ROLE OF OXIDATIVE STRESS IN PATHOGENESIS OF DIABETES AND ITS COMPLICATIONS

Amol Bhalchandra Deore*, Vinayak Dnyandev Sapakal, Nilofar S. Naikwade

Address: Department of Pharmacology, Appasaheb Birnale college of Pharmacy, South Shivajinagar, Sangli-416416. India.
Email: amoldeore22@gmail.com

Summary

This review article is comprised of pathogenesis and role of free radicals and oxidative stress in progression of diabetes and diabetic complications. Reactive oxygen species are formed disproportionately in diabetes by glucose autooxidation, impaired polyol pathway, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage cell structures, including lipids and membranes, proteins, DNA, increased lipid peroxidation, and development of insulin resistance. It has been suggested that enhanced production of free radicals and oxidative stress is central event to the development of diabetic complications. Changes in oxidative stress biomarkers including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia play major role in the pathogenesis of both types of diabetes mellitus as well as diabetic complications including diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, dyslipidemia, coronary heart disease, diabetic foot ulcer, diabetic ketoacidosis and peripheral vascular diseases. Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair caused by free radical induced cell damage.

Keywords: Reactive oxygen species; hyperglycemia; free radicals; Antioxidants; cell damage

Abbreviations

AGEs: advanced glycation end products; ALA: alpha lipoic acid; ATP: adenosine triphosphate; CHD: Coronary heart disease; DHLA: dihydrolipoic acid; ECD: endothelial cell dysfunction; GST: glutathione S-transferase; GPx: glutathione peroxidase; GSH: glutathione; GFAP: glial fibrillary acidic protein; H2O2: hydrogen peroxide; HDL: high density lipoprotein; IL: interleukin; IRS: insulin receptor substrate; JNK: Jun N terminal kinase; LDL: low density lipoprotein; MAPK: mitogen-activated protein kinase; NO: nitrous oxide; NF: necrosis factor; ˙OH: hydroxyl radical; PUFA: polyunsaturated fatty acids; PGI: prostaglandin I; PDGF: platelet derived growth factor; PKC: Protein kinase C; PKB: protein kinase B; ROS: reactive oxygen species; RAGE: receptor for advanced glycation end products; RNS: reactive nitrogen species; SOCS: suppressor of cytokine signaling proteins; SAPK: stress activated protein kinase; SOD: sulphoxide dismutase; TNFα: tumour necrosis factor alpha; TGF: transformine growth factor; VEGF: vascular endothelial growth factor; VLDL: vary low density lipoprotein; LOO˙: lipid peroxyl radicals.

Introduction

Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP production by the mitochondria. These by products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds. The delicate balance between their two antagonistic effects is clearly an important aspect of life. At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures [1-10]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and or supplements. Endogenous and exogenous antioxidants act as 'free radical scavengers' by preventing and repairing damages caused by ROS and RNS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [11-15].

Generation of free radicals

Free radicals can be produced by several different biochemical processes within the body including: reduction of molecular oxygen during aerobic respiration yielding superoxide and hydroxyl radicals; by products of chemistry such as oxidation of catecholamines and activation of the arachidonic acid cascade product electrons, which can reduce molecular oxygen to superoxide; production of superoxide hypochlorous acid (HOCl), a powerful oxidant, by activated phagocytes; nitric oxide production by vascular endothelium and other cells. In addition, free radicals can be produced in response to external electromagnetic radiation, such as gamma rays, which can split water to produce hydroxyl radicals [16].

Increased oxidative stress: Promoter of diabetes

There are many possible pathways of oxidative stress that can be associated with hyperglycemia [17-20].

Type I diabetes: Pancreatic β cell dysfunction

The common findings of prolong hyperglycemia and elevated lipid levels in the blood of diabetic patients led to the hypotheses of glucose toxicity [21], lipotoxicity [22] , and oxidative stress [23-25] . These include glycolysis and oxidative phosphorylation; methyl glyoxal formation and glycation, enediol and acetaldehyde formation (glucoxidation); diacylglycerol formation and protein kinase C activation, glucosamine formation; and hexosamine metablolism and sorbitol metabolism. Conceptually, as β cells are exposed to high glucose concentrations for increasingly prolonged periods of time, glucose saturates the normal route of glycolysis and increasingly is shunted to alternate pathways, such that ROS are generated from distinct metabolic processes within and outside the mitochondria. It has been proved that excessive levels of palmitate are associated with abnormal islet function (especially in the presence of high glucose concentrations), which leads to excessive lipid esterification that, in turn, can generate ceramide, thereby increasing oxidative stress [22, 26,27]. It seems unlikely; however, that circulating lipid itself, such as triglyceride or cholesterol, would be responsible for damaging islet tissue. It seems more likely that excessive circulating glucose levels lead to accelerated de novo synthesis of islet lipid. One mechanism by which glucose might contribute to liptoxicity is by virtue of its ability to drive

synthesis of malonyl CoA, which inhibits β-oxidation of free fatty acids. This in turn shunts free fatty acids towards esterification pathways, thereby forming triglyceride, ceramide and other esterification products [27, 28].

Oxidative stress is responsible for the decreased protein expression and levels of Pdx-1 and Maf-A. Both proteins are critical for normal insulin gene expression, as their absence or mutation of their DNA binding sites on the insulin promoter leads to decreased insulin mRNA levels, insulin content and insulin secretion. Chronic hyperglycemia leads to worsening of β cell function [29-34]. It was demonstrated that pancreatic islets contain relatively small amounts of the antioxidant enzymes CuZn-SOD, Mn-SOD, catalase, and glutathione peroxidase (GPx) [35]. Due to the low level of antioxidant enzyme expression and activity, the β cells are at greater risk of oxidative damage than tissues with higher levels of antioxidant protection [36].

Type II diabetes: Insulin resistance

Insulin rapidly interacts with its receptor at target tissues. The insulin receptor (IR, composed of two extracellular subunits and two transmembrane subunits linked by –S–S– bonds) possess an intrinsic tyrosine kinase activity. Tyrosine autophosphorylation of the IR subunit is induced following binding of insulin to the subunit [37]. The activated IR phosphorylates the insulin receptor substrate (IRS) proteins and other substrates. The process of phosphorylation leads to activation of different signaling pathways. While the ERK pathway is mainly involved in growth, the activation of phosphatidylinositol 3-kinase (PI 3-kinase), mainly through insulin receptor substrates 1 and 2 (IRS1, IRS2), is involved in the metabolic actions of insulin. IRS1 belongs to the IRS family and plays a key role in insulin signaling. While the phosphorylation of IRS1 on tyrosine residue is critical for insulin-stimulated responses, the phosphorylation of IRS1 on serine residues has a dual role: either to enhance or to terminate the insulin effects. The imbalance between the positive IRS1 tyrosine phosphorylation and the negative IRS1 serine phosphorylation is strongly stimulated by 'diabetogenic' factors including free fatty acids, TNFα and oxidative stress. Insulin activated protein kinase B (PKB) propagates insulin signaling and promotes the phosphorylation of IRS1 on serine residue, which in turn generates a positive-feedback loop for insulin action [38] . (Refer fig 1)

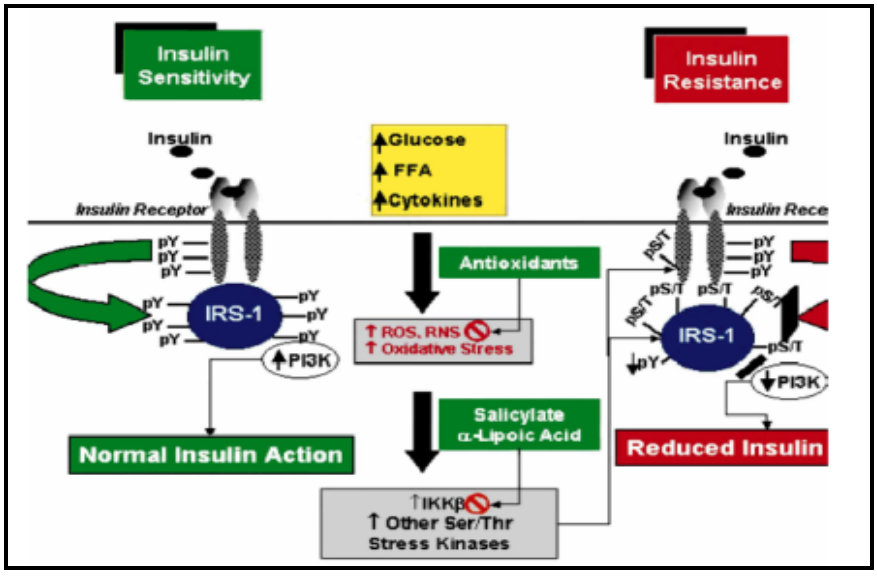

Fig 1: The role of serine kinase activation in oxidative stress-induced insulin resistance.

Various insulin resistance inducing agents such as angiotensin II, cytokines, free fatty acids, endothelin-1, cellular oxidative stress and hyperinsulinemia lead to both activation of several serine/threonine kinases and phosphorylation of IRS1 [39]. These agents are known to negatively regulate the IRS1 functions by phosphorylation, however also via other molecular mechanisms such as suppressor of cytokine signaling proteins (SOCS) expression, IRS degradation, and O-linked glycosylation. Understanding the mechanisms of IRS1 inhibition and identication of kinases involved in these processes may reveal novel targets for development of strategies to prevent insulin resistance [4].

Mechanisms for increased oxidative stress in diabetes

Autoxidative glycosylation

Autoxidative glycosylation is initiated by the oxidation of an aldose or ketose to a more reactive dicarbonyl sugar, which would then react with a protein. The reduced oxygen products formed in the autoxidation reaction include superoxide, the hydrogen radical and hydrogen peroxide, which, in the presence of metal ions, would cause oxidative damage to neighboring molecules. Therefore, autoxidative glycosylation is a possible mechanism for the production of free radicals, leading to fragmentation of proteins and oxidation of associated lipids during glycosylation reactions [17,40,41]. Autoxidative glycosylation is increased in the presence of hyperglycemia.

Sequential glycation, followed by free radical-mediated oxidation, will generate early glycosylation products. Some of these products undergo further chemical rearrangements to form irreversible, advanced glycosylation end products AGEs, which accumulate with aging and duration of diabetes in long-lived structural proteins, such as collagens. The formation of AGEs can induce covalent cross linking, resulting in hardening of the blood vessel walls with loss of their elasticity and increased vascular permeability [42]. Protein glycosylation is increased in diabetes and used as an index of long-term glucose control HbA1c. It correlates with diabetic complications. AGEs bind to a cell surface receptor known as receptor for AGE (RAGE), a multiligand member of the Ig super family. This binding initiates a cascade of signal transduction events involving p44/p42 MAPKs, nuclear factor-B, p21Ras, and other intermediates [43,44]. Interaction of AGEs with RAGE induces the production of ROS through a mechanism that involves localization of prooxidant molecules at the cell surface [45] and a key role for activated NADPH oxidase [46]. In neuronal cell lines, application of AGEs depletes GSH, but this is prevented in the presence of antioxidants [47]. Antioxidants or antibodies against RAGE prevent both oxidative stress and the downstream signaling pathways that can be activated by ligation of RAGE. AGE-mediated ROS production is particularly implicated in blood vessel endothelial activation and diabetic vascular complications [48,49] .

Lipid peroxidation

Oxygen radicals catalyse the oxidative modification of lipids. This peroxidation chain reaction is illustrated in Fig 2. Lipid peroxy radicals react with other lipids, proteins, and nucleic acids; propagating thereby the transfer of electrons and bringing about the oxidation of substrates. Cell membranes, which are structurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and, consequently, changes in membrane fluidity, permeability, and cellular metabolic functions result.

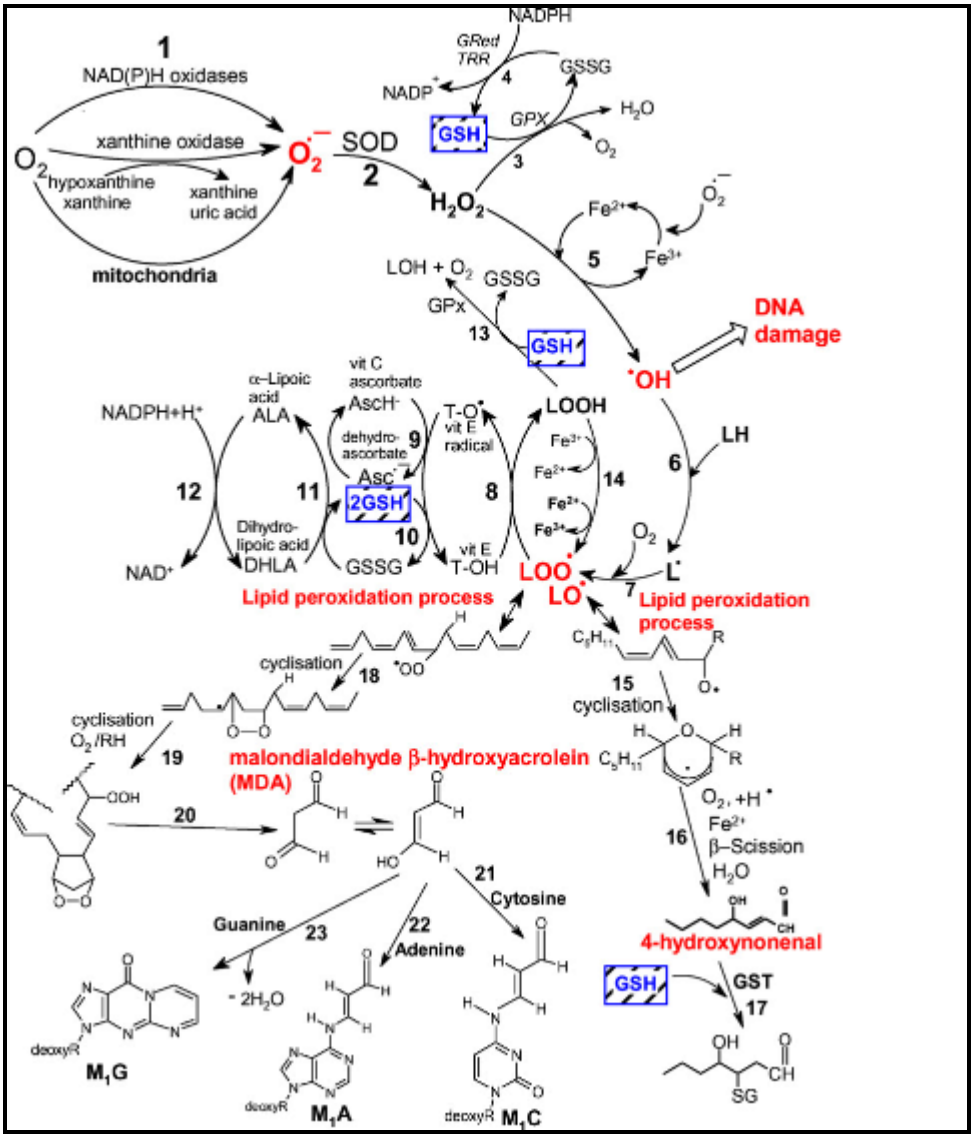

Fig 2: Pathways of ROS formation, the lipid peroxidation process and the role of glutathione (GSH) and other antioxidants (Vitamin E, Vitamin C, lipoic acid) in the management of oxidative stress. (Valko et al.,2007)

Reaction 1: The superoxide anion radical is formed by the process of reduction of molecular oxygen mediated by NAD(P)H oxidases and xanthine oxidase or non-enzymatically by redox reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain. Reaction 2: Superoxide radical is dismutated by the superoxide dismutase (SOD) to hydrogen peroxide. Reaction 3: Hydrogen peroxide is most efficiently scavenged by the enzyme glutathione peroxidase (GPx) which requires GSH as the electron donor. Reaction 4: The oxidised glutathione is reduced back to GSH by the enzyme glutathione reductase (Gred) which uses NADPH as the electron donor. Reaction 5: Some transition metals (e.g. Fe^{2+}, Cu^{+} and others) can breakdown hydrogen peroxide to the reactive hydroxyl radical (Fenton reaction). Reaction 6: The hydroxyl radical can abstract an electron from polyunsaturated fatty acid (LH) to give rise to a carbon-centred lipid radical ($L^{\bullet}$). Reaction 7: The lipid radical ($L^{\bullet}$) can further interact with molecular oxygen to give a lipid peroxyl radical ($LOO^{\bullet}$). If the resulting lipid peroxyl radical $LOO^{\bullet}$ is not reduced by antioxidants, the lipid peroxidation process occurs (reactions 18-23 and 15-17). Reaction 8: The lipid peroxyl

radical ($LOO^{\bullet}$) is reduced within the membrane by the reduced form of Vitamin E (T-OH) resulting in the formation of a lipid hydroperoxide and a radical of Vitamin E ($T\text{-}O^{\bullet}$). Reaction 9: The regeneration of Vitamin E by Vitamin C: the Vitamin E radical (T-O•) is reduced back to Vitamin E (T-OH) by ascorbic acid (the physiological form of ascorbate is ascorbate monoanion, $AscH^{-}$) leaving behind the ascorbyl radical ($Asc^{\bullet-}$). Reaction 10: The regeneration of Vitamin E by GSH: the oxidised Vitamin E radical ($T\text{-}O^{\bullet}$) is reduced by GSH. Reaction 11: The oxidised glutathione and the ascorbyl radical ($Asc^{\bullet-}$) are reduced back to GSH and ascorbate monoanion, AscH−,respectively, by the dihydrolipoic acid (DHLA) which is itself converted to α-lipoic acid (ALA). Reaction 12: The regeneration of DHLA from ALA using NADPH. Reaction 13: Lipid hydroperoxides are reduced to alcohols and dioxygen by GPx using GSH as the electron donor.

Lipid peroxidation process: Reaction 14: Lipid hydroperoxides can react fast with Fe^{2+} to form lipid alkoxyl radicals (LO•), or much slower with Fe^{3+} to form lipid peroxyl radicals ($LOO^{\bullet}$). Reaction 15: Lipid alkoxyl radical ($LO^{\bullet}$) derived for example from arachidonic acid undergoes cyclisation reaction to form a six-membered ring hydroperoxide. Reaction 16: Six-membered ring hydroperoxide udergoes further reactions to from 4-hydroxy-nonenal. Reaction 17: 4-hydroxynonenal is rendered into an innocuous glutathiyl adduct (GST, glutathione S-transferase). Reaction 18: A peroxyl radical located in the internal position of the fatty acid can react by cyclisation to produce cyclic peroxide adjacent to a carbon-centred radical. Reaction 19: This radical can then either be reduced to form a hydroperoxide (reaction not shown) or it can undergo a second cyclisation to form bicyclic peroxide which after coupling to dioxygen and reduction yields a molecule structurally analogous to the endoperoxide. Reaction 20: Formed compound is an intermediate product for the production of malondialdehyde. Reactions 21, 22, 23: Malondialdehyde can react with DNA bases Cytosine, Adenine, and Guanine to form adducts M1C, M1A and M1G, respectively [4].

The polyol pathway

The enzyme aldose reductase converts toxic aldehydes to inactive alcohols [50]. Glucose is a poor substrate for aldose reductase, but at high concentrations this enzyme converts glucose to sorbitol, initiating the polyol pathway of glucose conversion to fructose. Similar to GSH reductase, the enzyme aldose reductase is dependent upon NADPH as a cofactor. Therefore, excessive activation of the polyol pathway depletes cytosolic NADPH and subsequently depletes GSH, leaving the cell vulnerable to free radicals produced during normal cellular functions such as electron transfer. In addition, accumulation of sorbitol produces a cellular osmotic stress that also generates oxidative stress [51]. This pathway has been a target for therapies against diabetes complications including neuropathy. Recent human genetic and biochemical data link polymorphisms of the aldose reductase gene to increased risk of diabetic complications, with the principal allele associated with increased disease risk causing a 2- to 3-fold increase in aldose reductase gene expression [52].

Protein kinase C (PKC) activation

Prooxidants react with the regulatory domain to stimulate PKC activity, but antioxidants react with the catalytic domain of PKC and inhibit its activity [53]. Activity of PKC is increased in the retina, kidney, and microvasculature of diabetic rats, but there is no evidence for altered activity of any of the PKC isoforms in the peripheral neurons [54,55]. This suggests that the lipolytic pathway and production of diacylglycerol are the main causes of PKC activation in nonneuronal cell types [56]. Once activated, PKC activates the MAPKs that phosphorylate transcription factors and thus alter the balance of gene expression [57]. Specifically, it is the stress genes such as heat shock proteins and c-Jun kinases that increase

after PKC activation and can lead to apoptosis or vascular atherosclerosis. A role for PKC in inducing neuronal degeneration possibly at the level of the endothelial cell is implicated by three studies. Inhibition of PKCß reduces oxidative stress and normalizes blood flow and nerve conduction deficits in diabetic rats [56,58].High glucose causes nuclear factor-B activation in endothelial cells, leading to ROS formation, and cellular activation, an effect that is prevented in the presence of a PKC inhibitor [59] .

Reduced anti-oxidant defense

In addition to the increased generation of free radicals in diabetes, impaired generation of naturally occurring antioxidants also result in increased oxidative injury by failure of protective mechanisms [60]. Antioxidant defense system appears to be compromised in diabetic patients. It has been demonstrated that reduced scavenging of free radicals by SOD [61] and lake of GSH [62] and ascorbic acid [63] are associated with diabetic vascular pathology. Reduced other antioxidants, such as vitamin A and E, uric acid, reduced activity of catalase and GPx are also found in diabetes [64-66]. The mechanism by which the antioxidant reserve is reduced is not clear. Protein damage due to the protein glycosylation may be a mechanism that lowers the activities of primary antioxidant enzymes [61]. In addition, GSH deficiency may result from depletion of NADH in polyol pathway [67].

Oxidative stress and diabetic complications

Diabetic nephropathy

Hyperglycemia leads to increased accumulation of AGEs in the kidney, which in part, via enhanced free radical mechanisms, induces cross linking of proteins and membrane peroxidation. This results in increased membrane permeability and loss of integrity. Hyperglycemia-induced secondary mediator's activation such as protein kinase-C (PKC), mitogen-activated protein (MAP) kinases and cytokine lead to the production of predominantly vasodilatory prostaglandins. The increased glomerular permeability and glomerular blood flow initiate and maintain the early proteinuria and glomerular hyperfiltration which are also responsible for oxidative stress-induced renal injury in the diabetic condition [68]. In the late stage of diabetes, prostaglandines (F2 isoprostanes), formed by free radical catalysed lipid peroxidation, cause progressive vasoconstriction and systemic hypertension. The continued deposition of extracellular matrix proteins in the glomeruli and the reduced blood flow eventually lead to glomerulosclerosis and renal failure [69] .

A causal relationship between oxidative stress, ECD and diabetic nephropathy has been established [70] by observations that: a) High glucose can directly cause endothelial cell dysfunction (ECD) and increases oxidative stress in glomerular mesangial cells, a target cell of diabetic nephropathy. b) Lipid peroxides and 8-hydroxydeoyguanosine, indices of oxidative tissue injury, were increased in the kidneys of diabetic patient with albuminuria. The oxidized lipids are toxic to tissues especially the vascular endothelium, glomerular mesangial cells, smooth muscle cells and renal tubular epithelium. c) Oxidative stress induces mRNA expression of NFkB genes which in turn promotes production of proinflammatory proteins-TGF-B, fibronection, laminin, elastin, IL-1, IL-6, and PDGF, and d) Inhibition of oxidative stress ameliorates all the manifestation associated with ECD and diabetic nephropathy [71-74].

Diabetic neuropathy

One of the mechanisms by which hyperglycemia causes neural degeneration is via the increased oxidative stress that accompanies diabetes. Metabolic and oxidative insults often cause rapid changes in glial cells. Key indicators of this response are increased synthesis of glial fibrillary acidic protein (GFAP) and S100B, both astrocytic markers [75]. Hyperglycemia activates many signaling mechanisms in cells. Four major pathways that can lead to cell injury downstream of hyperglycemia are illustrated in fig 3.

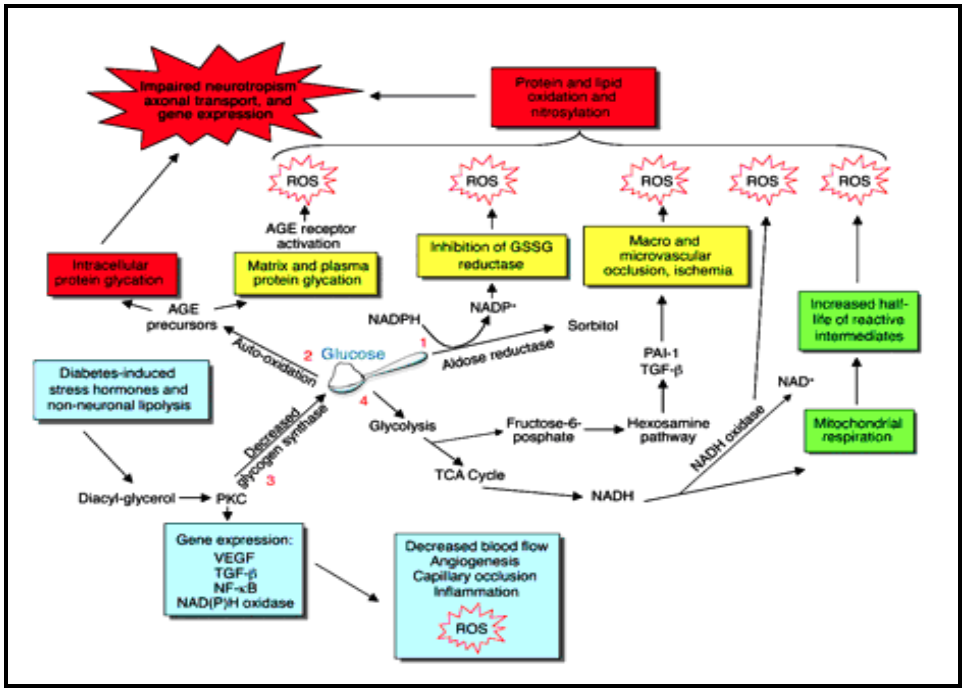

Fig 3: Four major pathways that can lead to diabetic neuropathy.

1) Excess glucose shunts to the polyol pathway that depletes cytosolic NADPH and subsequently GSH. In the development of neuropathy, the hyperglycemic state leads to an increase in action of the enzymes aldose reductase and sorbitol dehydrogenase. This results in the conversion of intracellular glucose to sorbitol and fructose.

The accumulation of these sugar products results in a decrease in the synthesis of nerve cell myoinositol, required for normal neuron conduction. Additionally, the chemical conversion of glucose results in a depletion of nicotinamide adenine dinucleotide phosphate stores, which are necessary for the detoxification of reactive oxygen species and for the synthesis of the vasodilator nitric oxide. There is a resultant increase in oxidative stress on the nerve cell and an increase in vasoconstriction leading to ischemia, which will promote nerve cell injury and death. Hyperglycemia and oxidative stress also contribute to the abnormal glycation of nerve cell proteins and the inappropriate activation of protein kinase C, resulting in further nerve dysfunction and ischemia [76-79]. 2) Excess glucose also undergoes autooxidation to produce AGEs that impair protein function and also activate RAGEs that use ROS as second messengers. 3) PKC activation both further increases hyperglycemia and also exacerbates tissue hypoxia. 4) Overload and slowing of the electron transfer chain leads to escape of reactive intermediates to produce $O_2^{-\cdot}$ as well as activation of NADH oxidase that also produces $O_2^{-\cdot}$. A unifying mechanism of injury in each case is the production of ROS that impair protein and gene function [80].

Diabetic retinopathy

Diabetic retinopathy develops in patients with both type 1 and type 2 diabetes and is the major cause of vision loss and blindness in the working population. The main risk factor of diabetic retinopathy is hyperglycemia accompanied by enhanced mitochondrial production of reactive oxygen species and oxidative stress, formation of advanced glycation end products (AGE) and hexosamines, increased polyol metabolism of glucose. The severity of vascular injury depends on the individual genetic background and is modified by other metabolic and haemodynamic factors influencing numbers of intracellular signalling molecules such as PKC, MAPK or NF-kappaB. In diabetes, damage to the retina occurs in the vasculature (endothelial cells and pericytes), neurons and glia, pigment epithelial cells and infiltrating immunocompetent cells: monocytes, granulocytes, lymphocytes [81]. The retina has high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders retina more susceptible to oxidative stress [82]. It has been suggested that the correlation between hyperglycemia, changes in the redox homeostasis, and oxidative stress are the key events in the pathogenesis of diabetic retinopathy. Animal studies have demonstrated that oxidative stress contributes not only to the development of diabetic retinopathy but also to the resistance of retinopathy to reverse after good glycemic control is reinstituted the metabolic memory phenomenon [83]. Resistance of diabetic retinopathy to reverse is probably attributed to accumulation of damaged molecules and ROS that are not easily removed even after good glycemic control is reestablished. Superoxide and hydrogen peroxide levels are elevated in the retina of diabetic rats and in retinal cells incubated in high glucose media [83-86]. Membrane lipid peroxidation and oxidative damage to DNA (indicated by 8-hydroxy-2'-deoxyguanosine, 8-OHdG), the consequences of ROS induced injury, are elevated in the retina in diabetes [87-90]. In diabetes, the activities of antioxidant defense enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, glutathione reductase, glutathione peroxidase, and catalase are diminished in the retina [91]. The levels of GSH intracellular antioxidant are decreased in the retina in diabetes [92], and the enzymes responsible for its metabolism are compromised [93,94] . Apart from the antioxidant defense enzymes, non- enzymatic antioxidants such as vitamin C, vitamin E, and β-carotene that exist biologically for the regulation of redox homeostasis are also depressed during hyperglycemia induced oxidative stress [95] .

Diabetic cataract is a major complication of diabetes mellitus, and is primarily caused by polyol accumulation and glycation within lens fibers and the epithelium. Blood sugar can passively diffuse into lens tissue insulin independently and then be converted by aldose reductase to polyols, which can not diffuse passively out of the lens, and thus they accumulate. This accumulation of polyols causes osmotic changes, which lead to lens hydration and swelling that are followed by biochemical and physiological damage to cell membranes [18] .

5.4 Coronary heart disease (CHD)

Hyperglycaemia could aggravate CHD by several mechanisms. Hyperglycaemia alters vascular reactivity, platelet aggregation, clot formation, lysis and foam cell formation. All these factors enhance atherosclerosis. Hyperglycaemia predisposes to formation of advanced glycation end products. Increased uptake of AGE in vascular wall has shown to increase vascular permeability and decrease its vasodilatory response to nitroglycerin and acetylcholine [96]. AGE products also activate leucocytes or vessel wall cells to enhance production of oxygen metabolites that can promote lipoprotein oxidation.

Glycation of matrix proteins also increases their stability, thus promoting their accumulation, which is characteristic of hyperplastic diseases [97]. Normal endothelium synthesises substances that contribute to maintenance of vascular tone such as PGI2 and endothelium derived relaxation factor (now identified as Nitric Oxide – NO). PGI2 also inhibits platelet aggregation. Synthesis of NO is decreased and its removal increased by advanced glycation end products. Its inactivation by oxygen free radicals is also enhanced and this leads to increased vascular resistance and platelet adhesion to endothelium [97]. Decreased synthesis of PGI2 also adds to vasoconstriction and vessel wall reactivity. Enhanced activity of protein kinase C in endothelial cells might contribute to synthesis of vasoconstrictor prostanoids [97]. Impaired endothelium mediated vasodilatation could result in hypertension and shear induced platelet aggregation. Reactivity of platelets and adhesion to vessel wall is enhanced by interaction with glycated LDL and increased levels of von Willebrand factor [98]. Altered metabolism due to decreased platelet polyphosphoinositide content causes enhanced release of thromboxane B2 which leads to increased platelet aggregation. Hyperglycaemia leads to glucose autooxidation and release of free radicals. Free radicals activate platelets and moreover, oxidative stress decreases the activity of antithrombin III [99]. This coupled with increased fibrinogen levels results in enhanced fibrin clot formation. Lipoprotein (a) is known to inhibit acitivity of plasminogen. Decreased fibrinolytic activity further increases the stability of the clot formed. Lipoprotein levels are also increased in diabetics [99]. The macrophage is the precursor of the cholesterol engorged foam cell, characteristic of atherosclerotic lesions. LDL and VLDL isolated from diabetic patients are taken up by the macrophages and they induce cholesterol ester synthesis and accumulation giving rise to foam cells [100]. Hyperglycaemia and oxidative stress can increase monocyte binding to endothelial cells and thus result in accelerated atherosclerosis [101] .

Insulin resistance and hyperinsulinaemia correlate strongly with VLDL-TG secretion rate and plasma TG concentration. Once the pool of VLDL-TG increases, HDL levels are decreased. The risk for Coronary artery disease (CAD) is further accentuated with increase in small dense LDL levels, which in turn is associated with insulin resistance. Another associated finding is that of postprandial lipaemia [102]. Hyperinsulinaemia inhibits the binding of HDL to its specific binding sites on human skin fibroblasts and thus retards the transfer of intracellular sterol to cell membrane and its transfer to HDL [103]. These actions may contribute to cholesterol accumulation and formation of foam cells. Plasminogen activator inhibitor-1 (PAI-1) levels vary directly with insulin resistance and are associated with recurrent myocardial infarction in younger men [99]. Hyperinsulinaemia and insulin resistance are associated with enhanced thrombus formation in response to endothelium injury: This coupled with low fibrinolytic activity accentuates the athero-thrombotic process.

Dyslipidemia

The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma [104]. Increased production of very low density lipoprotein (LDL) by the liver results from increased delivery of fatty acids because of decreased utilization by muscle and increased delivery of fatty acids from visceral abdominal fat to the liver via the portal circulation. Decreased catabolism of postprandial triglyceride rich lipoprotein particles because of reduced lipoprotein lipase activity accentuates diabetic dyslipidemia [105]. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart diseases [106] .

Diabetic foot ulcers

Neuropathy in diabetic patients is manifested in the motor, autonomic, and sensory components of the nervous system. Damage to the innervations of the intrinsic foot muscles leads to an imbalance between flexion and extension of the affected foot. This produces anatomic foot deformities that create abnormal bony prominences and pressure points, which gradually cause skin breakdown and ulceration. Autonomic neuropathy leads to a diminution in sweat and oil gland functionality. As a result, the foot loses its natural ability to moisturize the overlying skin and becomes dry and increasingly susceptible to tears and the subsequent development of infection. The loss of sensation as a part of peripheral neuropathy exacerbates the development of ulcerations. As trauma occurs at the affected site, patients are often unable to detect the insult to their lower extremities. As a result, many wounds go unnoticed and progressively worsen as the affected area is continuously subjected to repetitive pressure and shear forces from ambulation and weight bearing [107,108]. Neuropathy, mechanical stress, and macrovascular disease are involved in the pathogenesis of diabetic foot ulceration. Implicit in the development of gangrene and ulceration is the recognition that these factors interact with the microcirculation, resulting in the failure of skin capillary flow to meet nutritive requirements. These late functional abnormalities include loss of autoregulation and reduced hyperaemic responses which interact with loss of neurogenic flow regulation, disturbed endothelial function, and abnormal rheology to produce the familiar clinical picture of the diabetic foot [109] .

Peripheral vascular disease

Peripheral vascular disease is a contributing factor to the development of foot ulcers in up to 50% of cases [110,111]. It commonly affects the tibial and peroneal arteries of the extremities. Endothelial cell dysfunction and smooth cell abnormalities develop in peripheral arteries as a consequence of the persistent hyperglycemic state [76]. There is a resultant decrease in endothelium-derived vasodilators leading to constriction. Further, the hyperglycemia in diabetes is associated with an increase in thromboxane A2, a vasoconstrictor and platelet aggregation agonist, which leads to an increased risk for plasma hypercoagulability [112]. Platelet function is abnormal in diabetes as well. Expression of both glycoprotein Ib and IIb/IIIa is increased, augmenting both platelet von Willebrand factor and platelet–fibrin interaction [113].The intracellular platelet glucose concentration mirrors the extracellular environment and is associated with increased superoxide anion formation and PKC activity and decreased platelet-derived NO [113,114]. Hyperglycemia further changes platelet function by impairing calcium homeostasis and thereby alters aspects of platelet activation and aggregation, including platelet conformation and release of mediators [115] . In diabetes, plasma coagulation factors (eg, factor VII and thrombin) and lesion-based coagulants (eg, tissue factor) are increased, and endogenous anticoagulants (eg, thrombomodulin and protein C) are decreased [116-118]. Also, the production of plasminogen activator inhibitor-1, a fibrinolysis inhibitor, is increased [113, 116, 119-125]. Thus, a propensity for platelet activation and aggregation, coupled with a tendency for coaulation, is relevant to a risk of thrombosis. There is also the potential for alterations in the vascular extracellular matrix leading to stenosis of the arterial lumen [112]. Moreover, smoking, hypertension, and hyperlipidemia are other factors that are common in diabetic patients and contribute to the development of Peripheral arterial disease [126]. Cumulatively, this leads to occlusive arterial diseases that result in ischemia in the lower extremity and an increased risk of ulceration in diabetic patients.

Diabetic ketoacidosis

During the normal fed state, an increase in glucose concentration stimulates the pancreatic beta cells to secrete insulin and inhibits the pancreatic alpha cell production of glucagon. This increase in insulin and decrease in glucagon stimulates the liver to undergo glycolysis and glycogenesis of the ingested carbohydrates. The body is able to maintain the blood glucose levels within a very narrow range during both the fasting and fed state by increasing and decreasing the concentration of insulin. In diabetic ketoacidosis there is a decrease in the ratio of insulin to glucagon as well as an increase in the concentrations of the counter regulatory hormones. This decreased insulin and increased glucagons levels impairs entry of glucose into cells, which results in gluconeogenesis, glycogenolysis, and the breakdown of triglycerides (lipolysis) causing a complex metabolic disturbance of carbohydrate, protein, and lipid metabolism. As the glucose concentration and osmolality of extra cellular fluid increases, an osmolar gradient is created that draws water out of the cells leading to osmotic diuresis. With continued osmotic diuresis, hypovolemia eventually occurs which leads to a progressive decline in glomerular filtration rate, and hyperglycemia becomes more severe [127] .

The counter regulatory hormones play a key role by triggering pathways that are active when inadequate insulin is in the liver and peripheral tissues. This results in excessive lipolysis leading to the accumulation of free fatty acids. These free fatty acids are used for the formation of large amounts of acetyl Co A. This excess of acetyl Co A cannot enter the citric acid cycle, but is used for ketone body formation instead. Most of the acid produced in normal metabolism is in the form of carbon dioxide which is readily excreted from the lungs. In contrast ketone bodies cannot be excreted through the pulmonary system, which leads to metabolic acidosis [128,129].

Antioxidants

'Antioxidants' are substances that neutralize free radicals or their actions [130].Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. α-Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols, α-lipoic acid, glutathione etc.

Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase that catalyses the dismutation of superoxide to H_2O_2 and catalase that breaks it down to water [130,131]. Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of peroxyl radicals are effected. The effectors include various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. At the repair and reconstitution level, mainly repair enzymes are involved [132, 130, 131,133] .

Conclusion

The molecular mechanisms whereby oxidative stress causes insulin resistance are undefined. In a variety of tissues, hyperglycaemia and elevated free fatty acids result in the generation of ROS and RNS, leading to increased oxidative stress. In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive signaling pathways, such as NF-kB, MAPK, JNK/SAPK, PKC, AGE/ RAGE, sorbitol, and others. The consequence is the production of gene products such as VEGF and others that cause cellular damage, and are ultimately responsible for the long-term complications of diabetes. In addition, activation of the same or similar pathways appears to mediate insulin resistance and impaired insulin secretion. It is our view that there appears to be a common biochemical basis that involves oxidative-stress induced activation of stress sensitive signaling pathways.

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