57

Oxidative Stress and Antioxidants in Chronic Pancreatitis

Soumya Jagannath Mahapatra and Pramod Kumar Garg

Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, India

Introduction

Chronic pancreatitis (CP) is a progressive fibro-inflammatory disease of the pancreas characterized by repeated episodes of pancreatic inflammation and injury which result in pancreatic exocrine and/or endocrine insufficiency. Morphologically, it is characterized by parenchymal loss leading to atrophy, fibrosis, and ductal stricture and/or calcification causing ductal dilatation [1]. The pathophysiological mechanisms of CP are not well understood. Over the past few decades, oxidative stress has emerged as an important mechanism contributing to pancreatic inflammation and the role of antioxidants has been evaluated in patients with CP.

Free-radical production is an integral part of cellular physiology. These free radicals are neutralized by specific cellular enzymes and other small molecules called scavengers and antioxidants. In health, intricate balance exists between free radicals and antioxidants. However, excessive production of free radicals by activation of oxidative pathways or deficiency of antioxidants tilts the balance leading to "oxidative stress." It is formally defined as "an imbalance between oxidants and antioxidants in favor of oxidants leading to disruption of redox signaling and control, and/or molecular damage" [2]. This review focuses on oxidative stress and the role of antioxidants in mitigating oxidative stress, inflammation, and relieving pain in patients with CP.

Pro-Oxidants

Pro-oxidants are the molecules that favor formation of free radicals. Free radicals have one or more free electrons and usually are reactive oxygen species (ROS) or reactive nitrogen species (RNS) in the cellular environment. Free radicals and their reaction products are a part of normal cell physiology by acting as second messengers and they play an important role in cellular signaling [3]. Mitochondria are the site of physiological oxidative stress. Excessive production of ROS and RNS may damage biomolecules such as lipids, DNA, and proteins (Fig. 57.1).

Detoxification of xenobiotics is another source of production of free radicals. Xenobiotics are toxic substances that enter the body and undergo detoxification in two phases to a hydrophilic substance thus facilitating their excretion in the urine. In phase I metabolism, cytochrome P450 (CYP 450) oxidase system and hydrolyzing enzymes either cleave the parent molecule or result in oxidation or hydroxylation. In phase II metabolism, they are conjugated to a hydrophilic group (glucuronidation, acetylation, methylation, sulfation, or conjugation with glutathione) to form a polar water-soluble inactive compound that can be excreted by the body. Induction of phase I enzymes can itself result in generation of free radicals through the CYP450 system.

Various exogenous substances such as alcohol, tobacco, environmental pollutants, heavy metals, certain drugs, dietary toxins, and fumes lead to free radical generation. Alcohol metabolism can result in production of free radicals. Alcohol is converted to acetaldehyde, a toxic and reactive molecule, by alcohol dehydrogenase, and acetaldehyde is converted to acetate by aldehyde dehydrogenase. Each of the steps results in consumption of nicotinamide adenosine dinucleotide phosphate hydrogen (NADPH) and free radical formation.

Cigarette smoking similarly contains a lot of xenobiotics which can induce free-radical production and lipid peroxidation [4].

The Pancreas: An Integrated Textbook of Basic Science, Medicine, and Surgery, Fourth Edition. Edited by Hans G. Beger, Markus W. Büchler, Ralph H. Hruban, Julia Mayerle, John P. Neoptolemos, Tooru Shimosegawa, Andrew L. Warshaw, David C. Whitcomb, and Yupei Zhao.

Companion website: www.wiley.com/go/beger/thepancreas4e

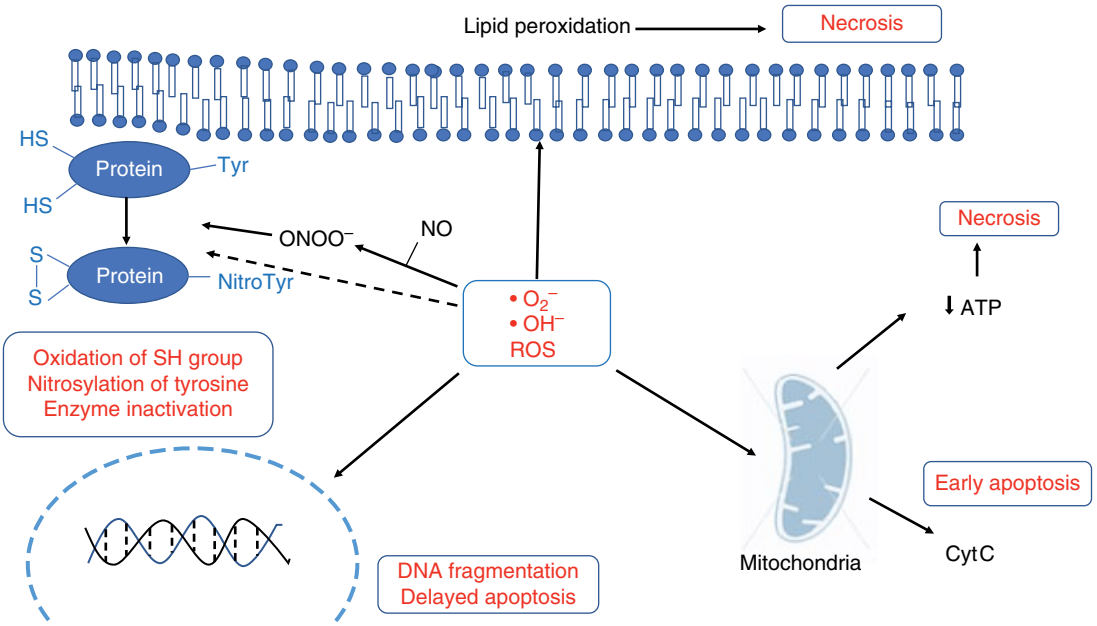

Figure 57.1 Intracellular free radicals can damage plasma membrane by lipid peroxidation. Mitochondria, which are the source of oxidative stress, get damaged which induces apoptosis by cytochrome c. Nitrosylation and oxidation of enzymes may result in their inactivation. Nuclear DNA may get fragmented inducing apoptotic pathways. ROS: reactive oxygen species.

Antioxidants

Antioxidants are substances that when present in low concentration compared to those of an oxidizable substrate significantly delay or inhibit oxidation of that substrate [5]. They can be classified into several groups: (i) cellular enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT); (ii) vitamins such as β-carotene, ascorbic acid, α-tocopherol; (iii) uric acid; and (iv) amino acids and proteins, which form complexes with transition metal ions. Cysteine is an important amino acid for the synthesis of glutathione. Methionine provides sulfur for the formation of cysteine and is thus an important dietary component for maintaining antioxidant defense. Selenium is a component of the GPx and Se-dependent enzyme thioredoxin reductase. These enzymes reduce hydrogen peroxide and lipid peroxides [6].

Primary defenses against oxidative stress include antioxidants vitamin E, vitamin C, β-carotene, glutathione, and uric acid. Second-line defense includes antioxidant scavenging enzymes that include SOD, CAT, and GPX. There exists a "redox homeostasis" within the cellular milieu and temporary exposure to ROS activate redox signaling (Fig. 57.2) [7].

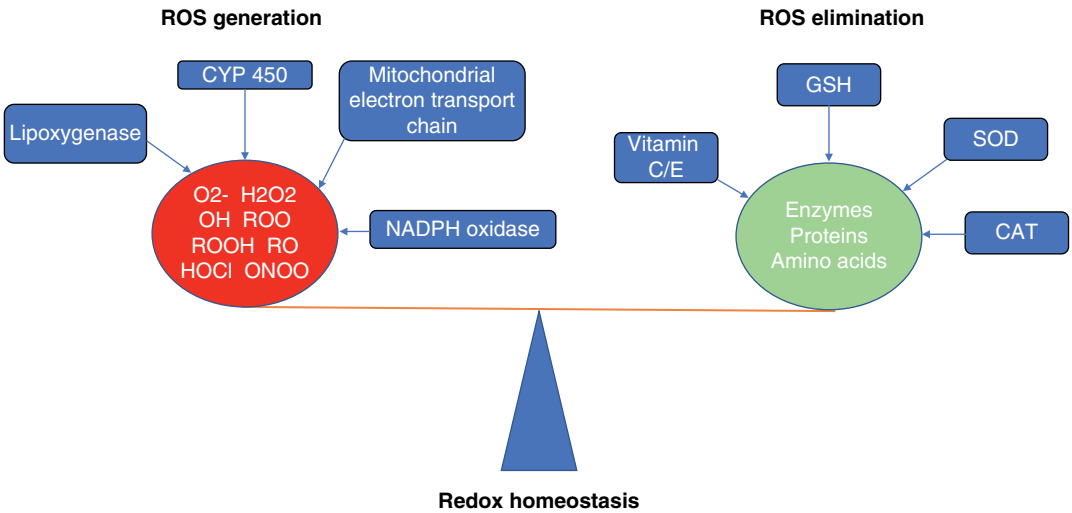

Figure 57.2 Redox homeostasis showing the balance between generation and elimination of reactive oxygen species (ROS).

Assessment of Oxidative Stress and Antioxidant Status

Oxidative Stress Markers

Measurement of thiobarbituric acid reactive substances (TBARS) is the most commonly used assay and is a marker of lipid peroxidation. Lipid peroxidation causes damage to the cell membrane. Malonaldehyde (MDA) and 4-hydroxynonenal (4-HNE) are the most important products of lipid peroxidation and can be used as markers of lipid peroxidation. Lipid peroxidation is a key step in oxidative stress mediated injury [8,9]. Some sugars may react with thiobarbituric acid resulting in assay interference [10].

Antioxidant Status Markers

Antioxidants work in tandem and complement each other. Hence, measurement of individual antioxidants may not be a direct measure of total antioxidant capacity (TAC) of the body. The ferric acid reducing ability of plasma (FRAP) measures the reducing capacity of blood from ferric to ferrous ion. FRAP is an easy and reproducible assay to measure TAC of the body [11].

Oxidative Stress in CP and its Consequences

The concept of "oxidative stress" in the pathogenesis of chronic pancreatitis can be credited to Joan M. Braganza, who proposed the hypothesis that products of hepatic detoxification may cause pancreatic diseases due to reflux of bile containing these substances into the pancreas. It was proposed that aberrant function of hepatic mixed function oxidase (MFO) may be the root cause of pancreatic diseases [12].

Rose et al. [13] first suggested that antioxidant deficiency may play a role in the pathogenesis of CP. Antioxidant deficiency was observed in patients with CP and oxidative stress was subsequently hypothesized as an important pathophysiological mechanism for the development of CP. It was supported by the observation that a high concentration of lipid-based free-radical oxidation products were detected in the serum and duodenal bile during the relatively asymptomatic phase between the recurrent attacks of pancreatitis suggesting persistent oxidative stress [14]. Three possible mechanisms were suggested: (i) CYP induction by cigarette smoking [15]; (ii) exposure to volatile petrochemical products in occupational environment [16]; and (iii) reduced dietary intakes of methionine and vitamin C in alcoholics [17,18].

Xenobiotic-mediated injury may perpetuate repeated/chronic inflammation in CP. The phase I enzymes such as CYP1A2, CYP3A, and NADPH-CYP oxidoreductase rather than phase II enzymes were found to be induced in the surgical biopsies of pancreas in a study of drug-metabolizing enzymes. Phase II enzymes facilitate removal of xenobiotics after conjugation with GSH, but phase I enzymes may produce toxic intermediates. In addition, pancreatic acinar cells showed evidence of oxidative stress [19].

ROS can lead to acinar cell death in pancreatitis. ROS is a key mediator of CCK-induced apoptosis in experimental pancreatitis. This is mediated by increasing intracellular calcium leading to release of mitochondrial cytochrome c, which activates caspases causing apoptosis [20,21]. ROS can also activate pancreatic stellate cells (PSC). PSC may also generate ROS by NADPH oxidase, which mediates activation of PSC [22].

Evidence for Oxidative Stress and Total Antioxidant Status in CP

Lipid peroxide activity was studied by Basso et al. [23] in 49 patients with CP, 28 patients with pancreatic cancer, 40 controls, and 53 patients with extrapancreatic diseases. It was observed that lipid peroxide activity was increased in patients with CP during disease relapse and correlated with the degree of inflammation.

Szuster-Ciesielska et al. [24], studied the ability of blood neutrophils to produce superoxide anion and hydrogen peroxide spontaneously and after stimulation and showed that the resting production of these free radicals was significantly higher in patients with pancreatitis compared with controls. Superoxide dismutase and catalase activities were greater in patients with alcoholic pancreatitis than controls.

Patients with CP and pancreatic cancer had higher levels of Cu/Zn superoxide dismutase in the pancreatic juices collected endoscopically compared with controls. Immunohistochemical studies of Cu/ZN-SOD in pancreatic tissue showed localization to ductal cells, islet cells, and centro-acinar cells but to a much lesser extent to acinar cells [25].

Schoenberg et al. [26] studied lipid peroxidation products in the tissue and serum of patients suffering from acute ($n = 9$) and chronic pancreatitis ($n = 11$). In patients with CP, the products of lipid peroxidation such as conjugated dienes and malonaldehyde were higher in pancreatic tissue compared to controls (organ donor). Reduced glutathione was significantly decreased suggesting oxidative stress. Increased levels of tissue lipid peroxidation products and altered glutathione

metabolism suggested ongoing peroxidation of lipids due to an enhanced generation of oxygen radicals.

Patients with alcoholic CP have low blood levels of many antioxidant factors despite adequate oral intake. In a study, serum levels of Vitamin A, Vitamin E, selenium, and glutathione peroxidase level were significantly lower compared to healthy controls despite no dietary differences in these micro-nutrients. The study hypothesized that such deficiency was probably due to pancreatic insufficiency and increased requirement due to oxidative stress [27].

In another study, Mathew et al. [28] evaluated antioxidants in patients with hereditary CP. Antioxidants levels in four groups were compared: hereditary CP, kindred but no pancreatitis, CP due to other etiology, and controls. Hereditary CP patients had lower antioxidant levels. Their kindred had higher vitamin E and selenium levels, which might have prevented them from having pancreatitis despite low glutathione peroxidase levels. Hence, the authors hypothesized that supplementation with vitamin E and selenium might be a good therapeutic option to decrease the frequency of pancreatitis in patients with CP.

Patients with CP in tropical areas had earlier onset of pancreatitis. In a study, Braganza et al. [29] showed that the bioavailability of beta-carotene and ascorbic acid was lower in tropical areas compared to temperate zones. The culinary practice that erodes bioavailability of these two antioxidants might have predisposed to pancreatic oxidative stress and earlier presentation of CP in tropical countries compared to temperate countries.

Whether presence of diabetes significantly modifies oxidative status in patients with CP was evaluated in another study. CP patients with or without diabetes were compared with type 1 diabetes and healthy controls for oxidant and antioxidant status as well as LDL oxidation status. Antioxidant status was altered in patients with CP particularly in those with diabetes. In these patients, vitamin E deficiency and elevated plasma glucose level were associated with significantly higher LDL oxidizability predisposing to atherosclerosis [9].

Uden et al. [17] found significantly low antioxidant levels of selenium, vitamin C, and vitamin E compared to controls in patients with idiopathic CP and suggested that selenium supplementation might be beneficial.

The question whether oxidative stress played any role in the progression of disease from recurrent acute to chronic pancreatitis was addressed in a recent study by Bopanna et al. [30]. The study included patients with idiopathic recurrent acute pancreatitis both in the acute phase and the quiescent phase to evaluate oxidative stress and found that patients had oxidative stress during the acute phase of illness and antioxidants levels were reduced. Taken together, the findings suggested that oxidative stress was associated with recurrent attacks of pancreatitis and might be contributing to the progression of recurrent acute pancreatitis to CP.

Hence, most studies have shown the presence of oxidative stress and decreased antioxidant levels in patients with CP. The low antioxidant levels may be due to dietary deficiency or impaired absorption resulting from pancreatic insufficiency or from increased consumption due to oxidative stress.

Role of Antioxidant Supplementation in CP

To mitigate oxidative stress and correct antioxidant deficiency, it was postulated that antioxidant supplementation might be beneficial. There are multiple randomized controlled trials (Table 57.1) and meta-analyses to address this issue.

The first RCT was conducted by Uden et al. [31]. It was a double-blind placebo-controlled crossover RCT. Twenty-three patients were recruited of whom only 20 were included in the final analysis (7 had alcoholic CP, 8 had idiopathic CP, and 5 had recurrent acute pancreatitis). One arm was given combination antioxidants (daily 600 µg selenium, 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, and 2 gm methionine) and the other arm was given placebo. Total duration of treatment was 20 weeks with crossover at 10 weeks. Six patients on placebo had recurrent attacks compared to none in the antioxidant arm. The authors concluded that active treatment was associated with clinical improvement over and above placebo effect.

Banks et al. [32] subsequently studied the antioxidant allopurinol in patients with painful CP and compared it with placebo. There was no decrease in visual analog scale (VAS) score or McGill score with the intervention though the study was limited by a small sample size of only 16 patients. Bilton et al. [33] also failed to show any improvement in VAS score in a double-blind placebo-controlled RCT of combination antioxidants (daily 800 mg SAMe, 600 µg selenium, and 9000 IU β-carotene) compared to placebo.

Curcumin supplementation for 6 weeks did not show any improvement in VAS score compared to placebo in an RCT. Though the study showed improvement in GSH level in red blood cells and decrease in MDA levels, there was no improvement in pain [34].

In a double-blind placebo-controlled crossover RCT, 36 patients with CP were randomized to receive combination antioxidants (4 times daily 75 µg selenium, 3 mg β-carotene, 47 mg d-α-tocopherol, 150 mg ascorbic acid, and 400 mg methionine) or placebo for 20 weeks with crossover at 10 weeks. There was significant improvement in pain component of SF 36 score with the combination antioxidant therapy [35].

Table 57.1 Summary of trials of antioxidant supplementation in chronic pancreatitis.

Author	Type of study	*N*	Intervention	Outcome measures	Results	Remarks
Uden 1990	Double-blind placebo-controlled crossover RCT	23	Combination antioxidants (daily 600 µg selenium, 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, and 2 gm methionine) vs. placebo Duration: 20 weeks, no washout period	• VAS • McGill pain score • Descriptive pain score	• 1.01 (0.16–4.26) vs. 1.88 (0.22–5.76), NS • No significant difference • No clear difference • 6 patients in placebo had attack compared to none with intervention	Low risk of bias
Salim 1991	3 armed, parallel, double-blind, placebo-controlled RCT	78 (25 vs. 26 vs. 27)	4 times daily 50 mg allopurinol vs. 500 mg dimethyl sulfoxide vs. placebo Duration: up to 24 hours pain-free (mean = 45 hours)	• Number of pain-free patients • Discharge days	• 13 vs. 12 vs. 4 • 3 days vs. 3 days vs. 5 days	Unclear risk of bias
Bilton 1994a	Double-blind placebo-controlled crossover RCT	30	3 daily doses of 800 mg S-adenosyl methionine (SAMe) vs. placebo Duration: 20 weeks (no washout)	• VAS	No difference	Data not shown
Bilton 1994b	Double-blind placebo-controlled crossover RCT	14	Combination antioxidants (daily 800 mg SAMe, 600 µg selenium, and 9000 IU β-carotene) vs. placebo	VAS	No difference	Data not shown
Banks 1997	Double-blind placebo-controlled crossover RCT	16	Allopurinol 300 mg/d vs. identical placebo Duration: 10 weeks with 2 weeks washout after initial 4 weeks	• VAS score (0–100): difference in mean decrease from baseline • McGill's score (0–45): difference in mean decrease	• 2.8, $P = 0.24$ • -0.3, $P = 0.75$	Low risk of bias
Nandi 2002	Parallel placebo-controlled RCT	25	Combination of antioxidants (daily 600 µg selenium, 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, and 2 gm methionine) vs. placebo Duration: 6 months	• Pain score (12 points) • Pain-free days/mo	• 1.25 vs. 3.62, NS • 3.75 vs. 4.12, NS	Unclear risk of bias
Durga Prasad 2005	Parallel single-blind placebo-controlled RCT	20	Combination antioxidants (3 times daily 500 mg curcumin and 5 mg piperine) vs. placebo Duration: 6 weeks	VAS score (after intervention)	5.81 (0.74) vs. 6.57 (0.74), NS	High risk of bias

(*Continued*)

Table 57.1 (Continued)

Author	Type of study	*N*	Intervention	Outcome measures	Results	Remarks
Kirk 2006	Double-blind placebo-controlled crossover RCT	36	Combination antioxidants (4 times daily 75 µg selenium, 3 mg β-carotene, 47 mg d-α-tocopherol, 150 mg ascorbic acid, and 400 mg methionine) vs. identical placebo Duration: 20 weeks (no washout)	• Daily VAS • SF 36: pain component (change from baseline)	• Not analyzed • +17 points vs. -7 points, $P < 0.05$	High risk of bias
Bharadwaj 2009	Parallel double-blind placebo-controlled RCT	127 (71 vs. 56)	Combination of antioxidants (daily 600 µg selenium, 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, and 2 gm methionine) vs. identical placebo Duration: 6 months	• Painful days/mo: decrease from baseline • Painful days/mo: after intervention • Pain-free participants	• 7.37 (6.75) vs. 3.21 (3.99), $P < 0.001$ • 1.68 (2.8) vs. 3.36(4.35), $P = 0.012$ • 23/71(32%) vs. 7/56 (13%), $P = 0.009$	Largest study to date with low risk of bias
Jarosz 2010	Open label parallel RCT	91 (46 vs. 45)	Combination antioxidants (vitamin C and vitamin E vs. standard treatment) Duration: 6 months	Pain-free participants	22/32 (68%) vs. 11/56 (31%), $P = 0.002$	High risk of bias
Siriwardena 2012	Parallel double-blind placebo-controlled RCT	70 (33 vs. 37)	Combination antioxidants (38.5 mg selenium, 113.4 mg d-tocopherol acetate, 126.3<tsmg ascorbic acid, and 480 mg l-methionine) vs. placebo Duration: 6 months	• Change in VAS • Average daily VAS • Pain-free participants	• -2.33 (2.09) vs. -1.97 (2.46), NS • 2.93 (1.96) vs. 3.05 (1.96), NS • 19 (58%) vs. 20 (54%), NS	Low risk of bias
Talukdar 2016	RCT	87	Antioxidant plus pregabalin vs. placebo for 2 months followed by open label antioxidants for 4 months in both groups	• Improvement in pain (VAS and Izbicki score)	• percent reduction of VAS (-50 [-80.0; -32.1] vs. -29.5 [-64.5; 0]; $P = 0.01$) • Izbicki score (14.5 [0; 21.3] vs. 30.0 [11.8; 41.3]; $P = 0.001$) • complete pain resolution (20 [47.6%] vs. 12 [26.7%]; $P = 0.04$)	Included patients had recurrence of pain after prior endoscopic or surgical therapy
Singh 2019	Parallel double-blind placebo-controlled RCT	107	Combination antioxidants (daily 600 µg selenium, 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, and 2 gm methionine) vs. placebo	• VAS • Pain-free participants	• No difference • No difference	The primary outcome of the study was improvement in pancreatic functions and pain assessment was a secondary outcome

NS: not significant; VAS: visual analog scale.

Table 57.2 Summary of meta-analyses on antioxidant supplementation in chronic pancreatitis.

Author	Study types included	Number of studies	Outcome measures	Conclusion
Ali et al. 2014 Cochrane systemic review	RCT	12 (585 participants)	Pain complaints pre- and post-intervention	Antioxidants can reduce pain slightly in CP. Adverse events in 1 in 6 patients may prevent use
Zhou et al. 2014	RCT	8 (573 participants)	Pain relief	Antioxidant administration effective in relieving pain
Rustagi et al. 2015	RCT	8 (446 participants)	Pain reduction	Benefit of antioxidant therapy for pain reduction (RR: 0.73; 95% CI: 0.58–0.91)
Mohta et al. 2020	RCT	4 (352 participants)	Pain relief Quality of life	No significant pain reduction or change in quality of life

The limitations of these studies were a small sample size, inclusion of predominantly alcoholic CP, nonstandardized quantification of pain, and short duration of antioxidant supplementation.

Bhardwaj et al. conducted a double-blind placebo-controlled RCT to study the role of antioxidant supplementation in patients with both alcohol-related and idiopathic CP. A total of 127 patients with CP (age 30.5 ± 10.5 years, 32 alcohol-related and 95 with idiopathic CP) were randomized to receive either antioxidants ($n = 56$; daily 0.54 gm ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol, 600 µg selenium, and 2 gm methionine) or placebo ($n = 71$), for 6 months. There was a significant reduction in number of painful days per month in the antioxidant group (7.37 [6.75] vs. 3.21 [3.99], $P < 0.001$). Twenty-three out of 71 patients were pain-free in the antioxidant group compared to 7 out of 56 in placebo ($P = 0.009$). The reduction in levels of TBARS and increase in levels of FRAP were also significantly higher in the antioxidant group, which was commensurate with the clinical observation [36].

Another trial randomized 70 patients with CP to receive either combination antioxidant, ($n = 33$; 38.5 mg selenium, 113.4 mg d-tocopherol acetate, 126.3 mg ascorbic acid, and 480 mg l-methionine) or placebo ($n = 37$) for 6 months [37]. The study did not find improvement in VAS score or number of pain-free patients with the antioxidant therapy. However, there were several limitations of the study which prevented its generalizability: all the patients were receiving a mean of 85 mg/day opioids suggesting chronic neuropathic pain, more than half of patients had failed prior medical or surgical therapy suggesting that they have a severe unresponsive disease, and they continued to drink alcohol and smoke during the study period [38].

One study evaluated the combined role of antioxidants and pregabalin in patients with recurrence of pain following surgical/endoscopic therapy and showed that the combination therapy significantly reduced pain [39].

Four meta-analyses [40–43] have been done to date to study the role of antioxidant supplementation in pain relief in CP (Table 57.2). Three out of four meta-analyses have shown benefit with antioxidant therapy. In the Cochrane systemic review and meta-analysis [40], which evaluated 12 RCTs including 585 participants, there was a slight reduction in pain with the antioxidant therapy. It showed that adverse events might prevent use of antioxidants in one out of six patients. Effects on other outcomes such as use of analgesics, exacerbation of pancreatitis, and quality of life remained uncertain.

Conclusion

Pain is the predominant symptom in patients with chronic pancreatitis. There is compelling evidence that oxidative stress is involved in the pathogenesis of CP. Patients with CP are deficient in antioxidants due to impaired absorption and increased demand. Supplementation with antioxidants is beneficial in reducing pain in patients with CP.

References

1 Singh VK, Yadav D, Garg PK. Diagnosis and management of chronic pancreatitis: a review. JAMA 2019;322:2422–2434.

2 Sies H, Jones D. Oxidative stress. In: Fink G, ed. *Encyclopedia of Stress*. 2nd edn. New York: Academic Press, 2007: 45–48. Available at: https://www.sciencedirect.com/science/article/pii/B9780123739476002853.

3 Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.

4 Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 1985;64:111–126.
5 Anon. Free Radicals in Biology and Medicine. 3rd edn. Sigma-Aldrich. Available at: http://www.sigmaaldrich.com/.
6 Howie AF, Arthur JR, Nicol F et al. Identification of a 57-kilodalton selenoprotein in human thyrocytes as thioredoxin reductase and evidence that its expression is regulated through the calcium-phosphoinositol signaling pathway. J Clin Endocrinol Metab 1998;83:2052–2058.
7 Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–162.
8 Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.
9 Quilliot D, Walters E, Bonte J-P et al. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. Am J Clin Nutr 2005;81:1117–1125.
10 Zwart LL de, Meerman JH, Commandeur JN et al. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 1999;26:202–226.
11 Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–76.
12 Braganza J. Pancreatic disease: a casualty of hepatic "detoxification"? Lancet 1983;322:1000–1003.
13 Rose P, Fraine E, Hunt LP et al. Dietary antioxidants and chronic pancreatitis. Hum Nutr Clin Nutr 1986;40: 151–164.
14 Guyan PM, Uden S, Braganza JM. Heightened free radical activity in pancreatitis. Free Radic Biol Med 1990;8:347–354.
15 Acheson DW, Hunt LP, Rose P et al. Factors contributing to the accelerated clearance of theophylline and antipyrine in adults with exocrine pancreatic disease. Clin Sci (Lond) 1989;76:377–385.
16 McNamee R, Braganza JM, Hogg J et al. Occupational exposure to hydrocarbons and chronic pancreatitis: a case-referent study. Occup Environ Med 1994;51:631–637.
17 Uden S, Acheson DW, Reeves J et al. Antioxidants, enzyme induction, and chronic pancreatitis: a reappraisal following studies in patients on anticonvulsants. Eur J Clin Nutr 1988;42:561–569.
18 Tandon RK, Garg PK. Oxidative stress in chronic pancreatitis: pathophysiological relevance and management. Antioxid Redox Signal 2011;15:2757–2766.
19 Norton ID, Apte MV, Haber PS et al. Cytochrome P4502E1 is present in rat pancreas and is induced by chronic ethanol administration. Gut 1998;42:426–430.
20 Halangk W, Lerch MM. A unique pancreatic mitochondrial response to calcium and its role in apoptosis. Gut 2009;58:328–330.
21 Odinokova IV, Sung K-F, Mareninova OA et al. Mechanisms regulating cytochrome c release in pancreatic mitochondria. Gut 2009;58:431–442.
22 Masamune A, Watanabe T, Kikuta K et al. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2008;294:G99–G108.
23 Basso D, Panozzo MP, Fabris C et al. Oxygen derived free radicals in patients with chronic pancreatic and other digestive diseases. J Clin Pathol 1990;43:403–405.
24 Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszeń M. Oxidative stress in blood of patients with alcohol-related pancreatitis. Pancreas 2001;22:261–266.
25 Hausmann DH, Porstmann T, Weber I et al. Cu/Zn-SOD in human pancreatic tissue and pancreatic juice. Int J Pancreatol 1997;22:207–213.
26 Schoenberg MH, Büchler M, Pietrzyk C et al. Lipid peroxidation and glutathione metabolism in chronic pancreatitis. Pancreas 1995;10:36–43.
27 Van Gossum A, Closset P, Noel E et al. Deficiency in antioxidant factors in patients with alcohol-related chronic pancreatitis. Dig Dis Sci 1996;41:1225–1231.
28 Mathew P, Wyllie R, Van Lente F, et al. Antioxidants in hereditary pancreatitis. Am J Gastroenterol 1996;91: 1558–1562.
29 Braganza JM, Schofield D, Snehalatha C et al. Micronutrient antioxidant status in tropical compared with temperate-zone chronic pancreatitis. Scand J Gastroenterol 1993;28:1098–1104.
30 Bopanna S, Nayak B, Prakash S et al. Increased oxidative stress and deficient antioxidant levels may be involved in the pathogenesis of idiopathic recurrent acute pancreatitis. Pancreatology 2017;17:529–533.
31 Uden S, Bilton D, Nathan L et al. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. Aliment Pharmacol Ther 1990;4:357–371.
32 Banks PA, Hughes M, Ferrante M et al. Does allopurinol reduce pain of chronic pancreatitis? Int J Pancreatol 1997;22:171–176.
33 Bilton D, Schofield D, Mei G et al. Placebo-controlled trials of antioxidant therapy including s-adenosylmethionine in patients with recurrent nongallstone pancreatitis. Drug Invest 1994;8: 10–20.
34 Durgaprasad S, Pai CG, Vasanthkumar et al. A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. Indian J Med Res 2005;122:315–318.
35 Kirk GR, White JS, McKie L et al. Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. J Gastrointest Surg 2006;10: 499–503.
36 Bhardwaj P, Garg PK, Maulik SK et al. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. Gastroenterology 2009;136:149–159.e2.
37 Siriwardena AK, Mason JM, Sheen AJ et al. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. Gastroenterology 2012;143:655–663.e1.

38 Garg PK. Antioxidants for chronic pancreatitis: reasons for disappointing results despite sound principles. Gastroenterology 2013;144:e19–20.
39 Talukdar R, Lakhtakia S, Nageshwar Reddy D et al. Ameliorating effect of antioxidants and pregabalin combination in pain recurrence after ductal clearance in chronic pancreatitis: results of a randomized, double blind, placebo-controlled trial. J Gastroenterol Hepatol 2016;31:1654–1662.
40 Ahmed Ali U, Jens S, Busch ORC et al. Antioxidants for pain in chronic pancreatitis. Cochrane Database Syst Rev 2014:CD008945.
41 Mohta S, Singh N, Gunjan D et al. Systematic review and meta-analysis: is there any role for antioxidant therapy for pain in chronic pancreatitis. JGH Open 2021;5:329–336.
42 Rustagi T, Njei B. Antioxidant therapy for pain reduction in patients with chronic pancreatitis: a systematic review and meta-analysis. Pancreas 2015;44:812–818.
43 Zhou D, Wang W, Cheng X et al. Antioxidant therapy for patients with chronic pancreatitis: a systematic review and meta-analysis. Clin Nutr 2015;34:627–634.