

Oxidative stress has been well implicated in the pathogenesis of various human diseases. Presently mechanistic considerations of the oxidative stress pathogenesis in most vital organ systems, e.g., nervous system, cardiovascular system, male/female reproductive system, and autoimmune disease-related systems, will be discussed.

Neurodegenerative Diseases: Parkinson's and Alzheimer's Diseases

The brain with major neurons and astrocytes is especially sensitive to the oxidative stress because of the lipid peroxidation in membranes containing high level of polyunsaturated fatty acids (PUFA). Oxidation of lipids, proteins, and DNA in neurons generates many by-products such as peroxides, alcohols, aldehydes, ketones, and cholesterol oxides which are toxic to the blood lymphocytes and macrophages, influencing the in vivo defense system (Ferrari 2000). ROS attacks proteins, oxidizing both the backbone and side chains, which in turn reacts with the amino acid side chain to form carbonyl functions. ROS attacks nucleic acids, causing DNA-protein cross-links and strand breaks, and modifies purine and pyrimidine bases resulting in the DNA mutations (Mattson 2003). ROS are particularly active in the brain and neuronal tissues as the

excitatory amino acids and neurotransmitters, whose metabolism produces ROS, which serve as the sources of oxidative stress and result in neural damage. Most significant ill effect on the neurons takes place by dysregulation of the intracellular calcium signaling pathways initiated by the ROS in neuronal cell death (Ermak and Davies 2002). Excitotoxic effects initiated by the ROS induce intracellular calcium influx, leading to the activation of glutamate receptors and apoptosis in the neurodegeneration. All these insults ultimately reflect into the specific disorders.

Oxidative stress has been linked to a range of chronic neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). In these conditions, nerve cells in the brain and spinal cord are damaged or lost, leading to either functional loss (ataxia) or sensory dysfunction (dementia). Mitochondrial dysfunctions and excitotoxicity and finally apoptosis result into the pathological conditions in each disease (Gandhi and Abramov 2012). Neurodegeneration mediates a number of factors including the environmental and genetic predisposition. Oxidative stress and additional free radical generation catalyzed by the redox metals play important role in the neurodegeneration. AD and PD being the main neurodegenerative disorders will be the special focus in the present write-up.

Role of Pathogenesis in AD and PD

Alzheimer's Disease (AD): AD is characterized by the loss of neurons or their synapses in the cerebral cortex and certain subcortical regions and in turn the progressive cognitive decline. Both amyloid plaques and neurofibrillary tangles, as clearly visible microscopically in the AD-affected brain (Tiraboschi et al. 2004), are due to the insoluble deposits of the extracellular amyloid ($A\beta$) peptide around the neurons. This small amyloid- β protein (39–43 amino acids) originates from a larger protein called amyloid precursor protein (APP), a transmembrane protein that penetrates through the neuron's membrane. APP is critical to the growth, survival, and post-injury repair of the neurons (Priller et al. 2006). Another protein named tau normally stabilizes the microtubules (supporting structures of the neurons guiding nutrients and molecules from the body of the cell to the end of the axon and back) on phosphorylation. In AD, tau proteins get hyperphosphorylated and then pair with other threads, creating neurofibrillary tangles and disintegrating the neuron's transport system (Hernandez and Avila 2007). Amyloid fibrils disrupt the cell's calcium ion homeostasis and induce apoptosis (Yankner et al. 1990).

Further, AD is characterized by the amyloid plaques deposition by chelating $A\beta$ with the transition metal ions (Cu^{2+} , Zn^{2+} , Fe^{3+}). In $A\beta$ the histidine residues at position 6, 13, and 14 coordinate with the transition metals. Binding of Cu^{2+} and Fe^{3+} results in a chemical reaction altering oxidation state of both the metals, producing H_2O_2 catalytically in the presence of transition metals, and finally giving toxic $OH\cdot$ free radicals (Opazo et al. 2002). AD brains show evidence of ROS-mediated injury. There is an increase in the levels of malondialdehyde and 4-hydroxynonenal in the brain and cerebrospinal fluid of AD patients compared to the controls.

Parkinson's Disease (PD): PD is clinically characterized by the progressive rigidity, bradykinesia, and tremor, whereas pathologically by a progressive degeneration of the dopaminergic neurons with age and deposition of inclusion bodies (Lewy bodies) of the protein α -synuclein in the

substantia nigra. In PD brain, the concentration of PUFA in the substantia nigra is reduced, while the levels of lipid peroxidation markers (malondialdehyde and 4-hydroxynonenal) are increased (Dalfo et al. 2005). Protein oxidative products as protein carbonyls are seen at high level in the PD brain compared to the controls, and also nitration and nitrosylation of certain proteins due to RNS in the PD brain are also observed (Brown and Borutaite 2004). Oxidative stress in the PD brain results in the increased levels of 8-hydroxydeoxyguanosine and also increase in the common deletions in mitochondrial DNA of the dopaminergic neurons in PD substantia nigra (Bender et al. 2006). Further, dopamine (neurotransmitter) is also a very good metal chelator and electron donor to generate toxic-free radicals. It has high tendency to coordinate with Cu^{2+} and Fe^{3+} and reduce metals to generate H_2O_2 (Gerard et al. 1994). Mutations in the α -synuclein protein modulate negatively the substantia dopamine activity that initiates neuronal cytoplasmic accumulation and interaction of dopamine with iron, producing ROS (Lotharius and Brundin 2002).

Oxidative Stress in the Pathogenesis of PD and AD: Neurons with long axons and multiple synapses require more energy for the axonal transport or long-term plasticity, resulting in mitochondrial dysfunction and further neurodegeneration. These features in different neuronal groups exhibit different degrees of oxidative stress. For example, in the hippocampus, CA1 neurons generate higher levels of superoxide anion than the CA3 neurons and exhibit higher levels of expression of both the antioxidant and ROS-producing genes (Wang and Michaelis 2010). Various sources of ROS production and their influence are shown in Fig. 2.1.

Mitochondria dysfunction and activation of ROS-producing enzymes (as discussed in Chap. 1), e.g., NADPH oxidase, xanthine oxidase, and monoamine oxidase, have been implicated in generating ROS and in turn neurodegeneration. Oxidative damage and the associated mitochondrial dysfunction may result in the energy depletion, accumulation of the cytotoxic mediators, and the cell death. Autophagic activity helps in the mitochondrial turnover, in which membrane autophagosomes

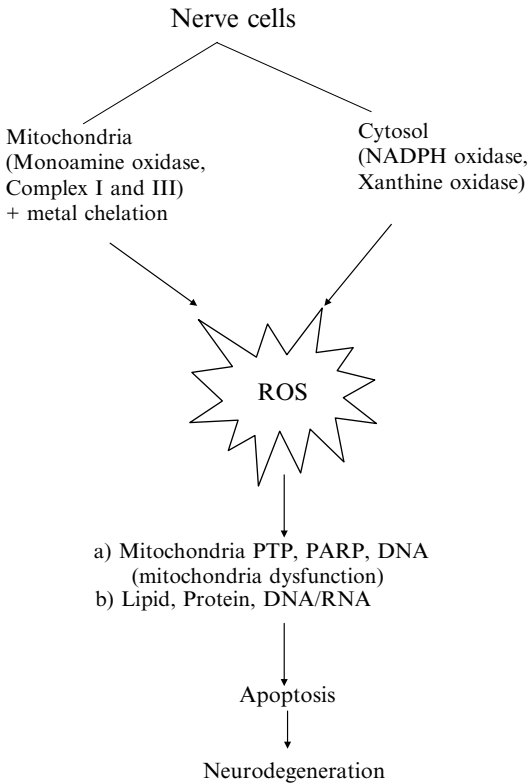


Fig. 2.1 ROS production and effects in nerve cells

sequester damaged/oxidized or dysfunctional intracellular components and organelles and direct them to the lysosomes for degradation. The absence of the autophagy (or mitophagy) may result in an abnormal mitochondrial function and oxidative or nitrative stress. The mitochondrial dysfunction includes the respiratory chain dysfunction and oxidative stress, reduced ATP production, calcium dysregulation, mitochondrial permeability transition pore (PTP) opening, and many more.

Mitochondrial pathology is evident in many neurodegenerative diseases including AD and PD. Mitochondrial dysfunction in the human brain is involved in the pathogenesis of PD and degeneration of dopaminergic neurons. The substantia nigra of PD patients shows reduced activity of the complex I. Complex I inhibitors such as rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and pesticides cause neurological changes similar to PD (Shapira

2008). Rotenone or MPP⁺ (active metabolite of MPTP) produces superoxide anions in the submitochondrial particles, and the neurotoxic effects (Lotharius and O'Malley 2000) of these is due to the production of oxidative stress as antioxidants prevents these changes. α -Synuclein, inner mitochondrial membrane-associated protein, interacts with the mitochondrial complex I function (Chinta et al. 2010). In the transgenic mice, over-expression of α -synuclein impairs mitochondrial function, increases oxidative stress, and enhances nigral pathology induced by MTPP (Song et al. 2004). Another protein, parkin, associated with the outer mitochondrial membrane, prevents cell death by inhibiting the mitochondrial swelling, cytochrome c release, and caspase activation (Darios et al. 2003). Parkin deficiency causes oxidative stress and mitochondrial impairment (Muftuoglu et al. 2004). Further, a protein PINK1 (phosphatase and tensin homologue, PTEN-induced kinase 1) is a mitochondrial kinase, and its deficiency results in impaired respiration with inhibition of complex I activity, reduced substrate availability, and rotenone-like increased production of ROS in mitochondria (Gandhi et al. 2009). PINK1 deficiency also results in an inability to handle cytosolic calcium challenges due to an impairment of the mitochondrial calcium overload. A combination of ROS production and mitochondrial Ca²⁺ initiates opening of the mitochondrial permeability transition pore (PTP), which allows translocation of the proapoptotic molecules from the mitochondria to the cytosol and that triggers apoptotic cell death.

A reduction in the complex IV activity has been demonstrated in mitochondria from the hippocampus and platelets of AD patients, as well as in the AD animal models. Accumulation of the A β leads to oxidative stress, mitochondrial dysfunction, and energy failure prior to the development of the plaque pathology. Deregulation of the calcium homeostasis has been demonstrated in AD, with A β causing increased cytoplasmic calcium levels and mitochondrial calcium overload, resulting in an increase in ROS production and opening of the PTP (Abramov et al. 2003). In addition to the alterations in mitochondrial bioenergetics, dysregulation of calcium homeostasis,

excitotoxicity, oxidative stress (inflammation), and other mechanisms involve also the protein misfolding leading to the aggregates, proteasome dysfunction, and neuroinflammation in PD (Hirsch et al. 2013).

In AD, the NADPH oxidase has been shown to contribute toward oxidative stress. Activation of NOX2 has been demonstrated in brains of AD patients, and also its deficiency has been shown to improve the AD in a mouse model (Park et al. 2008). At cellular level, amyloid- β induced activation of NADPH oxidase in rat primary culture of microglial cells and human phagocytes, through B-class scavenger receptor, CD36 (Wilkinson et al. 2006). A β also activates NOX by including calcium entry into astrocytes (Abramov et al. 2003) and induces opening of the mitochondrial permeability transition pore, mPTP (Abramov et al. 2004). This oxidative stress signal is passed on to the neighboring neurons, which is more damaging than to the astrocytes. In PD, in both the rotenone- and MPTP-induced toxin models, activation of NOX2 in microglia occurs (Gao et al. 2003). Genetic models of PD also exhibit increased oxidative stress. In one such model, loss of PINK1 function is associated with the increased ROS production by NADPH oxidase in the midbrain neurons. The NADPH oxidase is activated by the high cytosolic calcium concentration, leading to the overproduction of superoxide which inhibits the plasma-lemmal glucose transporter resulting in the deregulation of the mitochondria metabolism (Gandhi et al. 2009). The oxidative stress response by the microglial cells due to the NADPH oxidase plays a central role in the pathology of PD. This response in microglia occurs through the activation of the ERK signaling pathway by proinflammatory stimuli, leading to the phosphorylation and translocation of the p47 (phox) and p67 (phox) cytosolic subunits, the activation of membrane-bound PHOX, and the production of ROS (Peterson and Flood 2012).

A β is able to activate production of H₂O₂ in the cytosol of neocortical neurons (Kaminsky and Kosenko 2008). Inhibitor of XO, allopurinol, significantly suppressed OH^{*} generation in rat striatum of toxic models of PD induced by

paranonylphenol and MPP⁺ (Obata et al. 2001), suggesting a potential role for xanthine oxidase in the oxidative stress associated with PD. Monoamine oxidase A (MAOA) and monoamine oxidase B (MAOB), flavoenzymes, are located in the outer membrane of the mitochondria. They have a role in the oxidative catabolism of important amine neurotransmitters, including serotonin, dopamine, and epinephrine (Edmondson et al. 2009).

Electrical and Biological Effects

Direct electrical excitatory effect using low-frequency stimulation of the spinal cord or of the thalamus has been used for the diagnostic or even therapeutic applications. However, high-frequency stimulations (HFS) are considered for damaging and inactivating the neuronal structures, such as nuclei of the basal ganglia and also thalamus/sub-thalamic nucleus. Intracerebral recordings in the human patients tend to show the arrest of electrical firing in the recorded places. More recent data from the in vitro biological studies show that HFS profoundly affects the cellular functioning and particularly the protein synthesis, suggesting that it could alter the synaptic transmission by reducing the production of neurotransmitters (Benabid et al. 2005). Similarly, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), noninvasive cortical stimulation methods, have been successfully employed for the treatment of movement disorders (Wu et al. 2008). Studies show beneficial effects on the clinical symptoms in PD and support the effects on motor and nonmotor symptoms. Rebalancing of the distributed neural network activity and induction of dopamine release occur.

While exploring the oxidative signaling and inflammatory pathways in AD (Anderson et al. 2001), it was shown that activation of microglia with the beta-amyloid peptide activates the production of cyclooxygenase-2, iNOS, and TNF- α . These are considered as key mediators of the pathological cascade of AD. ox-LDL caused a sustained activation of the JNK that resulted in the phosphorylation of the transcription factor c-jun, which was abolished in neurons pretreated with

flavonoids. Furthermore, ox-LDL induced the cleavage of procaspase-3 and increased caspase-3-like protease activity in neurons and leading to apoptosis. Dietary flavonoids protect against neuronal apoptosis through selective actions within stress-activated cellular responses, including protein kinase signaling cascades (Schroeter et al. 2001). Guanosine protects human neuroblastoma cells against mitochondrial oxidative stress by inducing heme oxygenase-1 via PI3K/Akt/GSK-3 β pathway (Dal-Cim et al. 2012).

Cdk5 (cyclin-dependent kinase 5), a proline-directed serine/threonine kinase, plays multiple roles in neurons development, survival, phosphorylation of cytoskeletal proteins, and synaptic plasticity (Smith and Tsai 2002). Uncontrolled phosphorylation activity of Cdk5 has been closely associated well with AD and PD. Under oxidative stress condition, mitochondrial dysfunctions, excitotoxicity, A β exposure, calcium dyshomeostasis, and inflammation lead to rise in the intracellular Ca²⁺, activating calpain which cleaves p35 (activator of Cdk5) to p25 (Fig. 2.2) forming a more stable yet hyperactive Cdk5/p25 complex which aberrantly hyperphosphorylates various cytoskeletal proteins leading to neurodegeneration (Lee et al. 2000). Activation of Cdk by oxidative stress in AD causes hyperphosphorylation of τ , neurofilament, and other cytoskeletal proteins (Lee et al. 2000). Accumulation of A β in cortical neurons induces cleavage of p35 to p25 resulting in activation of kinases and inhibition of phosphatases proceeding NFT (neurofilament tangles) formation, primary markers of AD (Lee et al. 2000). Cdk5-mediated phosphorylation of peroxidases substrates reduces their enzymatic activities resulting in the ROS accumulation within cells (Sun et al. 2008).

HtrA2, a serine protease, was identified to be involved in the neuroprotection, and mutations adjacent to the two phosphorylation sites (S142 and S400) have been found in the PD patients. Cdk5 phosphorylates the HtrA2 at S400 in a p38-dependent manner in humans and mouse cell lines and brain (Fitzgerald et al. 2012). This phosphorylation is involved in maintaining mitochondrial membrane potential under stress conditions.

The activation of JNK pathways is critical for the naturally occurring cell death during development as well as for the pathological death associated with neurodegenerative diseases. Several in vitro and in vivo studies have reported alterations of JNK pathways potentially associated with the neuronal death in PD and AD. Also, Nrf2-ARE signaling pathway is an attractive therapeutic target for neurodegenerative diseases.

Cascades Leading to Dopamine Cell Degeneration

Metabolism of dopamine by the monoamine oxidase generates H₂O₂ and the auto-oxidation of dopamine generates superoxides. Thus, endogenous dopamine as well as exogenous treatment with levodopa (used in PD) may contribute additional oxidative stress insult, like mitochondrial dysfunction (Muller 2011). Also the monoamine oxidase (MAO)-induced metabolism of dopamine and production of H₂O₂ have an important role in the physiological calcium signaling in astrocytes (Vaarmann et al. 2010). In PD, adult substantia nigra pars compacta dopaminergic neurons create intracellular calcium oscillations through L-type calcium channels. This metabolic stress is counterbalanced by the ATP demanding pumps to restore the calcium concentration. It has been demonstrated that the opening of these L-type ion channels results in higher levels of oxidative stress in the mitochondria of such neurons (Surmeier et al. 2011).

Antioxidants Link in Neurodegenerative Disorders

The aim of using antioxidants in any pathology is to neutralize ROS and other kinds of free radicals produced as a consequence of the oxidative stress (Uttara et al. 2009). Brain cells and especially neurons require effective antioxidant protection because of the higher consumption of oxygen

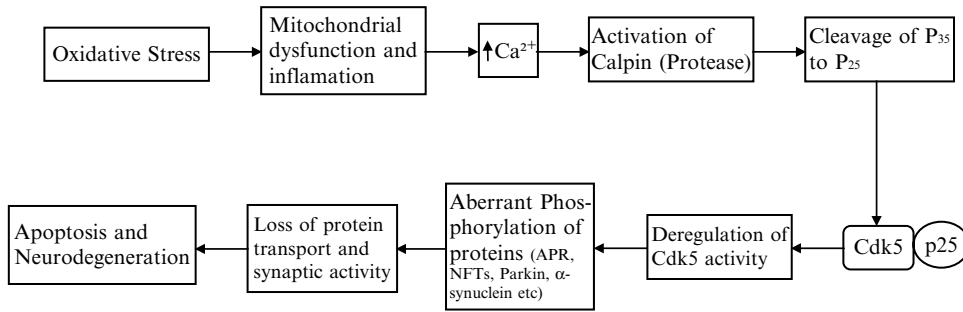


Fig. 2.2 Oxidative stress and Cdk5 in pathophysiology of neurons

(about tenfold), having long life duration and a prominent role of the nitric oxide to form RNS such as peroxynitrite. Glutathione peroxidase is known to localize primarily in glial cells, in which its activity is tenfold higher than in the neurons (Margis et al. 2008). Reduced glutathione (GSH, nonprotein thiol) is the main antioxidant in CNS (Dringen and Hirrlinger 2003) and non-enzymatically acts directly with free radicals. Glutathione peroxidase and glutathione reductase can act enzymatically to remove H_2O_2 and maintain glutathione in a reduced state (Dringen and Hirrlinger 2003).

Widely studied antioxidant therapies have been vitamin E (α -tocopherol, the major scavenger of lipid peroxidation in the brain), vitamin C (intracellular reducing molecule), and coenzyme Q10 (transfers electrons from the complexes I and II to complex III in the respiratory chain). Vitamin E supplementation in an AD mouse model resulted in the improved cognition and reduced A β deposition (Conte et al. 2004). The reduction of the amyloid deposition was particularly noted in young AD mice (Sung et al. 2004). Coenzyme Q10 has been shown to have multiple protective effects within the mitochondria. Administration of CoQ10 protects MPTP-treated mice from dopaminergic neuronal loss and also attenuated α -synuclein aggregation. Neuroprotection by CoQ10 in an MPTP-primate model has also been reported (Du and Yan 2010).

However, no antioxidant benefits of vitamin E and/or vitamin C in either AD or PD from large randomized controlled trials have been observed (Dumont et al. 2010). Furthermore, a large

meta-analysis of vitamin E clinical trials, CoQ10 trials, and a glutathione trial in PD concluded that there were only minor treatment benefits in the CoQ10 trials that may have been due to the improvement in the respiratory chain deficit rather than a direct antioxidant action (Weber and Ernst 2006). Animal experiments show that antioxidants are effective in the early stages of the disease. Other considerations are to regulate the bioavailability and the effective targeting of the antioxidants. This aspect is discussed in detail in Chap. 6.

Another recent and efficient consideration is of exploiting signaling pathways to mimic the antioxidant activity. Very recently, guanosine have been found protective against mitochondrial oxidative stress in human neuroblastoma cells by a signaling pathway that implicates P13K/Akt/GSK-3 β proteins and induction of antioxidant gene enzyme, heme oxygenase-1 (HO-1) (Dal-Cim et al. 2012). The importance of Nrf2-ARE signaling pathway has been well reviewed to be an attractive therapeutic target for neurodegenerative diseases with chemopreventive agents (vanMuiswinkel and Kuiperij 2005). Nrf2, a key redox regulatory factor, induces endogenous cytoprotective genes of antioxidant- and anti-inflammatory proteins. Dopamine-induced mPTP opening and dopamine-induced cell death could be prevented by inhibition of ROS production by provision of respiratory chain substrates and by alteration in calcium signaling, which suggest potential therapeutic strategies for neuroprotection in PD (Gandhi et al. 2012).

Cardiovascular Diseases

Hypercholesterolemia and Atherosclerosis

Events like intake of high-fat diet (HFD), hypercholesterolemia in the blood, and cholesterol deposition in the arterial wall are accepted as high risk factors for the development of atherosclerosis. This risk has been positively correlated with low-density lipoproteins (LDL), total cholesterol, and total cholesterol/high-density lipoprotein (HDL) ratio (Castelli 1986). In its initial stages, atherosclerosis lesions in the intima of the large, elastic, and muscular arteries consist of the fatty streak that is characterized by the lipid (principally cholesterol and its esters) accumulation in macrophages, T lymphocytes, and smooth muscle cells in addition to the ingested lipoprotein–proteoglycan complexes in more complex foam cells (Ross 1991). This further leads to the fibrous plaques resulting from the synthesis of collagen, elastin, and proteoglycans by smooth muscle cells and macrophages migrated to the intima (Sowers 1992). Qualitative changes in these fibrous plaques, at some later stage, may result in hemorrhage, ulceration, and/or thrombosis, leading subsequently to the arterial occlusions. This results in the ischemic necrosis of vital organs with far-reaching consequences. Peroxidation of polyunsaturated fatty acids (PUFA) gives rise to free radicals and endogenous peroxides, which are highly reactive and have both chemotactic and cytotoxic properties. Hypercholesterolemic atherosclerosis is associated with an increase in the blood and aortic tissue of the MDA content (a LPO product) and OFR producing activity of the polymorphonuclear leukocytes (Prasad and Kalra 1992).

Increased concentration of LDL cholesterol in the plasma constitutes a major risk factor for the atherosclerosis as is demonstrated by various clinical, epidemiological, and genetic studies (Jialal and Devaraj 1996). Both diet-induced hypercholesterolemia and LDL-receptor defective models are characterized by the alteration in the level and composition of the plasma lipoproteins.

Lipid-rich LDL and VLDL both have been shown to induce a dose-dependent increase in the monocyte adhesion to the endothelial cells (Endemann et al. 1987). Modified forms of LDL such as acetyl LDL or oxidized LDL are taken up by the scavenger receptor mechanism, resulting in cholesterol accumulation and subsequent foam cell formation (Brown and Goldstein 1983). Clinical and epidemiological studies show that increased levels of LDL cholesterol promote the atherosclerosis. LDL can be oxidatively modified by all major cells of the arterial wall and play a significant role in atherosclerosis in vivo. Macrophages play the role of scavengers as these cells have a large capacity to store altered LDL and diet-induced β -VLDL (Goldstein et al. 1980). Studies indicate that macrophages have only a limited number of receptors for the specific uptake of native LDL, but these can avidly take up certain chemically modified forms of LDL via an alternative specific, saturable receptor – the acetyl-LDL receptor (Parathasarathy et al. 1986).

Minimally oxidized LDL (MM-LDL), initially formed in the subendothelial space, can be taken up by the classical LDL receptor through the apoB and does not associate with the macrophages as normal LDL. However, a significant proportion of the unsaturated acyl chains of the cholesteryl esters and phospholipids in mid-oxidized LDL have been oxidized to hydroperoxides, isoprostanes, and short-chain aldehydes that have potent biological effects. This LDL stimulates production of the monocyte chemotactic protein-1 (MCP-1), resulting in monocyte binding to the endothelium and its subsequent migration into the subendothelial space where monocyte colony-stimulating factor (M-CSF) is also formed (Berliner et al. 1995). M-CSF promotes the differentiation and proliferation of monocytes into macrophages. These macrophages can in turn modify MM-LDL into a more oxidized form and are not recognized by the LDL receptor but become foreign and thus are taken up by the scavenger receptors pathway in macrophages leading to appreciable cholesterol ester accumulation and foam cell formation (Witztum and Steinberg 1991) and resulting in cholesterol accumulation (Fig. 2.3). Extensive oxidation up to 50 % of the cholesterol is

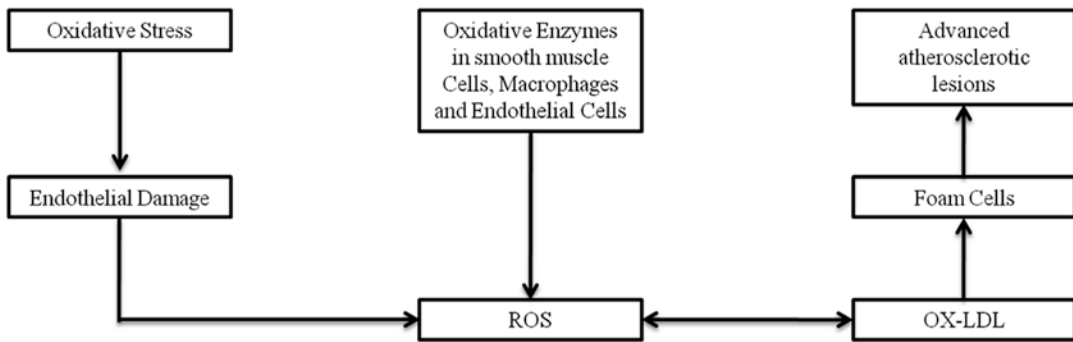


Fig. 2.3 Interaction of oxidative stress and atherosclerosis

converted into 7-ketocholesterol and other oxysterol and binds to the scavenger receptors such as SR-A and/or CD36. Further, most of the unsaturated fatty groups are oxidized to a complex mixture of products, and apoB is extensively fragmented, derivatized, and cross-linked and there is a substantial aldehyde modification to result in the products such as malondialdehyde and hydroxynonenal. Oxidatively modified LDL (ox-LDL) present novel properties, e.g., it is a potent chemoattractant for the monocytes, a potent inhibitor of macrophages mobility in the arterial wall (Jialal and Devaraj 1996), thus can promote its retention in the wall, is cytotoxic, which could promote endothelial dysfunction and atherogenesis by altering the expression of genes in the arterial wall (Jialal and Devaraj 1996).

Another lipoprotein, high-density lipoprotein (HDL), is known for its negative risk factor for the development of atherosclerosis. HDL apolipoprotein, ApoA1, promotes cholesterol efflux from the peripheral deposits within the vasculature and with subsequent transport to the liver for excretion (reverse cholesterol transport). HDL possesses the antioxidant activity that is primarily mediated via inhibition of the oxidation of LDL with a subsequent reduction of the cellular uptake by the monocyte–macrophage system and hence antiatherogenic effect (Nicholls et al. 2005). Antioxidant mechanism of HDL involves its chelation properties due to the presence of proteins such as ceruloplasmin on the surface of the lipoprotein (Kunitake et al. 1992), and also HDL has been demonstrated to accept

hydroperoxides from oxidized membranes in in vitro studies, which would potentially provide a pathway for the excretion or detoxification (Klimov et al. 2001). Additionally, Apo A-I has been demonstrated to reduce lipid hydroperoxides into redox-inactive compounds, which thus terminates the chain reactions of lipid peroxidation (Garner et al. 1998). The function of HDL is also due to its associated enzyme proteins exhibiting antioxidant activity. Paraoxonase 1 (PON 1) is an HDL-associated esterase/lactonase that exhibits the anti-inflammatory and antioxidant activity (Kaur and Bansal 2009; Precourt et al. 2011). It degrades oxidized fatty acids within the LDL particle which in turn exhibits an inhibitory effect on a variety of pro-atherosclerotic functions, including a decrease in binding of the circulating monocytes to the endothelium.

It is clear that ROS are responsible for the endothelial dysfunction and development of atherogenesis. Several enzymatic pathways contribute within the vessel wall for the production of different oxidants, such as NADPH oxidase, nitric oxide synthase, myeloperoxidase, xanthine oxidase, lipoyxygenase/cyclooxygenase, and mitochondrial respiratory chain/oxidative phosphorylation.

Endothelial NAD(P)H oxidase is a major source of ROS in the vasculature and can be activated by the stimuli such as angiotensin II, thrombin, platelet-derived growth factor, TNF- α , IL-1, and VEGF. C-reactive protein (CRP), a cardiovascular risk marker, has been reported to induce the superoxide production in human

aortic endothelial and smooth muscle cells (Venugopal et al. 2003) and may regulate NAD(P)H oxidase through the various activation pathways involving protein kinases and nitric oxide synthases (NOS). In active eNOS, reductase domain (containing the binding sites for NADPH, FAD, and FMN) and an oxygenase domain (containing Zn, tetrahydrobiopterin, BH₄, heme, and L-arginine) are linked by a hinge region to which calmodulin binds (Stocker and Kearney 2001). Under normal conditions, these enzymes transfer electrons from a heme group in the oxygenase domain to the substrate L-arginine to form L-citrulline and NO; BH₄ serves as a cofactor in this process (Bevers et al. 2006). If the availability of either BH₄ or L-arginine decreases, eNOS switches from a coupled state (generate NO) to an uncoupled state (generate O₂^{•-}) because the electrons from the heme reduce oxygen to form O₂^{•-}. NO[•] reacts rapidly with O₂^{•-} to generate ONOO⁻ which causes vascular dysfunction. iNOS is found in the vascular smooth muscle cells and also in activated macrophages in the atherosclerotic lesions. It is induced by the microbial endotoxins or cytokine stimulation (Murthy et al. 2004).

Myeloperoxidase (MPO), a heme-containing enzyme, catalyzes the conversion of Cl⁻ to the hydrochlorous acid (HOCl). Chlorinated biomolecules are considered specific markers of the oxidation reactions catalyzed by the enzyme. The MPO/H₂O₂/Cl⁻ system can give rise to 3-chlorotyrosine, chlorohydrins such as those of cholesterol and fatty acids, α-chloro fatty acid aldehydes, and free amino acid or protein-bound tyrosyl radicals. Tyrosyl radicals themselves may participate in the secondary oxidation reactions, including the oxidation of LDL. MPO/H₂O₂/Cl⁻ system and HOCl also oxidize nitrite to the nonradical oxidant, nitryl chloride (NO₂Cl) and the radical [•]NO₂, both of which promote nitration and can convert tyrosine into 3-nitrotyrosine. MPO plays a major role in the generation of nitrating species in vivo, and that formation of 3-nitrotyrosine is strictly dependent on the availability of [•]NO₂ (Carr and Frei 2001). MPO has been shown to co-localize with macrophages in the human artery wall, and its characteristic

oxidation products have been detected in atherosclerotic lesions (Malle et al. 2000). In hypercholesterolemic rabbits, atherosclerosis resulting from the diet was ascribed to the xanthine oxidase-induced oxidative stress (Ohara et al. 1993). Lipoxygenase (LPO) is another important source of ROS production in the vascular wall and these nonheme-containing dioxygenases oxidize PUFA to hydroperoxy fatty acids derivatives (Kuhn et al. 2005). The mitochondrial ROS have also been shown to be associated with the enhanced susceptibility to the atherosclerosis.

Hypoxia and Stroke

Hypoxia basically refers to a reduced supply of oxygen to the part of a tissue or organ, and when brain is involved, it is called the cerebral hypoxia. It is caused by any event that severely interferes with the brain's ability to receive or process oxygen. Prolonged hypoxia induces the neuronal cell death via apoptosis resulting in a hypoxic brain injury (Malhotra et al. 2001). When the brain is traumatized by the low oxygen levels by choking off the blood supply, this condition is called brain stroke. The widespread self-destruction takes place for days or even a week after the initial stroke.

Oxidative stress plays an important role in the acute ischemic stroke pathogenesis. Free radical formation and subsequent oxidative damage may be a factor in the stroke severity. In one of the studies, serum NO, MDA, and GSH levels were significantly elevated in the acute stroke patients compared to the control within 48 h of stroke (Ozkul et al. 2007). The “neurological deficit score” was negatively correlated with both MDA and NO levels; however, GSH levels were taken as an adaptive mechanism during this period. In ischemic stroke, the cerebral vasculature is a major target of the oxidative stress playing a critical role in the pathogenesis of ischemic brain injury following a cardiovascular attack. Superoxide and its derivatives have been shown to cause the vasodilation via opening of K⁺ channels and altered vascular reactivity, breakdown of the blood–brain barrier (BBB), and focal

destructive lesions in the animal models of the ischemic stroke (Allen and Bayraktutan 2009).

Among the several stress factors known to induce BBB breakdown, hypoxia is probably the most represented. Evidence of the oxidative stress occurring during hypoxia/ischemic situation raises its possible contribution to the barrier breakdown (Lochhead et al. 2010). Oxygen deprivation injury constitutes one of the most important pathophysiological mechanisms leading to the BBB breakdown. Oxidative stress occurring under O₂ deprivation insult (Chandel et al. 1998) raises the possible contribution of ROS signaling to the BBB breakdown. Involvement of ROS in the RBE4 ECs barrier function disruption during hypoxia was evidenced (AI Ahmad et al. 2009). Further, it was demonstrated that the oxidative stress significantly contributes to the barrier breakdown because artificial generation of the ROS decreased EC integrity (AI Ahmad et al. 2012) and also treatment of RBE4 monolayers with antioxidants during O₂ deprivation stress resulted in overall improvement of both barrier function and cell survival. This provides insight into the effect of oxidative stress on the BBB function during hypoxic insult.

Further, hypoxia-inducible factor 1 (HIF-1), a master regulator of hypoxia-responsive genes, regulates the expression of a broad range of genes that facilitate adaptation to the low O₂ conditions. Its targets include genes that code for the molecules that participate in the vasomotor control, angiogenesis, erythropoiesis, cell proliferation, and energy metabolism. All of these genes may potentially contribute to the recovery of neuronal cells following cerebral ischemia and reperfusion, and hence regulating HIF-1 induction and accumulation is a highly promising therapeutic approach for the cerebral ischemia. A number of mechanisms have been proposed to account for the neuroprotective effect of the HIF-1 (Guo et al. 2009): expression of its downstream gene product erythropoietin has been found to protect cells from hypoxic/ischemic injuries; VEGF expression (another downstream gene of HIF-1) counteracts detrimental ischemic injuries; prevents apoptotic cell death through inhibition of cytochrome c release, caspase activation, and

PARP cleavage; suppresses p53 activation; and thereby maintains cell survival.

HIF-1 may contribute to the cellular and tissue damage. It has been reported that the HIF-1 may mediate apoptosis during hypoxia/ischemia. HIF-1-induced apoptosis has been observed in the embryonic stem (ES) cells under hypoxic conditions (Carmeliet et al. 1998). The study indicates that in response to hypoxia, HIF-1 α (an inducible subunit) accumulates, associates, and stabilizes the active wild-type p53. It is possible that this increase in the p53 protein is responsible for the apoptosis reported in the hypoxia ES cells. The experimental observations support that HIF-1 α may induce cell death in a severe and prolonged ischemia and promote cell survival following mild ischemic insults (Baranova et al. 2007). Thus, HIF-1 plays an important role in the fate of ischemic insults with a double-edged sword effect. Its effects possibly depend on the degree of severity of the insult. Further explanations of the mechanism of the HIF-1 induction in ischemic neurons and its effect on the ischemic brain tissue are well reviewed by Shi (2009).

Further, considering the effective therapeutic targeting of the acute stroke, NOX4 is the most abundant vascular isoform, induced in stroke. Upon ischemia, NOX4 was induced in the human and mouse brain (Kleinschnitz et al. 2010). Mice deficient in the NOX4 (Nox4^{-/-}) of either sex were largely protected from the oxidative stress, blood–brain barrier leakage, and neuronal apoptosis, after both transient and permanent cerebral ischemia. Restoration of the oxidative stress reversed the stroke-protective phenotype in Nox4^{-/-} mice. NOX4 therefore represents a major molecular source of oxidative stress in cerebral ischemia including some cases of human stroke and novel class of drug target for stroke therapy.

ROS and Myocardial Infarction

Myocardial infarction (MI), commonly known as heart attack, results from the interruption of the blood supply to a part of the heart, causing heart cells to die. This is most common due to the occlusion of the coronary artery following rupture of

atherosclerotic plaque in the wall of an artery. The resulting ischemia and ensuing oxygen shortage, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle tissue (myocardium). Further, hypoxia and hypoxia–reoxygenation (H/R) are components of the tissue ischemia and reperfusion implicated in the myocardial infarction. Reperfusion (or reoxygenation) injury is the tissue damage caused when blood supply returns to the tissue after a period of ischemia or lack of oxygen.

The inflammatory response is partially responsible for the damage of the reperfusion injury. White blood cells, carried to the area by newly returning blood, release a host of inflammatory factors such as the cytokines (reviewed in Neri et al. 2013) as well as free radicals in response to the tissue damage. Such reactive species may also act indirectly in the redox signaling to turn on apoptosis. White blood cells may also bind to the endothelium of small capillaries, obstructing them and leading to more ischemia.

In prolonged ischemia (60 min or more), the hypoxanthine is formed as breakdown of ATP metabolism. The enzyme xanthine oxidase results in molecular oxygen being converted into the highly reactive superoxide and hydroxyl radicals. Xanthine oxidase also produces uric acid, which may act both as a prooxidant and as a scavenger of the reactive species such as peroxynitrite. Excessive nitric oxide produced during the reperfusion reacts with superoxide to produce the potent reactive species peroxynitrite. Such radicals attack the cell membrane lipids, proteins, and glycosaminoglycans, causing further damage. They may also initiate specific biological processes by the redox signaling. In the first few minutes after the reperfusion, a cascade of biochemical changes results in the opening of the mitochondrial permeability pore (MPT pore) in the mitochondrial membrane of cardiac cells, water enters into the mitochondria to make it dysfunctional and collapse, and calcium released to overwhelm the next mitochondria causes mitochondria energy to reduce or stop completely, resulting in cell death. So protecting the mitochondria is a viable cardioprotective strategy (Hausenloy and Yellon 2008). Cyclophilin D is a protein induced by the

excessive calcium flow to interact with other pore components and help in opening the MPT pore. Inhibiting cyclophilin D with the cyclosporine has been shown to prevent the opening of the MPT pore and protect the mitochondria and cellular energy production from the excessive calcium inflows (Javadov and Karmazyn 2007).

A number of studies have described the transplantation of the mesenchymal stem cells (MSCs) from the bone marrow as a strategy for the cardiac repair following myocardial infarction (Huang et al. 2010). However, the therapeutic efficacy of this procedure is greatly limited by the poor survival of the donor MSCs in the infarcted heart, especially because of the oxidative stress environment. It is widely reported that the HDL lowers the risks associated with the ischemic diseases (Duffy et al. 2012), especially because of the reverse cholesterol transport characteristic. In another study (Xu et al. 2012), preconditioning with the HDL resulted in the higher MSC survival rates, improved cardiac remodeling, and better myocardial function than in the MSC control group.

Studies have shown that the heat shock factor-1 (HSF1), a transcription factor for the heat shock proteins (HSPs), confers protection against the cardiovascular diseases, such as ischemia/reperfusion injury and myocardial infarction. HSF1 can prevent cardiomyocytes from apoptosis induced by the various stimulations and cytotoxic oxidative stress leads to apoptosis as a final event (Matsuzawa and Ichijo 2008). JNKs regulate the apoptosis of the H₂O₂-stimulated human pulmonary vascular endothelial cells and play an important role in regulating the left ventricular remodeling by promoting apoptosis (Yamaguchi et al. 2003). HSF1 and HSPs are protective against the oxidative damage (Yan et al. 2005), but also alleviates ischemia/reperfusion injury by prohibiting JNK activity (Zou et al. 2003). Thus, HSF1 may prevent the cardiomyocytes from apoptosis under the various stimulations via inhibition of the intracellular ROS production and then JNK activity. In a recent study, cultured cardiomyocytes of the neonatal rats were transfected with HSF1, ASK1, or both of them before exposure to the H₂O₂ and ROS generation, and JNK activity and apoptosis were examined (Zhang et al. 2011).

H₂O₂ increased intracellular ROS generation and apoptotic cells as expected, and all these cellular events were greatly inhibited by overexpression of HSF1.

Further, protein tyrosine phosphatase (PTP), important regulator in the cell signaling (as detailed in Chap. 5), serves as a molecular target for the ROS. Intermittent oxygenation of the cardiac tissue where reperfusion following ischemia is known to be an etiological factor for the tissue damage associated with the ischemic disease (Brandes et al. 2010). To explore the mechanism, both the respiratory system of mitochondria and NADPH oxidases have been implicated as sources of elevated ROS levels in situations of reperfusion or reoxygenation. To explain the impact of ROS in hypoxia–reoxygenation and ischemia/reperfusion on PTP activity/oxidation and its consequences for tyrosine signaling, a recent study was performed to investigate the potential effects of reoxygenation or reperfusion on PTP-oxidation and tyrosine kinase signaling using cell culture models and an ex vivo model of isolated perfused rat hearts (Sandin et al. 2011). This study demonstrated that the cultured cells exposed to hypoxia followed by reoxygenation and heart tissue subjected to ischemia/reperfusion are characterized by the increased oxidation of PTPs. Further analysis revealed that both cytosolic and receptor-like PTPs are susceptible to H/R-induced PTP-oxidation. Enhanced Erk1/2 phosphorylation was identified as PTP-oxidation sensitive signaling component, which was inactivated in a ROS-sensitive manner after treatment with antioxidant NAC. These findings have the general implication of hypoxia or ischemia affecting signaling processes under pathophysiological conditions.

Antioxidants and CVD

Seeing the association of oxidative stress with various CVDs, antioxidants were used to prevent these diseases in clinical trials with different formulations but produced mixed results. These are well reviewed (Singh and Jialal 2006; Vogiatzi et al. 2009). In contrast to the positive outcomes

from various trials with different formulations with vitamins, e.g., vitamins E and C, other antioxidant supplementation studies did not show any positive effect on the primary endpoints related to the cardiovascular events. One apparent reason considered the unexplored threshold doses of the type of the antioxidant with its formulation. Another important parameter considered was the knowledge of the redox reactions in *in vivo* conditions. For instance, vitamin C supplementation exerts prooxidant and antioxidant effects and at high doses exhibit DNA damage.

Complex informations on the experimental studies regarding antioxidant influence exist. In these studies, the statins increase catalase and BH₄ levels and in turn increase NO production and inhibit LDL oxidation while at the same time restoring vitamin C and E levels and endogenous antioxidants such as ubiquinone and glutathione. Vitamins C and E can inhibit the oxidative process for the prevention of atherosclerotic lesions. Vitamin C stimulates the increase of BH₄ levels and the activity of NO synthase and improves endothelial dysfunction (Lonn et al. 2001). Also vitamin C administration in patients with coronary syndromes, arterial hypertension, and hypercholesterolemia increases NO bioavailability. Vitamin E administration also reduces LDL oxidation and improves NO bioactivity and endothelial dysfunction owing to the malnutrition. Co-administration of vitamins C and E seems to improve endothelial function in hyperlipidemic patients (Engler et al. 2003). Various investigators have related the ability of dietary antioxidant to prevent the formation of highly oxidized LDL. Natural antioxidants such as polyphenols, which are found in fruits and vegetables, seem to be extremely useful, can improve lipid metabolism, and reduce ox-LDL (Wassmann et al. 2001).

Reproductive Systems Disorders (Male and Female)

The reproductive system in an organism, whether in male or female, works for the purpose of reproduction, which is a fundamental characteristic of life. Apart from the external organs of the

reproductive system, major internal organs include the gamete producing gonads (testicles or ovaries). Human reproduction takes place as internal fertilization of the female ovum with male sperms. Upon successful fertilization and implantation, gestation of the fetus then occurs within the female's uterus and finally birth of the child. The male reproductive system has one function, production of sperms, whereas the female reproductive system has two: the first is to produce egg cells and the second is to protect and nourish the offspring until birth.

Male Reproduction

The reproductive ability of sexually mature males is dependent upon the capacity of testes to produce large number of structurally and functionally active spermatozoa and maintenance of adequate levels of androgens (male sex hormones). Spermatogenesis is a precisely controlled process, occurring in the seminiferous tubules of testis, which gives rise to mature spermatozoa through a complex sequence of events that result in marked changes in the nuclei of the developing germ cells and finally formation of mature spermatozoa. The cycle of seminiferous epithelium of the testis is a dynamic and time-scaled phenomenon that forms well-defined cellular associations (or stages) within each tubule showing the various cell types in specific ratios to one another. Any alteration in these ratios indicates disturbance in the normal progression of spermatogenesis which can lead to male infertility.

Endocrinology and Gonadotoxicity

The principal androgen, testosterone, a steroid, is manufactured by the interstitial (Leydig) cells of the testes. Secretion of the testosterone increases sharply at puberty, and apart from the development of secondary sexual characteristics of men, testosterone is also essential for the production of sperms. Production of testosterone is controlled by the release of luteinizing hormone (LH) also called interstitial cell-stimulating hormone (ICSH) from the anterior pituitary gland, which

in turn is controlled by the release of the gonadotropin-releasing hormone (GnRH) from the hypothalamus. The level of testosterone is under the negative-feedback control from hypothalamus:

Hypothalamus → GnRH → Pituitary → LH
→ Testes → Testosterone

Of the many causes of gonadotoxicity in males, oxidative stress has been identified as one factor that affects fertility status and has been extensively studied. The generation of ROS can be exacerbated by a multitude of environmental, infectious, and lifestyle-related etiologies. A wide range of the industrial by-products and waste chemicals (polychlorinated biphenyls, nonylphenol, or dioxins) causes male infertility, both directly and indirectly. Increasing the presence of the by-products of manufacturing, such as lead, mercury, or cadmium in the environment, has been suggested to pose a serious threat to reproductive health. Lead has been reported to be gonadotoxic with a tendency of suppressing the LH and testosterone levels in animals (Taiwo et al. 2010). Sovol (a commercial mixture of polychlorinated biphenyls) was found to be gonadotoxic in male rat testis (decreased testis weight, sperm cell numbers in ejaculation, testicular weight, testosterone/estradiol in blood and increase in peroxidation) (Agletdinov et al. 2008). These results suggest that the disorders may play an important role in pathogenesis of the male infertility caused by the persistent organic pollutants. Also, with the advent of the modern cancer treatment, survival rates have improved substantially raising new concerns toward quality of life issues such as future fertility and offspring welfare. Chemopreventing agents act by hindering rapidly proliferating cells, hence exerting their gonadotoxic effect also (Ragheb and Sabanegh 2010). The extent of the damage to the germ cells and eventual fecundity depend on the class of chemotherapeutic agents, dosage, spermatogenetic stage targeted, as well as the original pretreatment fertility potential of the patients. In a study (Bahadur et al. 2005), semen quality from patients with leukemia, lymphoma, testicular cancer,

and other malignant neoplasms before and after gonadotoxic treatment were monitored. All categories of the patients displayed varying degrees of azoospermia and oligospermia, and recovery of the gonadal function was not significant. This highlighted the importance of ensuring sperm banking before treatment.

Infertility

Infertility has been a major medical and social pre-occupation; however, the past few decades have witnessed a remarkable decline in the fertility rates in the industrialized world. Out of the many well-known causes of male infertility, about 40–90 % of the cases are due to deficient and defective sperm production of unidentifiable origin. Oxidative stress is a common pathology seen in approximately half of all the infertile men. Oxidative injury to spermatozoa is considered as a major cause of the sperm dysfunction and the incidence of male infertility. Increased levels of ROS have been correlated with decreased sperm motility, increased sperm DNA damage, sperm cellular membrane lipid peroxidation, and decreased efficacy of oocyte–sperm fusion. All the cellular components, including lipids, proteins, nucleic acids, and sugars, are the potential targets of oxidative stress. The extent of oxidative stress-induced damage depends on the nature, amount, and the duration of the exposure of ROS and also on the extracellular factors such as temperature, oxygen tension, and the composition of the surrounding environment (Aitken and Fisher 1994). The following influences of ROS are observed:

Lipid Peroxidation (LPO): ROS attacks PUFA in sperm plasma membrane, leading to a cascade of chemical reactions called lipid peroxidation (Halliwal 1984). The free radicals react with fatty acid chains and release reactive lipid species, which further react with molecular oxygen to form the lipid peroxyl radical. Peroxyl radicals can react with fatty acids to produce lipid free radicals. Thus, lipid peroxidation in the spermatozoa is a self-propagating reaction.

Sperm Motility: The increased formation of ROS has been correlated with reduction of sperm motility (Armstrong et al. 1999). Decrease in motility is explained that H_2O_2 diffusion across the

membranes into cells inhibits the activity of vital enzymes such as glucose-6-phosphatase dehydrogenase (G6PD) that control the rate of glucose flux via hexose monophosphate shunt and in turn control the intracellular availability of NADPH. Another hypothesis involves a series of interrelated events resulting in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with the reduction in membrane fluidity that is necessary for sperm–oocyte fusion (deLamirande and Gagnon 1995).

DNA Damage: Exposing the sperm to artificially produced ROS causes DNA damage in the form of modification of all the bases, production of base-free sites, deletions, frame shifts, DNA cross-links, and chromosomal rearrangements. Oxidative stress also is associated with the high frequencies of single- and double-strand DNA breaks (Aitken and Krausz 2001). DNA bases and phosphodiester backbones are other sites that are susceptible to the peroxidative damage by ROS. High levels of ROS mediate the DNA fragmentation that is commonly observed in the spermatozoa of infertile individuals. Also, mutations in the mitochondrial DNA, which is also susceptible to oxidative damage, may cause defect of mitochondrial energy metabolism, and therefore lower levels of mutant DNA may compromise sperm motility in vivo (Spiropoulos et al. 2002).

Oxidative Damage to Protein: Oxidative attack on proteins results in the site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electric charge, and increased susceptibility or extreme tolerance to proteolysis. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain amino acids. Sulfur-containing amino acids and, specifically, thiol groups are very susceptible (Farr and Kogama 1991).

Apoptosis: ROS may also initiate a chain of reactions that ultimately lead to apoptosis. Apoptosis may help to remove abnormal germ cells and prevent their overproduction during spermatogenesis (Sakkas et al. 1999), thus maintaining the nursing capacity of the Sertoli cells. High levels of ROS cause DNA damage and disrupt the inner and outer mitochondrial

membranes, releasing cytochrome c and activating the caspases and at least apoptosis.

Free Radicals and Sperm Functions

ROS are generated mainly by the sperm and seminal leukocytes within semen (Garrido et al. 2004) and produce infertility by two key mechanisms. First, they damage the sperm membrane, decreasing sperm motility and its ability to fuse with the oocyte. Second, ROS can alter the sperm DNA, resulting in the passage of defective paternal DNA on the conceptus. Several studies show positive/negative correlation between seminal leukocytes numbers and ROS production. Activation state of the leukocytes was considered to play an important role in determining final ROS output. This is supported by the observations of a positive correlation between seminal ROS production and proinflammatory seminal plasma cytokines such as interleukins (IL-6, IL-8), and TNF α .

Small amounts of ROS produced by the spermatozoa are essential to many of the physiological processes such as fertilization, capacitation, hyperactivation, motility, and sperm–oocyte fusion (Agarwal et al. 2004). ROS such as nitric oxide or superoxide anion have also shown to promote capacitation and the acrosome reaction (Griveau et al. 1995). They also act as second messenger molecules and transmit signals by increasing the influx of calcium ions, which leads to increased production of ATP through a series of chain reactions. Capacitation has been shown to occur in the female genital tract, a process carried out to prepare the spermatozoa for interaction with oocyte. During this process, the levels of intracellular calcium, ROS, and tyrosine kinase increase, leading to an increase in cAMP (Aitken 1995). This facilitates hyperactivation of the spermatozoa, a condition in which they are highly motile. However, only capacitated spermatozoa exhibit hyperactivated motility and undergo a physiological acrosome reaction, thereby acquiring the ability to fertilize (deLamirande et al. 1997).

Most semen specimens contain variable number of the leukocytes, with neutrophils as the predominant type, and are considered potential sources of ROS (Aitken 1995). Activated neutrophils generate and release ROS in high concentrations to

form cytotoxic reactions against nearby cells and pathogens. Leukocytospermia has long been associated with decreased sperm concentration, motility, and morphology as well as decreased hyperactivation and defective fertilization (Moskovstev et al. 2007). Spermatozoa's own production of ROS is independent of the leukocytes and depends on the maturation level of the sperm.

During spermatogenesis, a defect of the cytoplasmic extrusion mechanism results in release of spermatozoa from germinal epithelium carrying surplus residual cytoplasm, and these cytoplasmic droplets are a major source of ROS (Gomez et al. 1996). The resulting spermatozoa are immature and functionally defective, and residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose-6-phosphate dehydrogenase (G6PD) (Aitken 1999). G6PD (NADPH oxidase, NOXs) at the sperm plasma membrane controls the glucose flux and intracellular production of β -nicotinamide adenine dinucleotide phosphate (NADPH) through the hexose monophosphate shunt. NADPH is used to fuel the generation of ROS via NADPH oxidase located within the sperm membrane. NADPH-dependent oxidoreductase (diaphorase) at the mitochondrial level also contributes ROS (Gavella and Lipovac 1992). As a result, teratozoospermic sperm produces increased amounts of ROS compared with morphologically normal sperm.

Further, one group investigated that NOX 5 enzyme of sperm is a calcium-dependent NADPH oxidase and is quite distinct from leukocyte NADPH oxidase, with NOX 5 activity not being controlled by protein kinase C as occurs in the leukocyte. While intrinsic (by sperm) and extrinsic (by leukocyte, 1000 \times) ROS production is negatively correlated with sperm DNA integrity, the relationship is significantly stronger for the intrinsic ROS production. The close proximity between intrinsic ROS production and sperm DNA makes it a more important variable in terms of fertility potential. Also spermatozoa are rich in mitochondria for the constant supply of energy for their motility. Unfortunately, when spermatozoa contain dysfunctional mitochondria, increased production of ROS occurs, affecting further

mitochondrial function. Such a relationship could be due to two mutually interconnected phenomena: ROS causing damage to the mitochondrial membrane and the damaged mitochondrial membrane further causing an increase in ROS production. Increased ROS levels have been correlated with decreased sperm motility. One hypothesis suggests that H_2O_2 diffuses across the membrane into the cells and inhibits the activity of some vital enzymes. Another theory involves a series of interrelated events resulting in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity that is necessary for sperm–oocyte fusion. Loss of motility observed when spermatozoa are incubated overnight is highly correlated with the lipid peroxidation status of the spermatozoa.

Varicocele patients (dilatation of testis veins) have increased ROS in serum, testis, and semen samples. Increased nitric oxide also has been demonstrated in the spermatic veins of patients with varicocele, which could be responsible for the spermatozoa dysfunction (Ozbek et al. 2000). ROS in patients with varicocele is due to the excessive presence of xanthine oxidase, a source of superoxide anion from the substrate xanthine and nitric oxide in dilated spermatic veins. On the other hand, it has been recorded that varicocelectomy increases the concentration of antioxidants such as SOD, catalase, GPx, and vitamin C, in seminal plasma as well as improves sperm quality (Mostafa et al. 2001). Patients with varicocele had increased 8-hydroxy-2-deoxyguanosine (8-OHdG), indicating oxidative DNA damage (Smith et al. 2006). Analysis conclude that oxidative stress significantly increased in infertile patients with varicocele as compared with normal sperm donors and antioxidant concentrations were significantly lower in infertile patients compared with controls.

Antioxidants' Role

Increased ROS generation in males with suboptimal sperm quality has been elucidated, offering multiple targets for a potential therapy. The high rate of mitosis and metabolic activity during spermatogenesis in the seminiferous tubules

makes the germ cells highly sensitive to the free radicals, thus creating a need for an effective antioxidant system. The germinal cells in the testis as well as the epididymal spermatozoa are equipped with enzymatic and nonenzymatic scavenger systems to prevent lipoperoxidative damage. Seminal plasma and sperm themselves also have an array of the protective antioxidants.

Three basic endogenous antioxidant enzymes (superoxide dismutase, SOD; catalase and glutathione peroxidase, GPx) play a significant role (Tremellen 2008). The cytosolic Cu/Zn-SOD is a remarkably dominant SOD isoenzyme in the seminal plasma as well as in spermatozoa (Peeker et al. 1997). Addition of SOD to sperm in culture has been confirmed to protect them from oxidative attack. The majority of evidence does support a link between deficient catalase activity and male infertility. Catalase with Cu/Zn-SOD removes O_2^- and may play an important role in decreasing lipid peroxidation and protecting spermatozoa during genitourinary inflammation (Sikka et al. 1995). Glutathione peroxidases (GPx 1–5) are a family of enzymes. This enzyme is located and is active in almost all the reproductive organs. Male factor infertility has been linked with a reduction in seminal plasma and spermatozoa GPx activity. The classic intracellular GPx1 is expressed in sperm/genital tract and a direct relationship has been demonstrated with sperm motility (Dandekar et al. 2002). More significantly, a direct relationship has been reported between male fertility and phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPx4), a selenoprotein that is highly expressed in testicular tissue. In addition coordinated activity of GPx, glutathione reductase (GR, regenerate glutathione), and glutathione clearly plays a pivotal role in protecting sperm from oxidative attack. Other enzymes, such as glutathione-S-transferases, ceruloplasmin, or heme oxygenase-1, may also participate in the enzymatic control of oxygen radicals and their products (Tremellen 2008).

The nonenzymatic antioxidants related to the male reproductive system include ascorbic acid (vitamin C), α -tocopherol (vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine, carotenoids, flavonoids, urate, coenzyme

Q-10, resveratrol, and prostasomes. These agents principally act by directly neutralizing free radical activity. Coenzyme Q-10 is an antioxidant that is related to low-density lipoproteins and protects against peroxidative damage. Since it is an energy-promoting agent, it also enhances sperm motility (Lewin and Lavon 1997). It is present in the sperm midpiece and recycles vitamin E and prevents its prooxidant activity (Aitken et al. 1993). Albumin also helps neutralize lipid peroxide-mediated damage to the sperm plasma membrane and DNA (Twigg et al. 1998). Extracellular organelles (prostrasomes) secreted by the prostate have been shown to fuse with leukocytes within semen and reduce their production of free radicals (Saez et al. 1998). A significant reduction in nonenzymatic antioxidant activity in seminal plasma of infertile compared with fertile men has been reported.

Vitamin E (tocopherol) is a major antioxidant in the sperm membranes and appears to have a dose-dependent effect and plays a vital role in protecting cell membranes from oxidative damage by scavenging all the three major types of free radicals (Suleiman et al. 1996). Vitamin C is an important water-soluble antioxidant, neutralizes hydroxyl superoxide and hydrogen peroxide radicals, and prevents sperm agglutination (Agarwal et al. 2004). It prevents lipid peroxidation, recycles oxidized vitamin E, and protects against DNA damage induced by H_2O_2 radicals (Kodama et al. 1997). Resveratrol is a potential lipid-soluble antioxidant that is commonly found in many plants. It inhibited lipid peroxidation of ram semen most effectively even when applied in low concentrations (Sarlos et al. 2002).

Female Reproduction

In mammals, oogenesis starts in the germinal epithelium in the development of the ovarian follicles, the functional unit of the ovary. Oogenesis consists of several subprocesses with final maturation to form an ovum. Folliculogenesis is a separate subprocess that accompanies and supports all oogenetic subprocesses.

Endocrinology and Gonadotoxicity

Ovaries of the sexually mature females secrete a mixture of estrogens (17β -estradiol is the most abundant and potent) and progesterone. Apart from the development of secondary sexual characteristics of the female, estrogens (steroids) are responsible for the monthly preparation of body for a possible pregnancy and its maintenance if it occurs. Progesterone is also a steroid and has a role in the menstrual cycle and pregnancy. Estrogens and progesterones are small hydrophobic molecules that are transported in the blood bound to a serum globulin. The hormone-receptor complex enters the nucleus (if it is formed in the cytoplasm) and binds to the specific sequences of DNA, called the estrogen (or progesterone) response elements. Response elements are located in the promoters of genes. The hormone-receptor complex acts as a transcription factor (often recruit other transcription factors for help) which turns on (or sometimes off) the transcription of the target genes.

The synthesis and secretion of estrogens are stimulated by FSH, which in turn is controlled by the hypothalamic gonadotropin-releasing hormone (GnRH). Progesterone production is stimulated by the LH, which is also stimulated by GnRH:

Hypothalamus – GnRH – Pituitary – FSH
– Follicle – Estrogen (negative feedback)

Hypothalamus – GnRH – Pituitary – LH – Corpus luteum – progesterone (negative feedback)

About every 28 days, some blood and other products of the disintegration of the inner lining of the uterus, endometrium, are discharged from the uterus, a process called menstruation. During this time, a new follicle begins to develop in one of the ovaries. After menstruation ceases, the follicle continues to develop, secreting an increasing amount of estrogen which causes the endometrium to become thicker and more richly supplied with blood vessels and glands. A rising level of LH causes the developing egg within the follicle to complete the first meiotic division (meiosis 1), forming a secondary oocyte. After about 2 weeks, there is a sudden surge in the production of LH which triggers ovulation: the

release of the secondary oocyte into a corpus luteum. Stimulated by LH, the corpus luteum secretes progesterone which continues the preparation of the endometrium for a possible pregnancy and inhibits the contraction of the uterus and development of a new follicle. If fertilization does not occur, the rising level of progesterone inhibits the release of GnRH which, in turn, inhibits further production of progesterone. As the progesterone level drops, the corpus luteum begins to degenerate and the endometrium begins to break down via apoptosis. The inhibition of the uterine contraction is lifted and the bleeding and cramps of menstruation begin.

Aggressive chemotherapy and radiotherapy used for the treatment of some cancers and autoimmune disorders are the most common causes of gonadotoxicity and subsequent infertility. Patients receiving chemotherapy are at risk of developing “premature ovarian failure” (POF, a well-known consequence of the exposure of the female gonad to chemotherapeutic drugs). The well-known gonadotoxic cyclophosphamide-based multiagent cytotoxic chemotherapy is one of the combinations of choice in treating female breast cancer (Kaufmann et al. 2003). Cancer of the cervix is another malignancy that affects the reproductive age of women, and some of those patients receive radiosensitizing chemotherapy which again might affect their gonads. Co-treatment with GnRH agonist may reduce ovarian damage significantly in the female patients treated for Hodgkin lymphoma and is considered in addition to assisted reproduction for women in the reproductive age receiving gonadotoxic chemotherapy (Blumenfeld et al. 2008). Also, in a study (Brougham et al. 2012) anti-Mullerian hormone (AMH), detectable in girls of all ages, falls rapidly during cancer treatment in both the prepubertal and pubertal age. Both fall during the treatment and recovery thereafter varied with the risk of gonadotoxicity. AMH is used as a marker of damage to the ovarian reserve in girls receiving treatment of cancer.

Oxidative Stress and Infertility

Oxidative Stress, Oogenesis, and Folliculogenesis: ROS may have a regulatory role in the oocyte

maturation, folliculogenesis, ovarian steroidogenesis, and luteolysis. Mammalian ovulation or follicular rupture results from the vascular changes and the proteolytic cascade. This is mediated by the cytokines, VEGF and ROS (both nitrogen and oxygen radicals). Interleukin-1 β causes nitrite to accumulate in the rat ovaries, demonstrating close interaction between the cytokines and NOS (Ben-Shlomo et al. 1994).

There is a delicate balance between the ROS and antioxidant enzymes in ovarian tissues. Expression of various markers of the oxidative stress have been demonstrated in normal cycling ovaries (Suzuki et al. 1999), and their concentrations have been demonstrated to be lower in the follicular fluid than in the blood, suggesting that follicular fluid contains highly active antioxidant system (Jozwik et al. 1999). Enhanced expression of the luteal Cu/Zn-SOD may be due to the hCG which may have an important role in the maintenance of the corpus luteal function in pregnancy. Also nitric oxide radical is one of the local factors involved in the ovarian folliculogenesis and steroidogenesis. NO binds to the heme-containing enzyme guanylate cyclase, which activates the cyclic-GMP (LaPolt et al. 2003). Plasma concentration of nitrate monitored during follicular cycle has revealed peak levels at ovulation (Ekerhovd et al. 2001). NO inhibits the ovarian and corpus luteum steroidogenesis (Seino et al. 2002) and has luteolytic action mediated through the increased prostaglandins and apoptosis (Vega et al. 2000). The preovulatory follicle has a potent antioxidant defense, which can be exhausted by the intense peroxidation (Aten et al. 1992). Transferrin, a blood plasma glycoprotein that binds the iron, is known to suppress ROS generation and has been proven an important factor for the successful development of the follicles.

Oxidative Stress, Endometrium, and Endometriosis: Oxidative stress is involved in the modulation of cyclic changes in the endometrium. There is a cyclical variation in the expression of SOD in the endometrium. Elevated lipid peroxidation and decreased SOD activity in the late secretory phase with increased ROS levels (Sugino et al. 2004) have been linked to be

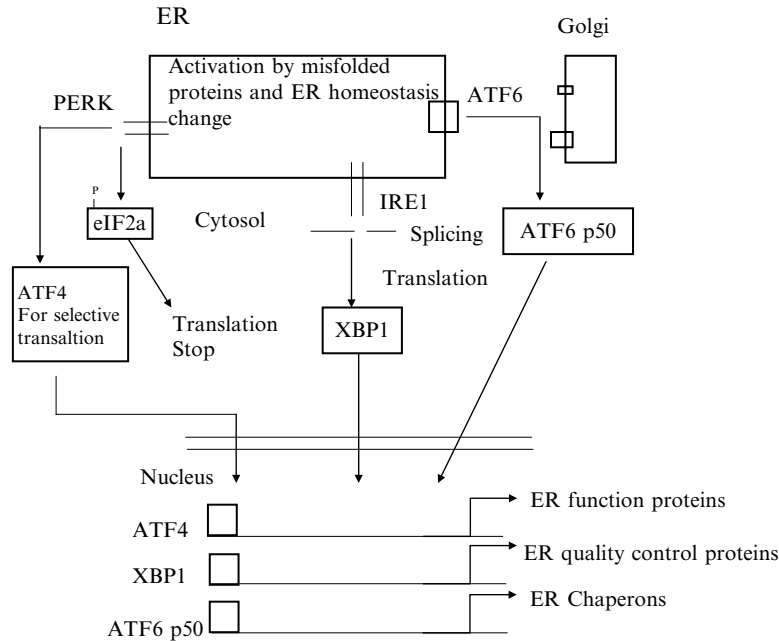


Fig. 2.4 Activation and response of unfolded protein response (UPR) pathways

important in the genesis of menstruation and endometrial shedding. The expression of eNOS and iNOS has been demonstrated in the human endometrium and endometrial vessels (Ota et al. 1998). NO is thought to regulate the microvasculature of endometrium. eNOS is also thought to bring about the changes that prepare endometrium for implantation. Stimulation of the cyclooxygenase enzyme is brought about by the ROS via activation of the NF- κ B, suggesting a mechanism for menstruation (Sugino et al. 2004). VEGF and Ang-2, key regulators of endometrial angiogenesis, are induced by hypoxia and ROS (Park et al. 2006), and their expression changes are thought to play an integral role in producing the abnormally distended and fragile vessels. Oxidative stress is thus implicated in the genesis of endometrial pathophysiology (Hickey et al. 2006). Endometriosis, blockage of sperm–egg union, is a complex phenomenon. Women with endometriosis have increased peritoneal fluid, macrophages, cytokines, and prostaglandins. ROS from macrophages may increase growth and adhesion of the endometrial cells in the peritoneal cavity, promoting endometriosis adhesions and infertility (Alpay et al. 2006). However, this etiology is controversial as others.

Further, the concentration of ROS plays a major role both in the implantation and fertilization of eggs (Sharma and Agarwal 2004). More severe attack by the ROS may lead to more extensive and irreparable cell damage, resulting ultimately in death through necrosis or apoptosis. These pathological effects are mediated by the opening of ion channels, lipid peroxidation, protein modifications, and DNA oxidation. ROS activate the calcium release channels in the ER membrane, which include the inositol-1,4,5-trisphosphate receptor, IP₃R, and the ryanodine receptor (Hool and Corry 2007). Ca²⁺ release activates diverse Ca²⁺-sensitive processes within the cell (Hool and Corry 2007), and loss of chaperone activity results in the accumulation of misfolded proteins within the lumen, leading to further generation of ROS as attempts are made to refold them (Tu and Weissman 2004). Accumulation also stimulates the unfolded protein response (UPR), a highly conserved set of signaling pathways (Fig. 2.4) that aim to restore homeostasis, but if this fails, it will stimulate apoptosis (Ron and Walter 2007). Rise in the cytosolic Ca²⁺ ion concentration will also adversely affect mitochondrial function, including an

increase in their own production of the ROS and opening of the permeability transition pore (PTP). As a result, the mitochondrial membrane potential, ATP synthesis, and ionic homeostasis fail and the cell undergoes necrosis or apoptosis (Leist et al. 1997).

A complex cytokine influence at the maternal–fetal interface creates conditions that are necessary to support the embryo implantation in the endometrium (Krussel et al. 2003). Critical changes occur in the vascular system which accompany follicular growth. As endometrium grows in the menstrual cycle, vessel regeneration occurs, i.e., spiral arteries and capillaries (Bausero et al. 1998). Estrogen promotes angiogenesis in the endometrium by controlling the expression of factors such as VEGF. ROS generated from the NADPH oxidase is critical for VEGF signaling in vitro and angiogenesis in vivo (Ushio-Fukai and Alexander 2004). Small amounts of ROS are produced from the endothelial NADPH oxidase activated by growth factors and cytokines.

Oxidative Stress, Pregnancy, and Placental Changes: Oxidative stress plays a role in both the normal development of placenta and in the pathophysiology of the complications such as miscarriage, preeclampsia, intrauterine growth restriction (IUGR), and premature rupture of the membranes. Development of placental hypoxia, reperfusion, and in turn oxidative stress triggers the release of cytokines and prostaglandins, which results in the endothelial cell dysfunction and plays an important role in the development of preeclampsia (Bilodeau and Hubel 2003). Activation of the mononuclear phagocytes can be triggered in the endometriosis by a number of factors including damaged RBCs and the apoptotic endometrial cells. A positive correlation between the concentrations of TNF- α in the peritoneal fluid and endometriosis has been reported (Bedaiwy and Falcone 2003).

The placenta at the start is supported by the secretions from the endometrial glands with low oxygen concentration which is more protective for the developing embryo rather than the maternal circulation (Burton et al. 2003). Maternal arterial blood is prevented from entering the intervillous space of the placenta by the plugs of

the extravillous cytotrophoblast cells that invade down the mouths of the uterine spiral arteries. The maternal intraplacental circulation recovers fully toward the end of the first trimester, when these plugs dislocate the circulation in the periphery of the placenta, where trophoblast invasion is least and progressively extends into the central region (Jauniaux et al. 2003). Onset of the circulation is associated with a threefold rise in the oxygen concentration within the placenta, stimulation of ROS generation, particularly in the critical syncytiotrophoblastic layer, which contains low concentrations of the principal antioxidant enzymatic defenses.

NO also regulates the microvasculature of the endometrium and is important in menstruation. Expression of iNOS was highest in patients with preterm pregnancy and not in patients in term labor. The expression of these enzymes decreased by 75 % at the term and was barely detectable in preterm in labor patients or term labor patients (Bansal et al. 1997), reiterating that NO has a role in the maintenance of uterine quiescence. Low levels of NO are important in ovarian function and implantation and cause relaxation of oviduct musculature. High levels of NO are reported as having deleterious effects on sperm motility, are toxic to embryos, and inhibit implantation (Lee et al. 2004). High levels of NO, such as those produced by macrophages, can negatively influence fertility. High levels of NO adversely affect sperm, embryos, implantation, and oviductal function, indicating that reduction in the peritoneal fluid NO production or blocking NO effects may improve fertility in women with endometriosis (Osborn et al. 2002).

Oxidative Stress and Spontaneous Miscarriage: Any imbalance between the cytokines and angiogenesis factors could result in the implantation failure and pregnancy loss (Choi et al. 2003). In cases of miscarriage, onset of the maternal intraplacental circulation is disorganized (Jauniaux et al. 2000), and it starts at an earlier stage and occurs randomly throughout the placenta. In 70 % of these cases, extravillous trophoblast invasion is superficial and consequently plugging of the spiral arteries is less complete. The apoptotic index is increased compared with control placentas

of a similar gestation age, and there is morphological evidence of degenerate syncytiotrophoblast sloughing off in some areas. In these cases, it seems that increased oxidative stress causes widespread destruction of the trophoblast. In confirmation of these findings, increased lipid peroxides in villous, decidual tissues and the serum of women undergoing pregnancy loss have been observed (Toy et al. 2010). High increase in the oxidative stress in the placenta takes place at 10–12 weeks of gestation on adapting to the maternal environment which causes increase in the expression and activity of the antioxidant enzymes (Jauniaux et al. 2000). Polymorphisms in the enzymes detoxifying ROS have been linked to an increased risk of miscarriage (Sata et al. 2003). Also the selenium deficiency with reduced activity of glutathione peroxidase is associated with miscarriage (Zachara et al. 2001).

Placental Oxidative Stress in Preeclampsia: Normal pregnancy is said to be a condition of the oxidative stress, as circulating levels of the ox-LDL increase and the total antioxidant capacity in pregnant women decreases compared with nonpregnant women (Belo et al. 2004). Pregnancy is also associated with a systemic inflammatory response, as evidenced by the activation of peripheral granulocytes, monocytes, and lymphocytes during the third trimester, all of which produce ROS. These states are observed to a much greater degree in preeclampsia. There is clear evidence of the placental oxidative stress in cases of the early onset preeclampsia, including increased concentrations of the protein carbonyls, lipid peroxides, nitrotyrosine residues, and DNA oxidation (Burton et al. 2009). The cause of the oxidative stress is thought to be vascular, because early onset of preeclampsia is associated with deficient conversion of the spiral arteries. In particular, the myometrial segments of the arteries are adversely affected. As the myometrial segment contains a highly contractile portion of the artery, it is proposed that failure to convert this section results in intermittent perfusion of the placenta and a low-grade ischemia–reperfusion-type injury (Hung et al. 2001). In support of this hypothesis, it is shown that hypoxia–reoxygenation *in vitro* is a potent inducer of the oxidative stress in term

placental explants, much more than hypoxia alone. Exposure of explants to changes in oxygenation causes generation of the ROS within the nitrotyrosine residues in a pattern matching closely to that seen in preeclamptic placentas. Furthermore, labor, in which the placenta is exposed to repeated episodes of ischemia–reperfusion, induces high levels of oxidative stress (Cindrova-Davies et al. 2007a).

Early onset of preeclampsia is associated with intrauterine growth restriction (IUGR) and high levels of ER stress in these placentas (Yung et al. 2008; Burton and Yung 2011). Induction of similar stress in trophoblast-like cell lines causes a reduction in their proliferation rate. In addition, the high levels of ER stress may contribute to the inflammatory response by stimulating the p38 and NF- κ B pathways. Hence, both ER stress and oxidative stress may contribute to the placental pathophysiology in preeclampsia (Burton et al. 2009). Increased phosphorylation of I κ B, an inhibitory subunit of NF- κ B, is observed in term placental explants subjected to hypoxia–reoxygenation *in vitro*, which provides a model for malperfusion of the placenta *in vivo* (Hung et al. 2001). Activation of the pathway is associated with increased tissue levels of the proinflammatory enzyme COX-2 and interleukin-1 β , increased secretion of TNF- α , and activation of the apoptotic cascade by the cleavage of caspase 3 (Cindrova-Davies et al. 2007b). Further, increased phosphorylation of p38 is observed in the term placenta after labor compared with control participants delivered by caesarean section (Cindrova-Davies 2009). ASK1 (upstream kinase of p38 and SAPK-JNK) is also activated in explants exposed to either hypoxia–reoxygenation or H₂O₂ (Cindrova-Davies 2009). Activation is associated with increased levels of the soluble receptor for VEGF, which has been implicated in the pathogenesis of preeclampsia.

Role of Antioxidants

Earlier well-known basic enzymatic and nonenzymatic antioxidants were suggested to protect the oocyte and the embryo from oxidative stress

by detoxifying and neutralizing the ROS production (Attaran et al. 2000). Considering antioxidants as a potential therapy for preeclampsia, vitamins C and E trials have not been successful (Roberts et al. 2010; Xu et al. 2010). However, in vitro experiments show positive results (Cindrova-Davies 2009). The difference may result from the ability of the vitamins to access the relevant trophoblast cell compartment in the necessary concentration in vivo. It is notable that the multivitamin usage during the pre-conceptional period is associated with a reduced risk of preeclampsia among lean or normal weight women (Catov et al. 2009). Conversely, women with a low dietary intake of vitamin C have been reported to have a trend toward increased risk (Klemmensen et al. 2009).

Autoimmune Diseases

Autoimmune disorder is a condition that occurs when the immune system mistakenly attacks and destroys the healthy body tissue. In patients with an autoimmune response, result in an hypersensitivity reaction, similar to the response in allergic conditions. In allergies, the immune system reacts to an outside substance, whereas with autoimmune disorders, the immune system reacts to normal body tissues. Organs and tissues commonly affected by the autoimmune disorders include blood vessels, connective tissues, endocrine glands such as the thyroid or pancreas, joints, muscles, red blood cells, and skin. Autoimmune diseases are multifactor diseases to which hereditary dispositions and environmental factors are related. Oxidative stress affects immune systems directly or indirectly. In the present write-up, three such disorders having link with oxidative stress have been discussed: HIV, colitis, and rheumatoid arthritis.

HIV

Long back it was proposed that the oxidative mechanisms are of critical significance in the genesis of AIDS (acquired immune deficiency

syndrome) and then further predicted that the mechanisms responsible for AIDS could be reversed by the administration of the reducing agents, especially those containing the sulfhydryl groups. The discovery of the HIV (human immunodeficiency virus) supported these as it considered the oxidative stress as a principal mechanism in both the development of AIDS and expression of HIV (Papadopoulos-Eleopoulos et al. 1989). In further experimentation, researchers found that the asymptomatic HIV-infected individuals and AIDS patients have decreased sulfhydryl and total glutathione and also the reducing agents suppress the expression of HIV. Since the viral production require thiols, which they obtain from the host, it may be assumed that the decreased SH level in the HIV-positive individuals may be the result of the HIV infection. However, for the HIV expression, oxidative stress is a prerequisite (Papadopoulos-Eleopoulos et al. 1991). The systemic decrease of the glutathione concentration in the HIV seropositive individuals may result from both decrease in synthesis and increased degradation. The oxidative stress to which the AIDS patients are subjected would lead to the cellular anomalies in many cells, including lymphocytes, resulting in the opportunistic infection, immunological abnormalities, and neoplasia. All these show in favor of the oxidation as being a critical factor in the pathogenesis of the AIDS and HIV expression.

HIV/AIDS patients suffer from several infections because of the poor immune system, especially as CD4-T cell immunodeficiency. Different factors released may trigger apoptosis in CD4⁺ T cell, including viral protein (i.e., gp 120, Tat), inflammatory cytokines from the activated macrophages (i.e., TNF- α), and toxins from microorganisms. In the HIV-infected patients, increased oxidative stress has been implicated in the increased HIV transcription through the activation of the NF-kB (Greenspan and Aruoma 1994). Glutathione (GSH) is a major intracellular thiol, which acts as a free radical scavenger and is thought to inhibit the activation of NF-kB (Sharon et al. 1997). NF-kB is involved in the transcription of HIV-1. Thus, ROS may potentially be involved in the pathogenesis of the HIV infection through

direct effects of the cells and through the interactions with the NF- κ B and activation of the HIV replication. The viral Tat protein liberated by the HIV-1-infected cells interferes with the calcium homeostasis, activates caspases, and induces mitochondrial generation and accumulation of the ROS, all being important events in the apoptotic cascades of several cell types. CD4⁺ T cell subset depletion in the HIV/AIDS patients is the most dramatic effect of the apoptosis mediated by redox abnormalities and induction of Fas/APO-1/CD95 receptor expression (Jaworowski and Crowe 1999). The proportion of the lymphocytes expressing Fas was shown to be elevated in the HIV-infected individuals. Some micronutrients play an essential role in maintaining the normal immune function and may protect immune effector cells from the oxidative stress (Meydani and Beharka 1998). Thus, infection by the HIV causes the persistent chronic inflammation through the intracellular increase of ROS, thus increasing the apoptotic index, mostly the one mediated by FAS/CD95, and depleting CD4⁺ T lymphocytes.

In the HIV/AIDS patients (Gil et al. 2003), the redox-related parameters and various types of the T lymphocytes load were studied and compared to the healthy subjects. Reduction of GSH levels and an increase in the MDA and total hydroperoxides levels were detected in the plasma of HIV⁺ patients. These patients also showed an increase of the DNA fragmentation in the lymphocytes as well as a significant reduction of the glutathione peroxidase and an increase in the SOD activity in erythrocytes. These results also show that the substantial oxidative stress occurs during HIV infection.

Since the discovery of HIV infection, numerous antiretroviral drugs that control the disease when administered in a potent combination are referred to as the highly active antiretroviral therapy (HAART). This therapy reduces the viral load and improves immune system reconstitution, leading to a significant reduction of the HIV-related morbidity and mortality (McArthur and Brew 2010). However, the HAART does not completely eliminate HIV and the treatment must continue for long, which has been related to the

long-term adverse events that can compromise the patient health. The prevalence of the HIV-associated neurocognitive disorder (HAND) is increasing as the HIV-infected individuals are living longer. HAND is manifested by the enhanced neuroinflammation, reactive astrocytes, formation of multinucleated giant cells, blood–brain barrier (BBB) damage, formation of microglial nodules, and neural apoptosis associated with increased viral replication and deterioration of the immune responses (Kanmogne et al. 2007). HAND has been characterized by the development of cognitive, behavioral, and motor abnormalities and occurs in about 50 % of the HIV-infected individuals (McArthur and Brew 2010).

As presented above, the oxidative stress is associated with HIV infection and therefore is true for the neurological disorders too. Studies have reported that HIV-1 viral proteins including gp120 and tat, released from the infected cells, induce oxidative stress in the CNS either directly or indirectly (Mollace et al. 2001). In vitro studies also demonstrated an increased ROS generation by gp120 exposure to the astrocytes (Reddy et al. 2012). Nrf2, ARE, and antioxidant genes, a well-known oxidative stress protective system, may have link with the inflammation and their role in the HAND. Further, the NOX2 subunit of NADPH oxidase was found involved in the HIV-1-mediated ROS generation (Williams et al. 2010). Inhibition of the NADPH oxidase with NOX2 knockdown and with pharmacological inhibitors, e.g., diphenyleneiodonium (DPI) and apocynin, significantly attenuated the HIV-1. Tat protein induces production of the inflammatory mediators such as TNF- α , IL-6, and MCP-1 in the microglia and macrophages (Mollace et al. 2001). Activation of NADPH oxidase in neurons can contribute to the cell death under stress stimuli.

Activation of the NF- κ B signaling has been shown to play an important role in inducing the oxidative stress and increased inflammation response mediated by the HAND (Shah and Kumar 2010), and also upregulation of the NF- κ B results in stimulating several inflammatory genes that play a vital role in the HAND (Williams et al. 2009). Upregulation of MMP-9 in the astrocytes treated with HIV-1 and its proteins such as

gp120 and Tat has been observed (Ju et al. 2009). MMP-9 has been reported to be increased in the CSF of HIV-infected neurologically impaired patients as well (Sporer et al. 1998). In addition, increased expression of the NF- κ B has been demonstrated to be mediated by the Nrf2 pathway, thereby resulting in increased MMP-9 expression (Mao et al. 2011). These findings suggest that the Nrf2 may be beneficial in preventing the oxidative stress and inflammatory cascades induced in the HAND and may provide insight in the development of novel therapeutic strategies against HAND.

Colitis

Colitis (pl. colitides) refers to an inflammation of the colon and is often used to describe an acute or chronic inflammation of the large intestine (colon, cecum, and rectum). The signs and symptoms of the colitides are quite variable and dependent on the etiology of the given colitis and factors that modify its course and severity. There are many types of colitis and classified by etiology: autoimmune inflammatory bowel disease (IBD, a group of chronic colitides), ulcerative colitis (UC, a chronic colitis that affects the large intestine), Crohn's disease (a type of IBD often leads to a colitis), idiopathic (microscopic colitis (a colitis diagnosed by microscopically), lymphocytic colitis, collagenous colitis), iatrogenic (diversion colitis, chemical colitis), vascular disease (ischemic colitis), and infectious colitis. Ulcerative colitis (UC) is idiopathic, chronic, and relapsing inflammatory bowel disease, which elicits the risk of colorectal cancer, the third most common malignancy in humans. Studies in the animal models of UC have helped to shed light on the mechanisms of the inflammation-driven colorectal carcinogenesis. The available evidence suggests that the DNA damage caused by the oxidative stress in the characteristic damage-regeneration cycle is a major contributor to colorectal cancer development in UC patients. Based on this concept, the dietary antioxidants are considered as the protective factors for the UC and associated carcinogenesis.

The colons of individuals with IBD are infiltrated with the neutrophils and activated macrophages that are capable of producing the high levels of ROS and RNS. In addition, inflammatory cytokines such as TNF- α and IFN- γ , which are overproduced in the IBD, are potent inducers of ROS and NO. Excessive production ROS and RNS leads to tissue damage of the host via oxidation of lipids, proteins, and DNA. Moreover, chronic inflammatory bowel diseases, both UC and Crohn's disease, are significant risk factors for the development of colon cancer. The cytokine, IL10 with potent anti-inflammatory and immune regulatory activity, inhibits the production of the inflammatory cytokines, such as IL1 and TNF- α , which stimulate the production of ROS. IL10 also inhibits the production of ROS in neutrophils and human monocytes (Kuga et al. 1996). It was reported that IL10-deficient mice (IL10^{-/-}) develop a spontaneous inflammatory bowel disease 3–6 months after birth (Berg et al. 1996). Further, study (Narushima et al. 2003) showed the presence of oxidative stress in the inflammatory bowel disease in nonsteroidal anti-inflammatory drug-(NSAID)-treated IL10^{-/-} mice and suggested a role for the oxidative stress in the pathophysiology of this model of the inflammatory bowel disease. The potential pathogenicity of the free radicals may have a pivotal role in the ulcerative colitis (UC). Fish oil omega-3 fatty acids exert anti-inflammatory effects on the patients with UC (Barbosa et al. 2003), by acting as free radical scavenger.

In further understanding the pathophysiology of the inflammatory diseases, endoplasmic reticulum (ER) stress has been linked. The synthesis, folding, and processing of the secreted and membrane proteins by the ER involve ER chaperones, maintenance of ER calcium pools, and an oxidative environment. A variety of stimuli, including the virus infections, endogenous imbalances in the cell, accumulation of the unfolded or misfolded proteins, loss of the calcium homeostasis, and glucose deprivation, can increase stress to the ER through a battery of UPR molecular pathways (shown in Fig. 2.4 in section “Female Reproduction”). In a recent study (Bogaert et al. 2011), involvement of the ER stress in IBD was studied at molecular level, and different implications of these were observed

in colonic and ileal disease which were related to the differences in the development of ileal or colonic disease.

The development of new diagnostic modalities at an early or precancerous stage is crucial to improve the prognosis of the UC-associated neoplasia (Fujii et al. 2008). Advanced oxidation protein products (AOPPs) are new protein markers of oxidative stress with proinflammatory properties, which accumulate in many pathological conditions (Wykretowicz et al. 2007). Being the products of oxidative imbalance themselves, AOPPs further participate in the potentiation and perpetuation of both oxidative stress and inflammation (Peng et al. 2006). Metallothioneins (MTs) have highly conserved number and position of cysteine residues, enabling them to incorporate monovalent and divalent metal atoms and to reduce ROS and RNS. MTs are known to participate in fundamental cellular processes such as cell proliferation and apoptosis (Cioffi et al. 2004). P53, tumor suppressor gene, mutations are the most frequently reported somatic gene alterations in human cancer, leading to accumulation of p53 gene products in tumor cells that can initiate an immune response with generation of circulating anti-p53 antibodies (p53Abs) (El-Sayed et al. 2003). Based on these informations, a recent study (Hamouda et al. 2011) was to exploit the use of p53Abs, MTs, and some oxidative stress markers in the early detection of dysplasia in chronic UC patients. Elisa of p53 antibodies (Abs) and MTs and spectroscopic analysis of AOPPs and GSH were carried. There was a positive correlation between AOPPs and both MTs and p53 Abs, and also between p53Abs and MTs. There was a negative correlation between AOPPs and GSH, and also between GSH and both MTs and p53Abs. In conclusion, oxidative stress and oxidative cellular damage play an important role in the pathogenesis of chronic UC and the associated carcinogenic process. P53Abs levels could help in early detection of dysplasia in these conditions.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many

tissues and organs, but principally attacks flexible (synovial) joints. The process involves an inflammatory response of the capsule around the joints (synovium), secondary to the swelling (hyperplasia) of synovial cells, excess synovial fluid, and the development of fibrous tissue (pannus) in the synovium. The pathology of the disease process often leads to the destruction of the articular cartilage and ankylosis (fusion) of the joints. RA can also produce diffuse inflammation in the lungs, membrane around the heart (pericardium), the membrane of the lung (pleura), and white of the eye (sclera), and also nodular lesions, most common in subcutaneous tissue. Although the cause of RA is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and it is considered a systemic autoimmune disease.

RA is a chronic multisystem disease with an unknown etiology. Increased oxidative stress and decreased antioxidant status are the hallmarks in patients of RA as compared to healthy individuals. A study (Karatas et al. 2003) indicates that increased oxidative stress and/or defective antioxidant status contributes to the pathology of RA. MDA levels in patients with RA were found to be significantly higher than controls, whereas levels of vitamins A, E, and C and activities of glutathione peroxidase and SOD were lower in the patients compared to controls. Plasma catalase had also been reported to be significantly lower in patients with RA (Kamanli et al. 2004). An epidemiological study (Knekt et al. 2002) suggested that low selenium status may be a risk factor for rheumatoid factor-negative RA. This shows that there is increased state of oxidative stress in RA, which proposes the use of antioxidants supplementation in such patients. In view of the animal studies strongly suggesting anti-inflammatory role of antioxidants like superoxide dismutase (Salvemini et al. 2001) and vitamin E (Behaska et al. 2002) in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA (Cerhan et al. 2003). Antioxidant implications and complications are reviewed by Mahajan and Tandon (2004).

RA being dependent on environmental factors and highly influenced by genetic composition,

Vasanthi et al. (2009) designed a study to generate data of the disease condition and the biochemical aspects in peripheral blood of population. Statistically significant changes were observed in the levels of MDA, vitamin E, total NO, and ESR in the patient group. Significant differences were also observed in ESR and vitamin E levels in patients with active disease. Increased oxidative stress status existed, which may lead to the connective tissue degradation leading to the joint and periarticular deformities in RA. In another study (Desai et al. 2010), oxidative stress was evaluated by measuring MDA and enzymatic antioxidant status by estimating the SOD and GR in the patients of RA. This study revealed that there was an increased oxidative stress and a decreased antioxidant defense in patients with RA as compared with healthy individuals. In extension to these, another study (Biniecka et al. 2011) was to assess the levels and spectrum of mitochondrial DNA mutations in synovial tissue from patients with inflammatory arthritis and to link these with oxidative stress status as assessed by analyzing in vivo tissue hypoxic status, lipid peroxidation, and cytochrome c oxidase expression levels. The effect of antioxidant treatment on the above processes was also examined. The findings demonstrate that hypoxia-induced mitochondrial dysfunction drives mitochondrial genome mutagenesis and antioxidants significantly rescue these events in synovial tissue from patients with inflammatory arthritis.

Further, seeing the increasing evidence that oxidative stress may play a key role in joint destruction in RA, the role of Nrf2, a transcription factor that maintains the cellular defense against oxidative stress, was studied (Wruck et al. 2011) in the synovial tissue from patients with RA using immunohistochemistry (IHC). Antibody-induced arthritis (AIA) was induced in Nrf2-KO (knock out) and Nrf2-WT (wild type) control mice. Nrf2 was activated in the joints of arthritic mice and of patients with RA. Nrf2-KO mice had more severe cartilage injuries and more oxidative damage, and the expression of Nrf2 target genes was enhanced in Nrf2-WT but not in KO mice during AIA. Both VEGF-A mRNA and protein expression was upregulated in Nrf2-KO mice during AIA. An unexpected finding

was the number of spontaneously fractured bones in Nrf2-KO mice with AIA. These results provide strong evidence that oxidative stress is significantly involved in cartilage degradation in experimental arthritis and indicate that the presence of a functional Nrf2 gene is a major requirement for limiting cartilage destruction.

To establish the correlation of the redox status in peripheral blood and the oxidative status at the site of inflammation in RA patients, spectrophotometry and/or flow cytometry analysis was carried (Kundu et al. 2012). The basal levels of total ROS, superoxide, and hydroxyl radicals were significantly raised in neutrophils sourced from peripheral blood and synovial infiltrate. However, there was no major increase in the RNS generated in monocytes from both sources. Furthermore, raised levels of superoxide in neutrophils of synovial infiltrate showed a positive correlation with NADPH oxidase activity in synovial fluid. Therefore, peripheral blood analysis directly correlates the inflammation status and in turn joint damage in RA patients and hence easy diagnosis. Staron et al. (2012) also generated biochemical analysis data in erythrocytes from RA patients. The level of the lipid peroxidation, antioxidant enzyme activities (CAT, SOD, GPx), level of the -SH groups, and GSH and Na⁺K⁺ ATPase activity in erythrocytes from patients with RA were estimated. There were no significant differences in CAT and GSH-Px activities. SOD activity is lower in RA patients than in the control group. Increase in the lipid peroxidation is observed in RA patients. Levels of the GSH and -SH groups are significantly lower in RA patients than in the control groups. Total ATPase and Na⁺K⁺ ATPase activities decreases in RA patients.

Inflamed synovium is infiltrated by neutrophils, macrophages, T cells, and B cells, which release a variety of proinflammatory mediators. Persistent inflammation results in destruction of the cartilage and bone. This occurs through a number of mechanisms, including oxidative and proteolytic breakdown of the collagen and proteoglycans (Wright et al. 2010). Once sequestered within the joint space, neutrophils degranulate and release a variety of potentially harmful

enzymes and peptides (Edwards and Hallett 1997). They may also undergo a respiratory burst and generate several ROS, including superoxide, H_2O_2 , hypohalous acids, and possible hydroxyl radical (Wright et al. 2010).

RA is a heterogeneous disease, in which MPO may play a role in the pathogenesis, severity, and/or outcomes. Indeed, MPO is present at high concentrations in SF of patients with RA. Also MPO is a marker of cardiovascular risk as discussed in CVD section in this chapter, and CVD is recognized as an important cause of death in patients with RA. Given the potential of MPO to contribute to both the pathology, a study was designed to determine whether MPO is active and promotes oxidative stress in SF through the production of hypochlorous acid and its relation with inflammatory activity in RA (Stamp et al. 2012). Plasma or SF was collected from RA patients and control individuals for analysis. Detection of 3-chlorotyrosine confirms that hypochlorous acid is produced in SF and reacts with proteins. There is no other known biological reaction that produces 3-chlorotyrosine and MPO is the only human enzyme capable of generating hypochlorous acid (Winterbourn and Kettle 2000). Hence, the strong correlation between 3-chlorotyrosine and the levels of MPO indicates that this enzyme catalyzes the production of hypochlorous acid in SF. Furthermore, the association of protein carbonyls with both MPO and 3-chlorotyrosine suggests that hypochlorous acid (strongest two-electron oxidant) is a major driver of the oxidative damage that occurs to proteins in the inflamed joint. It readily oxidizes cysteine and methionine residues as well as cross-linking and fragmenting proteins, inactivating α_1 -antiprotease inhibitor and adversely affecting the functions of LDL and HDL (Nicholls and Hazen 2009). Given its extreme and diverse reactivity, it is likely that hypochlorous acid contributes to the tissue damage that occurs in patients with RA. Thus, targeting specific inhibitors, such as 2-thioxanthines, against MPO would be expected to lower oxidative stress within the inflamed joint (Tiden et al. 2011).

References

Neurodegeneration Diseases

- Abramov AY, Canevari L, Duchen MR (2003) Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. *J Neurosci* 23:5088–5095
- Abramov AY, Canevari L, Duchen MR (2004) Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J Neurosci* 24:565–575
- Anderson I, Adinolfi C, Doctrow S, Huffman K, Joy KA, Malfrov B, Soden P, Rupniak HT, Barnes JC (2001) Oxidative signalling and inflammatory pathways in Alzheimer's disease. *Biochem Soc Symp* 67:141–149
- Benabid AL, Wallece B, Mitrofanis J, Xia C, Piallat B, Fraix V, Batir A, Krack P, Poliak P, Berger F (2005) Therapeutic electrical stimulation of the central nervous system. *C R Biol* 328:177–186
- Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T, Taylor RW, Turnbull DMJ (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* 38:515–517
- Brown GC, Borutaite V (2004) Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim Biophys Acta* 1658:44–49
- Chinta SJ, Mallajosyula JK, Rane A, Andersen JK (2010) Mitochondrial alpha-synuclein accumulation impairs complex I function in the dopaminergic neurons and results in increased mitophagy in vitro. *Neurosci Lett* 486:235–239
- Conte V, Uryu K, Fujimoto S, Yao Y, Rokach J, Longhi L, Trojanowski JQ, Lee VM, McIntosh TK, Pratico D (2004) Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury. *J Neurochem* 90:758–764
- Dal-Cim T, Motz S, Egea J, Parada E, Romero A, Budni J, Martin de Saavedra MD, Barrio LD, Tasca CL, Lopez MG (2012) Guanosine protects human neuroblastoma SH-SY5Y cells against mitochondrial oxidative stress by inducing Heme oxygenase-1 via PI3K/Akt/GSK-3 β pathway. *Neurochem Int* 61:397–404
- Dalfo EP, Portero-Otin MMP, Ayala VP, Martinez A, Pamplona M, Ferrer IM (2005) Evidence of oxidative stress in the neocortex in incidental lewy body disease. *J Neuropath Exp Neurol* 64:816–830
- Darios F, Corti O, Lucking CB, Hampe C, Muriel MP, Abbas N, Gu WJ, Hirsch EC, Rooney T, Ruberg M, Brice A (2003) Parkin prevents mitochondrial swelling and cytochrome release in mitochondria-dependent cell death. *Hum Mol Genet* 12:517–526

- Dringen R, Hirrlinger J (2003) Glutathione pathways in the brain. *Biol Chem* 384:505–516
- Du H, Yan SS (2010) Mitochondrial permeability transition pore in Alzheimer's disease cyclophilin D and amyloid. *Biochim Biophys Acta* 1802:198–204
- Dumont M, Lin MT, Beal MF (2010) Mitochondria and antioxidant targeted therapeutic strategies for Alzheimer's disease. *J Alzheim Dis* 20:5633–5643
- Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A (2009) Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochemistry* 48:4220–4230
- Ermak G, Davies KJ (2002) Calcium and oxidative stress from cell signaling to cell death. *Mol Immunol* 38:713–721
- Ferrari CKB (2000) Free radicals, lipid peroxidation and antioxidants in apoptosis: implication in cancer, cardiovascular and oxidants in apoptosis: implications in cancer, cardiovascular and neurological diseases. *Biologia* 55:581–590
- Fitzgerald JC, Camprubi MD, Dunn L, Wu HC, Ip NY, Kruger R, Martins LM, Wood NW, Plun-Favreau H (2012) Phosphorylation of HtrA2 by cyclin-dependent kinase-5 is important for mitochondrial function. *Cell Death Differ* 19:257–266
- Gandhi S, Abramov AY (2012) Mechanism of oxidative stress in neurodegeneration. *Oxidative Med Cell Longev*, PMID 22685618
- Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, Downara J, Latchman DS, Tabrizi SJ, Wood NW, Duchen MR, Abramov AY (2009) PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell* 33:627–639
- Gandhi S, Vaarmann A, Yao Z, Duchen MR, Wood NW, Abramov AY (2012) Dopamine induced neurodegeneration in a PINK1 model of Parkinson's disease. *Plos ONE* 7:e37565
- Gao HM, Liu B, Hong JS (2003) Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 23:6181–6187
- Gerard C, Chehal H, Hugel RP (1994) Complexes of iron (III) with ligands of biological interest dopamine and 8-hydroxyquinine-5-sulfonic acid. *Polyhedron* 13:591–597
- Hernandez F, Avila J (2007) Tauopathies. *Cell Mol Life Sci* 64:2219–2233
- Hirsch EC, Jenner P, Przedborski S (2013) Pathogenesis of Parkinson's disease. *Mov Disord* 28:24–30
- Kaminsky YG, Kosenko EA (2008) Effects of amyloid-beta peptides on hydrogen peroxide-metabolizing enzymes in rat brain in vivo. *Free Rad Res* 42:564–573
- Lee MS, Kwon YT, Li M, Peng J, Friedlander RM, Tsai LH (2000) Neurotoxicity induces cleavage of p35 to p25 by calpain. *Nature* 405:360–364
- Lotharius J, Brundin P (2002) Impaired dopamine storage resulting from α -synuclein mutations may contribute to the pathogenesis of Parkinson's disease. *Hum Mol Genet* 11:2395–2407
- Lotharius J, O'Malley KL (2000) The Parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation: a novel mechanism of toxicity. *J Bio Chem* 275:38581–38588
- Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M (2008) Glutathione peroxidase family—an evolutionary overview. *FEBS J* 275:3859–3970
- Mattson MP (2003) Will caloric restriction and folate protect against AD and PD? *Neurology* 60:690–695
- Muftuoglu M, Elilob B, Dalmazrak O, Ercan A, Kulaksiz G, Ogun H, Dalkara T, Ozer N (2004) Mitochondrial complex I and IV activities in leukocytes from patients with parkin mutations. *Mov Disord* 19:544–548
- Muller T (2011) Motor complications, levodopa metabolism and progression of Parkinson's disease. *Expert Opin Drug Metab Toxicol* 7:847–855
- Obata T, Kubota S, Yamanaka Y (2001) Allopurinol suppresses para-nonylphenol and 1-methyl-4-phenylpyridinium ion (MPP+)-induced hydroxyl radical generation in rat striatum. *Neurosci Lett* 306:9–12
- Opazo C, Huang X, Chemy R, Chemy R (2002) Metalloenzyme-like activity of Alzheimer's disease β -amyloid. Cu-dependent catalytic conversion of dopamine cholesterol, and biological reducing agents to neurotoxic H_2O_2 . *J Biol Chem* 277:40302–40308
- Park L, Zhou P, Pitstick R, Carbone C, Anrather J, Norris EH, Younkin L, Youkin S, Carlson G, McEwen BS, Ladecola C (2008) Nox2-derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein. *Proc Natl Acad Sci U S A* 105:1347–1352
- Peterson LJ, Flood PM (2012) Oxidative stress and microglial cells in Parkinson's disease. *Mediators Inflamm* 2012, 401264
- Priller C, Bauer T, Mitteregger G, Krebs B, Kretschmar HA, Herms J (2006) Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci* 26:7212–7221
- Schroeter H, Spencer JP, Rice-Evans C, Williams RJ (2001) Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-jun N-terminal kinase (JNK), c-jun and caspase-3. *Biochem J* 358:547–557
- Shapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol* 7:97–109, 68
- Smith DS, Tsai LH (2002) Cdk5 behind the wheel: a role in trafficking and transport? *Trends Cell Biol* 12:28–36
- Song DD, Shults CW, Sisk A, Rockenstein E, Masliah E (2004) Enhanced substantia nigra mitochondrial pathology in human α -synuclein transgenic mice after treatment with MPTP. *Exp Neurol* 186:158–172
- Sun KH, DePablo Y, Vincent F, Shah K (2008) Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. *J Neurochem* 107:265–278
- Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trajanowski JQ, Pratico D, Faseb J (2004) Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. *FASEB J* 18:323–325

- Surmeier DJ, Guzman JN, Sanchez-Padilla J, Goldberg JA (2011) The origin of oxidant stress in Parkinson's disease and therapeutic strategies. *Antioxid Redox Signal* 14:1289–1301
- Tiraboschi P, Hansen LA, Thal JJ, Corey-Bloom J (2004) The importance of neuritic plaques and tangles to the development and evolution of AD. *Neurology* 62:1984–1989
- Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuroparmacol* 7:65–74
- Vaarmann A, Gandhi S, Abramov AY (2010) Dopamine induces Ca^{2+} signaling in astrocytes through reactive oxygen species generated by monoamine oxidase. *J Biol Chem* 285:25018–25023
- van Muiswinkel FL, Kuiperij HB (2005) The Nrf2-ARE signaling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. *Curr Drug Targets CNS Neuro Disord* 4:267–281
- Wang X, Michaelis EK (2010) Selective neuronal vulnerability to oxidative stress in the brain. *Front Aging Neurosci* 2:12
- Weber CA, Ernst ME (2006) Antioxidants supplements and Parkinson's disease. *Ann Pharmacother* 40:935–938
- Wilkinson B, Koenigsknecht-Taboo C, Grommes C, Lee CYD, Landreth (2006) Fibrillar β -amyloid-stimulated intracellular signaling cascades require Vav for induction of respiratory burst and phagocytosis in monocytes and microglia. *J Biol Chem* 281:20842–20850
- Wu AD, Fregni F, Simon DK, Deblieck C, Pascual-Leone A (2008) Noninvasive brain stimulation for Parkinson's disease and dystonia. *Neurotherapeutics* 5:345–361
- Yankner BA, Duffy LK, Kirschner DA (1990) Neurotrophic and neurotoxic effects of amyloid beta protein reversal by tachykinin neuropeptide. *Science* 250:279–282
- Beyers LM, Braam B, Post JA, Zonneveld AJ, rbelink TJ, Koomans HA, Verhaar MC, Joles JA (2006) Tetrahydrobiopterin but not L-arginine, decreases NO synthase uncoupling in cells expressing high levels of endothelial NO synthase. *Hypertension* 47:87–94
- Brandes RP, Weissmann N, Schroder K (2010) NADPH oxidases in cardiovascular diseases. *Free Radic Biol Med* 49:687–706
- Brown MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophages. *Ann Rev Biochem* 52:223–261
- Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshorte E (1998) Role of HIF-1 α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394:485–490
- Carr AC, Frei B (2001) The nitric oxide congener nitrite inhibits myeloperoxidase/ $\text{H}_2\text{O}_2/\text{Cl}^-$ -mediated modification of low density lipoprotein. *J Biol Chem* 276:1822–1828
- Castelli WP (1986) The triglyceride issue: a view from Framingham. *Am Heart J* 112:432–437
- Chandel NS, Maitape E, Goldwasser E, Mathieu CE, Simon MC, Schumacbu PT (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A* 95:11715–11720
- Duffy D, Holmes DN, Roe MT, Peterson ED (2012) The impact of high-density lipoprotein cholesterol levels on long-term outcomes after non-ST-elevation myocardial infarction. *Am Heart J* 163:705–713
- Endemann G, Pronczuk A, Freidman G, Lindsey S, Alderson L, Hayes KC (1987) Monocyte adherence to endothelial cells in vitro is increased by β -VLDL. *Am J Pathol* 126:1–6
- Engler MM, Engler MB, Malloy MJ, Chiu EY, Schlotter MC, Paul SM, Shiehinger M, Lin KY (2003) Antioxidant vitamin C and E improve endothelial function in children with hyperlipidemia. *Endothelial Assessment of Risk from lipid in Youth (EARLY) trial. Circulation* 108:1059–1063
- Garner B, Cooke JP, Morrow JD, Ridker PM, Rifai N, Miller L, Witzthum JL, Mietus-Snyder (1998) Oxidation of high density lipoproteins. II, evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. *J Biol Chem* 273:6088–6095
- Goldstein JL, Ho YK, Brown MS, Innerarity TL, Mahley RW (1980) Cholesteryl ester accumulation in macrophages resulting from receptor mediated uptake and degradation hypercholesterolemic canine β -VLDL. *J Biol Chem* 255:1839–1848
- Guo S, Miyake M, Liu KJ, Shi H (2009) Specific inhibition of hypoxia inducible factor exaggerates cell injury induced by in vitro ischemia through deteriorating cellular redox environment. *J Neurochem* 5:1309–1321
- Hausenloy DJ, Yellon DM (2008) Time to take myocardial reperfusion injury seriously. *N Eng J Med* 359:518–520
- Huang J, Zhang Z, Guo J, Ni A, Deb A, Zhang L, Mirotso M, Pratt RE, Dzau VJ (2010) Genetic modification of

Cardiovascular Diseases

- AI Ahmad A et al (2009) Maintaining blood-brain barrier integrity pericytes perform better astrocytes during prolonged oxygen deprivation. *J Cell Physiol* 218:612–622
- AI Ahmad A, Gassmann M, Ogunshola OO (2012) Involvement of oxidative stress in hypoxia-induced blood-brain barrier breakdown. *Microvasc Res* 84:222–225
- Allen CL, Bayraktutan U (2009) Oxidative stress and its role in the pathogenesis of ischaemic stroke. *Int J Stroke* 4:461–470
- Baranova O, Miranda LF, Pichiule P, Dragatsis I, Johnson RS, Chavez JC (2007) Neuron-specific inactivation of the hypoxia inducible factor 1 α increases brain injury in a mouse model of transient focal cerebral ischemia. *J Neurosci* 23:6320–6332
- Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ (1995) Atherosclerosis: basic mechanisms, oxidation, inflammation and genetics. *Circulation* 91:2488–2496

- mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment and capillary density in the injured myocardium. *Circ Res* 106:1753–1762
- Javadov S, Karmazyn M (2007) Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. *Cell Physiol Biochem* 20:1–22
- Jialal I, Devaraj S (1996) Low density lipoprotein oxidation, antioxidants and atherosclerosis: a clinical biochemistry perspectives. *Clin Chem* 42:498–506
- Kaur HD, Bansal MP (2009) Studies on associated enzymes under experimental hypercholesterolemia: possible modulation on selenium supplementation. *Lipids Health Dis* 8:1–16
- Kleinschnitz C, Grund H, Wingler K, Armitage ME, Jones E, Mittal M, Barit D, Schwarz T, Geis C, Kraft P, Barthel K, Schuhmann MK, Herrmann AM, Meuth SG, Stoll G, Meurer S, Schrewe A, Becker L, Gailus-Durner V, Fuchs H, Klopstock T, de Angelis MH, Jandeleit-Dahm K, Shah AM, Weissmann N, Schmidt HH (2010) Post-stroke inhibition of induced NADPH oxidase type 4 prevents oxidative stress and neurodegeneration. *Plos Biol* 8:e1000479
- Klimov AN, Kozheynikova KA, Kuzmin AA, Kuzetov AS, Belora EV (2001) On the ability of high density lipoproteins to remove phospholipid peroxidation products from erythrocyte membranes. *Biochemistry (Mosc)* 66:300–304
- Kuhn H, Romisch J, Belkner J (2005) The role of lipoxygenase-isoforms in atherogenesis. *Mol Nutr Food Res* 49:1014–1029
- Kunitake ST, Jarvis MR, Hamilton RL, Kane JP (1992) Binding of transition metals by apolipoprotein A-1-containing plasma lipoproteins: inhibition of oxidation of low density lipoproteins. *Proc Natl Acad Sci U S A* 89:6993–6997
- Lochhead JJ, Mccaffrey G, Quigley CE, Finch J, DeMarco KM, Nametz N, Davis TP (2010) Oxidative stress increases blood-brain barrier permeability and induces alterations in occluding during hypoxia-reoxygenation. *J Cereb Blood Flow Metab* 30:1625–1636
- Lonn EM, Yusuf S, Dzavik V, Doris C, Yi Q, Smith S, Moore Cox A, Bosch J, Riley W, Teo K (2001) Effects of ramipril and vitamin E on atherosclerosis: the Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E (SECURE). *Circulation* 103:919–925
- Malhotra R, Lin Z, Vinconz C, Brosius FC 3rd (2001) Hypoxia induces apoptosis via two independent pathways in Jurkat cells: differential regulation by glucose. *Am J Physiol Cell Physiol* 281:C1596–C1603
- Malle E, Waeg G, Schreiber R, Grone EF, Sattler W, Grone HJ (2000) Immunohistochemical evidence for the myeloperoxidase/H₂O₂/halide system in human atherosclerotic lesions: colocalization of myeloperoxidase and hypochlorite-modified proteins. *Eur J Biochem* 267:4495–4503
- Matsuzawa A, Ichijo H (2008) Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim Biophys Acta* 1780:1325–1336
- Murthy KG, Szabo C, Salzman AI (2004) Cytokines stimulate expression of inducible nitric oxide synthase in DLD-1 human adenocarcinoma cells by activating poly(A) polymerase. *Inflamm Res* 53:604–608
- Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Tunilazzi E, Cerretani D (2013) Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. *Curr Vasc Pharmacol*, PMID 23716180
- Nicholls SJ, Dustina GJ, Cutri B, Bao S, Deummond GR, Rye KA, Barter PJ (2005) Reconstituted high-density lipoprotein inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. *Circulation* 111:1543–1550
- Ohara Y, Peterson TE, Harrison DG (1993) Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91:2546–2551
- Ozkul A, Akyol AL, Yenisey C, Arpacı E, Kyloglu N, Tataroglu C (2007) Oxidative stress in acute ischemic stroke. *J Clin Neurosci* 14:1062–1066
- Parathasarathy S, Printz DJ, Boyd D, Joy L, Steinberg D (1986) Macrophage oxidation of low density lipoprotein generates a modified form recognized by scavenger receptor. *Atherosclerosis* 65:505–510
- Prasad K, Kalra J (1992) Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. *Am Heart J* 125:958–961
- Precourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, Levy E (2011) The three-gene paraoxonase family: physiologic roles, actions and regulation. *Atherosclerosis* 214:20–36
- Ross R (1991) The pathogenesis of atherosclerosis. In: Braunward E (ed) *Heart disease*. W.B. Saunders Co., Philadelphia, pp 1135–1152
- Sandin A, Dagnell M, Gonon A, Pernow J, Stangl V, Aspenstrom P, Kappert K, Ostman A (2011) Hypoxia followed by re-oxygenation induces oxidation of tyrosine phosphatases. *Cell Signal* 23:820–826
- Shi H (2009) Hypoxia inducible factor 1 as a therapeutic target in ischemic stroke. *Curr Med Chem* 16: 4593–4600
- Singh U, Jialal I (2006) Oxidative stress and atherosclerosis. *Pathophysiology* 13:129–142. Review
- Sowers JR (1992) Insulin resistance, hyperinsulinemia, dyslipidemia, hypertension and accelerated atherosclerosis. *J Clin Pharm* 32:529–535
- Stocker R, Keaney JF (2001) Role of oxidative modifications in atherosclerosis. *Physiol Rev* 84:1381–1478
- Venugopal SK, Devaraj S, Jialal I (2003) C-reactive protein decreases prostacyclin release from human aortic endothelial cells. *Circulation* 108:1676–1678
- Vogiatzi G, Tousoulis D, Stefanadis C (2009) The role of oxidative stress in atherosclerosis. *Hellenic J Cardiol* 50:402–409. Review
- Wassmann S, Laufs U, Baumer AT, Muller K, Ahibary K, Linz W, Itter G, Rosen R, Bohm M, Nickenig G (2001) HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolaemic hypertension

- via reduced production of reactive oxygen species. *Hypertension* 37:1450–1457
- Witztum JL, Steinberg D (1991) Role of oxidized low-density lipoprotein in atherogenesis. *J Clin Invest* 88(1):785–1792
- Xu J, Qian J, Xie X, Lin L, Zou Y, Fu M, Huang z, Zhang G, Su Y, Ge J (2012) High density lipoprotein protects mesenchymal stem cells from oxidative stress-induced apoptosis via activation of the PI3K/Akt pathway and suppression of reactive oxygen species. *Int J Mol Sci* 13:17104–17120
- Yamaguchi O, Higuchi Y, Hirotani S, Kashiwase K, Nakayama H, Hikoso S, Takeda T, Watanabe T, Asahi M, Taniike M, Matsumura Y, Tsujimoto I, Hongo K, Kusakari Y, Kurihara S, Nishida K, Ichijo H, Hori M, Otsu K (2003) Targeted deletion of apoptosis signal-regulating kinase 1 attenuates left ventricular remodeling. *Proc Natl Acad Sci U S A* 100:15883–15888
- Yan LJ, Rajasekaran NS, Sathyanarayanan S, Benjamin J (2005) Mouse HSF1 disruption perturbs redox state and increases mitochondrial oxidative stress in kidney. *Antioxid Redox Signal* 7:465–471
- Zhang L, Jiang H, Gao X, Zou Y, Liu M, Liang Y, Zhu W, Chen H, Ge J (2011) Heat shock transcription factor-1 inhibits H₂O₂-induced apoptosis via down-regulation of reactive oxygen species in cardiac myocytes. *Mol Cell Biochem* 347:21–28
- Zou Y, Zhu W, Sakamoto M, Quin Y, Akazawa H, Toko H, Mizukami M, Takeda N, Minamino T, Takano H, Nagai T, Nakai A, Komuro I (2003) Heat shock transcription factor I protects cardiomyocytes from ischemia/reperfusion injury. *Circulation* 108:3024–3030
- spermatozoa movement and energy metabolism. *Free Radic Biol Med* 26:869–880
- Bahadur G, Ozturk O, Muneer A, Wafa R, Ashraf A, Jaman N, Patel S, Oyede AW, Ralph DJ (2005) Semen quality before and after gonadotoxic treatment. *Hum Reprod* 20:774–781
- Dandekar SP, Nandkarni GD, Kulkarni VS, Punekar S (2002) Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgrad Med* 48:186–189
- de Lamirande E, Gagnon C (1995) Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum Reprod* 10(suppl 1):15–21
- de Lamirande E, Leclerc P, Gagnon C (1997) Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Mol Hum Reprod* 3:175–194
- Farr SB, Kogama T (1991) Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. *Microbiol Rev* 55:561–585
- Gavella M, Lipovac V (1992) NADPH-dependent oxidoreductase (diaphorase) activity and isozyme pattern of sperm in infertile men. *Arch Androl* 28:135–141
- Garrido N, Meseguer M, Simon C, Pelliier A, Remohi J (2004) Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. *Asian J Androl* 6:59–65
- Gomez E, Buckingham DW, Brindle J, Lanzafame F, Irvine DS, Aitken RJ (1996) Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: correlation with biochemical markers of the cytoplasmic space, oxidative stress and sperm function. *J Androl* 17:276–287
- Griveau JF, Renard P, Le Lannou D (1995) Superoxide anion production by human spermatozoa as a part of the ionophore-induced acrosome reaction in vitro. *Int J Androl* 18:67–74
- Halliwal B (1984) Tell me about free radicals, doctor: a review. *J Roy Soc Med* 82:747–752
- Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T (1997) Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril* 68:519–524
- Lewin A, Lavon H (1997) The effect of coenzyme Q10 on sperm motility and function. *Mol Asp Med* 18(Suppl):S213–S219
- Moskovstev SI, Willis J, White J, Mullen BM (2007) Leukocytospermia: relationship to sperm deoxyribonucleic acid integrity in patients evaluated for male factor infertility. *Fertil Steril* 88:737–740
- Mostafa T, Anis TH, El-Nashar A, Iman H, Othman IA (2001) Varicocelelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl* 24:261–265
- Ozbek E, Turkoz Y, Gokdeniz R, Davarci M, Ozugurlu F (2000) Increased nitric oxide production in the spermatic vein of patients with varicocele. *Eur Urol* 37:172–175

Male Reproduction

- Agarwal A, Nallella KP, Allamaneni SS, Said TM (2004) Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online* 8:616–627
- Agletdinov EF, Kamilov FK, Alekhin EK, Romantsov MG, Bulygin KV, Makasheva LO (2008) Gonadotoxic effects of polychlorinated biphenyls in experiments on male rats. *Antibiot Khimioter* 53:15–18
- Aitken RJ (1995) Free radicals lipid peroxidation and sperm function. *Reprod Fertil Dev* 7:659–668
- Aitken RJ (1999) The Amoroso lecture. The human spermatozoon – a cell in crisis? *J Reprod Fertil* 115:1–7
- Aitken RJ, Fisher H (1994) Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioessays* 16:259–267
- Aitken RJ, Krausz C (2001) Oxidative stress, DNA damage and the Y chromosome. *Reproduction* 122:497–506
- Aitken RJ, Harkiss D, Buckingham DW (1993) Analysis of lipid peroxidation mechanisms in human spermatozoa. *Mol Reprod Dev* 35:302–315
- Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC (1999) Characterization of reactive oxygen species induced effects on human

- Pecker R, Abramson L, Marklund SL (1997) Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. *Mol Hum Reprod* 13:1061–1066
- Ragheb AM, Sabanegh ES Jr (2010) Male fertility-implications of anticancer treatment and strategies to mitigate gonadotoxicity. *Anticancer Agents Med Cem* 10:92–102
- Saez F, Motta C, Boucher D, Grizard G (1998) Antioxidant capacity of prostasomes in human semen. *Mol Hum Reprod* 4:667–672
- Sakkas D, Mariethoz E, Mnicsirdi G, Bizzaro D, Bianchi PG, Bianchi U (1999) Origin of DNA damage in ejaculated human spermatozoa. *Rev Reprod* 4:31–37
- Sarlos P, Molner A, Kokai M, Gabor GY, Ratky J (2002) Comparative evaluation of the effect of antioxidants in the conservation of ram semen. *Acta Vet Hung* 50:235–245
- Sikka SC, Rajasekaran M, Hellstrom WJ (1995) Role of oxidative stress and antioxidants in male infertility. *J Androl* 16:464–468
- Smith R, Kaune H, Parodi D, Madariaga M, Rios R, Morales I, Castro A (2006) Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress. *Hum Reprod* 21:986–993
- Spiropoulos J, Turnbull DM, Chinnerry PF (2002) Can mitochondrial DNA mutations cause sperm dysfunctions? *Mol Hum Reprod* 8:719–721
- Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E. *J Androl* 17:530–537
- Taiwo AM, Ige SO, Babalola OO (2010) Assessments of possible gonadotoxic effect of lead on experimental male rabbits. *Glob Vet* 5:282–286
- Tremellen K (2008) Oxidative stress and male infertility: a clinical perspective. *Hum Reprod Update* 14:243–258. Review
- Twigg J, Irvine DS, Houston P, Fulton P, Michael L, Aitken RJ (1998) Latrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma. *Mol Hum Reprod* 4:439–445
- Bausero P, Cavaille F, Meduri G, Freitas S, Perrot-Appplanat M (1998) Paracrine action of vascular endothelial growth factor in the human endometrium: production and target sites, and hormonal regulation. *Angiogenesis* 2:167–182
- Bedaiwy MA, Falcone T (2003) Peritoneal fluid environment in endometriosis. Clinopathological implications. *Minerva Ginecol* 55:333–345
- Belo L, Caslake M, Santos-Silva A (2004) LDL size, total antioxidant status and oxidised LDL in normal human pregnancy: a longitudinal study. *Atherosclerosis* 177:391–399
- Ben-Shlomo I, Kokia E, Jackson MJ, Adashi EY, Payne DW (1994) Interleukin-1 beta stimulates nitrite production in the rat ovary: evidence for heterologous cell-cell interaction and for insulin-mediated regulation of the inducible isoform of nitric oxide synthase. *Biol Reprod* 51:310–318
- Bilodeau JF, Hubel CA (2003) Current concepts in the use of antioxidants for the treatment of preeclampsia. *J Obstet Gynaecol Can* 25:742–750
- Blumenfeld Z, Avivi I, Eckman A, Epelbaum R, Rowe JM, Dann EJ (2008) Gonadotropin-releasing hormone agonist decreases chemotherapy-induced gonadotoxicity and premature ovarian failure in young female patients with Hodgkin lymphoma. *Fertil Steril* 89:166–173
- Brougham MF, Crofton PM, Johnson EJ, Evans N, Anderson RA, Wallace WH (2012) Anti-Mullerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. *J Clin Endocrinol Metab* 97:2059–2067
- Burton GJ, Yung HW (2011) Endoplasmic reticulum stress in the pathogenesis of early-onset pre-eclampsia. *Pregnancy Hypertens* 1:72–78
- Burton GJ, Hempstock J, Jauniaux E (2003) Oxygen, early embryonic metabolism and free radical-mediated embryopathies. *Reprod BioMed Online* 6:84–96
- Burton GJ, Yung HW, Cindrova-Davies T (2009) Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. *Placenta* 30(Suppl A):S43–S48
- Catov JM, Nohr EA, Bodnar LM (2009) Association of periconceptional multivitamin use with reduced risk of preeclampsia among normal-weight women in the Danish National Birth Cohort. *Am J Epidemiol* 169:1304–1311
- Choi HK, Choi BC, Lee SH, Kim JW, Cha KY, Baek KH (2003) Expression of angiogenesis- and apoptosis-related genes in chorionic villi derived from recurrent pregnancy loss patients. *Mol Reprod Dev* 66:24–31
- Cindrova-Davies T (2009) From placental oxidative stress to maternal endothelial dysfunction. *Placenta* 30(Suppl A):S55–S65
- Cindrova-Davies T, Yung HW, Johns J (2007a) Oxidative stress, gene expression and protein changes induced in the human placenta during labor. *Am J Pathol* 171:1168–1179
- Cindrova-Davies T, Spasic-Boskovic O, Jauniaux E (2007b) Nuclear factor-kappa B, p38, and stress-activated protein kinase mitogen-activated protein

Female Reproductive System

- Alpay Z, Saed GM, Diamond MP (2006) Female infertility and free radicals: potential role in adhesions and endometriosis. *J Soc Gynecol Investig* 13:390–398
- Aten RF, Duarte KM, Behrman HR (1992) Regulation of ovarian antioxidant vitamins, reduced glutathione, and lipid peroxidation by luteinizing hormone and prostaglandin F2 alpha. *Biol Reprod* 46:401–407
- Attaran M, Pasqualotto E, Falcone T, Goldberg JM, Miller KF, Agarwal A, Sharma RK (2000) The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. *Int J Fertil Womens Med* 45:314–320
- Bansal RK, Goldsmith PC, He Y, Zaloudek CJ, Ecker JL, Riemer RK (1997) A decline in myometrial nitric oxide synthase expression is associated with labor and delivery. *J Clin Invest* 99:2502–2508

- kinase signaling pathways regulate proinflammatory cytokines and apoptosis in human placental explants in response to oxidative stress: effects of antioxidant vitamins. *Am J Pathol* 170:1511–1520
- Ekerhovd E, Enskog A, Caidahl K, Klintland N, Nilsson L, Brannstrom M, Norstrom A (2001) Plasma concentrations of nitrate during the menstrual cycle, ovarian stimulation and ovarian hyperstimulation syndrome. *Hum Reprod* 16:1334–1339
- Hickey M, Krikun G, Kodaman P, Schatz F, Carati C, Lockwood CJ (2006) Long-term progestin-only contraceptive result in reduced endometrial blood flow and oxidative stress. *J Clin Endocrinol Metab* 9:3633–3638
- Hool LC, Corry B (2007) Redox control of calcium channels: from mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 9:409–435
- Hung TH, Skepper JN, Burton GJ (2001) In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. *Am J Pathol* 159:1031–1043
- Jauniaux E, Watson AL, Hempstock J (2000) Onset of maternal arterial blood flow and placental oxidative stress; a possible factor in human early pregnancy failure. *Am J Pathol* 157:2111–2122
- Jauniaux E, Hempstock J, Greenwold N (2003) Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. *Am J Pathol* 162:115–125
- Jozwik M, Wolczynski S, Szamatowicz M (1999) Oxidative stress markers in preovulatory follicular fluid in humans. *Mol Hum Reprod* 5:409–413
- Kaufmann M, von Minckwitz G, Smith R, Vekro Y, Gianni L, Eiermann W, Howell A, Costa SD, Beuzebec P, Untch M, Blohmer JU, Sinn HP, Sittek R, Soucho R, Tulusan AH, Volm T, Semu HJ (2003) International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer, review and recommendations. *J Clin Oncol* 21:2600–2608
- Krussel JS, Bielfeld P, Polan ML, Simon C (2003) Regulation of embryonic implantation. *Eur J Obstet Gynecol Reprod Biol* 110:S2–S9
- Klemmensen A, Tabor A, Osterdal ML (2009) Intake of vitamins C and E in pregnancy and risk of preeclampsia: prospective study among 57 346 women. *BJOG* 116:964–974
- LaPolt PS, Leung K, Ishimaru R, Tafoya MA, You-hsin Chen J (2003) Roles of cyclic GMP in modulating ovarian functions. *Reprod Biomed Online* 6:15–23
- Lee TH, Wu MY, Chen MJ, Chao KH, Ho HN, Yang YS (2004) Nitric oxide is associated with poor embryo quality and pregnancy outcome in in vitro fertilization cycles. *Fertil Steril* 82:126–131
- Leist M, Single B, Castoldi AF (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 185:1481–1486
- Osborn BH, Haney AF, Misukonis MA, Weinberg JB (2002) Inducible nitric oxide synthase expression by peritoneal macrophages in endometriosis-associated infertility. *Fertil Steril* 77:46–51
- Ota H, Igarashi S, Hatazawa J, Tanaka T (1998) Endothelial nitric oxide synthase in the endometrium during the menstrual cycle in patients with endometriosis and adenomyosis. *Fertil Steril* 69:303–308
- Park JK, Song M, Dominguez CE, Walter MF, Santanam N, Parthasarathy S, Murthy AA (2006) Glycodelin mediates the increase in vascular endothelial growth factor in response to oxidative stress in the endometrium. *Am J Obstet Gynecol* 195:1772–1777
- Roberts JM, Myatt L, Spong CY (2010) Vitamins C and E to prevent complications of pregnancy-associated hypertension. *N Engl J Med* 362:1282–1291
- Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8:519–529
- Sata F, Yamada H, Yamada A (2003) A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss. *Mol Hum Reprod* 9:725–728
- Sharma RK, Agarwal A (2004) Role of reactive oxygen species in gynecologic diseases. *Reprod Med Bio* 3:177–199
- Seino T, Salto H, Kaneko T, Takahashi T, Kawachi H (2002) Eight-hydroxy-2'-deoxyguanosine in granulosa cells is correlated with the quality of oocytes and embryos in an in vitro fertilization-embryo transfer program. *Fertil Steril* 77:1184–1190
- Sugino N, Karube-Harada A, Taketani T, Sakata A, Nakamura Y (2004) Withdrawal of ovarian steroids stimulates prostaglandin F_{2α} production through nuclear factor-kappaB activation via oxygen radicals in human endometrial stromal cells: potential relevance to menstruation. *J Reprod Dev* 50:215–225
- Suzuki T, Sugino N, Fukaya T, Sugiyama S, Uda T, Takayh R, Yajima A, Sasano H (1999) Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization. *Fertil Steril* 72:720–726
- Toy H, Camuzcuoglu H, Camuzcuoglu A (2010) Decreased serum prolidase activity and increased oxidative stress in early pregnancy loss. *Gynecol Obstet Invest* 69:122–127
- Tu BP, Weissman JS (2004) Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* 164:341–346
- Ushio-Fukai M, Alexander RW (2004) Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. *Mol Cell Biochem* 264:85–97
- Vega M, Urrutia L, Iniguez G, Gabler F, Devoto L, Johnson MC (2000) Nitric oxide induces apoptosis in the human corpus luteum in vitro. *Mol Hum Reprod* 6:681–687
- Xu H, Perez-Cuevas R, Xiong X, Reyes H, Roy C, Julien P, Smith G, von Dadelszen P, Leduc L, Audibert F, Moutquin JM, Piedboeuf B, Shatenstein B, Parra-Cabrera S, Choquette P, Winsor S, Wood S, Benjamin A, Walker M, Helewa M, Dubé J, Tawagi G, Seaward G, Ohlsson A, Magee LA, Olatunbosun F, Gratton R, Shear R, Demianczuk N, Collet JP, Wei S, Fraser WD, INTAPP Study Group (2010) An international trial of antioxidants in the prevention of preeclampsia (INTAPP). *Am J Obstet Gynecol* 202:239.e1–239.e10

- Yung HW, Calabrese S, Hynx D (2008) Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol* 173:451–462
- Zachara BA, Dobrzynski W, Trafikowska U (2001) Blood selenium and glutathione peroxidases in miscarriage. *BJOG* 108:244–247

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- Barbosa DS, Cecchini R, EIKadri MZ, Rodriguez MA, Burini RC, Dichi I (2003) Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. *Nutrition* 19:837–842
- Behaska AA, Wu D, Serafini, Meydani SN (2002) Mechanism of vitamin E inhibition of cyclooxygenase activity in macrophage from old mice: role of peroxynitrite. *Free Radic Biol Med* 32:503–511
- Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Helland G, Thompson-snipes L, Leach MW, Rennick D (1996) Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+)TH1-like response. *J Clin Invest* 98:1010–1020
- Biniecka M, Fox E, Gao W, Ng CT, Veale DJ, Fearon U, O'Sullivan J (2011) Hypoxia induces mitochondrial mutagenesis and dysfunction in inflammatory arthritis. *Arthritis Rheum* 63:2172–2182
- Bogaert S, De Vos M, Oliever K, Peeters, Elewaut D, Lambrecht B, Poullot P, Laukens D (2011) Involvement of endoplasmic reticulum stress in inflammatory bowel disease: a different implication for colonic and ileal disease. *Plos ONE* 6, e25589
- Cerhan JR, Sagg KG, Merlino LA, Mikuls TR, Criswell LA (2003) Antioxidant micronutrient and risk of rheumatoid arthritis in a cohort of older women. *Am J Epidemiol* 157:345–354
- Cioffi M, riegler G, Vietri MT, Pilla P, Caserta L, Carratu R, Sica V, Molinari AM (2004) Serum p53 antibodies in patients affected with ulcerated colitis. *Inflamm Bowel Dis* 10:606–611
- Desai PB, Manjunath DS, Kadi S, Chetana K, Vanishree J (2010) Oxidative stress and enzymatic antioxidant status in rheumatoid arthritis: a case control study. *Eur Rev Med Pharmacol Sci* 14:959–967
- El-Sayed ZA, Farag DH, Eissa S (2003) Tumor suppressor protein p53 and anti-p53 autoantibodies in pediatric rheumatological diseases. *Pediatr Allergy Immunol* 14:229–233
- Edwards SW, Hallett MB (1997) Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. *Immunol Today* 18:320–324
- Fujii S, Katsumata D, Fujimori T (2008) Limits of diagnosis and molecular markers for early detection of ulcerative colitis-associated colorectal neoplasia. *Digestion* 77(suppl 1):2–12
- Gil L, Martinez G, Gonzalez I, Tarinas A, Alvarez A, Giuliani A, Molina R, Tapanes R, Perez J, Leon OS (2003) Contribution to characterization of oxidative stress in HIV/AIDS patients. *Pharmacol Res* 47:217–224
- Greenspan HC, Aruoma O (1994) Could oxidative stress initiate programmed cell death in HIV infection? A role from plant derived metabolites having synergistic antioxidant activity. *Chem Biol Interact* 143:145–148
- Hamouda HE, Zakaria SS, Ismail SA, Khedr MA, Mayah WW (2011) p53 antibodies, metallothioneins and oxidative stress markers in chronic ulcerative colitis with dysplasia. *World J Gastrointest* 17:2417–2423
- Jaworowski A, Crowe SM (1999) Does HIV cause depletion of CD4⁺ T cells in vivo by the induction of apoptosis? *Immunol Cell Biol* 77:90–98
- Ju SM, Song HY, Lee JA, Lee SJ, Choi SY, Park J (2009) Extracellular HIV-1 Tat up-regulates expression of matrix metalloproteinase-9 via a MAPK-NF-kappaB dependent pathway in human astrocytes. *Exp Mol Med* 41:86–93
- Kamanli A, Naziroglu M, Aydilek N, Hacievliyagil C (2004) Plasma lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. *Cell Biochem Funct* 22:53–57
- Kanmogne GD, Schall K, Leibhart J, Knipe B, Gendehnan HE, Persidsky Y (2007) HIV-1 gp120 compromise es blood-brain barrier integrity and enhances monocyte migration across blood-brain barrier: implication for viral neuropathogenesis. *J Cereb Blood Flow Metab* 27:123–134
- Karatas F, Ozates I, Canatan H, Halifeoglu I, Karatepe M, Colakt R (2003) Antioxidant status and lipid peroxidation in patients with rheumatoid arthritis. *Ind J Med Res* 118:178–181
- Knekt P, Heliovaara M, Aho K, Aifthan G, Marniemi T, Aromaa A (2002) Serum selenium, serum alpha-tocopherol and the risk of rheumatoid arthritis. *Epidemiology* 11:402–405
- Kuga S, Otsuka T, Nitro H, Nunoi H, Nemoto Y, Nakano T, Ogo T, Umei T, Nihe Y (1996) Suppression of superoxide anion production by interleukin-10 is accompanied by a downregulation of the genes for subunit proteins of NADPH oxidase. *Exp Hematol* 24:151–157
- Kundu S, Ghosh P, Datta S, Ghosh A, Chattopadhyay S, Chatterjee M (2012) Oxidative stress as a potential biomarker for determining disease activity in patients with rheumatoid arthritis. *Free Radic Res* 46:1482–1489
- Mahajan A, Tandon V (2004) Antioxidants and rheumatoid arthritis. *J Ind Rheumatol Assoc* 12:139–142
- Mao L, Wang H, Qiao L, Wang X (2011) Disruption of Nrf2 enhances the upregulation of nuclear factor-kappa B activity, tumor necrosis factor-alpha and matrix metalloproteinase-9 after spinal cord injury in mice. *Mediators Inflamm* 2010:238321
- McArthur JC, Brew BJ (2010) HIV-associated neurocognitive disorders: is there a hidden epidemic? *AIDS* 24:1367–1370
- Meydani SN, Beharka AA (1998) Recent developments in vitamin E and the immune response. *Nutr Rev* 56:s49–s58
- Mollace V, Nottet HS, Clayette P, Turco MC, Muscoli C, Salvemini D, Perno CF (2001) Oxidative stress and

- neuroAIDS: triggers, modulators and novel antioxidants. *Trends Neurosci* 24:411–416
- Narushima S, Spitz DR, Oberley LW, Toyokuni S, Miyata T, Gunnett CA, Buettner GR, Zhang J, Ismail H, Lynch RG, Berg DJ (2003) Evidence for oxidative stress in NSAID-induced colitis in IL10^{-/-} mice. *Free Radic Biol Med* 34:1153–1166
- Nicholls SJ, Hazen SL (2009) Myeloperoxidases, modified lipo-proteins and atherogenesis. *J Lipid Res* 50(Suppl):S346–S351
- Papadopoulos-Eleopoulos E, Healand-Thomel B, Causer DA, Dufty AP (1989) An alternative explanation for the radiosensitization of AIDS patients. *Int J Radiat Oncol Biol Phys* 17:695–696
- Papadopoulos-Eleopoulos E, Healand-Thomel B, Causer DA, Turner VF, Papadimitriou JM (1991) Changes in thiols and glutathione as consequences of simian immune deficiency virus infection. *Lancet* 338:1013
- Peng KF, Wu XF, Zhao HW, Sun Y (2006) Advanced oxidation protein products induce monocyte chemoattractant protein-1 expression via p38 mitogen-activated protein kinase activation in rat vascular smooth muscle cells. *Chin Med J (Engl)* 119:1088–1093
- Reddy PV, Gandhi N, Samikkannu T, Saiyed Z, Agudelo M, Yndart A, Khatavkar P, Nair MP (2012) HIV-1 gp120 induces antioxidant response element-mediated expression in primary astrocytes: role in HIV associated neurocognitive disorder. *Neurochem Int* 61:807–814
- Salvemini D, Mazzoni E, Dugo L, Serrano I, De Sarro A, Caputi AP, Cuzzocrea S (2001) Amelioration of joint disease in a rat model of collagen induced arthritis by M40403, a superoxide dismutase mimetic. *Arthritis Rheum* 44:2909–2921
- Shah A, Kumar A (2010) HIV-1 gp120-mediated increases in IL-8 production in astrocytes are mediated through the NF-kappaB pathway and can be silenced by gp120-specific siRNA. *J Neuroinflammation* 7:96
- Sharon LW, Louise MW, Maureen LH, Jack PV, Peter GW (1997) Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications. *AIDS* 11:1689–1697
- Sporer B, Paul R, Koedel U, Grimm R, Wick M, Goebel FD, Pfister HW (1998) Presence of matrix metalloproteinase-9 activity in the cerebrospinal fluid of human immunodeficiency virus-infected patients. *J Infect Dis* 178:854–857
- Stamp LK, Khalilova I, Tarr JM, Senthilmohan R, Turner R, Haigh RC, Winyard PG, Kettle AJ (2012) Myeloperoxidase and oxidative stress in rheumatoid arthritis. *Rheumatology* 51:1796–1803
- Staron A, Makosa G, Koter-Michalak M (2012) Oxidative stress in erythrocyte from patients with rheumatoid arthritis. *Rheumatol Int* 32:331–334
- Tiden AK, Sjogren T, Svelsson M, Bemlind A, Senthilmohan R, Auchere F, Norman H, Markgren PD, Gustavsson S, Schmidt S, Landquist S, Forber LV, Maqon NJ, Paton LN, Jamerson GN, Eriksson H, Kettle AJ (2011) 2-Thioxanthines are suicide inhibitors of myeloperoxidase that block oxidative stress during inflammation. *J Biol Chem* 286:37578–37589
- Vasanthi P, Nalini G, Rajasekhar G (2009) Status of oxidative stress in rheumatoid arthritis. *Int J Rheum Dis* 12:29–33
- Williams R, Dhillon NK, Hegde ST, Yao H, Peng F, Callen S, Chebloune Y, Davis RL, Buch SJ (2009) Proinflammatory cytokines and HIV-1 synergistically enhance CXCL10 expression in human astrocytes. *Glia* 57:734–743
- Williams R, Yao H, Peng F, Yang Y, Bethel-Brown C, Buch S (2010) Cooperative induction of CXCL10 involves NADPH oxidase: implications for HIV dementia. *Glia* 58:611–621
- Winterbourn CC, Kettle AJ (2000) Biomarkers of myeloperoxidase-derived hypochlorous acid. *Free Radic Biol Med* 29:403–409
- Wright HL, Moots RJ, Bucknall RC, Edwards SW (2010) Neutrophil function in inflammation and inflammatory diseases. *Rheumatology* 49:1618–1631
- Wruck CJ, Fragoulis A, Gurzynski A, Brandenburg LO, Kan YW, Chan K, Hassenpflug J, Freitag-Wolf S, Varoga D, Lippross S, Pufe T (2011) Role of oxidative stress in rheumatoid arthritis: insights from the Nrf2-knockout mice. *Ann Rheum Dis* 70:844–850
- Wykretowicz A, Adamska K, Krauze T, Guzik P, Szczepanik A, Rutkowska A, Wysoki H (2007) The plasma concentration of advanced oxidation protein products and arterial stiffness in apparently healthy adults. *Free Radic Res* 41:645–649

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