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REVIEW

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Mitochondria: Oxidative stress, Dysfunction and Cell death

TA AJITH*
Department of Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur-680 555, Kerala, India.

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ABSTRACT

Mitochondrial research is presently one of the fastest growing disciplines in biomedicine. In mitochondria, reactive oxygen species (ROS) are generated as undesirable side products of the oxidative energy metabolism. It has been hypothesized that major factor in the dysfunction of mitochondria results from the defects in oxidative phosphorylation (OXPHOS) that results in the stimulation of the mitochondrial production of ROS and damage to mitochondrial DNA (mt DNA). Mitochondrial electron transport is an enzymatic source of oxygen radical generation and also a target against oxidant-induced damage. Recent experimental and clinical studies have suggested the increased productions of oxygen radicals in human diseases with or with out preserving the antioxidant status. Inhibition of oxidative stress and mtDNA damage could be novel and effective treatment strategies for many diseases including heart failure. There are evidences of beneficial effect of certain antioxidants such as Coenzyme Q10, selenium, carvediol, L-acetyl-carnitine, α-lipoic acid, vitamin E to alleviate the oxidative stress in mitochondria associated with many diseases. Over expression of the genes for peroxiredoxin-3, a mitochondrial antioxidant, or mitochondrial transcription factor A, could ameliorate the decline in mtDNA copy number. Based on the recent exciting developments in mitochondrial research, increasing pharmacological efforts have been made leading to the emergence of 'Mitochondrial Medicine'. The targeted and carrier-based delivery of drugs and DNA to mitochondria hardly constitutes a field of research on its own yet and is still in its infancy.

INTRODUCTION

Mitochondria are vital for the cell's energy metabolism and have central role in the regulation of programmed cell death. As the principal organelle for numerous fundamental metabolic pathways, mitochondrial dysfunction either causes or at least contributes to a large number of human diseases. According to Cadenas and Davies generation of superoxide radical ($O_2^{\bullet-}$) (approximately 160-420 mmol/day) by the inner mitochondrial membrane is the major source of intracellular oxygen radicals under physiological conditions [1]. The impaired intra mitochondrial metabolism with increased free iron levels and a defective mitochondrial respiratory chain, associated with increased free radical generation and oxidative damage may be considered possible mechanisms that compromise cell viability. It has been proposed that the symptoms of mitochondrial disease are the result of two pathophysiological effects of oxidative-phosphorylation (OXPHOS) deficiency: reduced mitochondrial energy (ATP) production and increased production and toxicity of mitochondrial ROS. OXPHOS deficiency results in a number of human diseases, affecting at least one in 5000 of the general population.

Despite the steady state level of reactive oxygen species (ROS) in mitochondria under physiological conditions, significant elevation of ROS with concomitant reduction of scavenging antioxidants have been observed in many pathological conditions. The oxidative damage in mitochondria is high

*Corresponding Author
TA Ajith
Professor Biochemistry,
Department of Biochemistry, Amala Institute of Medical Sceinces,
Amala Nagar, Thrissur, Kerala-680 555, India.
Email: taajith@rediffmail.com

relative to other organelles. Over the past few decades, the role of mitochondrial oxidative stress has been increasingly recognized in the pathophysiology of human diseases including in cardiovascular disease [2-4]. Chronic increases in oxygen radical production in the mitochondria can lead to a catastrophic cycle of mt DNA damage as well as functional decline, further oxygen radical generation, and cellular injury (Fig. 1). Decline in mitochondrial function may lead to cellular energy deficits, especially in times of greater energy demand, and compromise vital ATP-dependent cellular operations, including detoxification, repair systems, DNA replication, and osmotic balance. Mitochondrial decay may also lead to enhanced oxidant production and thus render the cell more prone to oxidative insult. Decline of mitochondrial energy status in advanced age due to lower activity of respiratory chain complexes, and Krebs cycle dehydrogenases has been reported in experimental animal models [5-7]. This article reviews an introduction to the basic mechanisms of free radical formation and their effect on the mitochondria.

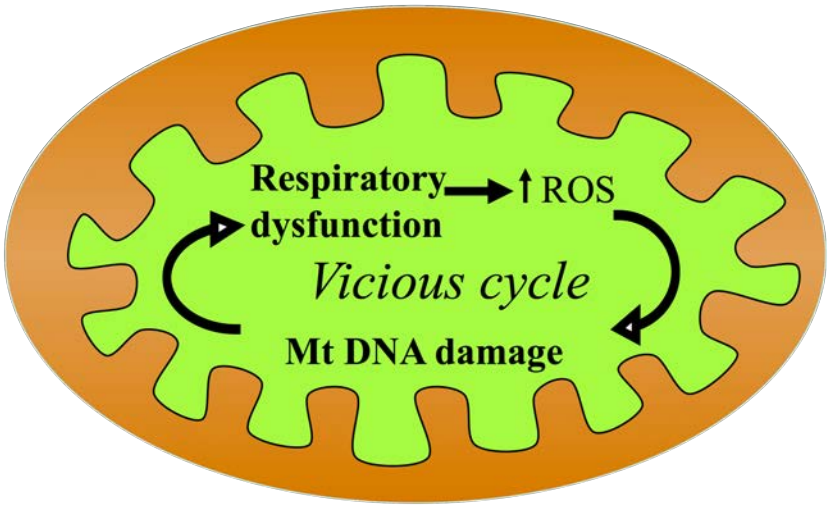

Figure 1. Mitochondrial ROS generation results in vicious cycle of mitochondrial functional decline.

Mitochondrial generation of reactive oxygen species

Reactive oxygen species (ROS) include free radicals (hydroxyl radical, $HO^{\bullet}$, and superoxide ion radical, $O_2^{\bullet -}$), non-radicals (hydrogen peroxide, H_2O_2, and singlet oxygen $^1O_2$), short lived lipid peroxidation products (peroxyl radical, $ROO^{\bullet}$, and alkoxyl radical $RO^{\bullet}$), and long lived secondary products (malondialdehyde, and 4-hydroxyalkenals). Mitochondrial electron transport chain (ETC) is one of the relatively well investigated sources of ROS. Approximately 95 percent of cellular oxygen (O_2) is reduced to water in stepwise electron carriers of the mitochondrial respiratory chain. Approximately 1-5 % of total O_2 consumption gives rise to potentially cytotoxic ROS such as $O_2^{\bullet -}$ and H_2O_2 (Fig. 2). Based on O_2 consumption of 6.4 l/kg/day, an 80 kg man would produce some 215-430 mmol and a 60 kg woman would produce some 160-320 mmol of $O_2^{\bullet -}$ in a day from the mitochondrial respiration [1].

Most of the electron carriers are thermodynamically capable of reducing oxygen to $O_2^{\bullet -}$. While it had been demonstrated that the isolated mitochondria generate $O_2^{\bullet -}$ derived H_2O_2, the concept of H_2O_2 generation from $O_2^{\bullet -}$ is induced in the presence of respiratory chain inhibitor e.g inhibition of complex III by antimycin A. Any factors adjusting the function of the respiratory chain affect ROS production. Later, it has been proved that a high mitochondrial membrane potential, except in aging where membrane potential declines, is one of the triggers that release H_2O_2 from $O_2^{\bullet -}$ [8]. According to Kadenbach and Arnold, the dephosphorelation, that inhibits cytochrome oxidase, the terminal electron acceptor of the respiratory chain, increases mitochondrial membrane potential and thus induces the $O_2^{\bullet -}$ generation [9].

The physiological rate of $O_2^{\bullet -}$ and H_2O_2 produced in the mitochondria is depending on the mitochondrial metabolic state. Relatively slow rate of respiration and no availability of ADP is associated with a relatively low rate of $O_2^{\bullet -}$ and H_2O_2 production, where as high rate of O_2 uptake and ample availability of ADP shows a relatively high rate of $O_2^{\bullet -}$ and H_2O_2 production. It has been demonstrated that mitochondrial ROS is produced by electron leakage from ETC complexes during normal respiration particularly in complex I and III [10]. $O_2^{\bullet -}$ generation is enhanced at complex III during hypoxia via autooxidation of ubiquinone on both sides of mitochondrial inner membrane. $O_2^{\bullet -}$ is the major source of intracellular oxygen radicals under physiological conditions [1].

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

The superoxide radical formed can also undergo spontaneous

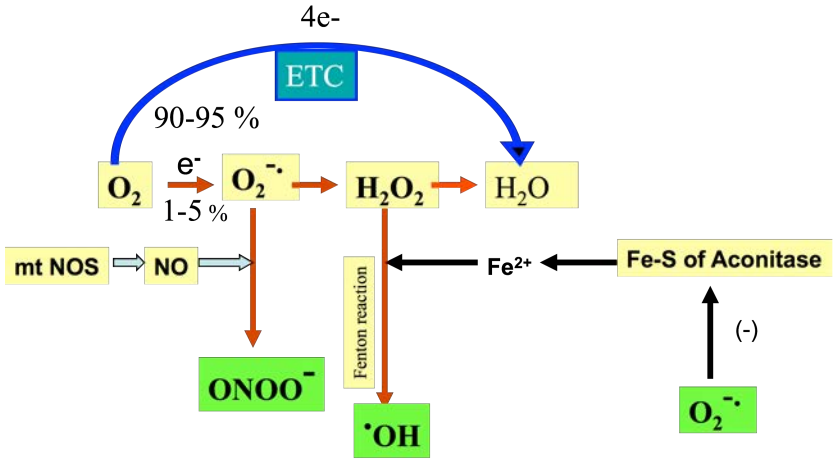

Figure 2. Fate of oxygen in mitochondria (ETC-electron transport chain)

The superoxide radical formed can also undergo spontaneous dismutation to produce H_2O_2.

$$O_2^{\cdot-} + e^- + 2\,H^+ \rightarrow H_2O_2$$

The dismutation product, H_2O_2 is permeable to cytosol. However, the generated $O_2^{\cdot-}$ is found to be remain mainly in the intermembrane space. Generation of H_2O_2 has also been observed in the oxidative deamination of biogenic amines by monoamine oxidases (MAO), a flavoprotein catalyzes the oxidative deamination of biogenic amines in the outer mitochondrial membrane.

The $O_2^{\cdot-}$ formed can also undergo spontaneous dismutation to produce singlet oxygen.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2\,H^+ \quad H_2O_2 + {}^1O_2$$

Generation of $^{\bullet}OH$, most oxidizing radical in a biological system from the Fenton's reaction had been well established. The accumulation of iron due to mutational inactivation of gene coding for the protein Frataxin, a mitochondrial protein involved in iron transport can leads to an autonomic recessive disease Friedreich's ataxia [11]. Similarly, in Wilson's disease mutations in copper P-ATPase, leading to increased intracellular copper and oxidative damage has been reported [12].

Fe (II) complex + H_2O_2 → Fe (III) complex + OH^- + $^{\cdot}OH$

The risk of ROS formation due to leakage of electron from ETC has been mainly observed under pathophysiological rather than physiological conditions, where alteration of membrane fluidity is one of the many reasons that eventually results in the generation of ROS. Mutations in genes that encode mitochondrial proteins could compromise mitochondria by altering components of the ETC, resulting in inefficient electron transport and increased $O_2^{\cdot-}$ production. Low level of nitric oxide (NO), produced by mitochondrial-specific nitric oxide synthase (mt NOS) partially inhibits respiratory chain. This partial inhibition increases mitochondrial ROS production in short term. $O_2^{\cdot-}$ and NO are readily converted either by enzymatic or non-enzymatic chemical reaction to non-radical species such as singlet oxygen, H_2O_2 or peroxynitrite (ONOO-). Tumor necrosis factor-α (TNF-α), a critical cytokine released during inflammation, infection, ischemia, oxidative stress and toxin exposure can also induces mitochondrial ROS production especially in liver, possibly through activation of acidic sphingomyelinase (ASM ase) and the release of ceramide , eventually results in block of electron transport at the complex III-ubiquinone cycle, leading to ROS generation [13].

Many mammalian mitochondrial enzymes can produce ROS by one electron reduction of O_2 are given in table 1. A wide variety of medicinally useful drugs can generate ROS when they undergo redox cycling with the mitochondrial ETC. One of the most widely studied drugs is an antitumor anthracyclin antibiotic doxorubicin. Other quinonoid compounds such as

Table 1: Mitochondrial enzymes can produce ROS by one electron reduction of O_2

Mitochondrial enzymes/complexes
Cytochrome b5 reductase
Monoamine oxidases (MAO)
Dihydroorotate dehydrogenase
Mitochondrial α-glycerophosphate dehydrogenase
Succinate dehydrogenase complex (SDH)
Aconitase
α-ketoglutarate dehydrogenase complex (α-KGDH)
Complex I
Complex III

daunorubicin, rubidazone and aclacinomycin cause cytotoxicity because of the ROS produced during the mitochondrial and cytochrome P-450 dependent redox cycling [14].

Mitochondrial antioxidants

Living tissues are endowed with innate antioxidant defense mechanisms, including the antioxidant enzymes SOD, CAT, and GPx. Antioxidant enzymes are considered to be the primary defense that prevents biological macromolecules from oxidative damage. The antioxidant enzymes, Manganese containing superoxide dismutase (Mn SOD) and GPx are recognized as primary defense against $O_2^{\cdot-}$ and H_2O_2 in eukaryotic cell mitochondria.

Mn SOD protects cells from $O_2^{\cdot-}$ attack by facilitating its dismutation into H_2O_2.. Though Mn SOD and GPx are recognized as primary defense against the ROS, experimental studies demonstrated the presence of a heme-containing CAT in mitochondria of various organs. Presence of a heme-containing CAT in the rat heart mitochondrial matrix was demonstrated by Radi et al. [15]. Nevertheless, the role of CAT in eukaryotic mitochondria, activity of glutathione peroxidase (GPx) significantly ameliorates the effect of H_2O_2.

Five different isoforms of GPx have been recently identified. They are GPx1, GPx2, GPx3, GPx4 and GPx6. The mitochondrial $O_2^{\cdot-}$ is dismutated to H_2O_2 by MnSOD, and the H_2O_2 is converted to H_2O by GPx-isoform 1 (GPx1) using reduced glutathione (GSH). Glutathione reductase (GR) reduces the oxidized form of glutathione (GSSG). Hence, the efficacy of GPx is mainly dependent on the availability of intracellular GSH and the ability of the cell to reduce the oxidized form, glutathione disulfide.

Thiol-containing compounds represent a major group of intracellular antioxidants. Glutathione, a water-soluble tripeptide, is the most abundant non-protein thiol molecule in tissues with predominant defense against ROS. It includes reduced glutathione (GSH) and oxidized glutathione (GSSG) (Fig. 3). Of the total cellular GSH pool, approximately 15 % is found in the mitochondria. GSH reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation, promotes the regeneration of ∞-tocopherol, and serves as a substrate for GSH-related enzymes, such as GPx and glutathione-s-transferase (GST). A small fraction of the total cellular pool of GSH is sequestered in mitochondria and the concentration of glutathione within mitochondria is in the range from 2 to 12 mmol/L. The fluidity of the inner mitochondrial membrane influences GSH transport. Therefore, increasing the amount of cholesterol has been shown to deplete the mGSH pool [16].

Thioredoxins (Trx), small proteins involved in the thiol-redox processes, containing two redox-active cysteine residues in their active sites. They are kept in their reduced state by a flavoenzyme, thioredoxin reductase (TrxR), in a NADPH-dependent reaction. Among the two forms of Trx and TrxR, mammalian mitochondria contain unique versions of Trx2, TrxR2, both of which are synthesized in the cytosol with mitochondrial targeting sequences and localized specifically in mitochondria. Trx supports GSH system, when glutathione reductase (GR) is deficient.

The newly identified Prx (peroxiredoxin) family of peroxidases (includes at least six isoforms in mammalian cells) reduces H_2O_2 to water using the reducing equivalents that are derived specifically from thiol-containing donor molecules, such as Trx [17]. Other antioxidants such as NAD(P)H, coenzyme Q10 and cytochrome c are also involved in the detoxification of mitochondrial ROS.

Oxidative stress and mitochondrial damage

The rate of free radical production and scavenging capacity of the antioxidants are essentially constant or balanced under normal homeostasis. ROS are produced in cells at relatively low steady state levels. While the changes in the oxidant-antioxidant balance can trigger redox-responsive signaling for homeostasis, the disruption of the delicate balance between generation ROS and antioxidant scavenging systems can lead to a shift towards oxidative cellular damage. This can either be due to an increase in ROS concentrations or due to a declined activity of one or

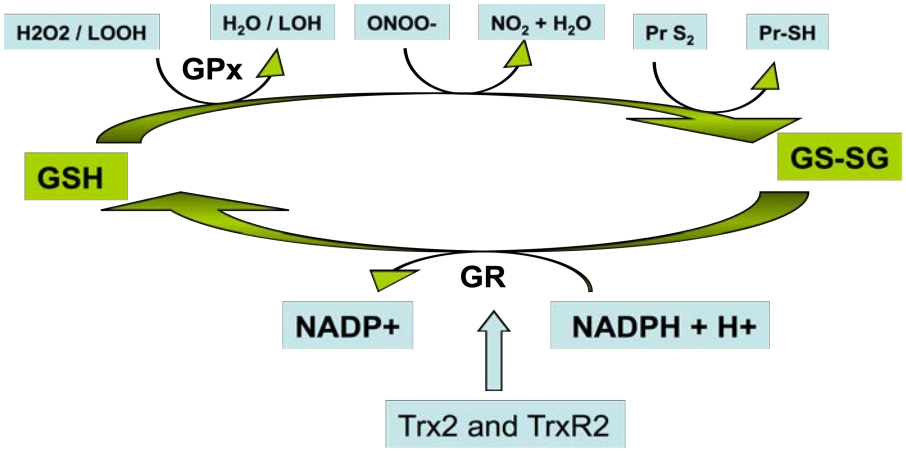

Figure 3. Cellular glutathione utilization and regeneration (GPx, Glutathione peroxidase; GR, glutathione reductase; Trx2 and TrxR2, Thioredoxines).

more antioxidant enzymes. If the initial increase of ROS is relatively small, the antioxidants may be sufficient to reset the original balance between the ROS production and scavenging. Mitochondrial components are constantly exposed to steady state concentration of ROS mainly $O_2^{\bullet-}$ and H_2O_2. However, under certain conditions, especially in pathological conditions, the production of ROS increased more strongly and persistently. The generated ROS have very short life span and can readily react with the macromolecules such as lipids, proteins and nucleic acids (Fig. 4).

Protein functional alteration

Proteins may differ strongly in their susceptibility to damage by oxidants. Practically all amino acids can serve as targets for oxidative attack while some of them such as tryptophan, tyrosine, histidine, and cysteine are particularly sensitive to ROS. Inner membrane of mitochondrion contains many iron and copper complexes, which can catalyze many reactions between $O_2^{\bullet-}$ and H_2O_2. Mn SOD and CAT are prone to age associated oxidative damage due to $^{\bullet}OH$, generated from Fenton's reaction. ROS-induced oxidative modification of many enzyme proteins results in structural alteration and their functional inactivation

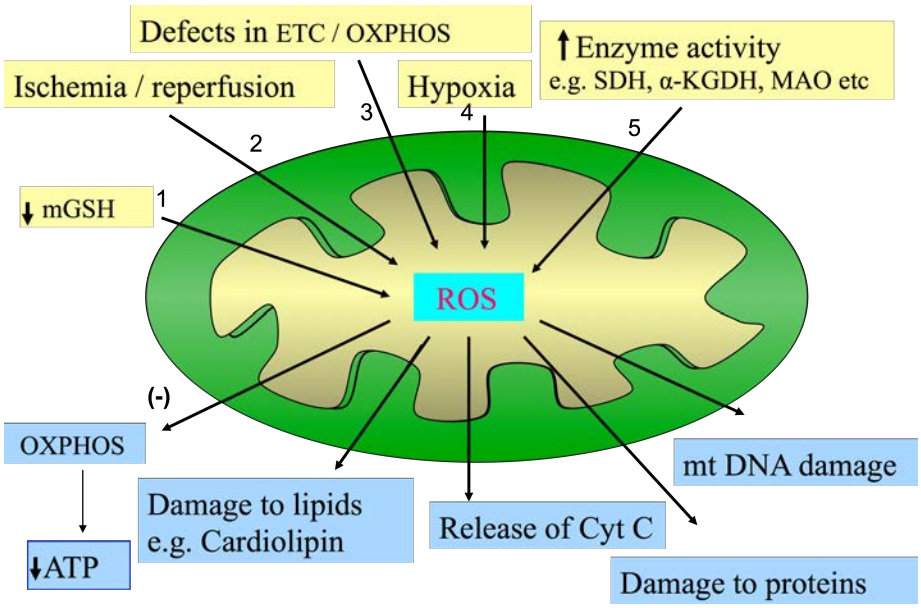

Figure 4. Schematic of ROS generation in mitochondria and damages caused by ROS

Damages to mitochondrial macromolecules are exaggerated during oxidative stress as is observed especially in pathological conditions. The pathological symptoms may result either from ROS and RNS mediated damage of macromolecules or from the changes in the gene expression.

Lipid peroxidation

The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation (LPO). Cardiolipin, an important phospholipid that serves as a cofactor for a number of critical mitochondrial transport proteins and retains cytochrome c at inner mitochondrial membrane through electrostatic interaction, declines due to oxidative damage. The oxidized cardiolipin is not only removed from the membrane but may also be decreased in level because of decreased *de novo* synthesis. Loss of cardiolipin, coupled with oxidation of critical thiol groups in key proteins, may adversely affect transport of substrates and cytochrome c oxidase activity that is necessary for mitochondrial function. Peroxidation of cardiolipin releases cardiolipin to execute the apoptotic cell death [18].

[19]. Deformation of enzyme structures may also lead to altered Km for the substrates. Oxidants may also cause increased damage or use of critical metabolites such as ubiquinone or small molecular weight antioxidants. Cadenas and Davies reported that activities of NADH dehydrogenase, NADH oxidase, succinate dehydrogenase, succinate oxidase and ATPase were rapidly inactivated by $^{\bullet}OH$ [1]. Moreover, $O_2^{\bullet-}$ can be a highly efficient inactivator of NADH dehydrogenase, NADH oxidase, and ATPase. ONOO- was found to be an inactivator of aconitase, an iron containing enzyme in the Krebs cycle. It has been demonstrated that under oxidative stress conditions the oxidized proteins instead of undergoing proteolytic digestion, aggregated by cross liking with one another and affect the normal cellular functions [19].

Damage to mitochondrial DNA

The mitochondrial DNA is a multicopy genome of 16.5 kb for 37 genes, which is transmitted only through the maternal line. The mtDNA is a double-stranded circle which encodes 13 polypeptides for the OXPHOS, 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). Both strands of the mt DNA tides involved in OXPHOS, including 7 subunits of rotenone-

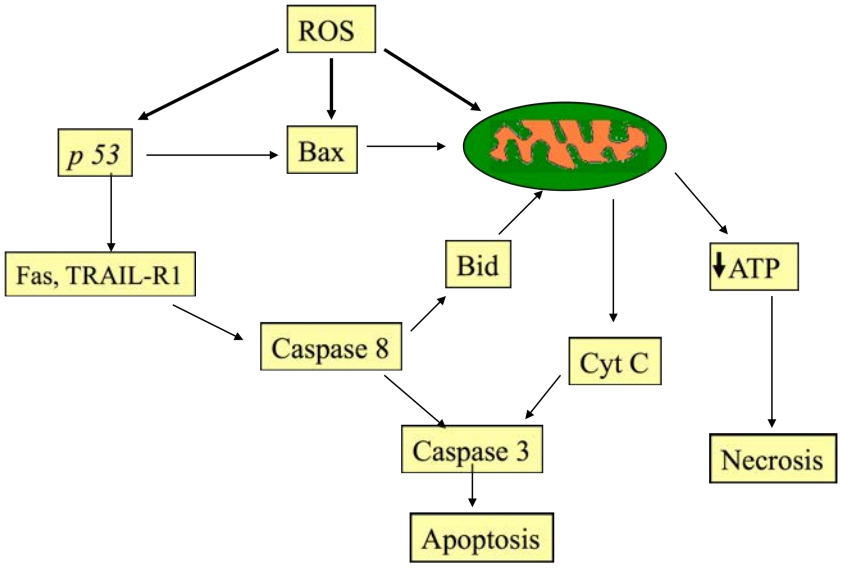

Figure 5. Schematic of ROS-induced apoptosis and necrosis.

sensitive NADH-ubiquinone oxidoreductase (complex I), 1 subunit (cytochrome b) of ubiquinol-cytochrome c oxidoreductase (Complex III), 3 subunits of cytochrome-c oxidase (complex IV), and 2 subunits of complex V (6 and 8 of ATP ases). Pol-γ is the only DNA polymerase found in the mitochondria, and is responsible for both replication and repair. Since 1988, when the first mutations in mt DNA were described, more than 400 mutations in mtDNA have been identified as being responsible for respiratory chain and OXPHOS diseases. Oxidative damage to mt DNA such as strand breaks and base modifications can occur either directly from ROS or from ROS derived lipid-hydroperoxide. For instance, •OH mediated modification of deoxyguanosine base of DNA to 8-hydroxydeoxyguanosine has been demonstrated. Gradual accumulation of somatic mt DNA mutations can finally cause mitochondrial dysfunction and loss of cellular energy production. mt DNA is highly susceptible to oxidative damage probably due to the (1) close proximity to the site of ROS/RNS production for being inside mitochondria, and that $O_2^{\cdot-}$ generated inside mitochondria is not permeable to cytosol (2) mt DNA lacks histone proteins (which protects nuclear DNA from oxidative damage); and (3) mitochondrial polymerases lack specificity for base excision repair, which is the major pathway eliminating oxidative DNA base lesions [20]. Many of these DNA damages are mutagenic, contributing to human diseases such as cancer, aging, cardiovascular, neurodegenerative diseases, chronic progressive external ophthalmoplegia, and the Kearns-Sayre syndrome [21-23]. Hereditary spastic paraplegia, dystonia, and Huntington's disease are other diseases in which mutations in gene related to mitochondrial function have been identified [24].

Apoptosis

Apoptosis, a special form of programmed cell death that plays an indispensable role in the development and homeostasis of multicellular organisms. Mitochondria contain several pro-apoptotic molecules that activate cytosolic proteins to execute apoptosis, block anti-apoptotic proteins in the cytosol and directly cleave nuclear DNA. Disruption of electron transport has been recognized as an early event of cell death. Mitochondrial permeability transition (MPT), a phenomenon characterized by mitochondrial swelling, uncoupling and inner membrane permeabilization to solutes of molecular mass up to 1500 Da plays an important role in initiating both apoptotic and necrotic cell death [25] . MPT is implicated in lethal cell injury from anoxia, ischemia/reperfusion, and oxidative stress to many cell types including heart [25]. ATP depletion caused by uncoupling of OXPHOS after the MPT leads to necrotic cell killing, where as cytochrome c release caused by mitochondrial swelling and outer membrane rupture after the MPT initiate apoptosis. An increase in cellular production of ROS is often observed in intrinsic (mitochondria mediated) pathway of apoptotic process triggered by various stimuli (Fig. 5).

The intrinsic pathway of apoptosis is mediated by the activation of proapoptotic members of the Bcl-2 family proteins ie. Bax, Bak. Activation of either Bax or Bak is required for apoptosis. Bax, a monomeric protein found in the cytosol, oligomerises on the outer mitochondrial membrane following apoptotic stimulus and causing mitochondrial membrane permeabilization. While Bak is an oligomeric integral mitochondrial membrane protein forms larger aggregate during apoptosis. Another Bcl-2 family protein, Bid provides crosstalk between the extrinsic (ie. death receptor pathway) and intrinsic pathways. Following this mitochondrial dysfunction, several apoptotic factors including cytochrome c (Cyt c), second mitochondria derived activator of caspase (Smac) are released from the mitochondrial intermembrane space into cytosol. Cyt c binds to the adapter apoptotic protease activating factor 1 (Apaf-1) that eventually results in the activation of cysteinyl aspartate specific proteases, caspases. Apoptosis-inducing factor (AIF), a proapoptotic

mitochondrial protein is also released from mitochondria whereupon it can translocate to nuclei and stimulates chromatin condensation and incomplete 50 kb DNA fragmentation (Stage I of apoptosis; caspase independent apoptotic stage). Smac contributes to caspase activation by binding and inactivating the endogenous inhibitor of caspases, IAPs. p53 can promotes apoptosis by up regulation of death receptor and death ligands including TNF-related apoptosis inducing ligand (TRAIL-R1), Fas and Fas L [26]. A transcriptional independent p53 mediated mitochondrial dysfunction and associated apoptotic mechanism has also been described.

Mitochondrial reactive oxygen species in cell signaling

Though the mitochondrial ROS are involved in the oxidative stress and pathophysiology of many human diseases, H_2O_2 was shown to be required for signaling by cytokine, growth factors, transcription factors such as activator protein 1 (AP-1), and nuclear factor –kappaB (NF- kB). Evidences suggest that mitochondrial ROS is involved in the signal transduction pathways that results in the cell differentiation, immune cell activation and metabolic adaptation of cells in hypoxic condition. H_2O_2 can oxidize thiol groups on cysteine residues of the target protein, phosphatases and can regulate its function in a signaling pathway including phosphatase and tensin homolog (PTEN), and mitogen activated protein kinase (MAPK) phosphatases [27]. Recently, Rawlands et al. suggests that exposure of microvascular endothelium to pro-inflammatory cytokine soluble TNF-α (sTNF-α) induced a Ca^{2+}dependent increase of mitochondrial H_2O_2, which results in shedding of TNFR1 from the endothelial surface and thereby regulate the severity of sTNF-α-induced microvascular inflammation [28]. Furthermore, under hypoxia, mROS have been shown to be required for activation of AMP-activated protein kinase (AMPK) and endocytosis of α subunit of Na/K ATPase [29].

mROS has also been involved in adaptive as well as innate immune cell functions Activation phenotype of T cells is controlled by a mitochondrial complex I-originated oxidative signal. T cell activation-induced expression of the cytokines IL-2 and -4 is determined by mitochondrial H_2O_2 and Ca^{2+} influx, which is, facilitated through the induction of the transcription factors NF-kB and AP-1 [30].

Future perspectives-mitochondrial pharmaceutics

Preservation of mitochondrial function is important for maintaining overall health. Several dietary supplements, including the mitochondrial cofactor and antioxidant lipoic acid (LA), increase endogenous antioxidants or mitochondrial bioenergetics [31]. Over expression of the genes for peroxiredoxin-3 (Prx-3), a mitochondrial antioxidant, or mitochondrial transcription factor A (TFAM), could ameliorate the decline in mtDNA copy number in failing hearts [32]. Over expression of TFAM may protect mtDNA from damage by directly binding and stabilizing mtDNA, which ameliorates mitochondrial dysfunction and thus the development and progression of heart failure. Similarly, over expression of GPx inhibits the development of left ventricular remodeling and failure after myocardial infarction. Co-enzyme Q10 (CoQ10) and L-acetyl-carnitine can be considered to be safe adjunct to standard therapies in cardiovascular disease [33]. Carvediol, cardiovascular drug has been proved to be effective in heart failure probably mediated through its potential antioxidant and antiapoptotic activities [34]. Though many of the drugs used in the treatment of cardiovascular diseases, mainly statins are proved to be antioxidants [35,36], data from well-designed randomized trials to issue the general recommendation for people to take antioxidant supplements in order to prevent the disease is insufficient.

Targeting of biologically active molecules to mitochondria in living cells will open up avenues for manipulating mitochondrial functions. Increasing pharmacological and pharmaceutical efforts have been undertaken to find effective therapies for disorders associated with malfunctioning mitochondria thus leading to the emergence of 'Mitochondrial Medicine'. The delivery of both, the small drug molecules and large macromolecules to and into mitochondria may provide the foundation for a large variety of future cytoprotective and cytotoxic therapies. The delivery of antioxidants may protect mitochondria from oxidative stress caused by a variety of insults; perhaps even contribute to slowing down the natural aging process. Attempts to achieve cell protection using antioxidants have already successfully been undertaken, many of them utilizing the avid reactivity of fullerene compounds with free radicals. The increase of mitochondrial concentrations of antioxidant drugs by selective targeting antioxidants to mitochondria in living cells should therefore be an effective therapy for a wide range of human diseases [37]. Two free radical scavengers, 4-hydroxy-2,2,6,6-tetramethylpiperidin-N-oxide (TEMPOL) and Salen-Mn (III) complex of o-vanillin (EUK-134) have been successfully synthesized and partially tested in term of their antioxidant and antiapoptotic properties [38].The mitochondrialy targeted version of vitamin E protected mitochondria from oxidative damage induced by iron/ascorbate far more effectively than vitamin E itself, as measured by the level of both, lipid peroxidation (thiobarbituric acid reactive species) and protein damage (protein carbonyls) [37]. However, the area of sub-cellular, i.e. mitochondria-specific delivery of drugs is still in its infancy.

Conclusion

Mitochondrial electron transport is an enzymatic source of ROS generation and also a target against ROS-induced damage. Chronic increase in ROS generation is observed as early event in many human diseases. Mutations in mt DNA and abnormalities in mitochondrial function are associated with common forms of diseases such as ischemic heart disease and neurodegenerative diseases. However, the strict causal relationships between abnormalities in mt DNA and many diseases have yet to be fully elucidated. Despite extensive experimental studies in animals using small molecular weight antioxidant to protect the mitochondrion from the oxidative insults and to improve the energy production, their clinical applications have not yet been fully elucidated. Increasing pharmacological and pharmaceutical efforts have been undertaken to find effective therapies for

disorders associated with malfunctioning mitochondria, mitochondria-specific delivery of drugs is still in its infancy. Therapeutic strategies to ameliorate the mitochondrial mediated oxidative stress should become a target of future research.

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Authors Column

Dr. Ajith T.A, received Master of Science in Medical Biochemistry from Kasturba Medical College, Mangalore, Karnataka, India in 1996 and Ph.D in Medical Biochemistry from Amala Cancer Research Centre, Thrissur, Kerala, India in 2004.

He has been PROFESSOR in Biochemistry at Amala Institute of Medical Sciences, Thrissur, Kerala. He has researched and authored 55 articles in peer-reviewed journals. His research is primarily focused on the role of natural compounds in cancer chemoprevention and their impact on the mitochondrial energy status. He serves as Editorial board member for nine international journals and reviewer for many National and International journals.