Cell Injury and Death: Oxidative Stress and Antioxidant Defense System: Review

Hücre Hasarı ve Ölümü: Oksidatif Stres ve Antioksidan Savunma Sistemi

Mukaddes EŞREFOĞLU, MD,[a]

[a]Department of Histology and Embryology, İnönü University Faculty of Medicine, Malatya

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Yazışma Adresi/*Correspondence:*
Mukaddes EŞREFOĞLU, MD
İnönü University Faculty of Medicine, Department of Histology and Embryology, Malatya, TÜRKİYE/TURKEY
drmukaddes@hotmail.com

ABSTRACT Cellular injury is defined as an alteration in cell structure or function resulting in stress that exceeds the compensatory ability of the cell. If the injury is severe enough, the cell suffers irreversible injury and dies. Cells death has historically been subdivided into necrosis and apoptosis. Actually, the simple apoptosis-necrosis classification does not adequately represent the complexity of cell death regulation. 'Autophagic cell death', 'mitoptosis', 'mitotic catastrophe', 'anoikosis' and 'oncosis' are the other expressions rarely used in order to define different types of cell death. Some mechanisms of apoptosis and necrosis are related to the process mediated by reactive oxygen and nitrogen species (ROS and RNS, respectively). Mitochondria are strong producers of ROS and at the same time, particularly susceptible to the oxidative damage produced by their action on lipids, proteins, and DNA. To protect cells from the damage caused by free radicals and related reactants, organisms have evolved several defense mechanisms to remove ROS from the intracellular environment. When free radical generation exceeds the defense capabilities of the organism, molecular damage is sustained. As this damage accumulates, cellular function gradually declines, eventually leading to death of cells, organs and the organism itself. Oxidative damage has been implicated in the pathogenesis of a range of diseases and ageing. Antioxidant and free radical scavenging agents against the destructive actions of free radicals are of obvious interest. In this paper, cell death concepts, their molecular mechanisms, histopathological aspects of oxidative stress related cell injury and death, and cellular anti-oxidative defense systems were reviewed.

Key Words: Antioxidants, cell death, oxidative stress, mitochondria, melatonin

ÖZET Hücresel hasar, hücrenin telafi edici yeteneğini aşan strese neden olan bir hücre yapı ve fonksiyon değişikliği olarak tanımlanır. Hasar yeterince ağırsa hücre, geri dönüşümsüz bir hasardan zarar görür ve ölür. Hücre ölümü tarihsel olarak nekroz ve apopitoz olarak ikiye ayrılır. Basit nekroz-apopitoz sınıflamasının hücre ölüm regülasyonunun karmaşıklığını yeterli şekilde temsil etmediği açığa çıkmıştır. 'Otofajik hücre ölümü', 'mitoptoz', 'mitotik katastrop', 'anoikoz' ve 'onkoz', farklı hücre ölüm şekillerini tanımlamak amacıyla nadiren kullanılan diğer terimlerdir. Nekroz ve apopitozun bazı mekanizmaları reaktif oksijen ve nitrojen türlerinin (sırayla ROS ve RNS) aracılık ettiği olayla ilişkilidir. Mitokondriyonlar güçlü ROS üreticileridir; aynı zamanda lipitler, proteinler ve DNA üzerindeki etkileri sonucunda üretilen oksidatif hasarlardan da özellikle sorumludurlar. Organizmalar kendilerini serbest radikal ve bağlantılı reaktantlardan korumak için, serbest radikalleri intraselüler ortamdan uzaklaştırmaya yönelik bir takım savunma mekanizmaları geliştirmiştir. Serbest radikal oluşumu, organizmanın savunma kapasitesini aştığı zaman moleküler hasar ortaya çıkar. Bu hasar biriktikçe, sonuçta hücrelerin, organların ve organizmanın bizzat kendisinin ölümüne neden olacak şekilde hücresel fonksiyon kademeli olarak azalır. Oksidatif hasar bir seri hastalığın ve yaşlanmanın patogeneziyle bağlantılı bulunmuştur. Serbest radikallerin yıkıcı etkilerine karşı serbest radikal süpürücüleri ve antioksidanlar büyük ilgi çekmektedir. Bu makalede hücre ölüm kavramı, moleküler mekanizmaları, oksidatif strese bağlı hücre hasarı ve ölümünün histopatolojik yönleri ve antioksidan defans sistemi gözden geçirilmiştir.

Anahtar Kelimeler: Antioksidanlar, hücre ölümü, oksidatif stres, mitokondriya, melatonin

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Cellular injury is defined as an alteration in cell structure or function resulting in stress that exceeds the compensatory ability of the cell through normal physiologic adaptive mechanisms.[1] Cells have limited ability to express their response to injury. They may respond (a) by dying, (b) by undergoing a transient or permanent morphological transformation (c) by mitotic activity that again may be transient or sustained, normal or abnormal, and may result (d) in either normal progeny or a modification thereof.[2] Cell injury is not always lethal. Agents that cause cell injury may lead to regeneration or adaptive metabolic or structural changes including atrophy, hypertrophy, hyperplasia, metaplasia or dysplasia.[1,2] On the other hand, excessive adaptive cellular changes are also markers for injury and diseases. Cell injury may be either reversible or irreversible. If the injury is severe enough and 'a point of no return' is reached, the cell suffers irreversible injury and dies.[1] It has been proposed that this 'a point of no return' step could be massive caspase activation, loss of the mitochondrial transmembrane potential, complete permeabilization of the outer mitochondrial membrane or exposure of phosphatidylserine residues that emit 'eat me' signals to neighboring cells.[3,4] 'Dead cells' would be different from 'dying cells' that are in process of cell death, which can occur through a variety of different pathways. The Nomenclature Committee on Cell Death (NCCD) suggest that a cell could be considered dead when any of the following molecular or morphological criteria are observed: 1) the cell has lost its integrity of the plasma membrane, 2) the cell has undergone complete fragmentation into discrete bodies; and/or, 3) its fragments have been engulfed by an adjacent cell in vivo.[5] Cell death has historically been subdivided into regulated and unregulated mechanisms. Apoptosis, a form of regulated cell death reflects a cell's decision to die in respond to cues and is executed by intrinsic cellular machinery. Unregulated cell death, often called necrosis, is caused by overwhelming stress that is incompatible with cell survival.[6] However, recently it has become clear that necrosis is a molecularly regulated event.[7,8] The clues about the concept of programmed course of necrosis are: 1) necrosis can occur during development and in adult tissue homeostasis; 2) susceptibility to necrotic cell death can be regulated by genetic and epigenetic factors; 3) the inhibition of some enzymes and processes can prevent necrosis; 4) inhibition of caspases can change morphological appearance of cell death from one type to another.[9-12] 'Necrosis' is usually considered a type of cell death with no signs of apoptosis or of autophagy.[12,13] A classical positive definition of necrosis is based on morphological appearance such as early plasma membrane rupture, dilatation of cytoplasmic organelles, in particular mitochondria.[12] Mitochondria, endoplasmic reticulum (ER) and lysosomes have essential roles in the control of necrotic cell death.[14] Necrosis is generally considered a passive process because it does not require new protein synthesis and has only minimal energy requirements. In humans, necrotic cell death occurs generally in response to severe physiological changes including hypoxia, ischemia, hypoglycemia, toxin exposure, exposure to reactive oxygen metabolites, extreme temperature changes and nutrition deprivation.[15,16] Several neurodegenerative syndromes and diseases, such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, vascular-occlusive diseases, infection and inflammatory diseases and cancer involves necrosis.[17-19]

The morphological appearance of necrosis is often that of oncosis. The expression 'oncosis' defines a cell death with cytoplasmic swelling, dilatation of cytoplasmic organelles, mechanical plasma membrane rupture and a moderate chromatin condensation. NCCD recommends limiting the use of the expression 'oncosis', as it overlaps with necrosis, and a partial apoptosis evolving into necrosis.[5] Oncosis is caused typically by ischemia that interferes with ATP generation or increases permeability of the plasma membrane. It is usually accompanied by karyolysis and evolves within 24 hours to typical necrosis.[18]

Apoptosis is a type of cell death that is accompanied by rounding-up of the cell, retraction of pseudopodes, reduction of cellular volume, condensation of the chromatin, fragmentation of the nucleus, little or no ultrastructural modification of

cytoplasmic organelles, plasma membrane blebbing, and maintenance of an intact plasma membrane until late stages of the process.[5,12] Detection of DNA fragmentation is currently one of the most frequently used techniques in the study of apoptotic cell death. Highly sensitive cytochemical techniques have been developed to visualize DNA fragmentation in individual nuclei. The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method utilizes the activity of the terminal deoxynucleotidyl transferase enzyme to label the 3' ends of DNA strand breaks, which may then be identified in individual nuclei by microscopy (Figure 1).[20]

'Autophagic cell death' 'mitoptosis', 'mitotic catastrophe', 'and 'anoikosis' are the other cell death types. These expressions are rarely used in order to define different types of cell death. Autophagic cell death is a type of cell death that occurs without chromatin condensation, accompanied by massive autophagic vacuolization of the cytoplasm. The double-membraned vacuoles contain degenerating cytoplasmic organelles or cytosol.[5,21] Normally, damaged or dysfunctional organelles are removed by autophagy.[22] It is an evolutionary-conserved intracellular catabolic mechanism that operates at low levels under normal conditions to mediate the degradation of cytoplasmic components, protein aggregates and expired intracellular organelles by forming autophagosomes. The contents of autophagosomes are degraded by lysosomal enzymes after autophagosomes fuse with lysosomes.[6] Autophagy can assume the killer role when apoptosis is unavailable.[22] Authophagy has a well-established role in defending against viral and bacterial invasion.[23] It is possible that autophagic cell death might be induced in a manner similar to that of apoptosis.

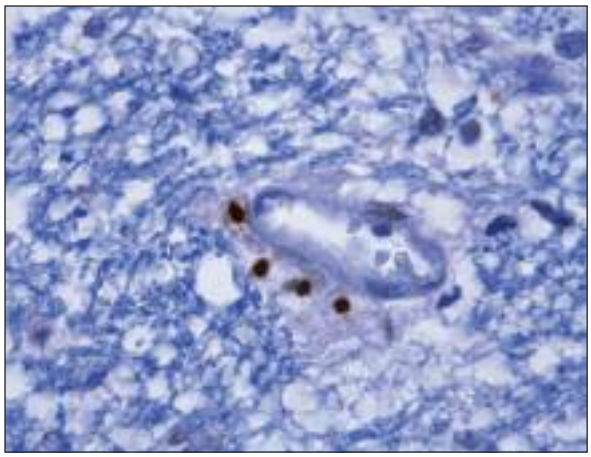

FIGURE 1: TUNEL + apoptotic cell nuclei around the vessels are observed in the brain of an aged rat; X 100.

The term, 'mitoptosis', was coined to describe suicide of the mitochondrion. Skulachev postulated that mitoptosis operated as a mechanism ridding the mitochondrial population in the cell from malfunctioning organelles [for example, from those that overproduce reactive oxygen species (ROS)].[24] The following chain events seem probable: ROS → opening of permeability transition pore → collapse of electric potential difference across the inner mitochondrial membrane → cessation of electric potential difference across the inner mitochondrial membrane-dependent import of mitochondrial protein precursors → death of mitochondrion which can not be repaired.[25]

Mitotic catastrophe is a cell death occurring during or shortly after a dysregulated or failed mitosis and can be accompanied by morphological alterations such as micronuclei and multinucleation.[26] Anoikosis is a classic apoptosis induced by loss of the attachment of cells form the substrate or the other cells.[5]

The distinction between cell death types is important, particularly because necrosis is often associated with unwarranted cell loss in human pathologies, and can lead to local inflammation (Figure 2).[8,27] Additionally apoptotic cells are engulfed completely by phagocytes; only parts of necrotic cells (which swell) are internalized by a macropinocytotic mechanism.[28] Apoptotic cells do not seem to attract neutrophils or lymphocytes. This could reflect a qualitative difference of apoptotic cell death, but it could also mean that cells dying singly release such small quantities of chemoattractans that not all the molecular species reach the vascular endothelium in effective concentrations.[18]

Necrosis can occur as a result of the activation of specific signal transduction cascades and subse-

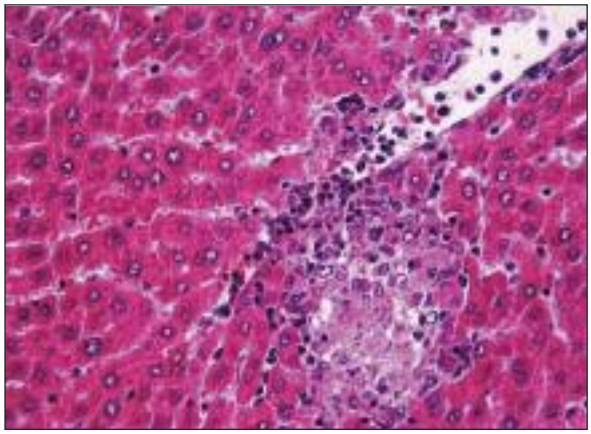

FIGURE 2: Necrotic area associated with inflammatory cell infiltration is observed in liver damage induced by acute pancreatitis in rats (HE, x40).

quently can be overt or revealed only by the inhibition of apoptosis and/or autophagy. Such a default occurrence of necrotic cell death and its unmasking by inhibition of apoptosis and/or autophagy might reflect its early emergency in evolution, perhaps as the early eukaryotic cell death pathway, with subsequent additions of other layers of cell death mechanisms such as apoptosis and autophagy.[12,29] The process leading to caspase activation or autophagy has acquired the capacity to 'decide' the cell death process and thus it starts upstream of the point of no return.[29] It is progressively becoming clear that what determines the cell fate is the intensity of an insult and the expression levels of the downstream signal transducers, as well as the extend of the calcium overload and the intercellular ATP levels.[30] The endogenously originated late serious energy depletion has been considered the trigger for the transition from the end of a fully developed execution phase of apoptosis to secondary necrosis.[31,32]

Recent studies have demonstrated that in response to a given death stimulus, there is often a continuum of apoptosis and necrosis. Many insults induce apoptosis at lower doses and necrosis at higher doses.[18] While mediators for necrosis such as calcium and ROS have been reported to contribute to apoptosis, recent studies have started to demonstrate that the ability of such mediators to initiate necrotic cell death depends on active participation by the dying cell. The core events of necrosis are bioenergetic failure and rapid loss of plasma membrane integrity.[8] Even in response to a certain dose of death-inducing agent, features of both apoptosis and necrosis may coexist in the same cell. In addition, if not engulfed by neighboring cells, dead cells in the late stage of apoptosis may present necrotic features due to the loss of cellular energy metabolism and plasma membrane integrity. This process is called 'apoptotic necrosis' or secondary necrosis.[18] Although apoptotic necrosis is activated by the same stimuli that initiate apoptosis, the morphological features such as organelle swelling, rapid mitochondrial dysfunction, plasma membrane permeabilization and lack of nuclear fragmentation are characteristic of necrotic cell death.[6]

MOLECULAR TARGETS OF CELLULAR INJURY

Any biologically important molecule in a cell can be the target of injury producing stress. However, the cell membrane, energy metabolism, protein synthesis and gene systems are particularly vulnerable. The integrity and function of the whole cell and the organelles depend on the integrity of their phospholipid membranes. By controlling the selective transport of molecules, the plasma membrane keeps the cell in osmotic equilibrium with extracellular fluid. Damage to plasma membrane increases the cell's permeability to sodium and water leading to swelling, and even disruption of the cell.[33] The core events of necrosis are bioenergetic failure and rapid loss of plasma membrane integrity. The inability to maintain the electrochemical potentials results in cytoplasmic swelling, rupture of plasma membrane, and cytolysis. The loss of membrane potentials may be the consequence of cellular energy depletion, damage to membrane lipids, and/or loss of function of homeostatic ion pumps/channels.[8]

As protons are pumped across the inner mitochondrial membrane, a membrane potential is generated. The resulting electrochemical gradient drives protons back into the matrix, generating ATP form ADP. Inhibition of mitochondrial respi-

ratory chain activity results in reduced cellular ATP and can induce cell death by necrosis.[8] It has been suggested that both apoptosis and necrosis can be mitochondria-dependent. In both cases, 'death proteins' are postulated to be released from the mitochondrial intermembrane space. However, in apoptosis this occurs due to an increase in permeability of the outer mitochondrial membrane for proteins (proapoptotic proteins induce the formation of pores); the inner membrane remains intact. As for necrosis permeability transition pore is formed in the inner membrane so the outer membrane is broken due to the matrix swelling.[34,35] Ca^{2+}, inorganic phosphate, alkaline pH, and ROS are a few of the many agents that promote the mitochondrial permeability transition.[36] As a consequence of permeability pore opening, solutes with a molecular mass of up to 1500 Da nonselectively diffuse across the mitochondrial inner membrane, leading to mitochondrial depolarization, uncoupling of oxidative phosphorylation, and large amplitude swelling, which in turn can lead to ATP depletion and cell death.[37] Mitochondrial damage and release of mitochondrial proteins amplifies apoptotic signaling in mammalian cells. Cytochrome c, released form damaged mitochondria, promotes the formation of heptameric 'apoptosome' megacomplex of APAF-1 (apoptotic protease activating factor-1) and caspase 9. Activated caspase-9 in turn cleaves and activates downstream caspases (caspase-3, -6, -7 etc) that carry out the execution phase of apoptosis.[38]

During the degradation phase of active necrosis several biochemical alterations have been described including mitochondrial dysfunction (production of ROS by mitochondria and swelling of mitochondria), high production of ROS, serious ATP depletion, intense ionic imbalance (failure of Ca^{2+} homeostasis), activation of a few proteases (particularly calpains and cathepsins), perinuclear clustering of organelles. These alterations ultimately converge in extensive lysosome rupture and ultimately plasma membrane rupture.[29,39] The important Ca^{2+} overload induces enhanced activation of hydrolyzing enzymes, including calpains and leads to exaggerated energy consumption and impairment of energy production; ATP depletion and activated calpains were shown to induce lysozome rupture, and released lysozomal cathepsins contribute to cytoplasmic membrane damage.[39-48] Calpains and cathepsins are the major proteases involved in necrosis.[8] In passive necrosis, cytoplasmic membrane damage is due to serious energy failure and to the cumulative activity of hydrolytic enzymes and of ROS. These events result in progressive membrane permeabilization to molecules of increasing size until the rupture of the membrane. This rupture is a consequence of the continuous cell swelling (oncosis or necrotic volume increase).[49]

Uncontrolled calcium entry can be a cause, as well as a result of, cell death. In stressed cells, progressive calcium entry occurs before the agonal contortion, blebbing, and swelling.[50] In viable cells, the plasma and intracellular membranes are virtually impermeable to Ca^{2+}. Most of the intracellular Ca^{2+} is stored in the ER. When the ER Ca^{2+} is released into the cytosol or the extracellular Ca^{2+} crosses the plasma membrane, cell death can be initiated due to the activation of Ca^{2+}-dependent proteases and/or mitochondrial overload.[51] Increased cytosolic calcium concentration results in the activation of some enzymes such as phospholipases, ATPases and proteases, which break down critical components of the cell.[1,52] Moreover, calcium is a key regulator of mitochondrial functions and acts at several levels within the organelle to stimulate ATP synthesis. The dysregulation of mitochondrial calcium homeostasis is now recognized to play a key role in several pathologies. For example, mitochondrial matrix Ca^{2+} overload can lead to enhanced generation of ROS, some of which are highly toxic and deleterious to cells and tissues.[53] Like many other insults, increased cytosolic Ca^{2+} can initiate either apoptosis or necrosis. The outcome of cell death is probably determined by the concentration of cytoplasmic Ca^{2+}. Whereas low to moderate Ca^{2+} triggers apoptosis, higher concentrations of Ca^{2+} is associated with necrosis.[54] The mitochondrial metabolic status may also affect the sensitivity of mitochondria to Ca^{2+} poisoning and contribute to the determination of cell death.[55]

Cells require a constant energy supply to drive metabolism and biosynthetic reactions. Depletion of ATP impairs the ability of the cell to synthesize structural and functional proteins, and shifts the energy metabolism towards anaerobic glycolysis accompanied by the accumulation of inorganic phosphate and lactic acid. The decrease of intracellular pH together with the increase in the concentration of cytosolic calcium alters the activity of many intracellular enzymes involved in homeostatic regulation. The 'acidosis' interferes with the enzyme functioning and can damage nuclear DNA. There is also reduction in protein synthesis.[1] However, after necrosis protein sythesis is sustained in the dying cell up to the point where the cell loses its membrane integrity.[56] Denaturation of cellular enzymes or structural proteins can severely impair cellular functions. Damage to structural proteins can impair the intracellular transport system and disrupt the supportive protein cytoskeleton of cells.[57] Additionally, decline in the function of the ATP-dependent ion pumps in the cytoplasmic membrane can lead to the opening of so called death channels in the cytoplasmic membrane that is selectively permeable to anions.[58] The opening of death channel results in colloid osmotic forces and entry of cations that drive the cytoplasmic membrane to swell and ultimately rupture.[8] Manipulating cell metabolism and ATP generation can regulate the cell fate. Because cells depend on so many ATP-dependent reactions, ATP levels can rapidly decline if the cell's ability to generate ATP through mitochondrial oxidative phosphorylation and/or cytosolic glycolysis is impaired.[59] For the normal progress of apoptotic pathways energy is required, and an initial increase in cytosolic ATP in apoptosing cells has been reported.[24,60] ATP is required for cell shrinkage, bleb formation, caspase activation, enzymatic hydrolysis of macromolecules, chromatin condensation, DNA fragmentation, nuclear fragmentation, and apoptotic body formation.[24,61] Thus, progression of apoptosis eventually leads to a late depletion of the intracellular ATP pool. This late serious energy depletion has been considered the trigger for the transition from the end of a fully developed phase of apoptosis to secondary necrosis.[62] It has been shown that lowering of [ATP] by 1/3 does not induce cell death whereas a transient decrease of more than 97% in [ATP] or its three-fold lowering for a longer period of time gives rise to necrosis.[63-65] Intracellular ATP levels appear to be a factor determining cell death fate by apoptosis and necrosis.

The first changes in the cell undergoing necrosis are mild cytoplasmic swelling, dilatation of smooth endoplasmic reticulum (SER), and loss of ribosomes from rough endoplasmic reticulum (RER) (Figure 3).[1,66] A further change is blebbing from the plasma membrane of cytoplasmic fragments that include cytosol but not the larger organelles such as mitochondria. All these changes also occur in sublethally injured cells. An important question therefore is the nature of the 'point of no return' at which there is irreversible commitment to necrosis. This commitment coincides closely with two mitochondrial changes: a violent dilatation and the appearance of matrix densities.[1] Mitochondria become edematous with increased translucence of the matrix, partial or total destruction of crests and sometimes the presence of myelin figures (Figures 4, 5).[67,68] The necrotic cells swell rapidly and both the plasma membrane and the internal membranes begin to rupture. Organelles spill out and are found in extracellular space (Figure 6). However, nuclear structures remain relatively intact.

Necrosis is associated with cell swelling and death. Apoptosis occurs where death is a part of re-

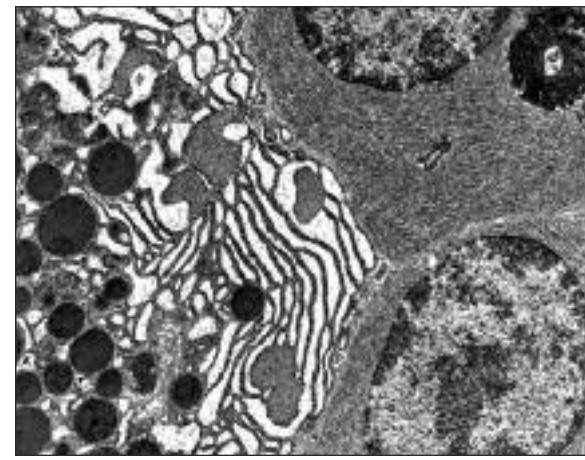

FIGURE 3: Prominent dilatation of granular endoplasmic reticulum in one of the pancreatic aciner cells (left one) is observed in acute pancreatitis in rats. Uranyl acetate and lead citrate X 8.000.

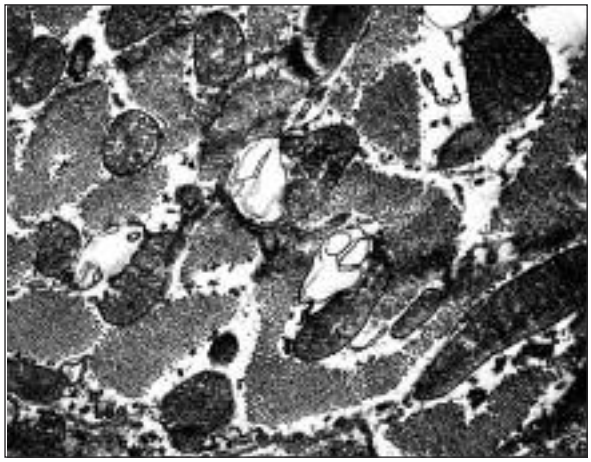

FIGURE 4: Many swollen, vacuolated and degenerated mitochondria are seen in heart muscle of an aged rat. Uranyl acetate and lead citrate X 8.000.

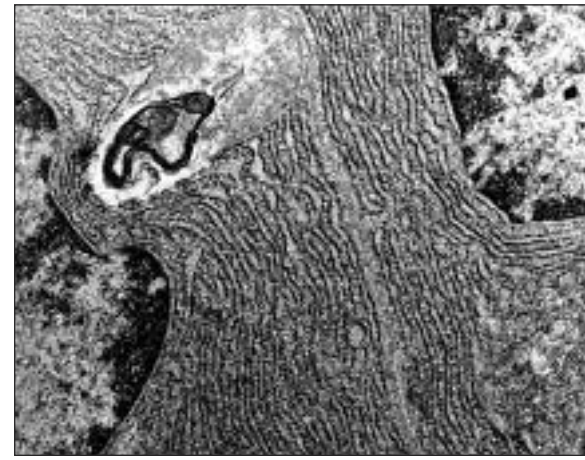

FIGURE 5: A swollen mitochondrion containing a dense myelin figure is observed in pancreatic aciner cell in acute pancretatitis in rats. Uranyl acetate and lead citrate X 20.000.

gulated process and is associated with cell shrinkage. In contradistinction to necrosis, mitochondria do not undergo swelling and rupture internal membranes.[1,18] The most striking morphological change is in nuclear chromatin condensation, in which chromatin condensation appears as dense hemilunar caps or complete toroids (margination of chromatin) (Figure 7).[1] The nucleus may also break up (karyorhexis) and the cell emits processes (the budding phenomenon) that often contain pyknotic nuclear fragments. These processes tend to break off and become apoptotic bodies, which may be phagocytized by macrophages or neighboring cells or remain free; however, the cell may also shrink into a dense, rounded mass, as a single apoptotic cell.[18] Biochemical events that are behind the apoptotic morphotype may include caspase activation, mitochondrial permeability transition with loss of membrane potential, moderate increased production of ROS, moderate cytosoloic Ca^{2+} overload and intranucleosomal DNA degradation.[24,61,69]

Phagocytosis is the process of engulfment of extraneous material by the cell.[2,33] At times, whole cells undergoing necrosis or apoptosis may be engulfed by their neighbors. The apoptotic cells and bodies into which it may fragment undergo progressive degenerative changes within the phagosome of the ingesting cell (Figure 8). There are important differences in the process of elimination of apoptotic and necrotic cells. Apoptosis is often

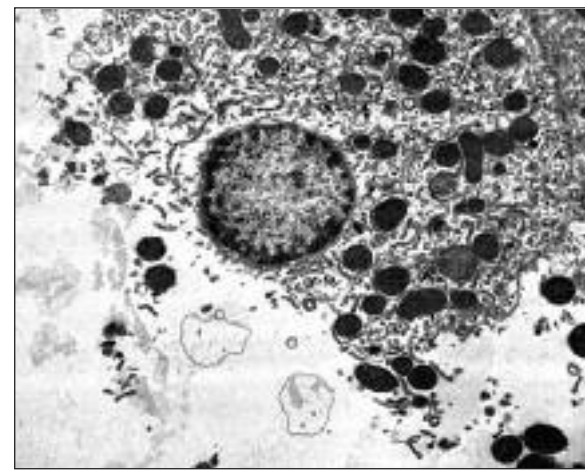

FIGURE 6: Hepatocyte necrosis induced by benzoprene administration in rat. Plasma membrane is ruptured, degenerated cytoplasmic organelles including mitochondria and fragmented membranes are spilled out. X 6.300.

considered a 'clean death' because it prevents the release of the intracellular content of the cell. In contrast, necrotic cell death and spilling of the intracellular content into the extracellular environment can trigger inflammation by providing 'danger' signals for the surrounding cells and immune competent cells.[70] The mechanisms by which apoptotic and necrotic cells are phagocytozed by macrophages are also different. Engulfment of cells under active necrosis is delayed and quantitatively and kinetically less efficient as compared to the uptake of apoptosing cells.[28,71] Engulfment of apoptotic cells occurs early, that is when the dying/dead

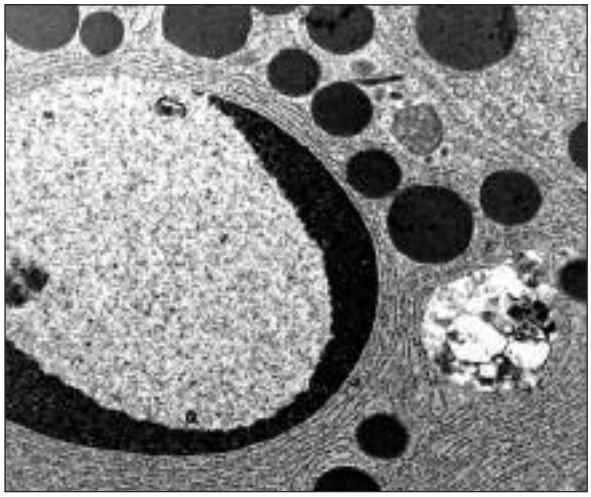

FIGURE 7: Acinar cell apoptosis induced by caerulein pancreatitis in rats. The nuclear chromatin condensation in apoptotic cell engulfed by its neighbor is highly characteristic. Uranyl acetate and lead citrate X 10.000.

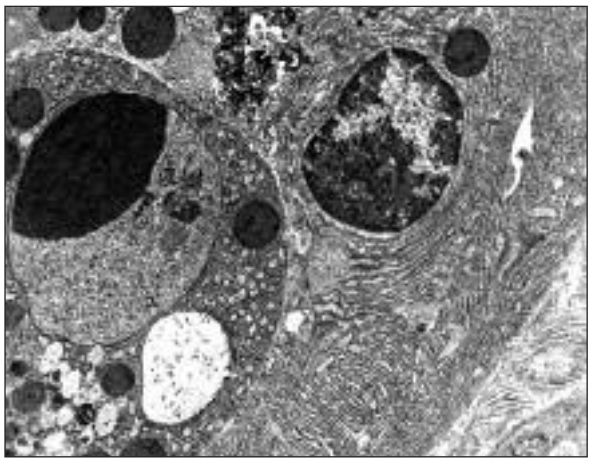

FIGURE 8: A aciner cell undergoing apoptosis is engulfed by its neighbor during acute pancreatitis in rats. Uranyl acetate and lead citrate X 8.000.

cells or apoptotic bodies still have near-to-normal cytoplasmic membranes whereas engulfment of necrotic cells occurs after cytoplasmic membrane damage so that fragments are engulfed.[62] Like apoptotic cells, apoptotic bodies also express signals for engulfment and are phagocytozed by scavengers or undergo secondary necrosis if not engulfed.[72,73] Autophagocytosis is the sequestration of damaged organelles within the lysozomes of the same cell (Figure 9).[33] As in active primary necrosis, serious energy depletion, high Ca^{2+} cytosolic overload and high ROS production will lead to activation of calpains and to lysozome rupture.[46,74] This process may allow cell damage to be confined and be repaired. Apoptosis, necrosis and autophagic response can all be initiated from the lysozomes. A critical point to determine the cell death mode is at the regulation of the lysozomal membrane permeabilization (LMP). Sphingosine, one of the sphingolipids of the structural component of cell membranes, has been shown to regulate LMP.[75] Sphingosine induces partial lysozomal rupture and apoptosis at low-to moderate concentrations, and extensive lysozomal rupture and necrosis at high concentrations.[76]

REACTIVE OXYGEN SPECIES

Aerobic organisms require ground state oxygen to live. However, the use of oxygen during normal metabolism produces ROS, some of which are highly toxic and deleterious to cells and tissues.[77-79] Physiologic levels of ROS can regulate transcription, serve as signal molecules, and defend against pathogen infection.[8] Free radicals are molecules that have one or more unpaired electrons.[80] The isolated preparations of mitochondria, ER, cytosol and nuclear membranes have all been shown to be sources of ROS.[1] The superoxide radical in the course of cellular metabolism is mainly produced during electron transport in the mitochondria.[77-79,81,82] During ageing and various stress conditions, peroxisomes may even become a source of ROS.[83] The primary function of electron transport chain (ETC) is ATP synthesis via oxidative phospohorylation.

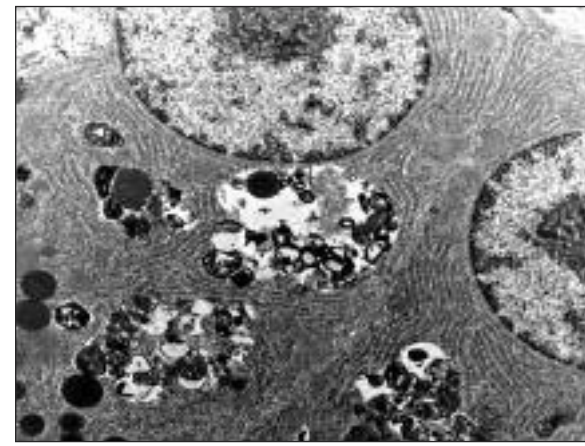

FIGURE 9: Caerulein-induced pancreatitis in rats. Many vacuoles containing granular or membraneous figures represent autophagocytosis of damaged organelles by lysosomal enzymes. X; 8.000.

The ETC, located in the inner mitochondrial membrane comprises a series of electron carrier enzymes.[84] Mitochondria generate superoxide from Complexes I and III in the ETC and release superoxide to both the mitochondrial matrix and intermembrane space.[82,85] The inability of superoxide to cross the mitochondrial inner membrane suggests that these two pools of superoxide affect mitochondrial properties and morphology, differently and more importantly depending on their location cause potential oxidative damage to different proteins and lipids, as well as DNA.[86,87]

The end product of the respiratory chain is water that is generated in a four-electron reduction of molecular oxygen (O_2). However, a small portion of O_2 is involved in generation of ROS including superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and the extremely reactive hydroxyl radical ($OH^{\cdot}$).[79,88,89] These free radicals are short-lived molecules containing an unpaired electron in an outer orbital-an electron that is not contributing to normal intermolecular bonding. These are essentially free chemical bonds, which are energetically unstable and highly reactive.[90] Mitochondria can also produce nitric oxide ($NO^{\cdot}$).[91,92] Nitric oxide can be converted to various reactive nitrogen species (RNS) such as nitroxyl anion (NO^-) and peroxynitrite ($ONOO^-$).[93] Free radicals can attack the double bond of unsaturated phospholipids in cell membranes, which eventually degrade the structural integrity of cell membranes. These double bonds are excellent targets for ROS attacks. Lipid oxidation can lead to the loss of the integrity of both plasma membrane and intracellular membranes such as that of lysozomes and the ER, leading to an intracellular leak of proteases or an influx of Ca^{2+} and resulting in necrosis.[94] They also impair the functions of enzymes by causing fragmentation of polypeptide chains or cross-linking sulfhydryl groups in proteins. In addition, they cause strand breaks or abnormal cross-linking in DNA.[90,95-97] Damage to DNA may lead to DNA-damage response, including activation of p53 and PARP (a nuclear enzyme). While activation of p53 causes apoptosis and cell cycle arrest, hyperactivation of PARP leads to necrosis.[98] ROS can initiate mitopotosis, which is postulated to rid the intracellular population of mitochondria from those that are ROS overproducing. Massive mitoptosis can result in cell death due to release to cytosol of cell death proteins normally hidden in the mitochondrial intermembrane space.[24]

Hydroxyl radical is the most reactive species that can attack and damage almost all molecules found in the living cell. Since it is so reactive, $OH^{\cdot}$ does not stay around more than a few microseconds before combining with a molecule. The reactions of $OH^{\cdot}$ leave behind the legacy in the cell in the form of propagating chain reactions. Thus it attacks DNA, free radical chain reaction propagate through the DNA and cause chemical alterations of the bases as well as strand breakage. The best-characterized biological damage caused by $OH^{\cdot}$ may be its ability to stimulate the free radical chain reaction known as lipid peroxidation. This occurs when $OH^{\cdot}$ is generated close to membranes and attacks the fatty acid side chains of the membrane phospholipids.[1,52,99] One $OH^{\cdot}$ can result in conversion of many hundred fatty acid-chains into lipid hydroperoxides. Accumulation of lipid hydroperoxides in a membrane disrupts its functioning and can cause it to collapse.[52,90] Some of the products of lipid peroxidation (malonaldehyde and 4-hydroxynonenal) increase the permeability and deformability of the membranes in which they are found. Mitochondrial membranes are particularly susceptible to this sort of damage, perhaps because they combine a high risk of ROS-mediated peroxidation.[1] We have shown mitochondrial degeneration in a vast array of oxidative stress conditions e.g., ageing (Figure 4), pancreatitis (Figure 5), and exposure to toxic agents (Figure 6).

Mitochondrial oxidative phosphorylation is the major ATP synthetic pathway in eukaryotes. Additionally, mitochondria are the site of steroid hormone and porphyrin synthesis, the urea cycle, lipid metabolism, and interconversion of amino acids.[100] They also play central roles in xenobiotic metabolism, glucose sensing/insulin regulation and cellular Ca^{2+} homeostasis.[53,101,102] Clearly, the development of mitochondrially-targeted drugs is an important research direction.[103,104]

Outside mitochondria, ROS are generated during biotransformation of various xenobiotics and drugs, inflammation, UV, and ionic radiation, etc.; and in conjunction with RNS by nitric oxide synthase activity.[105] Major extramitochondrial ROS sources are represented by cytochrome P450-dependent reactions.[106]

Mitochondria play an important role in controlling the life and death of a cell. Beyond a fundamental role in energy metabolism, they also play roles in thermogenesis, maintenance of cellular redox potential, Ca^{2+} homeostasis, ROS production, cell signaling and death.[107] In humans, numerous pathological conditions have been linked to mitochondrial dysfunctions. Cancer, diabetes, obesity, neurodegeneration, sepsis, and even ageing are all associated with mitochondrial dysfunction.[108-114]

OXIDATIVE STRESS RELATED PATHOLOGIES

It seems likely that tissue destruction and degeneration result in increased oxidant damage by the disruption of mitochondrial structure, so almost any disease is likely to be accompanied by increased free radical formation.[90] Considerable interest in oxidative stress comes from related pathologies including atherosclerosis, hypertension, ischemia-reperfusion, inflammation, cystic fibrosis, cancer, type-2 diabetes, Parkinson's and Alzheimer's disease, and other neurodegenerative diseases.[105] Indeed, defining the aspects of aging lies in the progressive vicious cycle in which oxidative stress plays a major role.[77,115] Oxidative stress arises from a significant increase in concentrations of ROS and RNS to the levels that are toxic to biomolecules, including DNA, lipids, and proteins. It is however known that ROS exert important regulatory functions.[116] Hence, a basal or tonal concentration of ROS, especially at the level of the mitochondrion, is essential for basic cell signaling processes.[89,117,118] In other words, all ROS are not created equal, and compartmentalization and concentration gradients are highly important.[89]

ANTIOXIDANT DEFENSE SYSTEM

To protect cells from the damage caused by free radicals and related reactants, organism have evolved several defense mechanisms to rapidly and efficiently remove ROS from the intracellular environment.[119] Molecules that directly neutralize (e.g., scavenge) free radicals as well as processes that otherwise rid cells of these reactive species are known as antioxidants.[120] Organisms endogenously generate a variety of antioxidants, such as glutathione whereas others are ingested, such as vitamin E.[115] When organisms are exposed to toxins; psychological and physical stress; and in advanced age, when free radical generation increases and exceeds the endogenous and exogenous defense capabilities of the organism; molecular damage is sustained.[121,122] Only excessive ROS or RNS production and/or decrease in detoxification mechanisms lead to oxidative stress and pathological conditions.[116] Thus, antioxidants may function to prevent the formation of or to detoxify free radicals, to scavenge ROS or their precursors. Halliwell has proposed this useful definition: 'an antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate'.[123]

Types of antioxidants include antioxidant vitamins (e.g., ascorbic acid, α-tocopherol, β-carotene), inorganic antioxidants (e.g., selenium), synthetic antioxidants (e.g., butylated hydroxyanisole), and a range of plant-derived polyphenols.[89] Organisms widely use glutathione, glutathione peroxidase, glutathione transferase, superoxide dismutase (SOD), catalase (CAT), and a variety of other antioxidants to protect themselves against generation of ROS.[90,124,125] Glutathione (GSH), most abundant nonprotein thiol in the cell, plays a critical role in antioxidant defense mechanism and in the detoxification of endobiotic and xenobiotic electrophiles.[126] Since mitochondria are not competent to synthesize glutathione, it is transported from the cytoplasm to the mitochondria.[126,127] Mitochondrial glutathione pool plays a critical role in cytoprotection.[116,128] Nevertheless, because the concentration of tripeptide glutathione (GSSG/ GSH) is so much higher than of any other system, the GSH/GSSG pool within the cell dominates the intracellular redox environment and comprises the

principal redox buffer of the cell. Hydrogen peroxide and other ROS are degraded by several selenium-dependent glutathione peroxidases (GPx), which, with glutathione, convert H_20_2 to water.[129,130] When GSH deficiency is produced in newborn rats or guinea pigs, the animal develops multiorgan failure and dies within a few days.[131] GSH functions directly in the destruction of ROS and as a substrate for GSH peroxidases (both selenium containing and others), which catalyze the reduction of hydrogen peroxide and other peroxides.[126] Another detoxification enzyme, catalase (CAT), can contribute to ROS detoxification. The activity of CAT converting H_20_2 to water is restricted in most tissues to peroxisomes. Actually, the heart mitochondria are the only mitochondria that also contain CAT. Catalase can operate elevated concentrations of H_20_2, e.g in skeletal muscle, erythrocytes.[132] Superoxide anion, $O_2^{\cdot-}$, in the mitochondrial matrix is converted to H_2O_2 by matrix MnSOD while $O_2^{\cdot-}$ released to the intramembrane space is partly dismuted by intermembrane space CuZnSOD.[133,134] Any residual $O_2^{\cdot-}$ which diffuses into the cytosol is similarly converted by cytosolic CuZnSOD. If any mitochondrial $O_2^{\cdot-}$ can reach the extracellular space, it then is detoxified by extracellular CuZnSOD. Once H_20_2 is produced, it can easily penetrate through membranes, owing to its uncharged property and poor reactivity.[105]

Melatonin (N-acetyl-5-methoxytriptamine) is the best known chemical mediator of the pineal gland.[135,136] It was first isolated from the bovine pineal gland and structurally identified by Lerner and colleagues in 1958.[137] In addition to the pineal gland, melatonin is synthesized by a number of extrapineal organs, which are regarded as non-endocrine organs and from which the release of this indoleamine is very low or is restricted to certain, very specific conditions. These include the retina, lens, bone marrow cells, gut, skin and possibly many other cells as well.[137-139] These organs are reported to contain the enzymatic machinery necessary for melatonin production. It remains unknown whether melatonin synthesized by extrapineal organs is the source of baseline melatonin levels in the blood. Certainly, pinealectomy is not accompanied by a total disappearance of blood melatonin. There is evidence claiming that during the day melatonin is released into circulation in very high amounts following a tryptophan load or at lower amounts after food intake.[140,141] Melatonin is able to directly scavenge a variety of toxic oxygen and nitrogen-based reactants, stimulates antioxidative enzymes, increases the efficiency of the ETC thereby limiting electron leakage and free radical generation, and promotes ATP synthesis.[67,84,119,142-145] Via these actions, melatonin preserves the integrity of the mitochondria and helps to maintain cell functions and survival.[84] It acts as an electron donor for molecules deficient in an electron.[146] Consequently, melatonin is able to detoxify highly reactive radicals directly.[147] On the other hand, it is a highly effective lipid antioxidant both in vitro and in vivo.[147,148] Melatonin seems to protect against lipid peroxidation via some mechanisms; one of these processes involve melatonin's ability to stabilize cellular membranes, an action that permits membranes to resist oxidative damage and allows cells to more optimally regulate their internal milieu.[80,149]

Melatonin is a highly lipophilic and hydrophilic agent.[150,151] Peripherally administered melatonin gains ready access to cells and seems to be especially highly concentrated in the nucleus.[152] Its apparently ubiquitous subcellular distribution, along with the fact that it easily crosses morphophysiological barriers, such as the blood-brain barrier, further emphasizes its potential importance as an antioxidant.[80] In addition to its antioxidative effects, melatonin acts through specific nuclear and plasma membrane receptors. In humans, melatonin receptors are detected in several organs, including the brain and retina, cardiovascular system, liver and gallbladder, intestine, kidney, immune cells, adipocytes, prostate and breast epithelial cells, ovary/granulose cells, myometrium, and skin.[153] The antioxidative beneficial effects of melatonin have been extensively studied in various pathological conditions associated with free radicals and related reactants, such as ischemia/reperfusion, inflammation, ionizing radiation ageing, age-related skin changes, carcinogenesis, etc.[67,68,139,144,154-158] Recent studies revealed that melatonin attenuated

acute gastric lesions induced by ethanol, stress, aspirin and ischemia/reperfusion because of its antioxidant activity and scavenging of free radicals.[159-162] It protects the gastric mucosa from oxidative damage in different experimental ulcer models.[159,163,164] We have recently demonstrated its beneficial effects on age-related gastric and intestinal oxidative damage induced by long-term pinealectomy (unpublished data). Melatonin is probably synthesized in the enterochromaffin cells of the gastrointestinal mucosa but the digestive tract may also take additional amounts of melatonin from the circulation and from the digestive tract after consumption of melatonin containing food.[59,93,159,165,166] Melatonin was found in high concentrations in numerous plants.[167] Thus eating vegetables or melatonin added foods might reduce free radical damage to tissues.

EFFECTS OF EXERCISE ON ANTIOXIDANT DEFENSE SYSTEM

Physical activity has been related to lipid peroxidation and antioxidant enzyme levels.[168-171] Experimental evidence indicates that moderate exercise has many beneficial effects, whereas acute and exhaustive exercise can produce damage in skeletal muscle and other tissues.[172,173] During exercise, the O_2 consumption is incremented in the whole body and in skeletal muscle. Performance of strenuous physical activity can increase oxygen consumption by 10- to 15-fold over rest to meet energy demands. The resulting elevated oxygen consumption produces an "oxidative stress" that leads to the generation of free radicals and lipid peroxidation. A defense system of free radical scavengers may minimize these dangerous radicals.[168] The increment of O_2 flux through the mitochondria (together with the inflammatory response at muscular level) leads to partial O_2 reduction, thereby triggering ROS production and antioxidant consumption.[174] Experimental evidence indicates that exhaustive exercise induces lipid peroxidation, DNA damage and alteration of antioxidant defense system.[175-177] Conversely, the protective effects of training are usually associated with up-regulation of endogenous antioxidant defense and repair systems, thus explaining why trained individuals display less cell damage than untrained subjects.[174,176,178,179] Mild stress derived by moderate training induces stimulation and maintenance of repair pathways.[173] However, the results of studies that addressed whether exercise increases oxidative stress are not consistent, perhaps because of the different levels of training of the subjects, the different exercises and intensities used, and the various measures of oxidative stress employed.[180] Despite the small availability of direct evidence for ROS production during exercise, there is an abundance of literature providing indirect support that oxidative stress occurs during exercise.

Although aerobic training has been found to increase antioxidant activity in animal models, the effects of aerobic training on antioxidant enzyme activity in humans are controversial.[181-183] Higher levels of antioxidant enzyme activity have been observed in trained subjects than in sedentary ones.[168,170,184] Some experimental studies report an increase of antioxidant enzyme activity after training, while others have documented no changes or even a decrease in circulating antioxidants.[168,185-189] Low levels of an antioxidant enzyme may result from reduced production of the enzyme or by inactivation related to their interaction with free radicals. The different results for the antioxidant enzyme system status may be the result of different assessment times, before or after training, and different exercise and intensities used.

Several studies have reported that activity of SOD, CAT, GPx and the content of GSH in various tissues were increased after exercise.[169,184,190-194] Changes in antioxidant scavengers and associated enzymes also provide clues about demands on the defense system. Increases in antioxidant enzyme activity correspond with enhanced resistance to oxidative stress. Finally, we can say that lifelong regular mild training exercise can improve the antioxidant defense in many tissues, by increasing the activity of antioxidant enzymes and reducing basal production of oxidants, without constituting any additional oxidant stress.

CONCLUSION

Oxidative stress plays an important role in cell injury and death. ROS are produced mainly by mitochondria during electron transport. Cell protect itself form oxidative stress via a series of antioxidants coming from endogenous or exogenous sources. Antioxidant agents such as melatonin, ascorbic acid, selenium can protect the cell from injury and death by maintaining the integrity and function of mainly mitochondria. Thus eating foods rich form antioxidant agents may reduce cell and tissue damage induced by ROS. Additionally, lifelong regular mild training exercise can improve the antioxidant defense in many tissues, constituting any additional oxidant stress.

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