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FORUM REVIEW ARTICLE

Oxidative Stress in Chronic Pancreatitis: Pathophysiological Relevance and Management

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Abstract

Significance: Chronic pancreatitis (CP) is a progressive, inflammatory disease of the pancreas leading to slow destruction of pancreatic parenchyma and progressive fibrosis. The pathophysiological mechanism of CP is not well understood. ***Recent Advances:*** A pathophysiologic role of oxidative stress in CP has, however, been suggested in recent years. Pancreatic acinar cells contain phase I cytochrome P450 (CYP 450) biotransforming enzymes and phase II conjugation reactions for the metabolism of xenobiotics. The oxidative stress in the acinar cell may result from generation of free radicals through CYP induction, concurrent exposure to a chemical that undergoes bioactivation, and insufficiency of micronutrients that are required to sustain antioxidant (AO) capacity. ***Critical Issues:*** Studies have shown that there is indeed a state of oxidative stress as evidenced by increased levels of products of oxidative stress and reduced AO capacity in patients with CP. A recent randomized, controlled trial has shown beneficial effect of AO therapy in CP; a combination of AOs (0.54 g ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, 600 μg organic selenium, and 2 g methionine per day in divided doses) led to significant reductions in pain and oxidative stress in patients with CP. ***Future Directions:*** Similar studies from other centers and multicenter studies should confirm that oxidative stress plays an important role in the pathophysiology of CP and supplementation with AOs leads to significant pain relief in patients with this disease. *Antioxid. Redox Signal*. 15, 2757–2766.

Introduction

Chronic Pancreatitis (CP) is a progressive, inflammatory disease of the pancreas leading to slow destruction of pancreatic parenchyma and progressive fibrosis (65). It causes exocrine and endocrine insufficiency and clinically results in malabsorption of dietary nutrients, diabetes mellitus, and severe unrelenting abdominal pain. The prevalence of CP is estimated to be 10–15/100,000 population but is much higher in southern India (24, 64). The causes of CP include toxic injury due to alcohol, hereditary and nonhereditary genetic predisposition, and metabolic derangements in the form of hypercalcemia and hypertriglyceridemia. Anatomical abnormalities such as pancreas divisum may give rise to CP but there is no causal evidence for this. In some cases, no clear etiological agent is found (idiopathic) (21). The diagnosis of CP is made in the presence of pancreatic calcification and/or pancreatic ductal changes (dilatation/irregularity). The diagnosis of calcification in best made on computed tomography scan or endosonography, and the ductal changes are best appreciated on either endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography (Figs. 1 and 2).

The most common types of CP are alcohol related and idiopathic. Alcoholic CP is the predominant type in countries such as the United States, United Kingdom, Germany, Brazil, and Japan, whereas idiopathic CP is the commonest type in countries such as India and China (24). Idiopathic CP that is prevalent in India is also known as tropical calcific pancreatitis (TCP). We have recently shown that this term is a misnomer and that idiopathic CP in India is akin to that seen in other parts of the world and has a strong genetic susceptibility (43). The cause of idiopathic CP was not known for a long time. The initial thinking regarding the pathogenesis of idiopathic CP revolved around multiple environmental factors such as diet and toxins, but such hypotheses were never proven in well-designed case–control studies. Protein calorie malnutrition and cassava consumption have also been considered as etiological factors for CP. However, we have shown that malnutrition is an effect and not a cause of idiopathic CP (42). During the last decade, attention has shifted largely toward underlying genetic susceptibility as a risk factor for CP. With regard to alcohol as the cause of CP, it is not known why only a minority of patients who abuse alcohol develop CP, suggesting the role of other important factors such as smoking and genetic predisposition.

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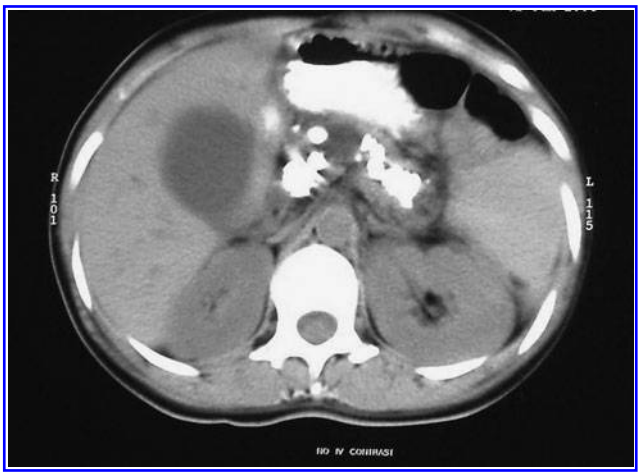

FIG. 1. A computed tomography picture of pancreatic calcification.

Various hypotheses have been proposed regarding the pathophysiological mechanisms in CP that include the "toxic–metabolic," "obstructive due to protein hypersecretion," "necrosis–fibrosis," and "oxidative stress." According to Bordalo *et al.* (9), ethanol induces a fatty degeneration of acini similar to that seen in the hepatocytes of the liver. Ethanol has either a direct or an indirect toxic effect, mediated by the ethanol metabolite, acetaldehyde, on the metabolism of pancreatic acinar cells. Sarles and Sahel (55) championed the obstructive hypothesis; they also emphasized that all cases of alcoholic pancreatitis are chronic in nature from the beginning. According to this hypothesis, alcohol stimulates increased secretion of proteins in the pancreatic juice, which leads to protein plug formation in the smaller pancreatic ducts. These protein plugs cause ductal obstruction and later become calcified. Ductal obstruction leads to acinar atrophy, periductular inflammation, and eventually, pancreatic fibrosis (57). A special pancreatic secretory protein (or lithostathine) normally prevents precipitation of calcium carbonate and is believed to play a pivotal role in the etiopathogenic process. Deficient secretion of lithostathine results in calcification of protein plugs (46). However, the unexplained points regarding this hypothesis are that (i) most patients with alcoholic pancreatitis present with clinical acute pancreatitis; (ii) autopsy studies have shown that approximately 50% of patients who die of alcoholic pancreatitis do not have histological evidence of chronicity (53); (iii) many patients with CP demonstrate signs of autodigestive necrosis of the pancreas; and (iv) altered lithostathine secretion has not been universally confirmed. The "necrosis–fibrosis" hypothesis is based on the concept that it is the recurrent episodes of acute alcoholic pancreatitis causing repeated pancreatic injury that ultimately result in CP (14). Resorption of areas of necrosis, as well as hemorrhagic necrosis within the pancreas, leads to perilobular fibrosis through the action of mediators such as transforming growth factor (TGF)-B. The repeated episodes of inflammation and fibrosis finally lead to CP.

Ammann and Muellhaupt (3) showed that progression of alcoholic pancreatitis correlated well with the incidence and severity of acute attacks of pancreatitis. Patients experiencing frequent attacks and severe pancreatitis with pseudocysts in the head of the pancreas were more likely to develop calcific pancreatitis; this supports the "necrosis–fibrosis" hypothesis. However, this hypothesis fails to explain: (i) how alcohol causes acinar dysfunction and necrosis; (ii) how primary painless CP occurs in 5%–10% of alcoholics; and (iii) why biliary pancreatitis virtually never progresses to CP, despite repeated attacks of severe pancreatitis.

Oxidative stress has emerged as one of the important pathophysiological mechanisms of CP. This review focuses on the role of oxidative stress and the effect of antioxidants (AOs) supplementation in ameliorating oxidative stress and relieving pain in CP.

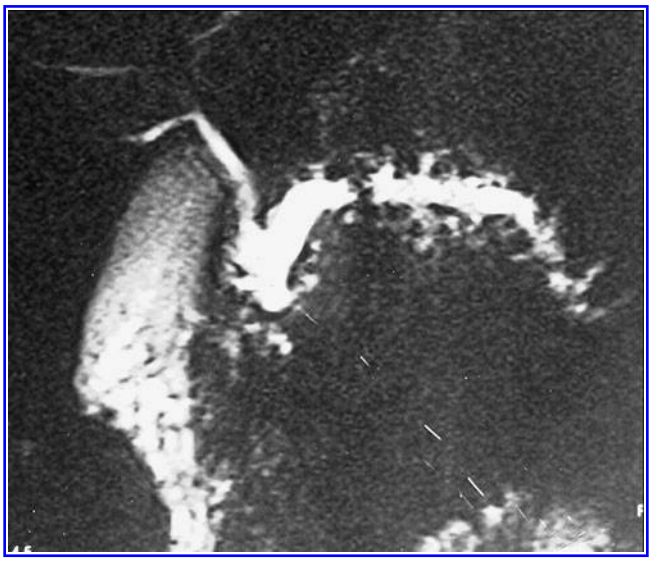

FIG. 2. A magnetic resonance cholangiopancreatography (MRCP) picture of pancreatic ductal dilatation.

Oxidative Stress in CP

Oxidative stress

Oxidative stress has been defined as an imbalance between pro-oxidants and AOs with increased free radical (FR) formation (35). Although the endogenous AO defense system is quite efficient, increased exposure to pro-oxidants or reduced AO capacity may lead to such an imbalance, resulting in oxidative stress. An increase in FR or reactive oxygen species (ROS) production may be attributed either to exogenous radicals such as radiation, pollutants, xenobiotics, and cigarette smoking or to endogenous sources such as inflammation and the respiratory burst. Cells or tissues are in a stable state if the rates of ROS production and scavenging capacity are constant and in balance, leading to redox homeostasis. If there is mild oxidative stress, cellular response through generating extra AOs is adequate (Fig. 3). If this balance is disturbed and ROS production is increased more strongly and persistently, the AO capacity may be overwhelmed. In such a situation, the system may still reach equilibrium but it may be associated with higher ROS concentrations. For example, such a shift to more oxidative conditions has been seen in aging. Pathological conditions may develop in extreme cases of persistently high ROS levels. These conditions do not

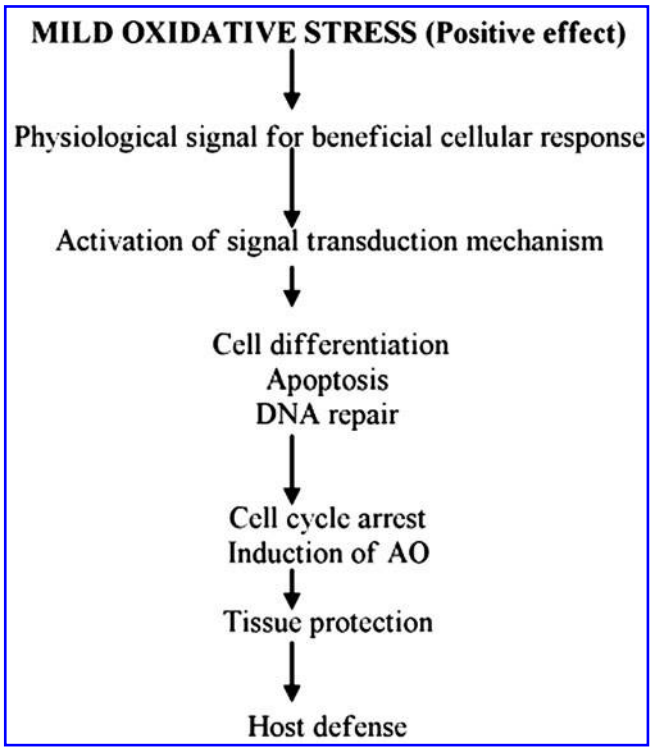

FIG. 3. Effect of mild oxidative stress for cellular protection.

necessarily lead to a loss of homeostasis but rather a chronic shift in the level of homeostasis. Pathological symptoms may thus result from both the damaging effects of ROS and the ROS-mediated changes in gene expression due to redox-sensitive signaling pathways. Severe oxidative stress can, however, cause cell injury and death. FR-induced death can lead to either necrosis or apoptosis.

Pro-oxidants

Pro-oxidants are compounds that favor the formation of FRs and increase the production of FR peroxidation products. Alcohol, tobacco, smoke, environmental pollutants, dietary toxins, fumes, certain drugs, and transition metal cations in free state, for example, iron and copper, are all pro-oxidants.

An FR is an atom or molecule that contains one or more unpaired electron (44). The most relevant radicals in biological regulation are superoxide (SO) and nitric oxide. These radicals are formed by two groups of enzymes, that is, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide synthase isoforms, respectively (Fig. 4).

Detoxification System as a Source of FRs

Xenobiotics are compounds that are foreign and hence toxic to the body. Detoxification of xenobiotics involves alteration of their structure to a more polar state, which allows their easy excretion. It can be divided into two types of reactions. In phase I metabolism, enzymes cleave the parent molecule into products that can be either more or less toxic, and in phase II metabolism, an endogenous molecule is attached to the phase I biotransformed compound to make it more polar and easier to excrete. Enzymes in phase I metabolism include cytochrome P450 (CYP 450) oxidase system and hydrolyzing enzymes. The P450 superfamily, with the CYP1, 2, and 3 families, is the major contributor to xenobiotic metabolism (4). Phase II reactions include glucuronidation and glutathione conjugation (Fig. 5).

Although induction of phase I enzymes, that is, CYP 450 enzymes, frequently assist the body in getting rid of xenobiotics, it can increase the toxicity of those compounds that are biotransformed to toxic metabolites, for example, alcohol can induce CYP2E1, which increases the biotransformation of acetaminophen to a toxic metabolite. The induction of CYP2E1 contributes to the metabolic tolerance to ethanol that develops in chronic and heavy drinkers. CYP2E1 is especially pertinent, because not only it is inducible by alcohol, but also it is known to generate ROS from ethanol itself.

Exogenous Sources of FRs

Alcohol

Alcohol-induced oxidative stress is linked to the metabolism of ethanol. Three metabolic pathways of ethanol have been described in the human body. These involve alcohol dehydrogenase, microsomal ethanol oxidation system, and catalase. Each of these pathways could produce FRs, which affect the AO system. The main effect of alcohol toxicity may be mediated *via* lipid peroxidation products (61). Alcohol is metabolized in two steps. First, the enzyme alcohol dehydrogenase converts alcohol to acetaldehyde, a toxic and reactive molecule. Next, the enzyme aldehyde dehydrogenase converts the acetaldehyde to acetate. Each of these reactions leads to formation of one molecule of nicotinamide adenine dinucleotide, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened O_2 use and ROS formation. The interaction of acetaldehyde with proteins and lipids can also lead to radical formation and cell damage. The damage to the mitochondria by alcohol results in decreased ATP production. As mentioned earlier, alcohol-induced increases in the activity of the

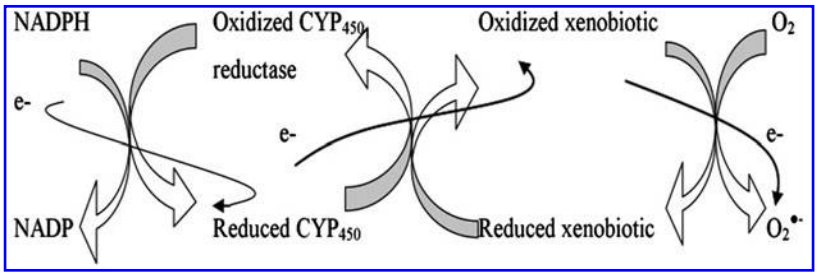

FIG. 4. Redox cycle generating free radical.

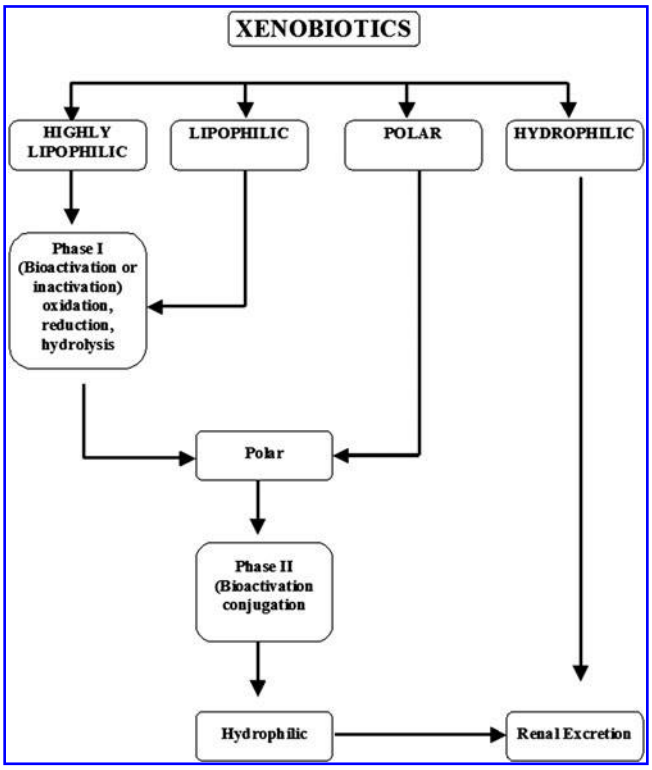

FIG. 5. Xenobiotic metabolism.

enzyme CYP 450 2E1 (CYP2E1), which metabolizes alcohol and other molecules, generates ROS in the process.

Cigarette smoking

Cigarette smoke contains a range of xenobiotics, including oxidants and FRs that can increase lipid peroxidation. One estimate suggests that it may contain 1014 FRs per inhalation (13). Cigarette smoke consumes the AOs vitamins C and E as well as other nutrients. Cigarette smoke has been suggested to be an active redox system that is capable of reducing molecular oxygen to produce SO, eventually leading to hydrogen peroxide and hydroxyl radicals. In addition, it has been shown that the principal radical in tar reacts with DNA *in vitro*, possibly by covalent binding. The gas phase of cigarette smoke contains small oxygen- and carbon-centered radicals that are much more reactive than the tar-phase radicals.

Antioxidants

AOs are the body's resource for protection against the diverse FRs and other oxidative stressors. Halliwell and Gutteridge (27) defined AO as "any substance that, when present in low concentration compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate." This definition includes the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) as well as nonenzymic compounds such as α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), and glutathione. In addition, there are compounds that have a relatively low specific antioxidative activity, but at high concentrations they can significantly contribute to the overall FR scavenging activity. The most prominent examples of such high-level, low-efficiency AOs are free amino acids, peptides, and proteins. Practically all amino acids can serve as targets for oxidative attack by ROS, although some amino acids such as tryptophan, tyrosine, histidine, and cysteine are particularly sensitive to ROS (16, 17) In mammals, cysteine is made from two other amino acids, methionine, which is essential in diet, and serine, which is not. Methionine furnishes the sulfur atom and serine furnishes the carbon skeleton in the synthesis of cysteine. Methionine is therefore the source of cysteine. Cysteine can become the rate-limiting factor in the synthesis of glutathione, which is the largest nonenzymatic AO defense. If the bioavailability of methionine is poor, it may result in low levels of glutathione. Therefore, dietary methionine is an important AO that participates in AO defense indirectly.

Selenium is a component of the GPx and Se-dependent enzyme thioredoxin reductase. Selenoenzyme thioredoxin reductase is involved in disposal of the products of oxidative metabolism (29). Se-dependent GPx plays an important role in preventing the accumulation of H_2O_2 and lipid peroxides. The conventional Indian diet provides about 48 μg Se/day, whereas vegan Indian diet provides 27 μg Se/day (37).

AOs can be classified into several groups:

(i) Cellular enzymes (SOD, CAT, GPX)
(ii) Uric acid, which is produced *via* metabolism of purines
(iii) Small molecules taken up in our diet (β-carotene, ascorbic acid, α-tocopherol)
(iv) Proteins, which form complexes with transition metal ions.

Primary defenses (71) include AOs vitamin E, vitamin C, vitamin A, glutathione, and uric acid. Then there are main AO-scavenging enzymes that include SOD, CAT, GPx, *etc*. The protective responses against oxidative damage and resetting the original state of "redox homeostasis" after temporary exposure to ROS are generally termed as "redox signaling."

Mechanism of Oxidative Stress-Mediated Injury

Alterations of cell membranes caused by FRs are lipid peroxidation, protein oxidation, nucleic acid modification, increase in intracellular Ca^{2+} concentrations, and signal transductions. The principal mechanisms for cell injury and tissue damage induced by FRs are direct attack, lipid peroxidation, DNA modification, and enzyme degradation/inactivation (Fig. 6).

Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (12). Amongst products of lipid peroxidation, malonaldehyde (MDA) and 4-hydroxynonenal (HNE) are the most important. These highly cytotoxic metabolites, produced in relatively large amounts, can diffuse from their site of origin to attack distant targets and form covalent links with various molecules (adducts) (20). Different cytotoxic effects of MDA and HNE formation have been reported *in vitro*, including enzyme inactivation and inhibition of DNA, RNA, and protein synthesis (51). Therefore, lipid peroxidation may cause severe damage and it has been assumed that this mechanism plays a key role in the pathogenesis of several human diseases. Further, recognition of lipid peroxidation involvement in the pathogenesis of a disease is of importance, because the deleterious effects of this process might be prevented by the administration of scavenging systems or AOs.

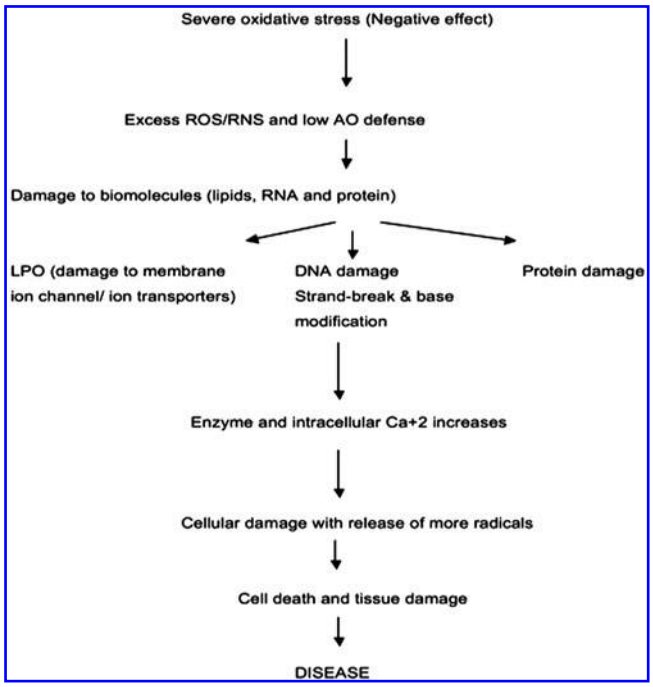

FIG. 6. Mechanism of oxidative stress-mediated tissue injury.

Assessment of Overall Oxidative Stress and AO Status

Lipid peroxidation products: thiobarbituric acid reactive substances

Various markers of oxidative stress and AO status have been tried and used to assess oxidative stress injury (18). Among various markers of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) are the most commonly applied assay (30). Although there may be a few interferences in its assay and some sugars also react with thiobarbituric acid (TBA) (34), it is of great utility as it can detect a range of lipoperoxidation aldehydes including MDA and HNE along with alkenals and alkadienals (36).

Total AO capacity

The ferric-reducing ability of plasma (FRAP) assay represents the total antioxidant capacity (TAC) of the body. This method gives an idea of TAC of the body that may not be reflected while estimating individual AOs and thus is a better indicator of the same, because AOs always work in conjunction and supplement each other. The FRAP assay is a fast, inexpensive, highly reproducible method (7).

Inflammation and Oxidative Stress

Oxidative stress and associated cellular injury promote inflammation, which is aggravated by increased production of the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) in the Kupffer cells. Initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na^+/K^+ ATP-ase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of ROS. All these toxicities are likely to play a role in the pathophysiology of shock, inflammation, and ischemia and reperfusion (Fig. 7) (15). The transcription factor, nuclear factor kappa B (NF-kappa B), is known to be a redox sensitive factor. NF-kappa B plays a central role in immune responses and inflammation, through regulation of the gene expression of a large number of cytokines and other immune response genes. NF-kappa B is present in the cytoplasm in stimulated cells and translocates into the nucleus in response to several stimuli, including oxidative stress. ROS enhance the signal transduction pathways for NF-kappa B activation in the cytoplasm and translocation to the nucleus (32). Adhesion of leukocytes to endothelial cells in postcapillary venules is an early step in chronic inflammation and depends on the expression of cell-surface receptors known as cell adhesion molecules (2). Cyclo-oxygenase-2 catalyzes the formation of prostaglandins and other eicosanoids from arachidonic acid. It is induced at the site of inflammation following stimulation with proinflammatory agents, such as interleukin (IL)-1, TNF-α, and lipopolysaccharides. Monocytes promote lipid peroxidation, through generation of ROS. Monocytes and macrophages secrete proinflammatory cytokines IL-1β, TNF-α, and IL-6, which augment monocyte endothelial adhesion. IL-1 requires TNF receptor factor for signaling. IL-1 activates mitogen-activated protein kinase, which is redox sensitive. Also, IL-1 or oxidants stimulate tyrosine phosphorylation and activate extracellular signal-regulated protein kinase 2 (ERK2) and c-Jun N-terminal kinase, which can be blocked by *N*-acetyl cysteine, indicating SO-stimulated activation of ERK. These reactions are inhibited by thiols and selenium (38).

Oxidative Stress in CP

Braganza *et al.* proposed the role of oxidative stress in the pathophysiology of CP, which was believed to be because of overactivity of mixed function oxidases (10). The FR overload in the pancreas may result from alcohol, smoking, petrochemical fumes, as well as dietary toxins.

The normal pancreas contains a dormant mechanism for metabolism of xenobiotics involving phase I CYP 450 biotransforming enzymes and phase II conjugation reactions that often involve GSH. The oxidative stress in the acinar cell may result from CYP induction, concurrent exposure to a chemical that undergoes bioactivation, and insufficiency of micronutrients that are required to sustain GSH stores. Inhaled toxins such as those from cigarette smoke cause CYP induction in islet cells. The suggestion that AO deficiency may play a role in the pathogenesis of CP was first made by Rose *et al.* (54). The AO deficiencies were observed in patients with pancreatitis and led to the hypothesis that CP was related to OS. The finding of high concentrations of lipid-based FR oxidation products in serum and also in duodenal bile, during the relatively asymptomatic interval between pancreatitis attacks, suggested persisting oxidant stress in patients with CP (25). Three causes have been identified for the problem: (i) CYP induction, especially of polycyclic aromatic hydrocarbon possessing enzymes, as by cigarette smoke (1); (ii) regular close exposure to volatile petrochemical products in the occupational environment (41); and (iii) above all, lower intakes of methionine and vitamin C, especially in alcoholics, which are required to sustain GSH stores (23, 66). Cigarette smoke is known to contain a large number of oxidants (13); thus it has been suggested to pose oxidative damage to critical

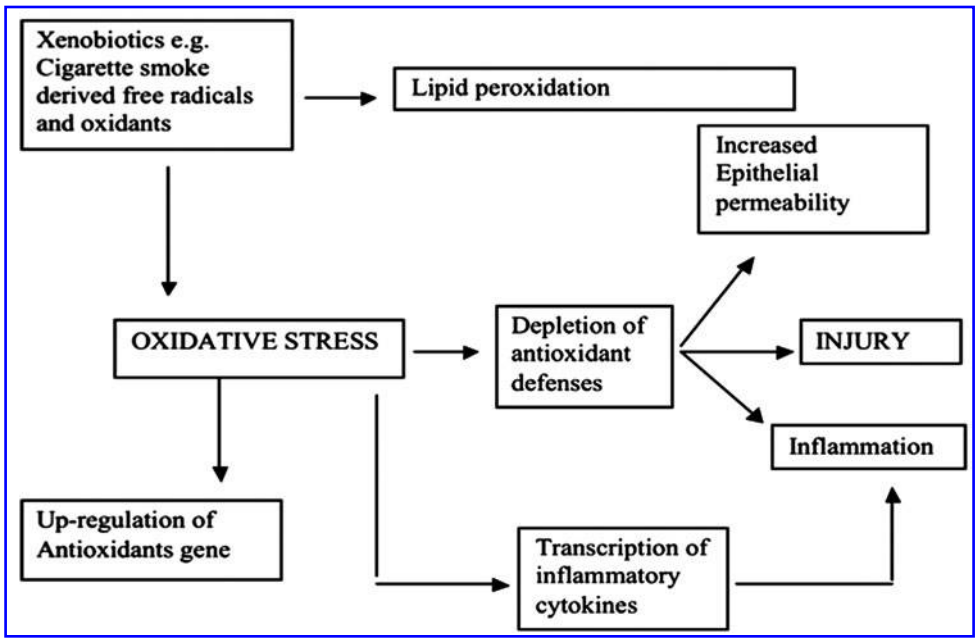

FIG. 7. Mechanism of injury through oxidative stress.

biological molecules. It has also been demonstrated that lipid hydroperoxides are formed after exposure of plasma to gas phase of cigarette smoke (22). Also, smokers have lower levels of vitamin C compared with nonsmokers (50). Cigarette smoking is an independent risk factor for pancreatitis in alcoholics (63).

The inference that xenobiotic-mediated injury in the pancreas itself is the real problem in CP was supported by two sets of observations. First, an immunological study of drug-metabolizing enzymes in surgical biopsies of the pancreas confirmed induction of the phase I enzymes CYP1A2, CYP3A, and NADPH-CYP oxido-reductase, but not phase II enzyme, glutathione-*S*-transferase, which facilitates the removal of toxic metabolites by conjugation with GSH. Second, the pancreatic acinar cells showed oxidant stress.

The oxidant stress starts in the acinar cells, increasing synthesis of heat shock proteins, cytokines, and also pancreatitis-associated protein (PAP). Pancreas has the capacity to metabolize ethanol *via* oxidative pathway. Chronic ethanol consumption induces the expression of CYP2E1 in rat pancreas (47), similar to that of liver (31). During nonoxidative pathway, fatty acid ethyl esters (FAEEs) accumulate in pancreas following the metabolism.

Mechanism of Cell Death Due to ROS in Pancreatitis

Two principal forms of cell death are apoptosis and necrosis. The proportion of apoptosis and necrosis in acute pancreatitis varies in mild and severe pancreatitis, with the former more pronounced in milder pancreatitis and the latter in severe form. The mitochondrial mechanisms related to cell death in pancreatitis are important as they might determine the relative extent of apoptosis and necrosis in pancreatitis. The intrinsic pathway of apoptosis involves release of mitochondrial cytochrome *c* into the cytosol. It has been shown that ROS is a key mediator of CCK-induced apoptotic responses in experimental pancreatitis. This effect is mediated by an increase in Ca^{2+} concentration, which leads to release of cytochrome *c* from the mitochondria, stimulating the intrinsic apoptotic pathway *via* caspases (48). On the other hand, it has also been shown that low micromolar concentration of Ca^{2+} leads to mitochondrial depolarization that induces necrosis due to depletion of ATPs. There appears to be a major role for mitochondria in the effects of Ca^{2+} and ROS on acinar cell death, at least in experimental pancreatitis (26).

Role of ROS in Pancreatic Stellate Cells

ROS are also involved in activation and cell function of pancreatic stellate cells (PSC) and thus might have a function in pancreatic fibrosis. PSCs express NADPH oxidase to generate ROS, which mediates key cell functions and activation of PSCs (39). It has been shown that ROS is important in pressure-induced PSC activation and extracellular matrix synthesis (5).

Oxidative Stress and AOs in CP

Szuster-Ciesielska *et al.* (62) measured levels of SO anion, H_2O_2 and SOD, CAT, and GPx levels in alcohol-related pancreatitis. Patients with alcoholic AP had significantly increased serum levels of CAT and SOD, but levels of GPx were comparable to that of controls. In sera of patients with CP, CAT and SOD were high, whereas GPx was significantly less when compared with controls and correlated negatively with alpha-amylase and lipase concentrations. This study also showed that neutrophils in patients with CP produced increased amounts of ROS and H_2O_2 without any induction *in vitro*.

Hausmann *et al.* (28) studied the distribution pattern of the cytosolic radical scavenging enzyme, Cu-Zn SOD, in the pancreatic juice and tissues in patients with CP or pancreatic malignancy and showed that the pancreatic juice contained higher levels than juice from controls without pancreatic disease.

In a study by Schoenberg *et al.* (58), lipid peroxidation was studied in 20 patients undergoing operative treatment for

chronic ($n=11$) and acute ($n=9$) pancreatitis. In CP, conjugated dienes as well as MDA concentrations in the tissues were significantly elevated. Reduced glutathione was significantly decreased, suggesting glutathione depletion due to oxidative stress.

In another study, van Gossum *et al.* (68) studied 35 patients with alcoholic CP. They concluded that patients with alcohol-related CP had low blood levels of many AO factors.

Basso *et al.* (6) studied lipid peroxide activity in the sera of 28 patients with pancreatic cancer, 49 with CP, 40 control subjects, and 53 with extrapancreatic disease and concluded that activity of oxygen-derived FRs occurred in CP and reflected the degree of inflammation.

A study by Mathew *et al.* (40) suggested that certain families have a genetically determined, low absolute concentration of GPx-red blood cells (RBCs) and thus may be predisposed to oxidative stress injury. In addition, patients with hereditary pancreatitis had significantly low levels of selenium and vitamin E. It was also seen that SOD was higher in patients with CP (hereditary) when compared with healthy nonrelated controls. It was also found in their study that SOD was higher in kindreds of patients with CP when compared with nonrelated healthy controls.

Morris-Stiff *et al.* (45) suggested that patients with CP had significantly reduced AO levels, compared with control subjects and patients with recurrent acute pancreatitis, but the AO profile of patients with recurrent acute pancreatitis was similar to those of the control subjects.

Pai *et al.* (49) studied 19 patients with CP (14 tropical, 5 alcoholic) and 19 age-matched controls with abdominal pain without any cause. MDA was measured in aspirated duodenal juice and found to be significantly higher in patients with CP.

In another study, Braganza *et al.* (11) measured AO levels in patients with tropical pancreatitis from southern India and showed that there was no significant difference in the levels between patients and controls, but when these samples were compared with those of healthy controls from Manchester, UK, it was found that both cases and controls from Southern India had significantly lower levels of AOs.

Segal *et al.* (59) from Soweto in South Africa compared AO levels of patients with healthy controls and found that there was a significant reduction in the levels of AOs in patients when compared with controls.

Uden *et al.* (66) studied AO levels in patients with idiopathic CP and found that they had significantly low levels of selenium, vitamin E, and vitamin C when compared with controls. Another study compared serum selenium levels in patients with CP and controls (69). It was observed that selenium levels were significantly lower in patients when compared with controls.

Quillot *et al.* (52) compared patients with only exocrine insufficiency and patients with exocrine as well as endocrine insufficiency. The controls groups were patients with type 1 DM and healthy subjects. They showed that there was a decrease in plasma levels of vitamin A, vitamin E, and carotenoids in both CP and DM, and in addition, CP was also associated with lower plasma selenium and zinc, lower CAT, and higher copper. No difference, however, was observed between type 1 DM and healthy controls. A recent study compared AO status in patients with CP and acute pancreatitis (56). The authors showed a significantly higher level of TBARS and lower plasma thiols.

Thus, most studies have shown that there is an increased oxidative stress in patients with CP.

AO Supplementation in CP

Uden *et al.* (67) performed a 20-week, double-blind, placebo-controlled cross-over trial using 600 μg organic selenium, 9000 IU β-carotene, 0.54 g vitamin C, 270 IU vitamin E, and 2 g methionine. This trial included 23 patients, of whom 20 were included in the final analysis (7 were alcoholic, 8 were idiopathic, and 5 had recurrent acute pancreatitis). Results showed that 6 patients on placebo had attacks compared with none on active treatment ($p=0.032$). They concluded that active treatment was associated with clinical improvement over and above placebo effect. Improvement was independent of diagnosis whether acute or CP, grade of pancreatogram abnormality in the chronic group, and putative etiological factor.

In an observational study, Whiteley *et al.* (70) studied the long-term impact of micronutrient supplementation on pancreatic pain in 103 patients with CP, 69 had large duct disease and 45 had pancreatic calculi. During a follow-up period of 9 years, 75 patients remained pain free and were fully rehabilitated socially. In 27 patients, there was substantial reduction in pain and the need for hospitalization. Only seven patients required surgery, six for cyst/pseudocysts drainage, and one for cholecystectomy.

In another study, Sharer *et al.* (60) studied the role of AO therapy in recurrent pancreatitis patients with very high serum levels of triglycerides from familial lipoprotein lipase deficiency. They concluded that despite unchanged triglyceride levels, AOs prevented recurrence of pancreatitis and controlled pain in patients followed up for at least 1 year, whereas each had at least three attacks per year preceding AO therapy.

One study involving 19 patients with tropical pancreatitis evaluated the role of curcumin—an AO compound purified from turmeric—supplementation for 6 weeks (19). They used 20 mg of curcumin with 5 mg of piperine for 6 weeks and studied its effect on the pattern of pain, RBC level of MDA, and GSH. They observed that there was a significant decrease in MDA with an increase in GSH. However, no corresponding improvement in pain was observed.

A recent randomized, placebo-controlled cross-over trial evaluated the efficacy of combined AO supplementation in patients with CP (33). The study included 36 patients and AO supplementation was given for 10 weeks. Final data were derived only from 19 patients who completed the 20-week trial. It was observed that there was a significant improvement in the quality of life and pain.

The limitations of these studies included small sample size, study of predominantly alcoholic CP, lack of evaluation of the effect of confounders, nonstandardized quantification of pain, mostly observational studies, and short duration of AO supplementation.

We conducted a randomized, controlled trial to study the role of AOs in relieving pain in patients with CP—both alcohol related and idiopathic (8). In a study of 127 patients with CP (age: 30.5 ± 10.5 years; 86 male, 35 alcoholic, and 92 with idiopathic CP) were randomized to receive either placebo ($n=56$) or AO ($n=71$) for 6 months. The AO supplementation used in the study was a combination of 600 μg organic selenium, 0.54 μg ascorbic acid, 9000 IU β-carotene, 270 IU

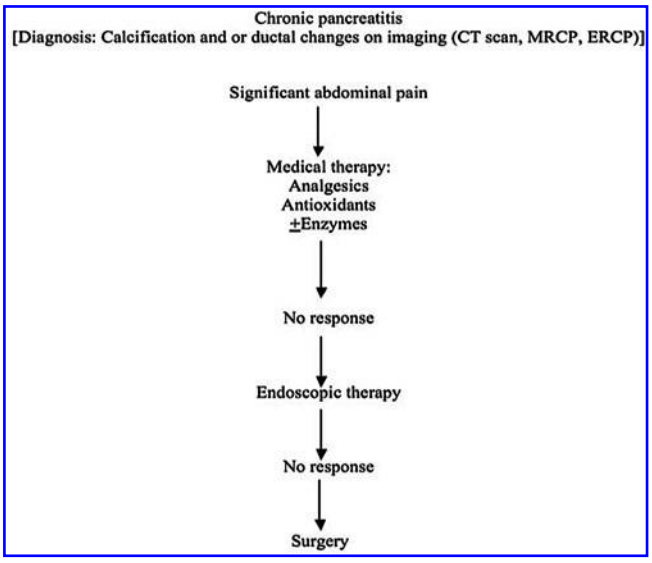

FIG. 8. Approach to a patient with chronic pancreatitis.

α-tocopherol, and 2 g methionine per day in divided doses (Betamore-G; Osper Pharmanautics, India). There was a significantly higher reduction in the number of painful days/month in the AO group compared with the placebo group (7.4 ± 6.8 *vs.* 3.2 ± 4, respectively; $p < 0.001$; 95% CI, 2.07, 6.23). The reduction in number of analgesic tablets/month was also higher in the AO group (10.5 ± 11.8 *vs.* 4.4 ± 5.8, respectively; $p = 0.001$; 95% CI, 2.65, 9.65). Further, 32% and 13% of patients became pain free in the AO and placebo groups, respectively ($p = 0.009$). Commensurate with the clinical improvement, there was a significant reduction in the level of TBARS and increase in FRAP in the AO group compared with the placebo group (TBARS: placebo 1.22 ± 0.7 *vs.* AO 3.5 ± 3.4 nmol/ml; $p < 0.001$; 95% CI, 0.96, 3.55; FRAP: placebo 5.6 ± 154.9 *vs.* AO 97.8 ± 134.9 μM Fe^{+2} liberated; $p < 0.001$; 95% CI, 44.98, 161.7).

However, pain in patients with CP is difficult to treat. The medical therapy requires a combination of analgesics, AOs, pancreatic enzymes, and nutritional support. If medical therapy fails, endoscopic therapy may be required in patients with dilated main pancreatic duct due to stones/stricture causing obstruction. Surgery is required if endoscopic therapy does not succeed. An algorithm of treating pain in patients with CP is given in Figure 8.

Conclusion

There is compelling experimental and clinical evidence about the important role oxidative stress plays in the pathophysiology of CP. Patients with CP may be deficient in their AO capacity. Supplementation with AOs leads to reduction in oxidative stress and can be associated with a reduction in abdominal pain in these patients.

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Abbreviations Used

AO = antioxidants
CAT = catalase
CP = chronic pancreatitis
CYP 450 = cytochrome P450
ERCP = endoscopic retrograde choangiopancreatography
ERK = extracellular signal-regulated protein kinase
FR = free radicals
FRAP = ferric-reducing ability of plasma
FROP = free radical oxidation product
GPx = glutathione peroxidase
HNE = 4-hydroxynonenal
IL = interleukin
MDA = malonaldehyde
MRCP = magnetic resonance cholangiopancreatography
NF-kappa B = nuclear factor kappa B
PSC = pancreatic stellate cells
RBC = red blood cells
ROS = reactive oxygen species
SO = superoxide
SOD = superoxide dismutase
TAC = total antioxidant capacity
TBARS = thiobarbituric acid reactive substances
TCP = tropical calcific pancreatitis
TNF-α = tumor necrosis factor-alpha

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