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Pathophysiology of Oxidative Stress and Antioxidant Therapy in Acute Pancreatitis

Susanta Meher, Satyajit Rath, Rakesh Sharma, Bikram Rout, Tushar Subhadarshan Mishra, Prakash Kumar Sasmal and Milthilesh Kumar Sinha

Department of General Surgery, AIIMS, Bhubaneswar, Odisha, India, 751019a, India

Abstract

Acute pancreatitis is an inflammatory disease of pancreas with varied clinical presentation ranging from mild self limiting disease to severe necrotising pancreatitis with high mortality. The exact pathogenesis of the disease is unclear despite extensive research. Recent studies have shown the role of oxidative stress in the pathogenesis of the disease. Many experimental studies have proven the role of oxygen free radicals in the initiation and progression of the disease. Antioxidant therapy has shown promising results in experimental animal models, whereas conflicting result has been seen in clinical studies in humans. This may suggest existence of different pathogenetic mechanism in humans. This review gives an overview of the role of oxidative stress in the pathophysiology of acute pancreatitis and outcomes of antioxidant therapy as a therapeutic agent in the treatment of acute pancreatitis.

Keywords: Oxidative stress; Reactive oxygen species; Antioxidants; Acute pancreatitis

Introduction

Acute pancreatitis as an inflammatory condition with varied clinical presentations, ranging from a self limiting condition to life threatening necrotising pancreatitis [1,2]. Around 10-20% of individual die from multi-organ failure even after best supporting care and pharmacologic therapy. High mortality rate is attributed to lack of specific theurapeutic interventions [2,3]. Acute pancreatitis develops after a cascade of multiple pathways leading to activation of trypsinogen which in turn activates other digestive enzymes. There occurs a surge in the oxidative stress leading to generation of free radical from oxidation of lipid and proteins, which disrupts the pancreatic membrane [4-6].

Perez et al. have shown in rat models that, cell necrosis and hemolysis can cause activation of the inflammatory cascade and oxidative stress [7]. Beneficial effects of anti-oxidant therapy in form of superoxide dismutase and catalase in acute pancreatitis in past trials thereby provide an indirect proof of role of oxidative stress in the disease pathogenesis [8]. They also suggested allopurinol, an inhibitor of xanthine oxidase as a potential therapeutic agent [8]. Several studies on oxidative stress, xanthine oxidase and other pro and anti-oxidants have shown that oxidative stress is not only a mediator in the early local events but also associated in systemic inflammatory response in acute pancreatitis [9,10].

Reactive oxygen species (ROS) activates the inflammatory cascade thereby recruiting inflammatory cells and cause tissue damage. Expression of inflammatory cytokine which is regulated by many signaling molecules such as Nuclear Factor (NF-kB) and Activator Protein-1(ap-1), Signal Transducer and Activator of Transcription 3 (STAT3), and Mitogen Activated Protein Kinases(MAPKs). ROS activates these various signaling molecules leading to activation of pro-inflammatory cytokines, which in turn amplifies the inflammatory cascade in acute pancreatitis [11]. Milnerowicz et al. have studied the degree of pro/antioxidative imbalance and have estimated the role of various antioxidants in maintenance of the balance of pro/antioxidants during acute pancreatitis. They have demonstrated that increase in IL-6 concentration in serum is correlated with Ranson criteria, and increase in Glutathione Peroxidase activity(GPx), levels of Metallothionein-1(MT-1), Thiobarbituric acid Reactive Substances(TBARS), or GGT, and NAG activities in patients groups compared with healthy subjects. They also noted a decrease in serum GSH levels in patients with acute pancreatitis suggesting oxidative stress. GPx/GSH(Glutathione Peroxidase/Reduced Glutathione) and MT-1 can be considered as agents of first line of defence against oxidative stress in acute pancreatitis [12].

Studies have shown significant consumption of antioxidant in patients with severe acute pancreatitis [13]. Oxidative stress, besides local effects also has metastatic effects on other organs such as lungs and it has been studied that the cytokine production and infiltration of inflammatory cells occur simultaneously both in lungs and pancreas during pancreatitis[14]. Antioxidant therapy and other ROS scavengers like Hydrogen rich saline, Emodin which is a component in Chinese herb, Diosmetin (3', 5, 7-trihydroxy-4'-methoxyflavone) the aglycone part of the flavonoid glycosides diosmin occuring naturally in citrus fruit may neutralize this imbalance of pro/antioxidant levels both in the pancreas as well as other organs and improve survival [15-17]. Kuliaviene et al. have demonstrated that the fatty acid composition of the erythrocyte membrane changes in acute pancreatitis which may be due to oxidative stress [18]. Necrosectomy done in severe necrotizing pancreatitis clears the lipid peroxidation products effectively leading to improved erythrocyte membrane fluidity and increased survival [19].

Oxidative Stress and Pathophysiology of Acute Pancreatitis

The exact pathogenesis of acute pancreatitis is still unclear despite extensive research worldwide [20]. The premature activation pancreatic zymogens particularly trypsinogen, leading to autodigestion of pancreatic tissue and subsequent development local and systemic inflammatory response is the most accepted theory till now [20]. Last

***Corresponding authors:** Susanta Meher, Department of General Surgery, AIIMS, Bhubaneswar, Odisha, India, 751019. Tel: 087630 26139; E-mail: chikusus@gmail.com

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decade has witnessed a lot of studies on the role of oxidative stress in pathogenesis of acute pancreatitis. In both experimental and clinical studies, it has been seen that oxidative stress plays a central role in the pathogenesis of AP.

Oxygen free radical (OFR)/ Reactive oxygen species (ROS) and oxidative stress

All aerobic organisms require ground state oxygen to survive [21]. During the process of oxygen metabolism some amount of oxygen free radicals are generated which are required to maintain body homeostasis [22,23]. At physiologic levels, these oxygen free radicals help in regulation of transcription, act as signalling molecules or stage a defence mechanism against microorganisms [24]. Normally a balance is maintained between the oxygen free radicals and the scavengers. Accelerated production of oxygen free radicals can occur in acute inflammatory disorder like acute pancreatitis which can cause tissue and cell damage and accentuate the inflammatory response.

Direct action of OFR includes lipid peroxidation in pancreatic membranes, oxidation of proteins and induction of DNA fragmentation [25]. OFR directly attacks the double bonds of unsaturated phospholipids leading to degradation of structural integrity of the cell membrane including both plasma membrane and membrane of intracellular organelles such as lysozomes and endoplasmic reticulum [26]. Accumulation of lipid degradation products, malonaldehyde (MDA) and 4-hydroxynonenol in the membranes cause increased permeability and deformability of the membranes [21]. As a consequence intracellular leakage of proteases and influx of Ca^{2+} occur, ultimately leading to tissue and cell damage [26]. Protein oxidation leads to fragmentation of polypeptide chains or cross linking of sulfhydryl groups resulting in impaired enzyme function [21]. OFR also cause abnormal cross linking and strand breaks in DNA [27]. DNA-damage response follows leading to the activation of p53 and poly-ADP ribose polymerase (PARP) a nuclear enzyme. Apoptosis and cell cycle arrest follows the activation of p53, whereas activation of PARP leads to cell necrosis [28].

Indirectly, OFR causes activation arachidonic acid cascade leading to increased production of thromboxanes and leukotriene B4 [21]. Thromboxanes because of their potent action on platelet aggregation and vasoconstricting effect, decrease microvascular tissue perfusion and enhance ischaemic injury [29]. On the other hand leukotriene-B4 promotes activation of polymorphonuclear leukocytes and discharge of lysosomal enzymes [30]. Activated polymorphonuclear leukocytes are responsible for respiratory burst that leads to increased production of reactive oxygen species and activated enzymes which contributes to further cell damage [8] (Figure 1).

Recently the role of oxidative and nitrosative stress in modulation of intracellular signaling by redox unbalance have been described. Up-regulation of pro-inflammatory genes occurs as a result of redox unbalance through activation of different pathways which ultimately leads to increased production of proinflammatory cytokines [31] (Figure 1).

ROS and NF-kB activation: The regulation of NF-kB occurs through oxidation and thiolation of upstream protein kinases and phosphatases [32]. ROS dependant tyrosine phosphorylation helps in NF-kappa B activation [33]. This ROS dependent activation seems to occur in the early phase of acute pancreatitis. Activation of NF-kB leads to increased production of proinflammatory cytokines and subsequent amplification of inflammatory response [31].

Disulfide stress: Oxidation of thiols in protein during acute inflammations leads to disulfide stress and inactivation of protein phosphatases, such as serine protein phosphatise 2A and tyrosine phosphatase SHP1 [31]. This favours mitogen activated protein kinase (MAPK) activation and amplification of the inflammatory cascade. Other relevant redox-signaling thiols targets of disulfide stress include thioredoxin-1, peroxiredoxin, Keap-1, disulfide isomerase, and endonuclease APE1/Ref1 [34]. Inhibition of MKPs via different signalling pathways leads to activation of MAPKs by ROS. MKPs belong to a large group of protein tyrosine phosphatases, its catalytic cysteine is much more sensitive to reversible oxidation than other cysteines which makes it more vulnerable targets of ROS [35]. Thus ROS governs the balance between MAPKs and protein phosphatases in controlling the inflammatory response by redox signalling [36].

Histone acetylation and redox signalling: In has been reported on previous studies that CBP/p300 histone acetyl transferase (HAT) complex regulates the expressions of inflammatory cytokines through activation of NF-kB and STAT-3 pathways [37]. This is required for activation of a number of inflammatory targets of NF-kB such as IL-6, IL-8, E-Selectin, and VCAM-1 [38]. STAT3 pathway is frequently used in acute in inflammatory reponse is regulated by acetylation and phosphorylation. p300 triggers the acetylation of STAT3 and formation of STAT 3 dimers which are critical in the transcriptional activation of IL-6 [39]. Activation of these phosphorylation acetylation pathways, NF- kB, MAPKs and STAT3 leads to changes in chromatin structure in order to trigger the expression of inflammatory genes and production of proinflammatory cytokines that subsequently leads to amplification of the inflammatory process and systemic inflammatory response syndrome [31].

Calcium signalling: Calcium homeostasis is highly sensitive to redox signalling. Disruption of this seems to be an important event in the pathogenesis of acute pancreatitis [40]. Ca^{2+} channels contain IP3R and RyR receptors which are rich in redox sensitive cysteine residues [41]. The thiol oxidation of these residues increases the activity of calcium channels of endoplasmic reticulum and consequent rise in cytosolic calcium. Intracellular hypercalcemia leads to premature activation of trypsinogen and subsequent cell and tissue damage [42]. Other redox signalling pathways involved in calcium homeostasis are STIM- Orai complex and plasma membrane Ca^{2+} ATPase pump [43]. The type of cell death depends on the duration and severity of the cytosolic calcium levels [40].

Reactive nitrogen species (RNS) and oxidative stress

RNS are also associated with oxidative stress and pathogenesis of AP [44]. During inflammatory process inducible nitric oxide synthase (iNOS) is the main source of NO [45]. It has been seen that up to a certain limit NO has beneficial effect. Uncontrolled over production of NO can be detrimental [46]. Endogenous NOS has shown beneficial effect on early phase of experimental pancreatitis [47]. Mice deficient in iNOS has shown less lipid peroxidation and tissue damage in experimental AP [31].

Glutathione and oxidative stress

Reduced glutathione (GSH) is the major non protein thiol which plays a central role as an antioxidant [31]. The ratio of reduced (GSH) and oxidised glutathione(GSSG) is a reliable indicator of oxidative status of the body [48]. During early phase of acute pancreatitis the level of reduced glutathione decreases, which is a hallmark of the disease [49]. Studies have shown that preteatment with glutathione mono ethyl ester exhibit beneficial effects in AP by increasing pancreatic GSH

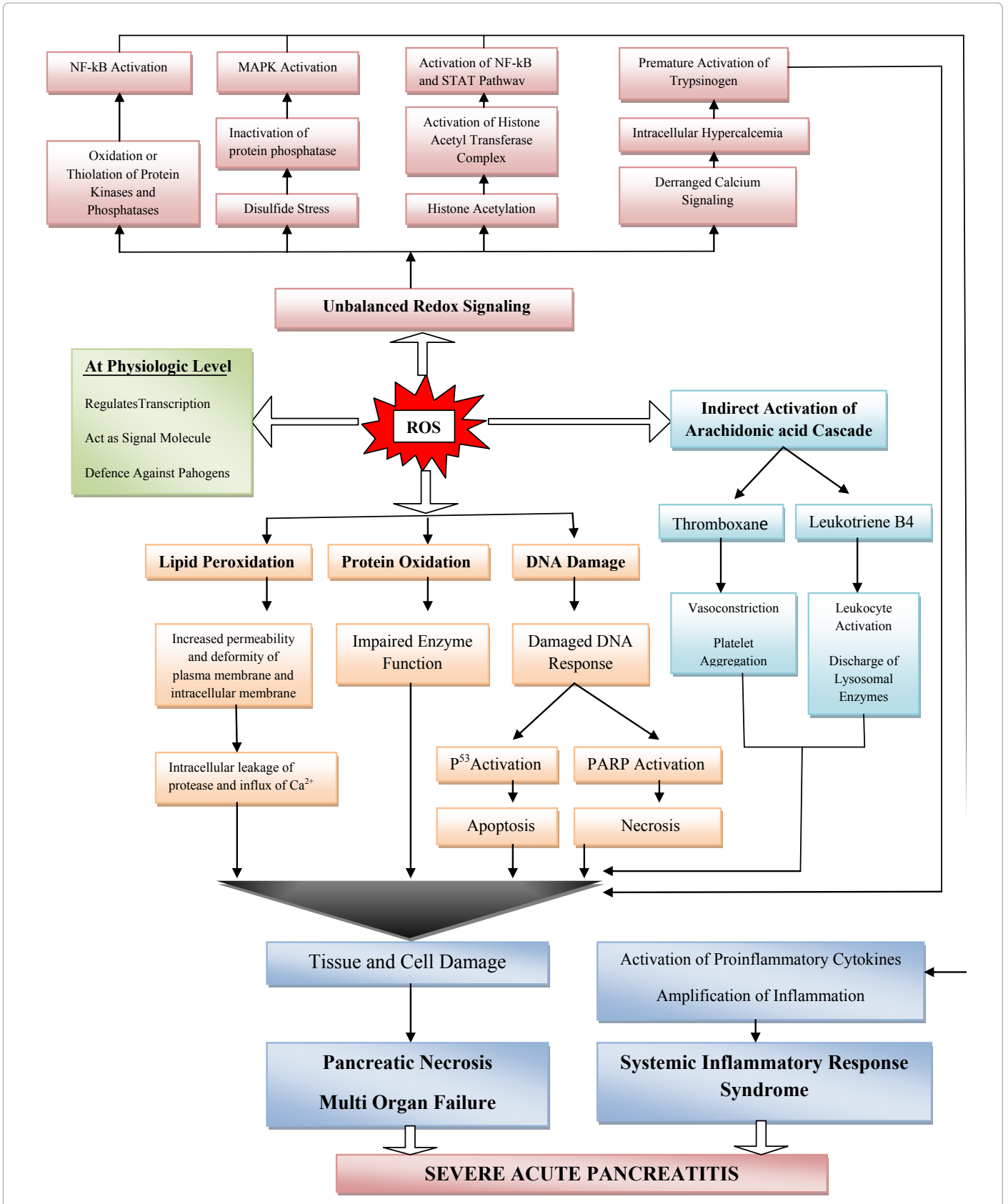

Figure 1: Central role of reactive oxygen species (ROS) in the pathogenesis of severe acute pancreatitis. NF-kB: Nuclear Factor- KappaB, MAPK: Mitogen activated protein kinase, STAT: Signal transducer and activator of transcription, PARP: poly-ADP ribose polymerase.

levels [31]. Whereas inhibition of GSH synthesis with L-buthionine-(S,R)-sulfoximine(BSO) led to more pancreatic necrosis and reduced survival in rats with experimental AP [50].

Xanthine Oxidase and oxidative stressors

Oxidation of the enzyme xanthine dehydrogenase(XDH) leads to the production of a large amount of free radicals [31]. Activation and conversion of this enzyme to xanthine oxidase (XO) occurs via two mechanisms. First,during conversion of chymotrymsinogen to chytmotrypsin; second, by oxidation of the thiol groups [51]. Studies have shown beneficial effect of allopurinol (inhibitor of XO) in experimental pancreatitis by reducing pancreatic edema, necrosis and systemic inflammatory response [52]. However effect of allopurinol in prevention of post ERCP pancreatitis and in treatment of AP is not clear [53].

Extracellular Haemoglobin

Recently extracellular haemoglobin in plasma that comes from pancreatic ascites has been found to be associated with severe acute pancreatitis [7]. Extracellular haemoglobin causes lipid peroxidation and activation of proinflammatory cytokines like TNF-α and IL-1β [7]. It also promotes leukocyte infiltration in lungs and induces hypoxia inducible factor (HIF)-vascular endothelial growth factor(VEGF) pathway [54]. Thus extracellular haemoglogin can contribute to pulmonary edema and acute respiratory distress by increasing vascular permeability in lungs through HIF-VEGF pathway [55].

Sanfey et al. were the first to show beneficial effects of anti-oxidant therapy in form of super-oxide dismutase and catalase in acute pancreatitis, thereby providing an indirect proof of role of oxidative stress in the disease pathogenesis [8]. Since then a lot of studies have demonstrated the role of oxidative stress in the pathogenesis of experimental pancreatitis [56]. It is well known that oxygen free radicals play a central role in initiation and accentuation of inflammatory process in acute pancreatitis. However most of the data are form experimental studies using animal models, only few have been derived from clinical studies [56]. The paucity of data from human studies is because of the unprecedented delay in patient presentation, which limits the investigations from the pathogenic mechanisms involved in initiation of the disease [57].

It is very difficult to measure oxygen free radicals directly because of its high reactivity [56]. For this reason stable metabolites of oxidative reactions have been accepted as the biomarkers of oxidative stress in acute pancreatitis. Malonaldehyde (MDA), protein carbonyls, thiobarbituric acid reactive substances (TBARS), pro-oxidative and anti-oxidative enzymes (Superoxide Dismutase (SOD), Catalase (CAT), myeloperoxidase (MPO), xanthine oxidase (XO), reduced glutathione(GSH) and the levels of natural antioxidants(Vitamin-C, Selenium) have been used as biomarkers of oxidative stress in most of the experimental studies [58]. All these biomarkers indirectly prove the role of oxidative stress in the pathogenesis of acute pancreatitis.

Antioxidant therapy

Halliwell in 1997 described antioxidants as, "any substance that, when present in low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substance" [59]. These molecules scavenge highly toxic free radicals and prevent tissue from oxidative damage. As oxidative stress plays a central role in pathophysiology of acute pancreatitis much emphasis has been given to antioxidant therapy in the treatment and prevention of the disease. Though various antioxidants have been studied in various experimental models only a few have been implemented clinically.

Vitamins C, A and E all have been extensively used as antioxidant in acute pancreatitis either as a single agent or in combination. In a randomized controlled trial in 2003 by Du et al. intravenous vitamin C was administered to 40 patients in dose of 10 g/day for 5 days with results of decrease in serum levels of TNF-alpha,IL-1, IL-8, CRP with increase in plasma levels of vitamin E, C, beta-carotene and lipid peroxide [60]. Length of hospital stay, deterioration of disease was reduced with overall improvement in disease course. In another similar study Sateesh et al. in 2009 used vitamin C in combination with N-acetyl cystiene and antoxyl forte and found similar results with decrease in the length of hospital stay and complications; whereas Bansal et al. in 2011 used all the three vitamins in comination without any significant differences in outcome in control and study group [61,62]. To our knowledge there has been no clinical studies on vitamin E or A as single agents.

N-acetyl cysteine (NAC), a thiol compound & a precursor of glutathione acts as a free radical scavenger. It interferes through its reducing capabilities in signaling pathways that regulate cell cycle, apoptosis, and inflammation. Siriwardena et al. in 2008 showed in their randomized, double blinded control trial using NAC along with vitamin C and selenium that serum levels of amylase, lipase and CRP were reduced where as no difference was noted in organ dysfunction, length of hospital stay and mortality rates [63]. Milewski et al. in 2006 used NAC in prevention of post-ERCP pancreatitis by giving oral and intravenous NAC both before and after ERCP without any positive outcome [64].

Selenium, a micronutrient found in trace amounts is a co factor of antioxidant enzyme glutathione peroxidase and helps in reduction of hydrogen peroxide as well as lipid hydroperoxidases. Kuklinski et al. in 1994 in their clinical study showed that early selenium therapy in acute pancreatitis may improve prognosis and outcome of the disease where as Lindner et al. in 2004 could not find any beneficial effect of sodium selenite in patients with acute pancreatitis [65,66].

Glutamine, semi essential amino acid and has antioxidant properties due to its capacity to normalize superoxide dismutase and blockage of nitric oxide overproduction [67]. Fuentes-orozco et al. in 2008 in their randomized control trial found administration of glutamine along with total parentral nutrition had favourable outcome in acute pancreatitis with reduced duration of shock, APACHE II scores and infectious morbidity in the course of disease [68]. Serum levels of IL-6, CRP were reduced where as IL-10, albumin, and protein were raised. Sahin et al. in 2007 had similar results with decreased complication rates with glutamine enriched total parentral nutrition [69].

Recent experimental studies and future perspectives on antioxidant therapy

Keeping in view the oxidative stress as the pathogenesis of acute pancreatitis a lot of experimental studies (Table 1) have been undertaken in past few decades using various antioxidant molecules. Almost all of these studies are based on chemically induced pancreatitis on rats by infusion of either cerulin, L-aghinine or sodium taurocholate. Infusion into peritoneal cavities or into ductal system induced pancreatitis in these rats that were pretreated with the antioxidant molecules. After sacrificing the animals, assays of oxidative stress markers were done both in serum as well as histopathological specimens. All of the studies reviewed by us showed promising results with chemo-preventive effects of the antioxidants on chemically induced pancreatitis.

Study	Antioxidant used	Pancreatitis inducing agent	Oxidative stress markers studied
Abdin et al. [70]	*Pentoxyphylline, alpha lipoic acid*	l-arginine	Amylase,CRP,IL-6, CAT,MDA
Abed et al. [71]	Lithium	Cerulin	Amylase, lipase, MPO
Akay et al. [72]	Taurine	Sodium taurocholate	Amylase, MDA,MPO
Akyol et al. [73]	Probiotic[saccharomyces boulardii], ciprofloxacin, meropenam	Sodium taurocholate	MDA,SOD
Cao et al. [74]	Sivelestat	Cerulin	Amylase, lipase, corticosterone, IL-1beta, TNF-alpha, NFkappaB
Carrasco et al. [75]	Resveratrol	Cerulin	Amylase, lipase, corticosterone, GPO, IL-1beta, IL-10
Chu et al. [76]	*Sesamol*	Cerulin	Amylase, LPO, GSH, NO
Cikman et al. [77]	Syringic acid	L-arginine	Oxidant status, oxidative stress index, lipid hydroperoxide
Inal et al. [78]	HBO+ 3-amino benzamide	Intraductal tarocholate	Tissue oxidative stress parameters, tissue histopathology scores, bacterial translocation
Kilic et al. [79]	Carvacrol	Cerulin	MDA, SOD, CAT, GSH, histopathology, 8-hydroxy-deoxyguanosine
Lee et al. [80]	ND 07	Cerulin +LPS	Amylase, lipase, MDA, NO, PGE2, TNF-alpha
Liang et al. [81]	Melatonin	L-arginine	TNF-alpha, IL-6, MDA, SOD
Lima et al. [82]	Eucalyptol[1,8 cineole]	Cerulin	Amylase, lipase, MPO, MDA, GSH, NF kappaB
Lv et al. [83]	Lycopene	---	MPO, LPO, SOD, TNF-alpha, IL-6
Onur et al. [84]	HBO+NAC	L-arginine	Amylase, calcium, LDH, MDA, SOD, GSH
Oztas et al. [85]	Lycopene	Ceruline	Amylase, lipase, TNF-alpha, IL-1, GSH,MDA, MPO, Na-K-ATPase
Peng et al. [86]	Micron Liu-He-Dan [MLHD]ointment	L-arginine	Amylase, TNF-alpha, IL-6, IL-10, SOD, MDA
Ren et al. [87]	Hydrogen rich saline infusion	High pressure air impact	Plasma enzymes, cytokines, oxidative stress molecules
Wu et al. [88]	Emodin	Sodium taurocholate	Amylase,TNF-alpha, IL-6
Yu et al. [11]	Diosmin	Cerulin	Amylase, lipase, TNF-alpha, IL-6, IL-10, MPO, iNOS, NF kappaB

CRP: C-reactive protein; CAT: Catalase; IL: Interleukin; MDA: Malonyldialdehyde; MPO: Myleperoxidase; SOD: Superoxide Dismutase; TNF: Tumor Necrosis Factor; NF: Nuclear Factor; GPO: Glutathione Peroxidase; LPO: Lipid Peroxidase; GSH: Glutathione; NO: Nitric Oxide; PGE2: ProstaglandineE2; iNOS: inducible Nitric Oxide Synthetase; HBO: Hyperbaric Oxygen

Table 1: List of recent experimental studies on various antioxidants on murine models.

Conclusion

Oxidative stress and reactive oxygen species, both play pivotal roles in pathophysiology of AP during the initial phase of the disease. Extensive research has been undertaken in the past and present involving various markers of OS. They have also provided an insight into the fairplay of numerous antioxidants in preventing and treating this dreaded condition. Though few clinical trials have showed promising results on this unconquered disease, still adequate number of large, multicenter, randomized, double-blinded clinical trials are lacking at this point of time to give a conclusive scenario on antioxidant therapy and its clinical efficacy. Novel targeted therapeutic options against various signaling molecules of oxidative stress needs further research and the spectrum of antioxidant therapy established through extensive human trials to gain an upper hand against the clinical course of the disease.

Conflict of Interest: Authors declare no conflict of interest

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