxide (NO) that originates from L-arginine due to the activity of constitutive (cNOS) or

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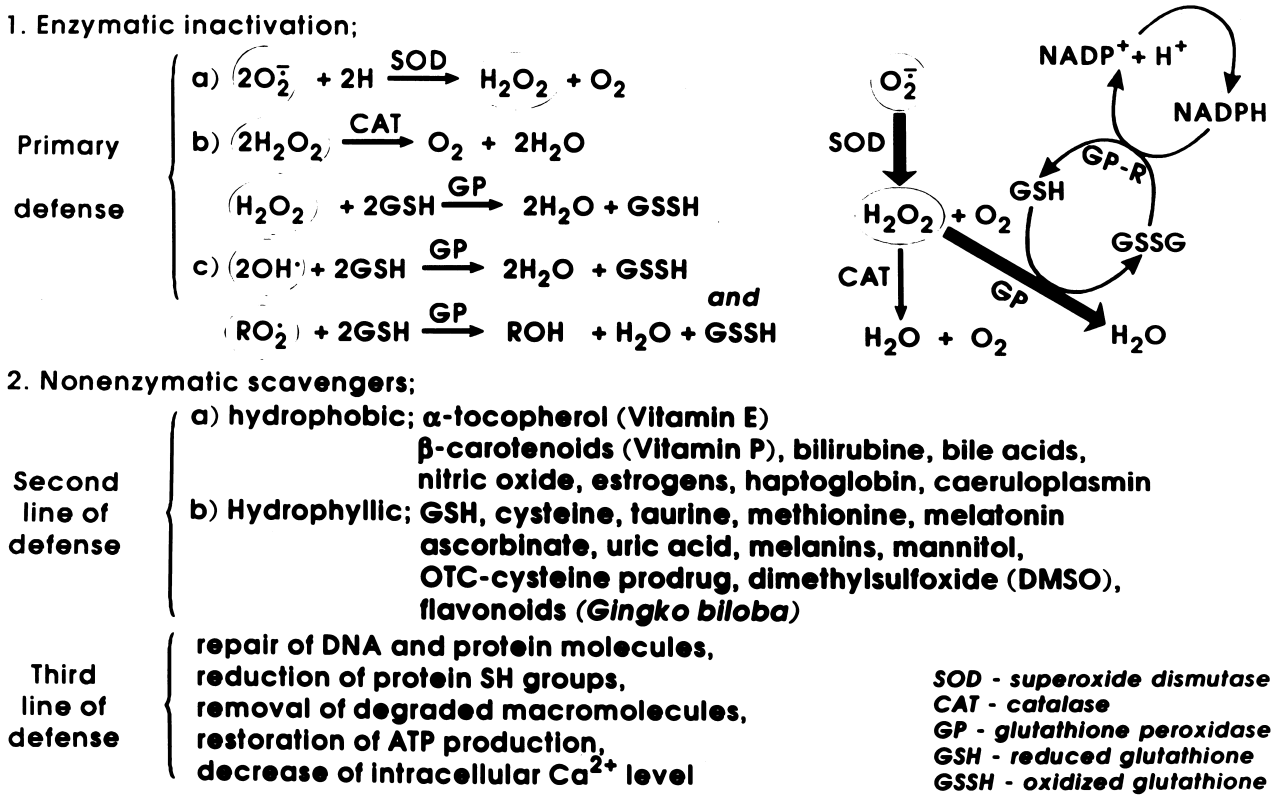


Fig. 1. Antioxidant mechanisms in primary, secondary and tertiary lines of defense against reactive oxygen species.

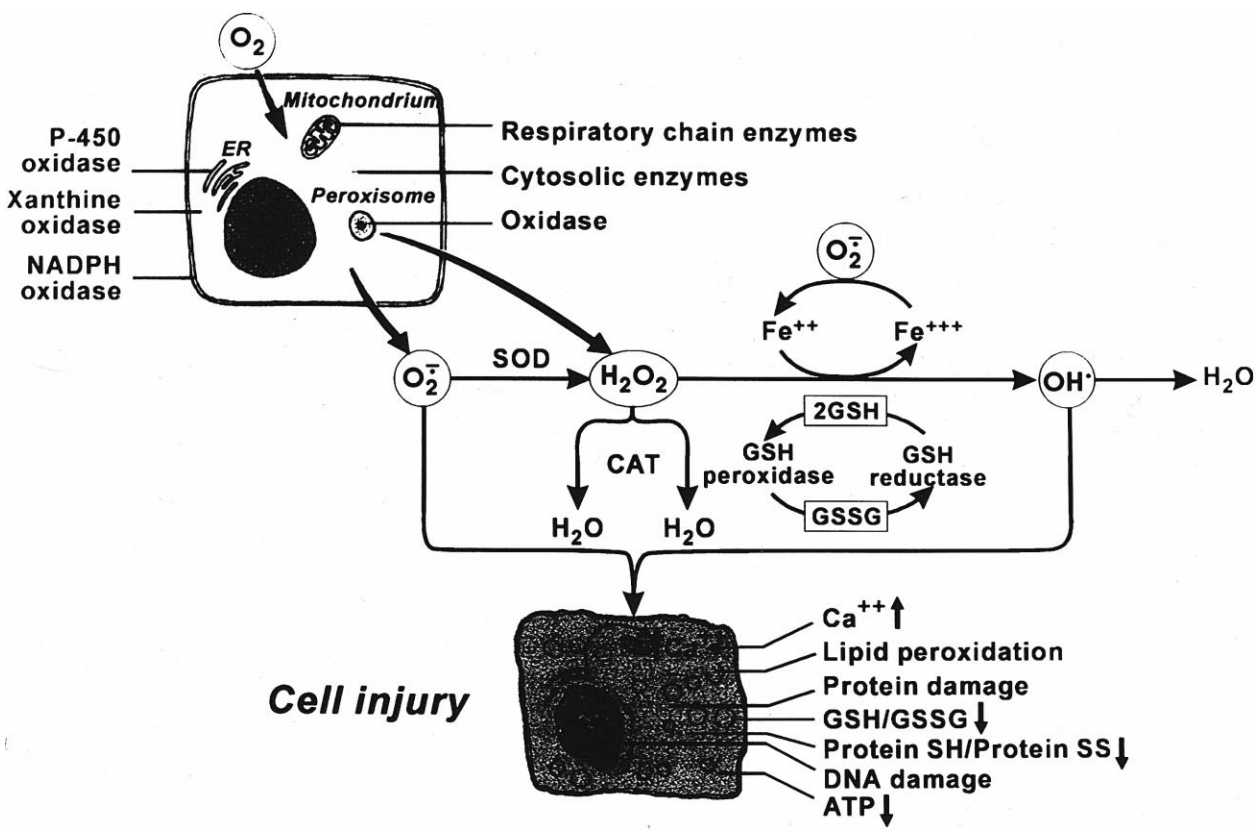


Fig. 2. Formation of reactive oxygen species and antioxidant mechanisms in biological systems.

inducible (iNOS) synthase in the pancreas may be produced by pancreatic nerves (Kirchgessner et al., 1994; Tay and Houles, 1994) or acinar cells (Wrenn et al., 1994). It may act, depending on the amounts released, either as biological scavenger and inactivator of reactive oxygen species (Rubanyi et al., 1991) or as toxic reactive oxygen species when combined with superoxide anion to form peroxynitrate (Beckman et al., 1990). If cellular antioxidants are low, or the rate of reactive oxygen species production exceeds the capacity of the endogenous antioxidant mechanisms, oxidative stress develops (Halliwel, 1991). The site of excessive reactive oxygen species production, that is whether it is predominantly extracellular or intracellular, may determine the degree of subsequent tissue damage (Fig. 2).

The role of reactive oxygen species has been studied in different experimental models of acute pancreatitis, but caerulein-induced acute pancreatitis has been the most widely explored. Rapid induction, a mild and highly reproducible course, and easily detected changes of acute interstitial pancreatitis have made this secretagogue-induced model a favorite for investigations of pathophysiological events in this disease (Lerch and Adler, 1993, 1994). It is suggested that the histological picture of caerulein-induced pancreatitis resembles the early phase of acute edematous pancreatitis in humans. This review attempts to provide a broad analysis of available data about the role of reactive oxygen species in the pathogenesis and treatment of caerulein-induced acute pancreatitis.

2. The effects of scavenger treatment on acute pancreatitis — the indirect evidence for the role of reactive oxygen species in the pathogenesis of this disease

The possible involvement of reactive oxygen species in acute pancreatitis was first reported in 1984. Sanfey et al. (1984) utilized an isolated perfused ex vivo canine pancreas preparation for the initiation of pancreatitis by ischemia, intravenous (i.v.) free fatty acid infusion, or partial pancreatic duct occlusion. When scavenger enzymes such a superoxide dismutase and catalase were added to the perfusate prior to exposure to the injurious stimuli, less pancreatic edema accumulation and serum amylase elevation were evident. In other experiments, allopurinol, a specific inhibitor of xanthine oxidase, gave similar results when given before the injury (Sanfey et al., 1985) (Fig. 3).

Two years later, using a similar approach, Guice et al. (1986) studied the possibility of a role of reactive oxygen species in caerulein-induced acute pancreatitis in the rat. A combination of superoxide dismutase and catalase was given i.v. as a bolus, 30 min after a 12-h continuous i.v. infusion of caerulein for induction of acute pancreatitis. This combined treatment prevented pancreatic edema formation, as assessed by pancreas weight gain, and diminished the ultrastructural injury expressed by intense infiltration by inflammatory cells, vacuolization, and dilation

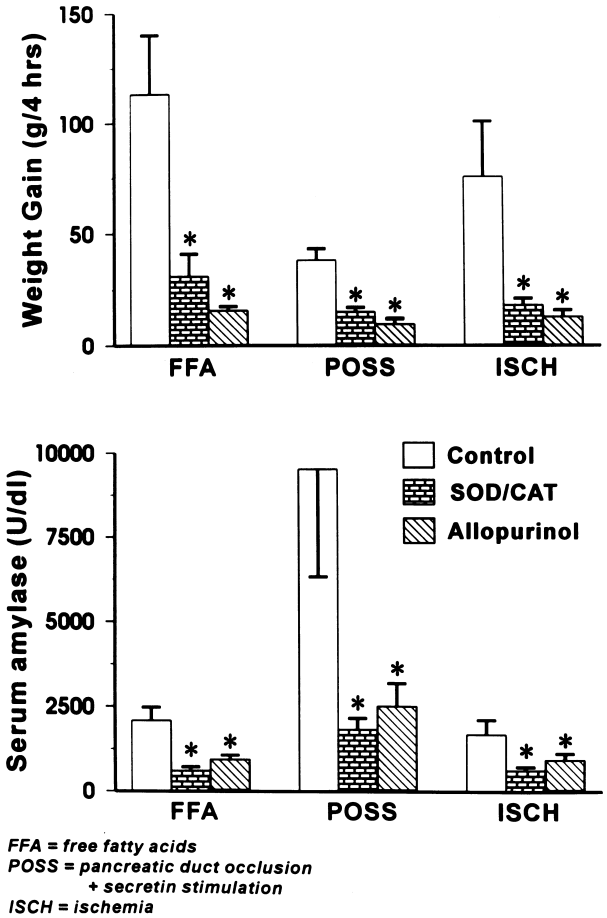


Fig. 3. Reactive oxygen species mediate a common essential step in the pathogenesis of all forms of acute pancreatitis. According to Sanfey et al. (1984, 1985), acute pancreatitis in ex vivo perfused pancreas induced by i.v. infusion of fatty acids (FFA), pancreatic duct occlusion combined with secretin stimulation (POSS) and ischemia (ISCH) can be attenuated by pretreatment of animal with superoxide dismutase and catalase as well as allopurinol.

of rough endoplasmic reticulum and Golgi apparatus in the acinar cell cytoplasm at 24 h compared with those in animals given caerulein alone. The authors hypothesized that caerulein causes changes within the acinar cell that lead to the formation of oxygen-derived radicals. These radicals are produced in amounts that exceed the capacity of intrinsic scavengers and cause membrane damage. This damage results in leakage of excess of free radicals, pancreatic enzymes and cellular debris into the interstitium. These events are then propagated by the production of additional free radicals and injury to other cellular membranes, such as the capillaries. In this model, a single injection of exogenous scavengers given after the injury appears to be sufficient to break this hypothetical cycle and provides protection to the pancreas (Guice et al., 1986). In a subsequent study, Guice et al. (1987) showed that both neutrophil depletion and complement depletion prevent early edema formation in caerulein-induced acute pancreatitis. Using specific oxygen radical scavengers they found that H_2O_2 or H_2O_2-derived oxygen radicals were

responsible for the early edema formation. Specifically, native catalase was protective against edema formation. However, the possibility of a critical role of complement activation in the pathogenesis of caerulein-induced acute pancreatitis in the rat was recently ruled out by Weiser et al. (1996). In this latter study, soluble complement receptor type 1 failed to induce either pancreatic edema, or to increase the hematocrit and serum amylase.

Wisner et al. (1988) were unable to show a protective effect of administration of native superoxide dismutase on caerulein-induced acute pancreatitis. They attributed this lack of an effect of native superoxide dismutase to its extremely short circulating half-life in the rat (<6 min). Therefore, in their study, they used polyethylene glycol-linked superoxide dismutase (polyethylene glycol:superoxide dismutase) with a greatly extended half-life in the rat circulation (>35 h). They found that a single i.v. bolus injection of 4×10^4 U/kg of polyethylene glycol:superoxide dismutase before caerulein treatment significantly reduced hyperamylasemia by approximately 25% and that a continuous 6 h i.v. infusion of 4×10^4 U kg^{-1} h^{-1} of polyethylene glycol:superoxide dismutase produced significant reductions of approximately 25%, 35% and 50% in pancreas edema, serum amylase, and acinar cell vacuolization, respectively, compared with the values in rats given caerulein alone. The authors admitted that, on the basis of their work, it was not clear whether the protective effects of exogenous polyethylene glycol:superoxide dismutase on caerulein-induced acute pancreatitis are caused by the enzyme acting intracellularly or extracellularly on the pancreas. It is known, however, that some reactive oxygen species such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) can pass through cell membranes by either diffusional processes or transport through anionic membrane channels, suggesting that, in their studies exogenous polyethylene glycol:superoxide dismutase could be acting extracellularly through catalytic inactivation of O_2^- diffusing into the interstitium from acinar cells. The enzyme could also be acting extracellularly by scavenging O_2^- in the interstitium generating chemotactic factors for neutrophils (Rinderknecht, 1988), thereby preventing leukocyte infiltration into the pancreas.

Interestingly, Guice et al. (1989) found that treatment with polyethylene glycol-conjugated catalase was not protective against pancreatic edema formation in caerulein-induced acute pancreatitis in the rat. The relatively low levels of polyethylene glycol-catalase found in the pancreas outside the vascular compartment suggest that the failure to prevent edema formation may result from inability of polyethylene glycol-catalase to reach extravascular sites of injury because of its large molecular size (490 vs. 240 kDa for the native catalase).

Since the molecular weights of superoxide dismutase (30–80 kDa) and of catalase are too large to enable them to pass through biomembranes, Nonaka et al. (1992) evaluated the effect of a new synthetic ascorbic acid derivative, 2-octadecylascorbic acid, $C_{24}H_{44}O_6$ (CV-3611) on the development of caerulein-induced acute pancreatitis in mice. CV-3611 is an agent that scavenges several reactive oxygen species, including superoxide radical anion (O_2^-), hydroxyl radical (OH), and lipid peroxides. It is formed by the introduction of lipophilic groups on the hydroxyls of the 2- or 3-carbon in ascorbic acid, and has a high affinity for biomembranes. Treatment with this low molecular weight antioxidant (428.6 Da) resulted in a significant reduction in pancreatic edema formation as well as of serum amylase and lipase levels, especially in the early phase of the disease.

Niederau et al. (1991) studied the effects of another low molecular weight antioxidant seleno-organic substance, Ebselen [2-phenyl-1,2-benzisoselenazol-3(2*H*)-one], known to catalyze GSH peroxidase-like reactions and to inhibit lipid peroxidation. Ebselen is a substance with a relatively small molecular weight (274.18 Da). It is well absorbed by the intestine and, inside the cells, has a high affinity to lipophilic membranes such as the microsomes. Because of the tightly bound selenium moiety, Ebselen is not available for direct utilization in biosynthesis of GSH peroxidase. In the caerulein-induced acute pancreatitis in mice, Ebselen at doses of 10–500 mg/kg, given 5 min prior to each caerulein injection, slightly attenuated the development of pancreatic edema, but did not reduce the increase in serum amylase and had no effect on the intensity of histological alterations, necrosis, vacuolization and inflammation.

In another study, but with the same model of pancreatitis, Niederau et al. (1992) studied the effects of catalase, superoxide dismutase, deferoxamine (Desferal) which sequester iron and thus curb the transition metal-catalyzed production of reactive oxygen species, dimethyl sulfoxide — $^{\cdot}OH$ scavenger, and allopurinol. All compounds failed to markedly ameliorate hyperamylasemia and the degree of necrosis or intracellular vacuolization. The highest dose of Desferal (100 mg/kg, given s.c. seven times at hourly intervals, a half hour before each of the caerulein injections), which tended to cause a slight reduction in serum amylase and some inflammation, was associated with diminished acinar cell vacuolization. The increase in pancreatic weight, which reflects the formation of edema, was significantly reduced by both doses of catalase (20 or 100 mg/kg, i.v.) as well as by the highest doses of superoxide dismutase (80 mg/kg, i.v.) and Desferal. The other doses and compounds did not significantly alter the increase in pancreatic weight.

The effect of another low molecular weight scavenger was tested by Neuschwander-Tetri et al. (1992). The authors found that treatment with GSH monoethyl ester has an ameliorating effect in caerulein-induced acute pancreatitis. Mice treated with 20 mmol/kg, given i.p. 1 h before caerulein, followed by 10 mmol/kg injections 3 and 7 h after starting caerulein, were found to have diminished histological evidence of pancreatitis (necrosis, inflamma-

tion, and vacuolization) and serum hyperamylasemia reduced by 40%. Since GSH monoethyl ester is not easily taken up into the cell (Anderson et al., 1985), Neuschwander-Tetri et al. (1994) expected better results in the next study after administration of the cysteine prodrug, L-2-oxothiazolidine-4-carboxylate. L-2-Oxothiazolidine-4-carboxylate is transported into cells and then cleaved by 5-oxoprolinase in an ATP-dependent reaction to form L-cysteine and subsequently increase GSH peroxidase levels. Surprisingly, the biochemical and histological evidence of caerulein-induced acute pancreatitis was not altered by treatment with this drug. In contrast, the usefulness of treatment with L-2-oxothiazolidine-4-carboxylate, in caerulein-induced acute pancreatitis in mice was recently reported on by Lüthen et al. (1997). In their hands, s.c. administration of the cysteine prodrug, L-2-oxothiazolidine-4-carboxylate, at the dose of 20 mmol/kg, given 30 min before the first injection of caerulein, and then repeated every 2 h, exerted spectacular beneficial effects on the severity of the disease. The beneficial action was expressed by significant reduction (~ 50%) in serum amylase and histopathological damage to the pancreas. In view of these contradictory findings of two groups using the same experimental model of pancreatitis, the therapeutic potential of cysteine prodrugs requires further investigation.

In one of the recent attempts to blunt reactive oxygen species-mediated injury in acute pancreatitis, Wang et al. (1996) evaluated the role of metallothionenin. Metallothionenin is a heavy-metal-binding small, low-molecular weight cytoplasmic protein composed of 61 or 62 amino acid residues 30% of which are cysteine residues (Kägi and Schäffer, 1988). Therefore, it has been suggested that metallothionenin may scavenge endogenous free radicals and improve the course of certain free radical-induced diseases such as streptozotocin-induced diabetes in rats (Yang and Cherian, 1994). In a study performed by Wang et al. (1996), a single i.p. injection of Zn (5 mg/kg of $ZnSO_4 \cdot 7H_2O$) resulted in significant (83-fold) elevation of normal rat pancreatic metallothionenin, measured after 24 h, and partially prevented some biochemical and histological features of caerulein-induced acute pancreatitis. In these rats, the increase in serum amylase was reduced by 25%, while inflammatory cell infiltration and vacuolization were diminished by ~ 50%. Simultaneously, only slight reduction in pancreatic edema was observed.

The superoxide dismutases catalyze the dismutation of O_2^- to H_2O_2, which is then metabolized to water by GSH peroxidase or catalase (Freeman and Crapo, 1982; Halliwel, 1991). The superoxide dismutases in eukaryotes are classified into two types: Cu^{2+}/Zn^{2+} superoxide dismutase which is constitutively expressed in cytosol, and Mn-superoxide dismutase which is induced transcriptionally in mitochondria on exposure of cells to endotoxins and cytokines (Wong and Goeddel, 1988). It was previously reported that Mn-superoxide dismutase is induced in the pancreas of rats by very low doses of lipopolysaccharide, and that the increased activity of this enzyme in the pancreas correlates with the decreased severity of caerulein-induced acute pancreatitis (Abe et al., 1995). Recently, to evaluate further the role of reactive oxygen species and superoxide dismutases in pancreatitis, the same research group used a transgenic mouse strain overexpressing Cu^{2+}/Zn^{2+}-superoxide dismutase (Kikuchi et al., 1997). The Cu^{2+}/Zn^{2+}-superoxide dismutase transgenic mouse strain, TgHS-SF218/10, carried a stable multiple copy insertion of a native human Cu^{2+}/Zn^{2+}-superoxide dismutase gene. The activity of Cu^{2+}/Zn^{2+}-superoxide dismutase in the basal state pancreas of the transgenic mice was 1.7-fold higher than that of the nontransgenic littermates, but the activity of Mn-superoxide dismutase did not differ between the two groups. Both the elevation of serum amylase and the development of pancreatic edema in the course of caerulein-induced acute pancreatitis were significantly reduced in the transgenic mice compared to those in the nontransgenic littermates. In the transgenic mice, the pancreatitis-associated reduction in Cu^{2+}/Zn^{2+}-superoxide dismutase activity in the pancreatic tissue was significantly less than that in the nontransgenic mice. These results demonstrate directly the importance of intracellular reactive oxygen species in the pathogenesis of acute pancreatitis.

Another piece of evidence of oxidative stress implication in the pathogenesis of caerulein-induced acute pancreatitis was recently provided by Sato et al. (1997). The authors addressed the issue of adaptive responses to oxidative stress in the pancreas and found that expression of the stress protein, heme oxygenase-1, in this gland was enhanced 12–24 h after initiation of caerulein-induced acute pancreatitis.

In a similar approach, Fu et al. (1997) demonstrated that oxidative stress-responsive genes, including c-*fos*, heme oxygenase-1, and metallothionein-I are similarly and rapidly upregulated in the pancreas during caerulein-induced acute pancreatitis in mice. This was accompanied by the accumulation of antioxidant proteins — heme oxygenase-1 and metallothionenin-I. In addition, pancreatic cytokine genes, including interleukin 1β, interleukin-6, and tumor necrosis factor-α, were rapidly upregulated in the pancreas with a subsequent rapid intrapancreatic accumulation of interleukin-1β and interleukin-6. These data suggest that oxidative stress and inflammation both occur in the pancreas during the early stages of acute pancreatitis. However, the authors found that acute induction of pancreatic oxidative stress by diethylmaleate injection and/or acute induction of an inflammatory response by lipopolysaccharide injection did not cause the changes characteristic of acute pancreatitis. It was finally suggested that simply inducing oxidative stress and/or inflammation may be insufficient to initiate acute pancreatitis.

In the last decade evidence has been provided for the generation of NO in the pancreatic nerves and acini due to

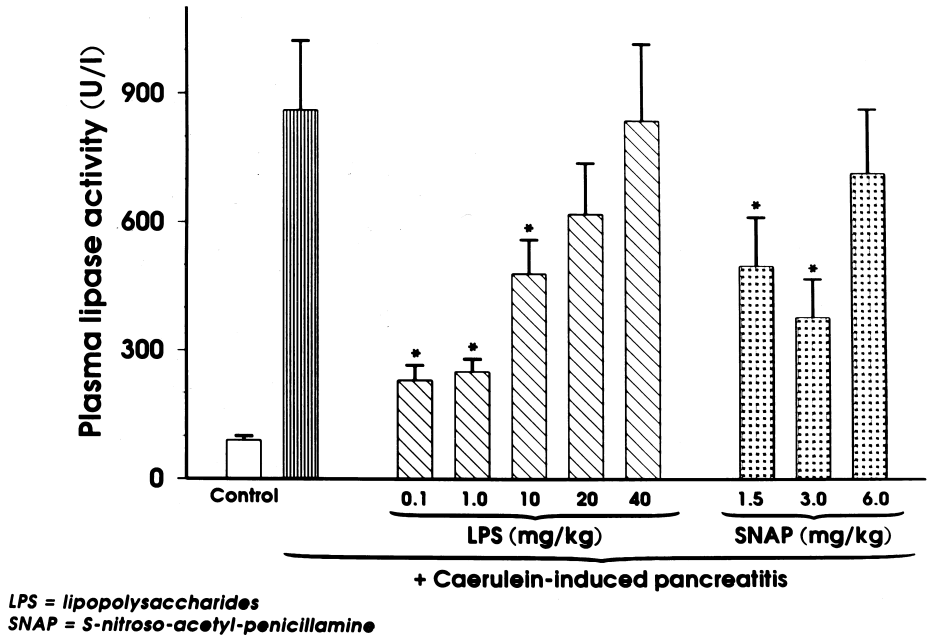


Fig. 4. Effects of lipopolysaccharides or SNAP on pancreatic weight and pancreatic blood flow (presented as percent control) in rats infused with caerulein (5 μg kg^{-1} h^{-1}) to induce pancreatitis (Konturek et al., 1994).

the cNOS activity. We found that following induction of acute pancreatitis with caerulein, the iNOS may also be expressed and activated, leading to excessive production of NO in the pancreas. Administration of L-arginine, a substrate of NOS, was found to ameliorate the caerulein-induced acute pancreatitis in rats and to reverse, at least in part, the potentiation of pancreatic inflammatory changes caused by N^G-nitro-L-arginine (L-NNA), a blocker of NOS activity (Konturek et al., 1994; Molero et al., 1995). It is of interest that bacterial lipopolysaccharides which activate pancreatic tissue cNOS protect from rather than augment the inflammatory reactions induced in the pancreas by caerulein infusion and that this is accompanied by an enhanced generation of NO and attenuation of the fall in pancreatic blood flow (Jaworek et al., 1999). Similar effects were obtained using potent NO donors such as *S*-nitroso-acetyl-penicillamine. These results indicate that NO released endogenously or released from exogenously applied NO donors protects the pancreas against the damage provoked by caerulein overstimulation of this organ and that the L-arginine-NO system is involved in the pancreatic protection mediated mainly by the enhancement of the pancreatic circulation (Fig. 4).

3. The evidence of reactive oxygen species-mediated pancreatic tissue damage and direct measurements of reactive oxygen species in acute pancreatitis

Lipid peroxidation is an autocatalytic free radical-mediated destructive process whereby polyunsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides. These latter compounds spontaneously rearrange to produce multiple degradation products, including malondialdehyde (Chance et al., 1979). Measurement of end products of lipid peroxidation is perhaps the most widely used assay for oxidative damage (Liu et al., 1997).

The evidence of excessive lipid peroxidation within pancreatic tissues in the course of caerulein-induced acute pancreatitis, was first reported by Dabrowski et al. (1988). Intravenous infusion of caerulein in a supramaximal dose (5 μg kg^{-1} h^{-1}) resulted in a significant elevation of pancreatic tissue malondialdehyde concentration, detected at 3 and 12 h as a thiobarbituric acid-reactive substance. Severe oxidative stress was additionally manifested by a concomitant dramatic depletion of pancreatic superoxide dismutase, to 16% of normal activity, at 3 h. At 12 h, pancreatic superoxide dismutase activity was so low that it could not be detected. On the contrary, a slight (by 17%) increase in superoxide dismutase activity was found in the serum of rats with 3-h caerulein-induced acute pancreatitis. In rats with a 12-h infusion of caerulein, serum superoxide dismutase had returned to its normal level, but was present in ascitic fluid. Therefore, the authors suggested that, in this experimental model, an oxygen radical-mediated alteration in cell membranes facilitates the leakage of scavenger enzyme, superoxide dismutase, from the pancreas to circulating blood and into the peritoneal cavity, thus contributing to the oxidative stress (Fig. 5). These findings were confirmed and extended by Schoenberg et al. (1990), who showed that peroxidation products, conjugated dienes and malondialdehyde, in pancreatic tissue are increased

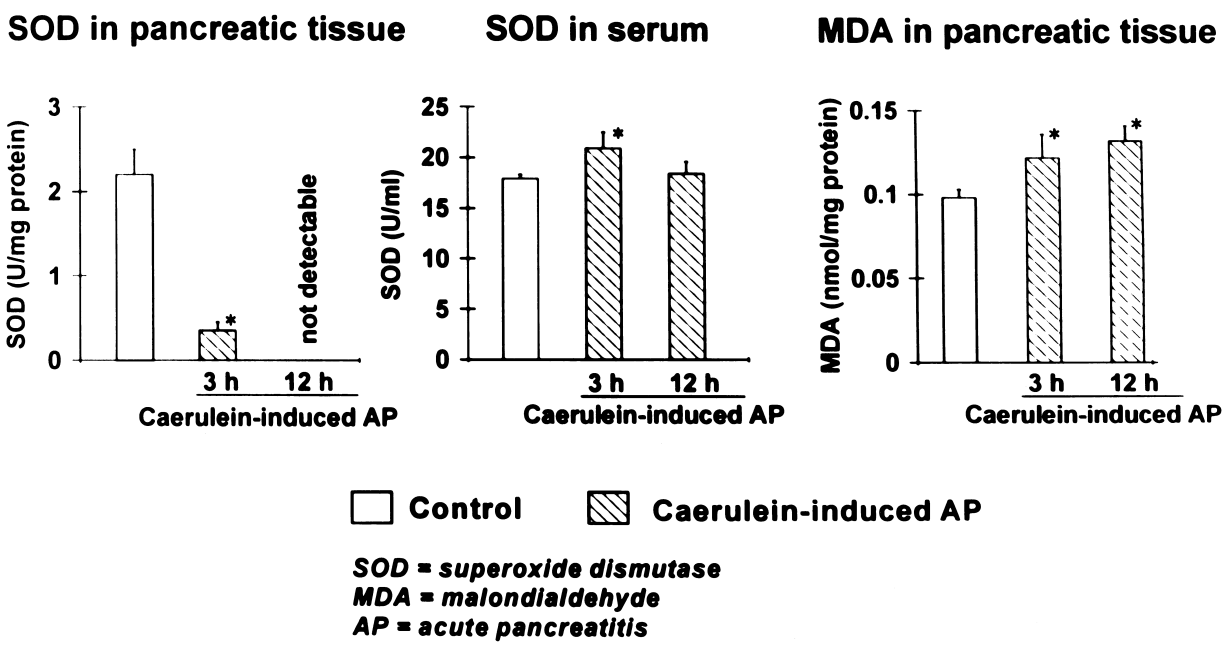


Fig. 5. First evidence for the pancreatic tissue decrease of superoxide dismutase and excessive lipid peroxidation (malondialdehyde) in caerulein-induced acute pancreatitis in rats (Dabrowski et al., 1988).

even after 30 min of caerulein infusion, reach their highest level after 3.5 h, and decrease to normal levels after 12 h. Nonaka et al. (1990) observed similar changes in pancreatic tissue of mice with caerulein-induced acute pancreatitis. They showed an early and distinct (7-fold) increase in pancreatic lipid peroxidation products after 3.5 h, then a gradual decrease in spite of which, after 12 h, lipid peroxidation products were still highly elevated (by ~ 300%). In the same study, concomitantly with the rise in lipid peroxidation products, pancreatic tissue superoxide dismutase activity started to decrease in an early phase of the disease and after 12 h had diminished to 40% below the normal level. Pancreatic catalase activity remained unchanged up to 6 h, and then gradually decreased to 60% of the normal level. Interestingly, another scavenger enzyme, GSH peroxidase, activity was not significantly altered in the course of the disease. In another study, Dabrowski and Chwiecko (1990), found that in 6-h caerulein-induced acute pancreatitis in rats, a significant (70%) increase in pancreatic malondialdehyde concentration is associated with a profound (70%) depletion of total pancreatic sulfhydryl compounds, which comprise protein, and non-protein thiols, represented predominantly by GSH peroxidase. These findings were subsequently confirmed and extended by Neuschwander-Tetri et al. (1992), who showed the time-course of changes in pancreatic tissue GSH peroxidase in mice given various doses of caerulein. Treatment with the 50 μg/kg dose (hourly-repeated seven s.c. injections) resulted in acute pancreatitis of maximal severity for this model, accompanied by an early and rapid depletion of pancreatic GSH peroxidase. Treatment with the 5 μg/kg dose produced less GSH peroxidase depletion and less severe pancreatitis. Treatment with the 0.1 μg/kg dose of caerulein resulted in much less GSH peroxidase depletion and did not induce pancreatic injury. These observations suggest that GSH peroxidase consumption occurs not only in the course of pancreatitis, but also in the secretory state after physiological stimulation of the pancreatic acinar cell. Because cysteine availability can be rate-limiting for GSH peroxidase synthesis, in another study, Neuschwander-Tetri et al. (1994) measured the pancreatic content of cysteine in mice during caerulein treatment. The authors found that the pancreatic cysteine content decreased to 42% of normal after 4 h of caerulein treatment. Administration of the cysteine prodrug, L-2-oxothiazolidine-4-carboxylate, more than doubled the pancreatic cysteine content at 4 h and prevented pancreatic cysteine depletion after caerulein treatment. However, L-2-oxothiazolidine-4-carboxylate neither prevented pancreatic GSH peroxidase depletion nor altered the biochemical and histological evidence of caerulein-induced acute pancreatitis. On the contrary, Lüthen et al. (1997) have reported that L-2-oxothiazolidine-4-carboxylate causes a 60% increase in pancreatic GSH peroxidase. Furthermore, in the same model of pancreatitis, L-2-oxothiazolidine-4-carboxylate administration, 20 mmol/kg, attenuated the decrease of pancreatic GSH peroxidase and protein thiol for up to 8 h and blunted the caerulein-induced increase in amylase activity and histopathologic damage. The authors assumed that thiols (e.g., GSH peroxidase) and their corresponding disulfides are critically involved in the pathophysiology of caerulein-induced acute pancreatitis.

In an interesting approach, Gough et al. (1990) used chemiluminescence to provide direct evidence of genera-

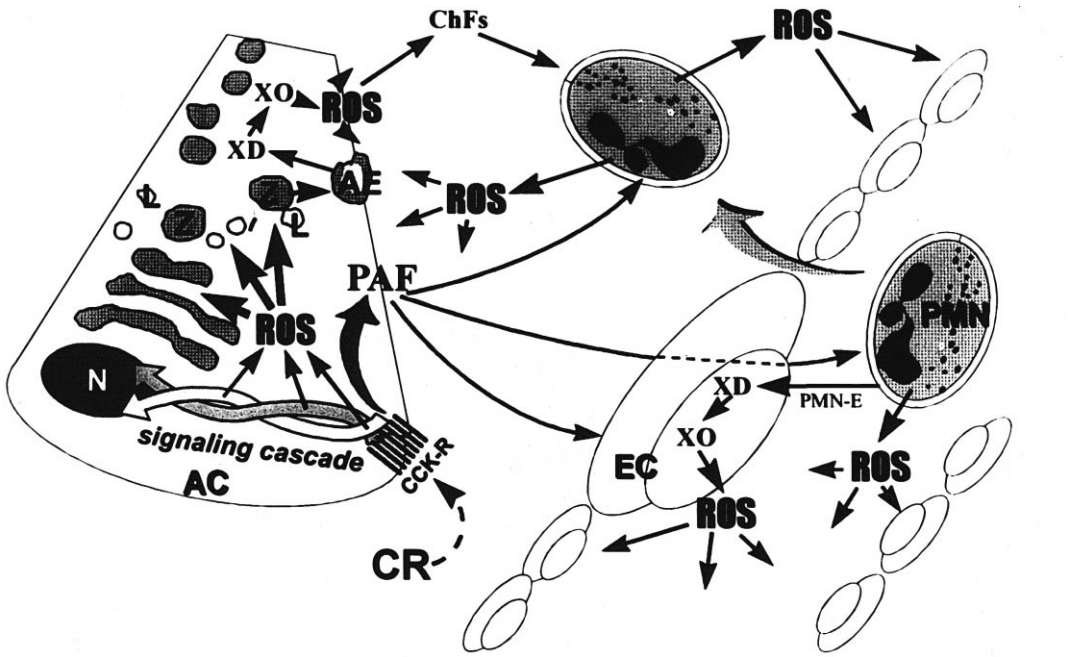
tion of reactive oxygen species in acute pancreatitis. Chemiluminescence, a phenomenon based on the emission of light during reactive oxygen species-mediated reactions, was measured in pancreatic tissue samples at 5-min intervals. In caerulein-induced acute pancreatitis, chemiluminescence reached a peak, 32-fold the normal level at 20 min, and then gradually declined but, at 60 min, it was still above the normal range.

It is known that pentane and ethane are formed during the process of lipid peroxidation and are exhaled. In contrast to pentane, which is extensively oxidized in the liver, ethane undergoes little hepatic metabolism, therefore its exhalation reflects mainly its formation rate. In their recent study, Lévy et al. (1997) assessed lipid peroxidation in rats with caerulein-induced acute pancreatitis, by in vivo measurement of ethane exhalation. Early in the course of the disease, 1 h after the last of four i.p. injections of 20 μg/kg caerulein, exhalation of ethane was significantly (3-fold) elevated and remained at the same level for up to 5 h of observation.

4. What is the source of the enhanced reactive oxygen species generation in caerulein-induced acute pancreatitis?

Of the multitude of potential sources of reactive oxygen species, xanthine oxidase and activated leukocytes have been implicated in the tissue damage associated with ischemia-reperfusion and inflammation (Forman and Thomas, 1986; Parks, 1989). In normal, non-ischaemic tissues, xanthine oxidoreductase exists predominantly as an innocuous, NAD^+-reducing dehydrogenase which can be converted to an O_2^- producing oxidase (xanthine oxidase) through oxidation of essential sulphydryl groups (reversible) or by limited proteolysis (irreversible). Recent evidence suggests that leukocytic elastase, tumor nercosis factor alfa, or the chemotactic oligopeptide, *N″*-formyl-Met-Leu-Phe, can also convert xanthine dehydrogenase into xanthine oxidase (Ward, 1991). Since the xanthine oxidase inhibitor, allopurinol, has been shown to attenuate pancreatic damage in a canine model of acute pancreatitis, presumably by preventing the generation of cytotoxic O_2^- (Sanfey et al., 1985), Wisner and Renner (1988) examined the effect of allopurinol in caerulein-induced acute pancreatitis. In their study, a continuous i.v. infusion of allopurinol (20 mg kg^{-1} h^{-1}) for 6 h along with an acute pancreatitis-producing dose of caerulein (10 μg kg^{-1} h^{-1}) reduced pancreatic edema by approximately 45% and hyperamylasemia by approximately 60% compared with those in rats i.v. infused with either caerulein alone or caerulein plus a lower dose (10 mg kg^{-1} h^{-1}) of allopurinol. Recently, in caerulein-induced acute pancreatitis in rats, treatment with allopurinol and, most of all, pretreatment with this drug was shown to protect from severe subcellular alterations characteristic of this model of pancreatitis (Brunelli and Scutti, 1998). Interestingly, in a similar model of pancreatitis, using mice with caerulein-induced acute pancreatitis, two other groups found no beneficial effect of allopurinol on pancreatic edema and hyperamylasemia (Devenyi et al., 1987; Niederau et al., 1992). Therefore, the role of xanthine oxidase as a potent source of reactive oxygen species in caerulein-induced acute pancreatitis remains controversial.

The plasma membrane-associated NADPH oxidase of neutrophils, monocytes, eosinophils and macrophages is another potential source of reactive oxygen species (Forman and Thomas, 1986; Parks, 1989). The NADPH oxidase reduces molecular oxygen to O_2^- with the concomitant oxidation of cytosolic NADPH. Unlike the xanthine oxidase system which generates approximately 20% O_2^- and 80% H_2O_2 under normoxic conditions at a physiological pH, NADPH oxidase generates predominantly O_2^- with H_2O_2 as a secondary product through spontaneous dismutation of O_2^-. Neutrophils also contain high concentrations of myeloperoxidase, which can use the H_2O_2 generated by NADPH oxidase to oxidize halides and consequently produce hypochlorous acid, known as a very potent oxidant (Forman and Thomas, 1986; Ward, 1991). Our knowledge about the potential role of leukocytes as a source of reactive oxygen species in caerulein-induced acute pancreatitis is sparse and is based, as far as we know, on two publications (Guice et al., 1987; Dabrowski et al., 1991). The first report was from Guice et al. (1987), who showed that both neutrophil depletion and complement depletion or catalase injection prevented early edema formation in caerulein-induced acute pancreatitis. Later, Dabrowski et al. (1991) showed that a platelet-activating factor antagonist (BN52021) prevented the activation of leukocytes and therefore significantly ameliorated caerulein-induced acute pancreatitis in rats. The positive effect of BN 52021 was expressed by prevention of pancreatic tissue damage, edema and hyperamylasemia, as well as prevention of oxidative stress as assessed from the lack of both superoxide dismutase depletion and concomitant elevation of lipid peroxidation products in pancreatic tissue. One of the most interesting findings in this latter study was that the treatment with platelet activating factor antagonist completely protected pancreatic tissue from infiltration with leukocytes. However, it is necessary to emphasize that those spectacular effects of platelet activating factor-antagonist were observed only in the early (at 3 h) phase of the disease. At 6 h the effect of BN 52021 was much less expressed, most likely due to involvement of other than platelet activating factor inflammatory mediators in the later phase of the disease. The findings about the important role of platelet activating factor and platelet activating factor-activated neutrophils in the pathogenesis of caerulein-induced acute pancreatitis were recently confirmed and extended by others (Konturek et al., 1992; Zhou et al., 1993; Sandoval et al., 1997), who provided strong evidence that, upon in vivo stimulation with



AC - *acinar cell*; CCK-R - *CCK receptor*; ChFs - *chemotactic factors*;
CR - *caerulein*; AE - *activated enzymes*; EC - *endothelial cell*; L - *lysosomes*; N - *nucleus;*
PMN - *polymorphonuclear cell;* PMN-E - *PMN-elastase*; PAF - *platelet-activating factor;*
ROS - *reactive oxygen species*; XD - *xanthine dehydrogenase*; XO - *xanthine oxidase*; Z - *zymogens*

Fig. 6. Potential sites of generation of reactive oxygen species in caerulein-induced acute pancreatitis.

caerulein, platelet activating factor is being produced in pancreatic tissue (Fig. 6).

5. Concluding remarks

The data available on the role of reactive oxygen species in biology and medicine along with the data presented here led us to show a schematic diagram explaining how reactive oxygen species can participate in the pathogenesis of caerulein-induced acute pancreatitis. Supramaximal stimulation with a cholecystokinin analogue, caerulein, activates cholecystokinin receptors in pancreatic acinar cells. Since it is known that reactive oxygen species play a very important role in signal transduction (Lander, 1997), it is possible that hyperstimulation of cholecystokinin receptors may result in excessive intracellular generation of reactive oxygen species (see Gough et al., 1990), i.e., maximal reactive oxygen species generation at 20 min. This, in turn, may cause alterations in cytoskeleton function, which is an early phenomenon previously postulated by Braganza (1991), and recently reported upon by others (Fallon et al., 1995; Jungerman et al., 1995). This disruption of the cytoskeleton offers an explanation for the disturbances of intracellular transport of digestive enzymes, leading to their premature intracellular activation. In acinar cells, activated proteases may convert xanthine dehydrogenase into xanthine oxidase, providing an additional source of reactive oxygen species. Intracellularly generated reactive oxygen species, in concert with digestive enzymes, can increase cellular permeability and allow the access of both activated pancreatic enzymes and reactive oxygen species-induced chemotactic factors to the interstitial and vascular spaces. Simultaneously, caerulein-induced production and secretion of platelet activating factor and NO by pancreatic acinar cells provides an additional inflammatory mediator and contributes to microcirculation disarrangement. Consequently, invading leukocytes activate endothelial xanthine oxidase and produce themselves huge amount of reactive oxygen species.

All these findings prove strongly that oxidative stress occurs as an early phenomenon in pancreatic tissue in the course of caerulein-induced acute pancreatitis. Oxidative stress was documented in pancreatic tissue by means of methods showing generation of reactive oxygen species and accumulation of products of reactive oxygen species-mediated lipid peroxidation, with concomitant depletion of enzymatic and low molecular weight antioxidants. Features of acinar cell injury and inflammation, especially pancreatic edema, show a marked improvement following treatment with a broad spectrum of antioxidants, nitric oxide or platelet activating factor-antagonists. Unfortunately, in most cases these beneficial effects are temporary and generally restricted to an early phase of the disease. However, results of well-designed clinical trials will help to evaluate the importance of oxidative stress-oriented treatment in acute pancreatitis in humans.

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