

Oxidative Stress in the Pathophysiology of Diabetic Neuropathy: Mechanisms to Management

Geeta Negi¹, Ashutosh Kumar¹, Shyam S Sharma*¹

¹Department of Pharmacology and Toxicology
National Institute of Pharmaceutical Education and Research
Sector 67, S.A.S. Nagar, Punjab, India-160062

Neuropathy represents the main cause of morbidity and mortality among diabetic patients. Accumulating evidences insinuate that changes in cellular function resulting in oxidative stress act as a cardinal factor in the development and progression of diabetic neuropathy. Since all of the mechanisms of neuronal injury converge upon oxidative stress, this appears an exigent target for therapeutic intervention, particularly as an adjunct to other targeted strategies. However, despite positive preclinical evidences a mystery still remains as to why antioxidants per se have not demonstrated neuroprotection in humans. A comprehensive understanding of the fundamental mechanism of antioxidant action may open up new roads for drug development. A growing body of evidences now suggests that strategies utilizing a more targeted antioxidant approach may be the elusive additive therapy required to further optimize neuroprotection in diabetes.

Introduction

Diabetes, characterized by persistent hyperglycemia, is one of the most prevalent lifestyle diseases affecting people across the globe. Diabetic population of the world in 2000 was 171 million and it has been projected to rise to 366 million by the year 2030¹. In India currently 19 million people are affected with diabetes and this figure may reach as high as 60 million by the year 2025. The human and economic costs of this epidemic are enormous and health care cost incurred by diabetes all over world is gigantic. Mortality and morbidity associated with diabetes is mainly due to complications arising from it which include neuropathy, nephropathy, vasculopathy and retinopathy. Diabetic neuropathy (DN) is the most important of them affecting around 50 % of the patients. A detailed definition of DN says "diabetic neuropathy is a descriptive term meaning a demonstrable disorder, either clinically evident or subclinical, that occurs in setting of diabetes mellitus without other causes for peripheral neuropathy. The neuropathic disorder includes manifestations in somatic and/or autonomic parts of peripheral nervous system²."

The hyperglycemic environment is the back bone of the pathophysiology of diabetic neuropathy leading to the development of complications through many intertwined cellular pathways: polyol pathway; hexosamine shunt; AGE-RAGE interactions; endoneurial dysfunction; impaired

neurotrophic support (Nerve growth factor-NGF and neurotrophin-3)³; protein kinase C (PKC) pathway; Poly ADP-Ribose Polymerase (PARP) overactivation and nuclear factor kappa B pathway leading to cell death; impaired insulin/C peptide action etc. Inhibiting any one of them does not halt the progression of DN nor does it reverse the disease process completely. However it has already been shown that all of the pathways coalesce into a common fate i.e. oxidative stress⁴. Oxidative stress may be defined as a condition in which overwhelming production of reactive oxygen species (ROS) annihilate the innate antioxidant defense capacity of the body. Excess production of ROS is considered to alter neuronal membrane permeability and configuration inter alia functional modification of various cellular proteins. Thus targeting this single pathway can crystallize all the efforts made to ameliorate the epidemic of diabetic neuropathy.

Innate antioxidant mechanisms

Antioxidants are defined as substances which inhibit or impede the oxidative damage to subcellular proteins, carbohydrates, lipids and DNA. In response to excess ROS production during respiration and metabolism mammals have evolved numerous antioxidant systems. In order to maintain the levels of antioxidants in the cell; dietary uptake or *de novo* synthesis is necessary. Even fleeting episodes of acute hyperglycemia can blunt the antioxidant capacity of plasma and increase oxidative stress in diabetics. The warriors of body's antioxidants defense are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). SOD catalyses the dismutation of

*Corresponding author

S.S Sharma, Ph.D., Associate Professor
Dept. of Pharmacology & Toxicology,
NIPER, S.A.S. Nagar; e-mail: sssharma@niper.ac.in

superoxide (O_2^-). Since in the process of dismutation hydrogen peroxide (H_2O_2) is generated, this enzyme bands together with other two antioxidant enzymes catalase and glutathione peroxidase, the H_2O_2 removing enzymes to protect cellular damage by H_2O_2 . Three isoenzymes of SOD are known: the Cu/Zn SOD² localized in the cytosol, Mn/SOD present in mitochondria and extracellular Cu/Zn SOD which differs from intracellular Cu/Zn SOD in molecular mass and possesses attached carbohydrate. Criticality of this enzyme for survival is highlighted by the fact that complete knockout of SOD is lethal within days of birth in mice⁵. Catalase and glutathione peroxidase are other antioxidant enzymes that detoxify H_2O_2 to water, and therefore their activity needs to be present when SOD is active. Glutathione peroxidase equally protects against the oxidation of dihydrorhodamine 123 (an indicator dye) by peroxynitrite ($OONO^-$), requiring glutathione as reductant indicating that it also acts as a defense line against peroxynitrite-mediated oxidations⁶.

In addition to the antioxidant enzymatic defense, mammalian cells also possess small non protein molecules which quench free radicals and dampen the injurious effects of ROS; these include glutathione (reduced form), thioredoxin, vitamin C and Vitamin E. Of particular mention among these molecules is glutathione, a tripeptide (γ -Glu-Cys-Gly) which is present ubiquitously in mammalian cells. Depletion of glutathione stores in the cell draws it indefensible to oxidative injury. In diabetic neuropathy ischemia and excitotoxicity leads to neuronal apoptosis. Glutathione has shown to prevent this event. In another study it has been demonstrated that neuroblastoma cells show magnified resistance to oxidative stress when glutathione-S-transferase is overexpressed⁷.

Oxidative stress: a real miscreant in pathophysiology of DN

It is now an article of faith that ROS contribute to cell and tissue dysfunction and damage in diabetic neuropathy. The mechanisms of oxidative stress leading to the development of neuropathy have been explored in various animal models. Hyperglycemia unleash multiple pathways such as redox imbalances secondary to enhanced aldose reductase activity, increased advanced glycation end products and altered protein kinase C activity to induce oxidative stress. Oxidative stress is associated with the development of apoptosis in neurons and glial cells and so could be the unifying mechanism that leads to nervous system damage in diabetes. Many up-to-the-minute studies have supported this hypothesis, including *in vivo* and *in vitro* measurement of oxidative stress in sensory neurons and dorsal root ganglion (DRG).

There are many polymorphous pathways that lead to oxidative stress in the peripheral nervous system in chronic

hyperglycemia but mitochondrial metabolism and the cascade of oxidative phosphorylation are underscored as key contributors. To have a better understanding of a large and abstruse field, it is helpful for the reader to first consider the mechanisms for the endogenous generation of ROS.

Generation of ATP through oxidative phosphorylation and oxygen consumption is major function of mitochondria. Intermediates of the tricarboxylic acid cycle such as nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide (FAD) reducing equivalents are fuels for mitochondrial ATP production. Electrons generated through these reducing equivalents are passed along the electron transport chain (ETC) in the inner mitochondrial membrane. The ETC comprising of oxidoreductase complexes I, II, III and IV (cytochrome c), shuttles the electrons through these molecular complexes which are ultimately donated to molecular oxygen to form water.

Sometimes during shuttling of electrons, single electron escapes and results in a partial reduction of molecular oxygen to form a superoxide anion (O_2^-). Mitochondrial O_2^- generation acts as a catalyst for production of other ROS such as hydrogen peroxide (H_2O_2), which can, via Fenton reaction, result in highly reactive hydroxyl radicals (OH^\cdot). Under normoglycemic conditions the levels of ROS produced are negligible, and they are surmountable by cellular antioxidants such as glutathione, catalase and superoxide dismutase. However when mitochondrial antioxidant machinery is overwhelmed by these ROS, oxidative damage and cell death is bound to occur⁸.

The hyperglycemic cell metabolizes more glucose, increasing the turnover of the mitochondrial energy-generating complexes occurs, which increases the production of free radicals. Oxidative stress critically alters energy regulation and survival in the mitochondria through following mechanisms:

- Nitric oxide (NO) reversibly competes with molecular oxygen for binding to cytochrome *c* oxidase, producing its reversible inhibition at physiological levels and act as a regulator for electron transfer. However, with excess O_2^- , NO forms $ONOO^-$ which binds irreversibly to cytochrome *c* oxidase.
- Oxidative stress due to massive production of O_2^- and $ONOO^-$ inhibits the import of essential proteins² from cytosol to the mitochondria.
- Damage of inner membrane proteins by ROS induces membrane permeability transition, which is followed by release of cytochrome *c* and apoptosis⁹.

To cap it all, ROS might compromise the integrity of the mitochondrial genome. ROS plays a role in local apoptosis in peripheral neurons via actions of caspases and proapoptotic Bcl proteins that damage mitochondria, and

Review Article

ultimately impair neuronal function and viability. Prolonged hyperglycemia, through overproduction of ROS, is likely to damage dorsal root ganglion mitochondrial DNA, adding to long-term nerve dysfunction.

Convergence of various pathogenetic pathways with oxidative stress

Multiple pathogenic factors are interrelated in diabetic neuropathy. Although hyperglycemia is considered to be a major pathogenic factor in the development of diabetic neuropathy, the mechanisms associated with this are not yet fully understood. The pathophysiology of diabetic neuropathy includes increased oxidative and nitrosative stress yielding advanced glycosylated end products (AGEs)¹⁰, polyol accumulation¹¹, hexosamine flux¹², decreased nitric oxide/impaired endothelial function, PARP over activation, mitogen-activated protein kinase (MAPK) and cyclooxygenase-2 activation and impaired (Na⁺/K⁺)-ATPase activity. Although specific inhibitors of any of these mechanisms ameliorate various diabetes-induced abnormalities *in vitro* and *in vivo*, common element linking all these pathways of hyperglycaemia-induced damage was

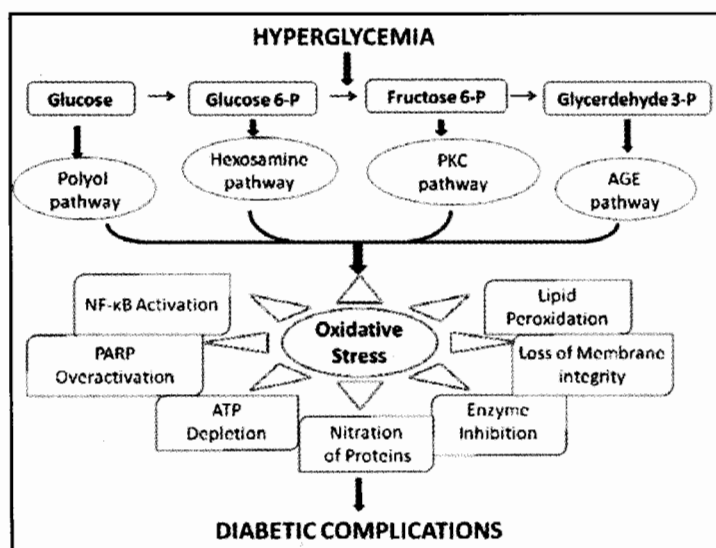


Fig 1: Various biochemical pathways leading to diabetic complication. Under normoglycemic conditions, glucose is metabolized via glycolysis. But in diabetes where chronic hyperglycemia is present glucose enters various other biochemical pathways. These include- polyol pathway, hexosamine flux, increased intracellular formation of AGE (advanced glycosylated end products), PARP overactivation and PKC activation. All these pathways culminate to produce oxidative and nitrosative stress and ultimately cell death

a missing piece of puzzle till now. However, this mystery has now been resolved by some of the recent findings that each of these pathogenic mechanisms proclaim a single process induced by hyperglycemia: overproduction of reactive oxygen species.

a) Polyol pathway

Aldose reductase (alditol NAD(P)⁺-1-oxidoreductase,

EC1.1.1.21) is a widely expressed aldehyde-metabolizing enzyme. It catalyzes NADPH dependent reduction of a number of carbonyl compounds like glucose.

Under normoglycemia, most of the glucose is phosphorylated to glucose-6-phosphate by hexokinase while a part of it (3%) is converted to sorbitol via polyol pathway. This reaction is catalyzed by aldose reductase requiring NADPH as cofactor. Sorbitol is subsequently oxidized to fructose by sorbitol dehydrogenase and requires NAD⁺ as cofactor. In hyperglycemia, hexokinase is saturated and excess glucose is metabolized by polyol pathway (30%). This leads to overflow of products of polyol pathway along with depletion of reduced form of nicotinamide dinucleotide phosphate (NADPH) and oxidized form of nicotinamide adenine dinucleotide (NAD⁺), the cofactors of polyol pathway. This leads to metabolic imbalance in tissues which undergo insulin independent uptake of glucose: lens, retina and sciatic nerve, the major target organs of diabetic complications^{4, 13}.

Accelerated polyol pathway leads to a change in redox status of cells by depleting NADPH and NAD⁺ resulting in altered activity of glutathione reductase, an important enzyme required to maintain cellular levels of glutathione, an endogenous antioxidant. The overall result is increased susceptibility to oxidative stress^{14, 15}. Increased ratio of NADH/NAD⁺ in cytosol inhibits the activity of the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and increases the concentration of methyl glycerol and diacylglycerol which serve to activate two other pathways: increased AGE formation and PKC activation respectively.

Moreover converse proof of relation between polyol pathway and oxidative stress is obtained by the observation that the biomarkers of oxidative stress were blunted by genetic ablation of aldose reductase inhibitors, including DNA damage (8-hydroxydeoxyguanosine excretion), poly(ADP-ribose) polymerase (PARP) activation and activation of c-Jun NH₂-terminal kinase (JNK). However complete inhibition of aldose reductase to prevent polyol pathway is also not desirable as it is involved in detoxification of lipid peroxidation products¹⁶.

b) AGE mediated ROS generation

Reducing sugars like glucose undergo non-enzymatic reactions with the primary amino groups of proteins to form glycated residues called "Amadori products". These early glycation products undergo further complex reactions such as dehydration, condensation, and crosslinking to form stable covalent adducts called advanced glycated end products (AGE).

During euglycemia, glyceraldehyde 3-phosphate, a glycolysis intermediate, undergoes oxidation by GAPDH. In presence of high glucose, GAPDH mediated catabolism of

glyceraldehydes and dihydroxyacetone phosphate is impaired which undergoes autooxidation to generate two potentially toxic substances, α -ketoaldehydes and H_2O_2 . As described under preceding section, increased formation of sorbitol via polyol pathway and oxidation of sorbitol by NAD^+ increases the cytosolic $NADH:NAD^+$ ratio which tends to inhibit GAPDH activity. This leads to increased level of triphosphate, methylglyoxal and diacylglycerol (DAG). Also the inhibition of GAPDH is mediated by poly(ADP-ribose) by PARP which is activated in hyperglycemia induced oxidative stress^{15, 17}.

Increased non enzymic glycation alters the function and structures of various macromolecules in tissues causing basement membrane thickening, demyelination and impaired axonal transport as a result of glycation of myelin, tubulin and neurofilaments. Extracellular matrix component modified by AGE precursor interact abnormally with other matrix components and with the receptors for matrix protein on cells. Plasma proteins modified by AGE precursors bind to AGE receptors on endothelial, mesangial cells and macrophages, inducing receptor mediated production of ROS. Interaction of AGE with receptors for AGE (RAGE) activates the pleiotropic transcription factor NF- κ B, which causes pathological changes in gene expression.

The transgenic and knockout animal models have strengthened the concept that the AGE-RAGE interaction plays a crucial role in the development and progression of diabetic neuropathy. Also the fact that agents that modify the AGE formation process improve some of the symptoms of diabetic neuropathy supports the role of AGE formation in its pathogenesis. LR-90 [4-(2-chlorophenylureido)phenoxyisobutyric acid] a scavenger of AGE precursor and ALT-711 (alagebrium chloride) an agent that disrupt the cross-links have been shown to reduce AGE formation and oxidative stress in streptozotocin induced diabetes rat model¹⁸.

c) Protein kinase C (PKC) activation

Excess dihydroxyacetone formed during hyperglycemia undergo reduction to glycerol-3-phosphate and subsequent acylation to yield diacyl glycerol (DAG). DAG then activates PKC. PKC has a unique structural feature that facilitates its regulation according to redox status of cell. Prooxidants react with regulatory domain to stimulate its activity while antioxidant reacts with catalytic domain and inhibits its activity. On activation, it activates stress genes such as MAPK that phosphorylates transcription factors and thus alters the balance of gene expression. It activates stress genes such as hsp and c-jun kinases that can lead to apoptosis and vascular atherosclerosis^{17, 19}. As with some aldose reductase inhibitors, some of the PKC inhibitors have been shown to exhibit antioxidants effects. The bisindolylmaleimide, LY333531, ruboxistaurin, vitamin E,

antisense oligonucleotides or peptide fragment inhibitors have demonstrated potential benefits in diabetic cardiovascular diseases²⁰.

d) Hexosamine pathway

During hyperglycemia fructose 6-phosphate is converted to glucosamine 6-phosphate by an enzyme- glutamine; fructose 6-phosphate aminotransferase (GFAT). Further processing to UDP-N-acetylglucosamine aids proteoglycan synthesis and formation of O-linked glycoproteins. This pathways leads to increased transcription of TGF- α , TGF- β 1, PAI-1 and has been implicated in insulin resistance. Inhibition of GFAT blocks the transcription of TGF- α , TGF- β and PAI-1. Glucosamine has been shown to elevate H_2O_2 levels experimentally and antioxidants tends to inhibit this effect^{4, 17, 21}.

e) PARP overactivation

Poly (ADP-ribose) polymerase (PARP EC 2.4.2.30) is a nuclear enzyme that act as DNA nick-sensor and facilitates DNA repair. This enzyme catalyzes the addition of ADP-ribose units to DNA, histones, and various DNA repair enzymes, which affects cellular processes such as replication, transcription, differentiation, gene regulation, protein degradation, and spindle maintenance. On activation it cleaves nicotinamide adenine dinucleotide (NAD^+) resulting in the formation of nicotinamide and ADP-ribose residues, which are attached to nuclear proteins forming poly (ADP-ribose)ated protein polymers. The potential role of PARP in DNA repair mechanisms is evident from the fact that PARP deficient cells are more prone to DNA damage by various ionizing radiations and alkylating agents²².

Role of PARP in cell death is also very important. When PARP is over activated, it metabolizes NAD^+ into polymers of ADP-ribose and nicotinamide, which leads to the depletion of the pyridine nucleotide pool. Therefore, cellular metabolic pathways which depend upon NAD^+ as cofactor, such as anaerobic glycolysis and mitochondrial respiration, are impaired²³.

Recently it has been proved in many findings that PARP overactivation and oxidative stress are two inseparable pathways. Under normal conditions, PARP activity is relatively quiescent. However, under conditions of oxidative stress, excessive DNA single-strand breakage is triggered by ROS leading to overactivation of PARP. Activated PARP initiates an energy consuming cycle resulting in rapid depletion of the intracellular pools of NAD^+ and ATP, hampering glycolysis and mitochondrial respiration, eventually leading to cellular energy crisis and cell death through necrotic route²⁴. Oxidative stress also stimulates activation of redox-sensitive transcription factors such as NF κ B and AP-1, key regulators of inflammatory cytokines and chemokines.

Drug	Exp. Model	Parameter	References
FeTMPyP and FeTPPS	STZ induced diabetes in rats	Corrected MNCV and NBF deficits. Protection against nitrosative stress	26
Resveratrol	STZ induced diabetes in rats	Ameliorated the alterations in MNCV and NBF, significant reduction in DNA fragmentation	27
Edaravone	STZ induced diabetes in rats	Protection against MNCV and NBF deficits, restored antioxidant enzyme levels	28
Trolox	STZ induced diabetes in rats	Ameliorated the alterations in MNCV, NBF, hyperalgesia, MDA levels and antioxidant enzymes in diabetic rats	29
U83836E	STZ induced diabetes in rats	Ameliorated the alterations in MNCV, NBF, hyperalgesia, MDA levels and antioxidant enzymes	30
Oleuropein	Alloxan induced Diabetes in rabbits	Restored MDA and Blood glucose levels to normal	31
Apocynin	STZ-induced diabetes in rats	Protection against MNCV and NBF deficits, restored blood glucose	32
Garlic oil and melatonin	STZ-induced diabetes in rats	Normalized total thiol levels, ceruloplasmin uric acid, blood glucose, total lipid, TG and cholesterol level	33
Tempol	STZ-induced diabetes in rats	Corrected MNCV, NBF and SNCV deficits	34
DL- α -Lipoic acid	STZ-induced diabetes in rats	NBF and MNCV deficits restored	25
Vitamin E	STZ-induced diabetes in rats	Normalized ATPase, Myo-inositol and taurine level	35

f) Miscellaneous

Oxidative stress has an important role in diabetes induced impairment of neurotropic support, which is closely associated with schwann cell injury. Numerous studies indicates ROS as powerful activators of three subfamilies of MAPK's i.e. JNK/SAPK, ERK and p-38 MAPK. In addition, oxidative stress affects multiple signal transduction pathways- arachidonic acid cascade, phosphoinositide, Ca^{2+} signalling as well as neurotransmission. Oxidative stress has also been implicated in myelin fiber atrophy and other morphological changes characteristic of advanced diabetic peripheral neuropathy ¹⁷.

Current status of antioxidant therapy

Keeping in view that oxidative stress plays a pivotal role in the pathogenesis of neuropathy, many antioxidant trials are ongoing. A number of antioxidants have reached phase 3 of clinical trials. These include vitamin E, curcumin, ascorbic acid and lipoic acid. Mice (2-4 months old) treated with ascorbic acid once a week during three months showed an increase in the percentage of myelinating nerve fibers and showed better results in locomotor tests. Treatment with lipoic acid has improved nerve conduction velocity during studies in diabetic animals ²⁵.

Based on these preclinical findings these antioxidants vitamins are expected to perform same in human trials. A

combination of allopurinol, alpha lipoic acid and nicotinamide is under phase 3 clinical trials for diabetic autonomic neuropathy.

However, no antioxidant therapy is approved by the FDA for diabetic neuropathy in the USA. Lipoic acid is only member of this list which has been approved for the treatment of diabetic neuropathy in Germany. Some novel antioxidant molecules have been tested for their potential to prevent cardiovascular defects in diabetic rodents such as metallothionein, edaravone, tempol, and melatonin. The following compounds could make their way to clinical trials as adjunct therapies for diabetic neuropathy:

Many *in vivo* and *in vitro* studies have explicitly identified ROS as a key player in the pathophysiology of diabetic neuropathy; the clinical outcomes of antioxidant therapy have been disheartening. A number of explanations have been put forth to account for the failure of antioxidants at the level of clinical trials. Antioxidant administration to patients with established neuropathy does not provide coverage during the progression of the disease, when ROS production is increased. Specific ROS may vary in their extent of contribution to the activation of various pathogenetic pathways, thereby putting a check on the effectiveness of antioxidants which scavenge a particular reactive species. For example, the antioxidant vitamins do not scavenge H_2O_2 , which may be more important than

O²⁻ in the development of endothelial injury and tissue hypoxia. Finally, the antioxidants tested until now do not match the efficacy of the endogenous antioxidant defense systems. A more comprehensive research at preclinical as well clinical levels must be designed to conquer this failure.

Future vistas

Targeted antioxidants

Mitochondria, being foremost in the regulation of energy metabolism, ROS production and apoptosis, can be targeted for therapeutic benefit in many pharmacological conditions including diabetic neuropathy. Mitochondria-targeted antioxidants have displayed the protective abilities against toxic oxidative stress in cell culture studies. These agents selectively concentrate in the inner membrane of mitochondria and thus scavenge ROS at the site of production thereby curbing mitochondrial oxidative damage and death of neuron.

MitoQ, a triphenyl-phosphonium cation (TPP⁺)-linked antioxidant, has been tried to directly target mitochondria while also obviating solubility problems associated with the natural antioxidant coenzyme Q (CoQ10). When compared to nontargeted CoQ10 analogue decylubiquinone, MitoQ has been shown to be a more potent antioxidant and moreover it concentrate several 100-fold within mitochondria ³⁶.

A novel class of cell-permeable antioxidant peptides that selectively partition into the inner mitochondrial membrane has been reported. These peptides, known as Szeto-Schiller (SS) peptides, are nontoxic and have been shown to protect against oxidative stress in a range of neurodegenerative diseases ³⁷.

Drug targeted at mitochondria

Target	Molecule targeted	Potential disease treated
$\Delta\Psi_m$	MitoQ	Neurodegenerative disease, diabetes
$\Delta\Psi_m$	MitoPBN	IR injury and diabetes
$\Delta\Psi_m$	MitoPeroxidase	IR injury and diabetes
$\Delta\Psi_m$	GSH-choline ester	IR injury and diabetes
$\Delta\Psi_m$	NAC-choline ester	Stroke and diabetes
inner membrane	SS31	Neurodegenerative disease
inner membrane	SS01	Neurodegenerative disease

Abbreviations: ETC, electron transport chain; IR, ischemia and reperfusion; ROS, reactive oxygen species; $\Delta\Psi_m$ mitochondrial membrane potential; MitoPBN, triphenyl-phosphonium cation (TPP⁺)-linked phenyl tert-butyl nitron; MitoQ TPP⁺-linked coenzyme Q; NAC, N-acetylcysteine; SS, Szeto-Schiller peptides

Although mitochondria-targeted antioxidants are in the embryonic stage of their development, they do vouch for potential therapy for the treatment of not only diabetic neuropathy but also of other diseases associated with oxidative stress. A myriad of preclinical studies support their potential use for ischemia-reperfusion injury and neurodegenerative disorders

Increasing the expression of antioxidant enzymes

Expression and induction of enzymes that protect against ROS induced damaging effects play an important role in determining the risk of neuropathy in human. Many experimental evidences have thrown light on the potential of innate antioxidant enzyme system against oxidative stress induced cellular damage. A torrent of scientific groups is studying about possibilities for such an antioxidant therapy. Adenovirus containing manganese superoxide dismutase cDNA (AdMn-SOD) are being tried *in vitro* and *in vivo* in the treatment of various cancers. Gene therapy with organ-specific targeting of Mn-SOD plasmid liposome accords a valuable technique for increasing the levels of SOD in specific organs at high risk of oxidative damage ⁶. Zwacka *et al.* have shown that the increasing the expression of SOD reduced the degree of apoptosis, providing the grounds for redox gene therapies ³⁸.

However experimental evidences that overexpression of these enzymes can protect neurons against oxidative injury are still lacking. Moreover whether this approach can be exploited clinically in neuropathic condition is still under the layers of doubt as at the time of diagnosis of neuropathy in diabetic patients massive turnover of ROS had already occurred. Under such a heavy load of ROS whether these antioxidant enzymes will be able to surmount oxidative stress is a major question.

External supply of antioxidants

One of the most applicable approaches of combating oxidative stress is to increase antioxidant defense of the body by supplying them externally. Although antioxidants are already in clinical use, but limitations encountered with conventional antioxidant therapy calls for some more effective alternative. SOD which is frontline defense against H₂O₂ has been tested but was found inadequate as being a peptide it was unstable, did not permeate cell membrane, and provoked an immune response. SOD liposome infusions have been reported to render protection against O₂ toxicity. A plethora of Cu, Zn-SOD conjugates are available, including polyethylene glycol (PEG)-SOD, Ficoll-SOD, lecithinized SOD, polyamine conjugated SOD, cationized SOD, genetically engineered SOD polymers, pyran-SOD and albumin-SOD complexes. These conjugates offer an advantage of longer half-lives than the unconjugated SOD molecules ⁶.

Review Article

References

1. Wild S, Roglic G, Green A, *et al.*, *Diabetes care.* 2004, 27: 1047-53.
2. Vinik AI, Maser RE, Mitchell BD, *et al.*, *Diabetes care.* 2003, 26: 1553-79.
3. Anand P, *Prog Brain Res.* 2004, 146: 477-92.
4. Vincent AM, Russell JW, Low P, *et al.*, *Endocr Rev.* 2004, 25: 612-28.
5. Lebovitz RM, Zhang H, Vogel H, *et al.*, *Proc Natl Acad Sci U. S. A.* 1996, 93: 9782-7.
6. Mate's JM, *Toxicology.* 2000, 153: 83-104.
7. Xie C, Lovell MA, Xiong S, *et al.*, *Free radical biology & medicine.* 2001, 31:
8. Fariss MW, Chan CB, Patel M, *et al.*, *Molecular Interventions.* 2005, 5: 94-111.
9. Nicholas DG and Budd SL, *Physiological Reviews.* 2000, 80: 315-60.
10. Schmid U, Stopper H, Heidland A, *et al.*, *Diabetes Metab Res Rev.* 2008,
11. Oates PJ, *Current drug targets.* 2008, 9: 14-36.
12. Du XL, Edelstein D, Rossetti L, *et al.*, *Proc Natl Acad Sci U S A.* 2000, 97: 12222-6.
13. Robertson RP, *J Biol Chem.* 2004, 279: 42351-4.
14. Obrosova IG, Pacher P, Szabo C, *et al.*, *Diabetes.* 2005, 54: 234-42.
15. Yabe-Nishimura C, *Pharmacol Rev.* 1998, 50: 21-33.
16. Pieper GM and Siebeneich W, *J Cardiovasc Pharmacol.* 1997, 30: 734-8.
17. Evans JL, Goldfine ID, Maddux BA, *et al.*, *Endocr Rev.* 2002, 23: 599-622.
18. Huebschmann AG, Regensteiner JG, Vlassara H, *et al.*, *Diabetes care.* 29: 1420-32.
19. Sima AA, *Cell Mol Life Sci.* 2003, 60: 2445-64.
20. Das Evcimen N and King GL, *Pharmacological Research.* 2007, 55: 498-510.
21. Brownlee M, *Nature.* 2001, 414: 813-20.
22. Jagtap P and Czabo C, *Nature Reviews.* 2005, 4: 421- 40.
23. Liu X, Luo X, Shi Y, *et al.*, *Cancer biology & therapy.* 2008, 7:
24. Obrosova IG, Drel VR, Pacher P, *et al.*, *Diabetes.* 2005, 54: 3435-41.
25. Stevens MJ, Obrosova I, Cao X, *et al.*, *Diabetes.* 2000, 49: 1006-15.
26. Arora M, Kumar A, Kaundal RK, *et al.*, *European journal of pharmacology.* 2008, 596: 77-83.
27. Kumar A, Kaundal RK, Iyer S, *et al.*, *Life Sci.* 2007, 80: 1236-44.
28. Saini AK, Kumar HSA and Sharma SS, *European journal of pharmacology.* 2007, 568: 164-72.
29. Sharma SS and Sayyed SG, *Clin Exp Pharmacol Physiol.* 2006, 33: 1022-8.
30. Sayyed SG, Kumar A and Sharma SS, *Life Sci.* 2006, 79: 777-83.
31. Al-Azzawie HF and Alhamdani MS, *Life Sci.* 2006, 78: 1371-7.
32. Cotter MA and Cameron NE, *Life Sci.* 2003, 73: 1813-24.
33. Anwar MM and Meki AR, *Comp Biochem Physiol A Mol Integr Physiol.* 2003, 135: 539-47.
34. Coppey LJ, Gellert JS, Davidson EP, *et al.*, *Free Radic Res.* 2003, 37: 33-40.
35. Van Dam PS, Van Asbeck BS, Bravenboer B, *et al.*, *Metabolism.* 1999, 48: 442-7.
36. Armstrong JS, *British Journal of Pharmacology.* 2007, 151: 1154-65.
37. Szeto HH, *The AAPS Journal.* 2006, 8: E521-E31.
38. Zwacka RM, Dudus L, Epperly MW, *et al.*, *Hum. Gene Ther.* 1998, 9: 1381-6.

Current Research & Information on Pharmaceutical Sciences

Subscription & Advertisement Details

ANNUAL SUBSCRIPTION

Subscription of CRIPS for four issues (January-March, April-June, July-September, October-December).

INSTITUTION : Rs. 750/- INDUSTRY : Rs. 2000/-

Advertisement

FULL PAGE	HALF PAGE
B & W Rs. 2500/-	B & W Rs. 1500/-
Colour Rs. 6000/-	Colour Rs. 4500/-
Back Page (Colour)	: Rs. 10,000/-
Inside Back Cover (Colour)	: Rs. 7,000/-
Inside Front Cover (Colour)	: Rs. 8,000/-

A copy of journal can be sent on request. All the payment shall be made by demand draft in favour of Director, NIPER, S.A.S. Nagar, Punjab 160062. Payment may be sent to The Editorial Office - CRIPS, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160062.