

Oxidative Stress and Alcoholic Pancreatitis

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ABSTRACT

Alcoholic pancreatitis is common in the Western world, yet the exact mechanism by which alcohol induces pancreatic damage remains uncertain. Genetic predisposition, pro-inflammatory to inflammatory signal transduction pathways as well as apoptotic properties and oxidative stress have been among the proposed mechanisms, yet the exact mechanism remains the topic of investigation. Reactive oxygen species (ROS) are highly reactive molecules that are generated as metabolic products that react with and damage cellular components. Alcohol promotes the production of ROS while lowering cellular antioxidant levels thereby altering the state of balance between pro and anti-oxidant status leading to oxidative stress in many tissues including the pancreas. Several lines of evidence indicate that oxidative stress plays an important role in the development of alcoholic pancreatitis. Here we review the hypothesis and proposed mechanisms of oxidative stress in alcoholic pancreatitis, the evidence supporting the role of alcohol-induced oxidative stress within the pancreas and the role of emerging antioxidant treatments.

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Key words: Oxidative stress; Alcoholic pancreatitis; Reactive oxygen species (ROS)

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INTRODUCTION

Alcohol is a well-known cause of pancreatitis and accounts for the majority of observed cases in Western societies^[1]. Genetic predisposition, pro-inflammatory to inflammatory signal transduction pathways as well as apoptotic properties and oxidative stress have been some of the main underlying mechanisms suggested for alcoholic pancreatitis^[2], yet the exact etiology and molecular mechanism still remains a point of debate and significant investigation. One factor playing a major role in alcohol-induced pancreatitis is the state of oxidative stress as a result of excessive free radical generation and antioxidant depletion^[3].

The role of reactive oxygen species (ROS) and oxidative stress in the pathogenesis of various human diseases has been well documented. Reactive oxygen species have also been implicated in pancreatic damage in various forms of experimental and human pancreatitis^[4-8]. Although ethanol is known to be the most common cause of pancreatitis in the Western world, relatively few studies have dedicated exclusive focus on ethanol and its induction of oxidative stress within the pancreas. Here we review the proposed mechanisms of ethanol-induced oxidative stress as it takes place in the pancreas, the evidence for the role of oxygen radicals in alcohol-related pancreatitis, and potential therapeutic implications.

WHAT IS OXIDATIVE STRESS?

Free radicals are molecules or compounds that contain unpaired electrons in their atomic or molecular structure which renders them unstable. Because of the instability in their structure, these molecules are highly reactive as they attempt to pair with other molecules to create a more stable compound. The most common free radicals include the superoxide (O₂⁻), hydrogen peroxide (H₂O₂), peroxide (O₂⁻), hydroxyl (.OH), and nitric oxide (NO.) radicals which are considered primary ROS^[9]. Under ethanol exposure, ROS production is enhanced while the level of antioxidants is reduced; this resulting state of imbalance between ROS production and reduced antioxidants is called oxidative stress^[3].

SOURCES OF REACTIVE OXYGEN SPECIES

The mitochondrion is a major source of ROS, with the majority of oxygen radicals produced in a biological system being derived from the mitochondrial respiratory chain^[9]. In a biological system, molecular oxygen undergoes reduction through the addition of four electrons in the mitochondrial respiratory chain and cytochrome oxidase system to form water, generating ATP in the process. Continued oxygen consumption leads to sequential, univalent reduction to produce a partially reduced, reactive oxygen-derived species within the mitochondria that is initiated by the generation of superoxide anion^[10]. The conversion of superoxide anion into hydrogen peroxide by the enzyme superoxide dismutase (SOD) within the mitochondria leads to the generation of hydroxyl radical and other reactive species which damage constituent cellular macromolecules^[11].

In addition to the mitochondrial respiratory chain are numerous biological sources of ROS, most important of which include the Cytochrome P450 oxidase system (P450), the xanthine oxidase system and activated leukocytes. The P450 system which includes the CYP1, CYP2, and CYP3 families, is the major enzyme superfamily involved in metabolizing compounds such as fatty acids, cholesterol, steroids and bile acids. More importantly, these enzymes are involved in removing and detoxifying ethanol, a foreign and hence toxic agent in the body. ROS are generated by the P450 system in the biochemical process of detoxifying ethanol^[12].

Xanthine oxidase (XOD) is found as a dehydrogenase (XOH) under physiological conditions transferring hydrogen from xanthine to nicotinamide adenine dinucleotide (NAD), thereby generating NADH. Under ethanol exposure, however, its oxidase form is promoted where the ROS superoxide and hydrogen peroxide are generated^[13]. Alcohol consumption promotes the formation of xanthine oxidase which is a primary source of ROS causing injury within the pancreas^[14,15].

Activated macrophages and neutrophils contain the NADPH oxidase system which when activated generates superoxide and hydrogen peroxide radicals; this normally allows leukocytes to protect against invading microorganisms. Excess ROS generated by these cells, however are deleterious to host cells and tissues in the presence of pancreatitis^[16].

ANTIOXIDANTS

Antioxidants (AO) are the body's main resource for protection against ROS and oxidative stressors and biological systems have developed antioxidant defense mechanisms to counter cytotoxicity from ROS. Mammalian cells are primarily protected against oxidative damage by natural antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase which act as oxygen radical scavengers^[17].

Superoxide dismutase (SOD) is responsible for the detoxification and removal of superoxide radical and is found in three different forms within the mitochondria^[18]. A copper-zinc form of SOD is found within the cytosol and the space surrounding the mitochondria, while a manganese-containing form is present in the mitochondrial matrix, both of which are important for prevention of ROS-induced toxicity^[19]. The main function of catalase (CAT) involves the removal of hydrogen peroxide but can also promote the interaction of hydrogen peroxide with other hydrogen donors, such as ethanol, forming water, O₂, and an oxidized hydrogen donor product^[17].

The most important enzymatic system may be the Glutathione peroxidase (GPx) system which comprises several enzymes and cofactors including NADPH and the endogenous antioxidant glutathione, in its reduced form (GSH), to remove hydrogen peroxide species^[20]. As an essential part of this system, GSH helps

remove exogenous drugs and chemicals as well as various reactive molecules from within a cell^[11]. Because mitochondria lacks catalase, mitochondrial GSH in association with GPx is the major mechanism by which hydrogen peroxide H₂O₂ is detoxified by the mitochondria^[11,20].

Further defense is provided by numerous non-enzymatic compounds that are taken up in the diet and react with oxygen radicals and disrupt the self-perpetuating cycle of oxygen radical production. The most prominent of these antioxidants include Vitamin E (α -tocopherol), Vitamin C (ascorbate), β -carotene, selenium, bilirubin, uric acid and cysteine^[21].

PROPOSED MECHANISMS OF ALCOHOL-INDUCED OXIDATIVE STRESS IN THE PANCREAS

Although a clear link between ethanol consumption and pancreatitis has been well established, the majority of ethanol consumers do not develop pancreatitis^[1]. This observation has led investigators to question the potential role of genetic predisposition in certain individuals to alcohol or alcohol metabolites and the induction of oxidative stress as a risk factor for developing pancreatitis. One area of investigation involves polymorphisms in genes encoding for inflammatory cytokines and subsequent pathways involved in alcoholic pancreatitis that have yet to find a direct correlation^[22-24]. Others have investigated the association of alcoholic pancreatitis and genetic polymorphisms in detoxification enzymes that are involved in the metabolism of ethanol and oxidative compounds as possible susceptibility factors. Studies investigating an association between genetic polymorphisms for the enzyme glutathione transferase and alcoholic pancreatitis have similarly not found a significant association^[25-27]. One study reported a potential protection against pancreatitis in alcohol users with this mutation, though the investigators admitted limitations including uncontrolled confounding factors^[28]. Similar investigations of polymorphism in SOD have not found a significant link to pancreatitis^[29,30]. Some studies have also suggested that the type and amount of alcohol consumed can be a determinant of oxidative stress and subsequent pancreatitis, although not all these studies found an association^[31-33]. This issue remains a topic of controversy, however, as studies reporting a positive correlation have often not corrected for the total amount of alcohol consumed.

Recent clinical data lends support to the notion that antioxidants are beneficial for patients with acute pancreatitis with studies suggesting its benefits to stem from inhibition of NF-kappa B activity as well as reduction of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-1 which have been found to play active roles in ethanol induced oxidative stress in the pancreas^[34,35]. Ultimately, alcohol-induced oxidative stress mediates its damage through mechanisms that alter the state of balance between antioxidant status and increased ROS (Figure 1).

Alcohol-induced oxidative stress leading to pancreatitis is partly linked to the metabolism of ethanol itself within the pancreas^[36]. Ethanol is metabolized by three different pathways which involve the enzymes alcohol dehydrogenase, microsomal ethanol oxidation system and catalase. Each of these pathways generates free radicals which alter the state of imbalance leading to oxidative stress^[37]. Alcohol is first converted to acetaldehyde by alcohol dehydrogenase (ADH). Aldehyde dehydrogenase (ALDH) then converts acetaldehyde to acetate. Each of these reactions produces one molecule of NADH, which provides more reactive material and thus enhanced activity of the mitochondrial respiratory chain^[38].

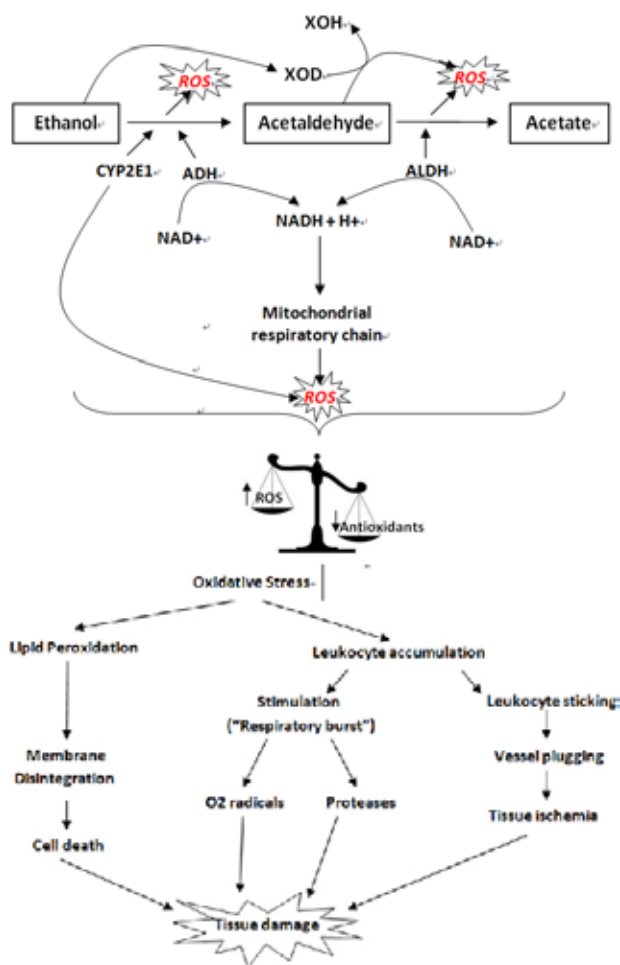


Figure 1 Mechanisms of alcohol-induced oxidative stress-mediated injury.

Acetaldehyde (AA) produced during alcohol metabolism is itself a toxic metabolite which reacts with proteins and lipids leading to further radical formation and cell damage^[38]. Acetaldehyde is also an excellent substrate for the enzymes xanthine oxidase (XOD) which generates more toxic oxygen radicals during its oxidation^[39], leading to pancreatic injury^[14,15]. Alcoholic pancreatitis induced by ischemia followed by AA can be inhibited by pretreatment with SOD and CAT, while ameliorated with the XO inhibitor allopurinol supporting the role of AA and xanthine oxidase as sources of ROS in alcoholic pancreatitis^[40].

Alcohol further induces damage via the cytochrome P450 system which is a common source of ROS^[12]. Alcohol induces the CYP2E1 form of the cytochrome P450 system which metabolizes ethanol itself to more toxic products while generating ROS in the process^[41]. Increased ROS production and lipid peroxidation found in chronic ethanol treated rats was blocked by chemical inhibitors of CYP2E1 and anti-CYP2E1 immunoglobulin G in two experiments^[42,43]. Other studies have demonstrated increased CYP2E1 activity in the rat as well as the human pancreas after ethanol exposure leading to pancreatic injury^[44,45].

Studies also suggest that the mitochondrion which are involved in the generation of ROS are themselves targets of oxidative stress and also contribute to the mechanisms by which oxidative stress mediates injury. Ethanol depletes GSH in the liver, particularly in the mitochondria, which is normally characterized by high levels of GSH needed to eliminate ROS generated during activity of the respiratory chain^[20]. NADH generated during ethanol metabolism is shuttled into the respiratory complexes of the mitochondria by the

malate-aspartate shuttle, which is enhanced by ethanol treatment^[46]. These excess reducing equivalents facilitate transfer of electrons to molecular oxygen generating more superoxide anions in the process, propagating the cycle of ROS generation and cell toxicity^[47]. Ethanol also decreases overall mitochondrial respiration and a decrease in rate of ATP synthesis^[20,48]. This further inhibits the mitochondria's ability to import GSH needed to prevent an ongoing cycle of oxidative damage. Although these findings have mainly been observed in hepatic tissue, studies on pancreatic tissue have confirmed similar findings in alcoholic pancreatitis^[49].

One common effect of increased oxidative stress and imbalance from ethanol exposure is lipid peroxidation^[50]. Free radicals readily react with biological substances including proteins, polysaccharides, and nucleic acids. However, most readily affected are polyunsaturated fatty acids which are present in high concentrations in the cellular membrane. The resulting damage causes lipid peroxidation and alteration of the cell membrane which leads to disintegration of the cell and ultimately cell death^[43].

Free radicals can also induce injury through promoting migration and activation of leukocytes in the pancreas^[51]. Activated polymorphonuclear (PMN) leukocytes produce ROS and also secrete various enzymes (i.e. myeloperoxidase, elastase and acid proteases), leukotrienes, prostaglandins which lead to a propagating cycle of tissue injury and severe inflammation (and generation of more ROS) within the pancreas^[52].

MEASUREMENT OF OXIDATIVE STRESS IN ALCOHOLIC PANCREATITIS

A single reliable measurement to determine the involvement and extent of tissue damage by oxygen radicals has been difficult because of their high reactivity. As a result, the assessment of tissue damage in alcoholic pancreatitis has been accomplished in a variety of ways. One method relies on measuring the effects of radical interactions with biological substrates to form products such as lipid peroxidation products that include malondialdehyde (MDA) and 4-hydroxynonenol (HNE). Another measure of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) is commonly used as it can detect a range of lipoperoxidation aldehydes including MDA, HNE and other conjugated dienes^[53]. Other methods have included measurements of antioxidant enzymes and changes in glutathione metabolism via measurements of its reduced and oxidized forms, and treatment with radical scavengers that detoxify radical species.

EVIDENCE OF OXIDATIVE STRESS IN ALCOHOLIC PANCREATITIS

Animal models showing the role of oxidative stress in alcoholic pancreatitis

Several experimental studies (Table 1) have demonstrated that oxidative stress plays a role in the pathogenesis of both acute and chronic alcoholic pancreatitis by altering the balance between ROS generation and antioxidant capacity to remove these excess reactive species. Animals treated with ethanol acutely show significant reduction in SOD and GSH while MDA activity is significantly increased^[54,55].

A study by Wittel *et al.*^[56] investigated the ability of pancreatic acinar cells to generate ethanol-dependant ROS *in vivo* and *in vitro*. In an *in vivo* model of pancreatitis, rats were treated with ethanol alone and with duct obstruction or pancreatic stimulation. Animals treated with ethanol alone showed significantly lower levels of reduced glutathione

Table 1 Measurements of oxidative stress in animal models of alcoholic pancreatitis.

| Reference | Method | Tissue measurement | Results |
|---|--|-----------------------------|---|
| Weber <i>et al</i> ^[54] | Acute EtOH 13.7 mmol/kg, analysis at 1,2,6,12,16,20,24 h | SOD, GPx, MDA, XOD, Protein | ↑MDA at 1 & 2 h, ↓GPx at 20 h, ↓SOD at 16 h |
| Altomare <i>et al</i> ^[55] | Acute EtOH 25%, analysis at 6 h | GSH, GSSH, MDA, CP | ↑MDA, ↓GSH, ↑GSSG |
| Wittel <i>et al</i> ^[56] | <i>In vivo</i> : 40% for 90 min, analysis at 30 min, 1,2,4,6,12,24 h <i>In Vitro</i> : 0.3% EtOH incubation | GSH, GSSH, MDA, CD, MPO | ↑MDA, ↓GSH, ↑GSSG, ↑CD ↓GSH, ↑GSSG |
| Palimieri <i>et al</i> ^[57] | Acute EtOH 25%, analysis at 5 h | GSH, GSSH, MDA, CP | ↑MDA, ↓GSH, ↑GSSG |
| Andican <i>et al</i> ^[58] | Acute EtOH 48%, analysis at 24 h | NO, GSH, TBARS | ↑NO, ↓GSH, ↑TBARS |
| Norton <i>et al</i> ^[59] | 4 weeks EtOH 36% pair fed | GSH, MDA | ↑MDA, ↓GSH |
| Grattagliano <i>et al</i> ^[49] | 8 weeks EtOH 3% pair fed | GSH, MDA, CP, ATP | ↑MDA, ↓GSH, ↓ATP |
| Aleynik <i>et al</i> ^[60] | 8 weeks EtOH 36% pair fed | HNE, F2-IP | ↑HNE, ↑F2-IP, both decreased with PPC |
| McKim <i>et al</i> ^[61] | 3 weeks EtOH 35-40% continuous intragastric infusion | HNE | ↑HNE |
| Kono <i>et al</i> ^[62] | 4 weeks or 8 weeks 35-40% EtOH continuous enteral infusion | HNE, AHR | ↑HNE and AHR with 8 wk treatment |
| Limuro <i>et al</i> ^[63] | 4 wks 35% EtOH continuous intragastric infusion | AHR | ↑AHR |

(GSH) and increased levels oxidized glutathione (GSSG). Ethanol treated animals were also found to have significantly increased levels of MDA and conjugated dienes. In the *in vitro* model, isolated pancreatic acini incubated with ethanol had decreased concentration of reduced glutathione, GSH. Interestingly, ROS was shown to be generated independent of leukocyte infiltration by showing histological pancreatic injury without significant leukocyte infiltration in the tissue, further supporting the role of ethanol in generating free radicals at the pancreatic acini.

Palimieri *et al*^[57] conducted a study where the content of antioxidant enzymes and markers of lipid peroxidation were measured in isolated rat pancreatic acinar cells after an acute oral ethanol load. Similar to previous studies, pancreatic acinar cells isolated from ethanol-treated rats showed a significantly lower GSH and higher GSSG concentrations as well as elevated levels of MDA. Similar results were reported by Andican *et al*^[58] in which the levels of the reactive free radical nitric oxide (NO), TBARS and GSH were investigated. They found significantly higher levels of NO metabolites as well as TBARS in the systemic circulation of ethanol treated rats. In addition, GSH concentration was again found to be lower in pancreatic tissue as well as erythrocytes isolated from the ethanol treated group, indicating that the effects of ethanol induced oxidative stress are not limited to pancreatic cells but may involve other cellular components that interact within the pancreatic tissue to propagate cellular damage.

Evidence also indicates chronic ethanol exposure have significant impact on oxidative stress generation in the pancreas. In a study by Norton *et al*^[59] chronic ethanol administration produced a 46% increase in MDA content in the pancreas of ethanol-fed rats after 4 weeks of ethanol administration. Interestingly, this biochemical change was observed in the absence of histological evidence of inflammation or necrosis, implying that the observed changes were a primary phenomenon rather than part of the inflammatory response.

Chronic ethanol administration also produces progressive oxidative modification and impairment of mitochondrial function in pancreatic tissue^[47]. These effects were also observed in the pancreas by low dose chronic ethanol administration in a study conducted by Grattagliano *et al*^[49]. Ethanol treatment induced a significant decrease in both pancreatic (36%) and mitochondrial (44%) GSH concentrations compared to controls while increasing levels of MDA in both whole pancreas and mitochondrial fractions. Low levels of pancreatic ATP was associated with a fall in mitochondrial ATP synthesis in ethanol treated animals. Since GSH is not synthesized by the mitochondria, this decreased production of energy impairs the mitochondrial capacity to import GSH from the cytosol contributing to further propagation of ROS formation^[20].

In the course of lipid peroxidation, polyunsaturated fatty acids have been thought to be the most probable target of free radical attack leading to cell wall comprise and death. In a novel experiment, Aleynik *et al*^[60] studied whether polyenylphosphatidylcholine (PPC)

administration, a mixture of polyunsaturated phosphatidylcholines extracted from soybeans, inferred protection against alcohol-induced oxidative stress and lipid peroxidation in the rat pancreas. Their study found 70% higher lipid peroxidation products in pancreatic tissue isolated from ethanol treated rats compared to controls. Ethanol fed animals that were then concomitantly administered PPC had reduced levels of peroxidation while the values remained unchanged in control groups not treated with PPC. GSH levels were significantly decreased in pancreatic tissue of alcohol fed animals, which was also circumvented by PPC administration suggesting the potential role of therapeutic oral PPC therapy in attenuating ethanol induced oxidative pancreatic damage. Similarly elevated levels of HNE and as an index of oxidative stress pancreatitis tissue were supported in two other studies utilizing an enteral feeding model of ethanol in rats^[61,62].

Although the aforementioned studies lend strong evidence and support to the role of oxidative injury induced by ethanol on the pancreas, it can be argued that that measured markers of oxidative stress are the result of injury rather than preceding and hence being a source of injury. This question was addressed in a study by Limuro *et al*^[63] where rats were chronically fed ethanol using a model of continuous enteral infusion. By administering [¹³C]-ethanol, the investigators were able to show significantly elevated carbon centered radical adducts, specifically α -hydroxyethyl radical, demonstrating that the radical adducts were formed in the pancreas from [¹³C]-ethanol. Additionally, these findings were found in the absence of significant histological damage and normal levels of amylase and lipase (which were similar to the study by Norton *et al*^[59]), supporting the concept that enhanced oxidative stress is a primary phenomenon in alcoholic pancreatitis.

While most studies have focused on radical induced changes at the pancreatic acinar cells, it is important to note that ethanol may also alter oxidative stress balance in pancreatic stellate cells (PSC). A recent study from our laboratory investigated the NADPH oxidase system, which generates superoxide radicals and ROS, within pancreatic stellate cells^[64]. Stimulation with platelet-derived growth factor significantly increased NADPH oxidase activity which was measured by detecting production of ROS using the lucigenin-derived chemiluminescence method. Ethanol treatment markedly augmented the NADPH oxidase activity in pancreatic stellate cells generating increased ROS. These observed effects were attenuated by antioxidant administration, supporting the role of ethanol induced oxidative stress in pancreatic stellate cells. Masamune *et al*^[65] similarly demonstrated the role of NADPH oxidase as a source of ROS in pancreatic stellate cells. In one of their studies, administration of antioxidants including Vitamin E and N-acetyl-cysteine inhibited ethanol induced damage at the pancreatic acinar cells^[66]. In an earlier study, Apte *et al*^[67] demonstrated the capacity of PSCs to metabolize ethanol when activated. Their study further demonstrated that ethanol exposure leads to PSC activation via the metabolism of ethanol to acetaldehyde

and subsequent oxidant stress generation.

Recent experimental studies have also implicated the potential therapeutic effect of suppression of inflammatory cytokine, for instance with monoclonal TNF antibody (infliximab) in animal studies with necrotizing pancreatitis^[68]. Although these findings may provide a new target for therapy, large clinical trials are needed to study the clinical utility and efficacy of such treatments.

Human studies

A number of human studies have assessed the direct role of ethanol in inducing oxidative stress and subsequent pancreatic damage. The increase in lipid peroxidation due to enhanced generation of oxygen radicals by ethanol exposure is paralleled by severe depletion of physiological antioxidant status. In a small study, Van Gossum *et al*^[69] measured antioxidant parameters in 35 patients with alcoholic chronic pancreatitis compared to healthy controls. Significantly lower levels of antioxidants Vitamin A, Vitamin E, selenium and GSH were observed in patients with alcoholic pancreatitis though dietary intakes did not differ in patients and controls.

In a similar study, Szuster-Ciesielska *et al*^[70] investigated the role of oxidative stress in patients with both acute and chronic alcoholic pancreatitis (AP) by measuring the ability of peripheral blood neutrophils isolated from patients with proven alcoholic pancreatitis to produce superoxide and hydrogen peroxide radicals; SOD, CAT and GPx levels were also measured. Patients with acute AP had significantly increased levels of CAT and SOD while levels of GPx were comparable to controls. Neutrophils in patients with chronic AP produced increased amounts of ROS and peroxide without any induction *in vitro*, indicating the insult to be ethanol-induced.

Ample evidence indicates the association of oxidative stress and chronic alcoholic pancreatitis. Verlaan *et al*^[71] demonstrated significantly lower levels of GSH and anti-oxidative thiols cysteine and cysteinylglycine in the sera of patients with pancreatitis. Plasma levels of oxidative protein damage and lipid peroxidation were established by measuring concentration of protein carbonyls and MDA which were both significantly higher in patients with alcoholic pancreatitis. In addition, the generation of ROS measured by the chemiluminescence technique in whole blood was slightly higher in alcoholic patients.

In a study evaluating the role oxidative stress in patients with chronic alcoholic pancreatitis, Casini *et al*^[72] investigated the contribution of pancreatic periacinar stellate cells in the synthesis of extracellular matrix components causing pancreatic fibrosis. Patients with chronic alcoholic pancreatitis had significantly increased formation of HNE-protein adducts within pancreatic acinar cells compared to healthy controls, which was associated with an active synthesis and deposition of collagen leading to pancreatic fibrosis.

Though observations made in these studies gives support for the role of oxidative stress in alcoholic pancreatitis, these studies were limited by elements such as a small sample size and an observational method of study lacking control for confounding many factors. These limitations may have been addressed by randomized-controlled studies evaluating the role of oxidative stress via measurement and/or treatment with antioxidants. In an early study, Uden *et al*^[73] investigated the effects of antioxidant therapy for recurrent pancreatitis in a 20-week double-blind cross-over placebo-controlled trial which enrolled 28 patients. Of the 20 patients who adhered to the study, six patients had recurrent attacks while on placebo treatment compared to none while on active antioxidant therapy which included a mixture organic selenium, β -carotene, vitamins C, E and methionine. Biochemical analysis demonstrated baseline serum concentrations

of selenium, β -carotene and Vitamin E to be significantly lower in patients with pancreatitis compared to healthy controls. These levels were unaltered by placebo treatment but normalized by antioxidant treatment^[74]. Although this study endorsed the potential beneficial effect of treatment with antioxidants, its generalizability was limited again by a small sample size which has been addressed by more recent studies.

In a randomized controlled trial, Bwardwaj *et al*^[75] enrolled 147 patients with alcoholic or idiopathic chronic pancreatitis who were randomized to groups that were given placebo ($n=71$) or antioxidants selenium, ascorbic acid, β -carotene, α -tocopherol and methionine ($n=56$) for 6 months. Their study found antioxidant supplementation to relieve pain and reduce the level of oxidative stress in patients with chronic pancreatitis. As a secondary outcome measurement, markers of oxidative stress including serum levels of SOD and TBARS, Vitamins A, C and E levels as well as total antioxidant capacity were evaluated by the Ferric Reducing Ability of Plasma (FRAP) technique. At baseline, patients with pancreatitis were found to have significantly higher levels of oxidative markers as well as lower levels of antioxidants compared to controls. There were no significant differences observed at 1 month, but at 6 months following intervention, lipid peroxidation products (TBARS) and SOD levels had decreased significantly in the antioxidant group compared to placebo while there was an significant increase in FRAP in the antioxidant group. Vitamin A, C and E levels were similarly elevated in the antioxidant groups. Contradicting results were reported by Burton *et al*^[76] in which AO showed less effect in alcoholic pancreatitis than idiopathic or obstructive pancreatitis though these findings were from an observational cohort study.

The emerging role of antioxidant therapy in acute and chronic pancreatitis and its potential mechanism has been described by Jha *et al*^[34]. Resveratrol, a Chinese herb medicine, showed positive clinical effect on acute pancreatitis including alcoholic pancreatitis. Its mechanisms have been suggested to involve suppression of the NF-kappa B, pro-inflammatory cytokines such as TNF-alpha, IL 6 and 1 as well as anti-apoptotic effects through regulating apoptotic mediators such as Bax, Bcl-2 and caspase-3.

A more recent clinical trial evaluating the effect of antioxidant treatment for acute pancreatitis was conducted by Bansal *et al*^[77]. Patients presenting with mainly acute alcoholic pancreatitis were randomized to treatment with antioxidant Vitamins A, C, and E in addition to standard therapy ($n=19$) or standard therapy alone ($n=20$). No significant differences were found in organ dysfunction or length of hospital stay, although two patients died in the control groups compared to no deaths in the treatment group. No significant differences in MDA, SOD, or GSH levels were observed between the two groups at seven days, though this study was limited by a short observational period.

The role of antioxidants in pancreatitis continues to be a source of debate and investigation, however, with more recent studies on find mixed results with respect to benefits. Some studies have shown improved pain and quality of life in patients treated with antioxidants, which is equated to decreased oxidative stress, while others found no benefit to treatment^[78-82]. These studies, however, contained mainly non-alcohol induced pancreatitis. Therefore, up to date, there is no solid clinical evidence to support the clinical benefit of antioxidant in treatment of pancreatitis.

CONCLUSIONS

Both experimental and clinical evidence indicate that oxidative stress plays an important role in the development of alcoholic pancreatitis.

The discovery of generation of ROS generated by pancreatic stellate cells may provide new direction to investigate the contribution of pancreatic stellate cells and ROS generated through the NADPH system on the pathogenesis of alcoholic pancreatitis. Clinical trials have found mixed results for treatment with antioxidants in patients with pancreatitis though these studies did not select for alcohol-related cause of pancreatitis. Furthermore, clinical trials studying these effects are conducted on patients who present with already established pancreatitis, and are hence treatments that are given after-the-fact when damage may already be induced. Although antioxidants may be implicated as a potential modality for treatment of alcoholic pancreatitis, large clinical trials have yet been done to evaluate the outcome and efficacy of treatment with antioxidants specifically in this population. Potential future directions should include clinical trials with antioxidant therapy as prophylactic treatment in chronic alcoholics who may be prone to developing pancreatitis. Prophylactic treatment in this manner may hence prevent oxidative stress that leads to pancreatic injury. Given the considerable evidence suggesting the role of oxidative stress in alcoholic pancreatitis and no available effective medical treatment, further studies on the clinical outcome with antioxidants may shed a light on the future management of alcoholic pancreatitis.

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