

Role of Oxidative Stress in the Pathogenesis of Pancreatitis: Effect of Antioxidant Therapy

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Abstract

Oxidative stress plays an important role in the pathogenesis of acute pancreatitis. The exact pathogenesis of pancreatitis remains unknown but several mechanisms related to oxidative and inflammatory stress are implicated. It is reasonable to surmise that antioxidants would play a protective role in ameliorating the deleterious effects of pancreatitis. We have a wealth of data from animal models that reveal a positive correlation between antioxidant drugs and improved outcomes in experimental pancreatitis. Human clinical trials with antioxidants however, have disclosed conflicting results. We review the existing pathogenesis of pancreatitis related to oxidative stress and provide of a review of current trials with antioxidant therapy.

Keywords: Oxidative stress; Acute pancreatitis; Human clinical trials

Introduction

Pancreatitis is a complex disorder the exact mechanism of which remains controversial. Injury to the Pancreatic Acinar cells causes a complex cascade of events that includes increased production of reactive oxygen species (ROS) resulting in the oxidation of lipids and proteins and disruption of the pancreatic membrane [1]. This disorder can range from a mild ailment to a fatal attack. Although the mortality rate from acute pancreatitis (AP) has decreased over the last decade due to improvements in critical care, the incidence of AP remains high worldwide [2]. Recent reports have revealed an increase in the incidence of pancreatitis and the corresponding rise in the cost of the related inpatient care in the developed countries including the United States [3-6]. A study by Fagenholz et al. concluded that total costs of hospitalization for AP exceeded 2 billion US dollars annually in the United States [3]. Currently, treatment for this disorder is aimed at supportive care.

Pancreatitis and oxidative stress

Oxidative stress and its constant companion inflammation play critical roles in the pathogenesis of pancreatitis and its numerous complications. Oxidative stress is caused by a combination of increased production of reactive oxygen species (ROS) [7-11] and impaired antioxidant capacity [12-16]. ROS consist of a group of highly reactive intermediary oxygen metabolites which are generated in the course of oxygen metabolism. ROS have many important biological functions, such as regulation of redox-sensitive transcription factors, redox-sensitive signal transduction pathways and direct interaction with various molecules. In addition, ROS produced by activated leukocytes and macrophages are powerful weapons against invading microorganisms. Under normal conditions, ROS are safely neutralized by the antioxidant defense system. However, when ROS production exceeds the capacity of the antioxidant defense system, the uncontained ROS cause cellular injury and dysfunction by attacking biomolecules, and modulating redox-sensitive signal transduction pathways and transcription factors [17-19].

AP result in an oxidative stress which amplifies the inflammatory process through the recruitment and release of proinflammatory mediators, leading to a systemic inflammatory response [20]. Two striking features determine the clinical course and the severity of AP: (1) Development of systemic inflammatory response syndrome in the

early phase of the disease, and (2) Development of pancreatic necrosis leading to infection, sepsis, multi-organ system failure and death.

Alcoholic pancreatitis

AP can be caused by various etiological factors however about 80% of all cases are related to either bile stones or excessive alcohol consumption. Alcohol induced damage is, therefore, a relevant model to study the mechanism and pathophysiology of pancreatitis. Alcohol's toxicity is mediated through the action of alcohol itself or through its oxidative and non-oxidative metabolism [21-24]. In oxidative metabolism, alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde followed by chemical transformation of acetaldehyde to acetate by isoform 2 of aldehyde dehydrogenase (ALDH2), which is specifically localized in the mitochondria. These reactions are coupled with depletion of oxidized nicotinamide adenine dinucleotide (NAD⁺) and accumulation of reduced nicotinamide adenine dinucleotide (NADH) since both ADH and ALDH consume NAD⁺ and produce NADH. In non-oxidative metabolism, fatty acid ethyl ester (FAEE) synthase converts alcohol to FAEE [21,22]. Thus, alcohol metabolism results in accumulation of acetaldehyde, acetate, NADH, and FAEE, as well as changes in cellular and mitochondrial NAD⁺ and NADH levels. Excessive alcohol consumption results in pancreatic damage through a number of potential mechanisms. Oxidative metabolism of alcohol has been shown to produce ROS [25,26] and inhibit secretion from pancreatic acinar cells, events that trigger oxidative stress and inflammation through activation of NF- κ B, and formation of TNF- α , IL-6, and other inflammatory mediators. It also alters cell permeability, increases cell fragility and promotes necrosis by inhibiting apoptosis.

Cigarette smoking has recently been implicated as a risk factor for the development of pancreatitis [27-30]. A Danish population based

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Received April 01, 2013; Accepted April 23, 2013; Published April 25, 2013

Citation: Robles L, Vaziri ND, Ichii H (2013) Role of Oxidative Stress in the Pathogenesis of Pancreatitis: Effect of Antioxidant Therapy. *Pancreatic Dis Ther* 3: 112. doi:10.4172/2165-7092.1000112

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cohort study identified that smoking as an independent risk factor for the development of pancreatitis. Smoking alone was attributed to pancreatitis development in approximately 46% of the cases [29]. The North American Pancreatitis study was a multicenter prospective trial of 20 United States centers looking at the incidence of pancreatitis in a population with self-reporting tobacco smoking and alcohol consumption. After controlling for various other risk factors, cigarette smoking was found to be a dose dependent risk factor for chronic pancreatitis and recurrent AP [30]. Smoke and tar from tobacco smoke contain a large concentration of ROS. Furthermore, rodent studies showed that cigarette smoke extract caused a significant increase in proinflammatory cytokines, including IL-1 β , IL-6 and TNF- α , in vascular endothelial cells [31].

Nrf2- Keap1 pathway

The pathogenesis of pancreatitis clearly indicates that ROS play a critical role in activation of the inflammatory cascade, recruitment of inflammatory cells and tissue damage. Oxidative stress has been implicated in the pathogenesis of numerous disorders. For this reason, considerable attention is currently focused on exploring the potential efficacy of antioxidant or antiinflammatory mediators in the prevention and treatment of various disorders including pancreatitis. The Nrf2-keap1 pathway is the master regulator of the endogenous antioxidant defense system as it regulates expression of hundreds of genes encoding antioxidant and cytoprotective enzymes and related molecules. Consequently the Keap1-Nrf2-ARE pathway is critical in counteracting oxidative stress and inflammation. In fact dysregulation of Nrf2 pathway contributes to the pathogenesis of many disorders including: cancer, neurodegenerative diseases, chronic obstructive pulmonary disease, asthma, atherosclerosis, diabetes, inflammatory bowel disease, rheumatoid arthritis, chronic kidney disease and aging [32]. Several studies by Jha et al. in China have examined the effects of a naturally occurring Nrf2-keap1 pathway activator, resveratrol, on a rodent model of severe AP induced by sodium taurocholate [33-38]. The first report in 2005 demonstrated the effect of resveratrol on nuclear factor Kappa-B (NF- κ B) and the inflammatory response in a rat model of severe acute pancreatitis. The rats treated with resveratrol were reported to have significantly lower levels of NF- κ B, Tumor Necrosis Factor- α (TNF- α), and Interleukin-8 (IL-8) expressions compared to controls. Moreover, histological examination of the pancreas showed less hyperemia, necrosis, and edema in the treated group [35]. Likewise, resveratrol therapy significantly reduced serum amylase level and lowered lipid peroxidation, intracellular calcium overload, myeloperoxidase, and Ca²⁺-Mg²⁺-ATPase in the pancreas of the rats with acute pancreatitis [34,38]. Other studies have shown significant attenuation of the systemic inflammation, lung and liver injury with resveratrol treatment in animals with severe acute pancreatitis [33,36,37]. The studies further showed significant reduction of the cytochrome c in the mitochondria as well as reduced apoptotic related proteins, Bax, Bcl-2, and caspase-3, in the treated rats [33,36,37].

Antioxidant therapy

Since oxidative stress plays a pivotal role in the pathogenesis of pancreatitis, agents that can ameliorate oxidative stress should improve outcomes. There is a substantial amount of data on the effect of antioxidants in the treatment of oxidative stress in rodent models. Although, resveratrol has proven effective in ameliorating pancreatitis in rodent models, no clinical trials have been conducted using Nrf2 activator to treat pancreatitis in humans. Some antioxidants have undergone trials but yielded mixed results.

Since 1990, two regions in Germany began introducing selenium routinely into their treatment strategy for patients with acute pancreatitis. In retrospective analysis investigators found that early administration of Selenium reduced mortality, decreased complications, and reduced the number of surgical interventions. Of note, no patient had died due to pancreatitis since implementation of the selenium in one region of Germany however; complications occurred in patients who were given the antioxidant late in the course of the disease [39]. Another retrospective study from Germany examined the effect of combination of selenium and D- α -tocopherol in a cohort of 99 patients. Investigator found a significant decrease in the mortality rate in the treatment group (34% vs. 1.1%) as well as improvement in the clinical course without the need for surgical intervention [40]. Only one study has examined the effect of antioxidant therapy on recurrent pancreatitis. Uden et al. [41] randomized 20 patients during a 20 week period to receive placebo or a combination of selenium, Vitamin C, β -carotene, Vitamin E, and methionine. In the placebo group 6 patients experienced a recurrent attack during the observation period while none of the patient in the antioxidant-treated group suffered a recurrence. Moreover, pain scores were significantly reduced in the antioxidant-treated group compared to the placebo-treated group [41]. In contrast, a randomized placebo-control trial of selenium enrolling 70 patients found no significant difference in 17 different clinical parameters between placebo and treatment groups followed for up of 90 days after hospital discharge [42]. In a randomized placebo-controlled trial from China investigators examined the effect of intravenously-administered ascorbic acid (vitamin C) delivered in patients with acute pancreatitis. The study revealed significant reduction in superoxide dismutase, erythrocyte catalase, CD4 positive cells, and CD4/CD8 cell ratio in the treatment group. Moreover, fever and emesis resolved and leukocyte count and urinary amylase normalized faster, and serum TNF- α , IL-6, and IL-8 were significantly reduced in the treated group [43]. Melatonin is highly effective in neutralizing oxygen radicals, activate antioxidant enzymes, and suppress cytokine production [44]. In one study endogenous melatonin was measured in the serum of patients with AP and compared to median levels in healthy volunteers. The findings suggested that high endogenous serum levels of melatonin in the first 24 hours after the onset of AP correlated with a milder course of the disease, especially in younger patients [45]. In contrast to cases of spontaneous AP, antioxidants therapy does not seem to confer protection in patients with post endoscopic retrograde cholangiopancreatography (ERCP)-induced pancreatitis. In a double-blinded trial, patients were given a single dose of beta-carotene 12 hours prior to an ERCP. The investigators found no difference in the incidence of acute pancreatitis between the patients who received antioxidant (9.4%) with those who had received placebo and developed (10%). Of note, 4 patients developed severe AP in the placebo group compared to no patients in the treatment group; however, this was not statistically significant (20). In another study, Milewski et al. [46] investigated the effects of N-acetylcysteine (NAC), on prevention of post-ERCP pancreatitis. NAC is a free radical scavenger that has already shown to confer a protective effect clinically and is commonly used in hospitalized patients for protection against oxidative stress to the kidney, lungs, and liver. In this study, one hundred and six patients were randomly assigned to receive NAC orally and intravenously before and after ERCP. NAC failed to prevent post-ERCP pancreatitis in the treatment group and amylase levels revealed no differences between treatment and control groups [46]. Gu et al. [47] performed a meta-analysis of randomized controlled trials using antioxidant supplementation for the prevention of post- ERCP pancreatitis. Eleven studies, from 1999-2011 with over 3,000 patients were included in the analysis. The antioxidants analyzed

in this series included selenium, allopurinol, β -carotene, NAC, and pentoxifylline. Based on the evidence, the authors concluded that there was no benefit to antioxidant supplementation in the prevention or severity of post-ERCP pancreatitis [47]. A multidrug approach was investigated in a randomized control trial using intravenous NAC, selenium, and Vitamin C in 43 patients with severe AP. While markers for oxidative stress were lower in the treatment group there was no significant difference in patient outcomes [48]. In an observational study performed on 46 patients, the role of multiple anti-oxidant therapies (selenium, NAC, Vitamin C, β -carotene, and α -tocopherol) was investigated in patients with severe AP. Only 25 patients were included in the final analysis which showed that multiple antioxidant drug treatment confers no benefit in their patients [49]. Sateesh performed a randomized study on 53 patients receiving placebo or Vitamin C, NAC, and antoxyl forte daily within 72 hours of onset of AP. The investigators found that antioxidant treatment resulted in a statistically non-significant reduction of hospital stay (10.3 vs. 7.2 days) and a similar complication rate between the two groups [50]. In another randomized study looking at multiple antioxidants, 39 patients with severe AP were randomized to standard treatment or standard treatment and Vitamin A, E and C for 14 days. No significant difference was demonstrated in the two treatment groups regarding, length of hospital stay, and organ dysfunction. However, all patients survived in the antioxidant group, while two patients died in the standard care group and univariate analysis showed a marginal benefit with antioxidant treatment ($p=0.034$) [51].

Conclusion

Some antioxidants, mostly naturally-occurring compounds, have undergone trials, with some agents conferring beneficial effects in patients with pancreatitis; however there is conflicting and insufficient clinical data to support their routine use in humans at this point. Except for the meta-analysis which included 3,000 patients, few of these trials were sufficiently powered and most were conducted in a single institution. Large multicenter randomized placebo-controlled trials are needed to determine the efficacy of antioxidant therapy in the management of AP. The currently-available data on the efficacy of the traditional antioxidant compounds in the treatment for AP are inconclusive. However, use of novel and more potent oxidative stress modulators may change the treatment paradigm for AP. Pancreatitis remains a common problem in the United States and although the mortality rate has declined, the incidence continues to remain constant. Despite recent advancements in the understanding of the pathophysiology of AP the precise mechanisms and optimal treatment of this serious condition remain elusive and await further investigations.

Acknowledgement

This work was supported in part by NIH-NCRR UL1 TR000153, KL2 TR000147 and the Juvenile Diabetes Research Foundation International 17-2011-609.

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