On the Role of Energy Decline in Muscle Cell Apoptosis

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Abstract

This article emphasizes the importance of mitochondria and the cellular ATP level for cell death. Oxidative stress and an altered cellular calcium homeostasis, considered to be mediators of apoptosis, synergistically decrease the mitochondrial membrane potential, lower the cellular ATP level, and thereby cause cell death. Conversely, stabilization of the mitochondrial membrane potential, *e.g.*, by the protooncogene *bcl-2*, prevents cell death. Energy decline induced by oxidative stress may also bring about death in muscle cells, as indicated by the link between myopathies and mutations in mitochondrial DNA as well as by the induction of myopathies by the anti-AIDS drug azidothymidine. Oxidative stress is also causally involved in the etiology of Alzheimer's disease. Recent evidence suggests that the β-amyloid precursor protein, thought to play an important role in the pathogenesis of Alzheimer's disease and inclusion-body myositis, causes mitochondrial abnormalities in cultured human muscle cells. Counteracting oxidative stress in muscle cells promises to be useful in preventing their death.

Key words: Alzheimer's disease, ATP, calcium, membrane potential, reactive oxygen.

BAM 6 (4): 215-219, 1996

Apoptosis is an evolutionarily conserved form of physiological cell death important for tissue development and homeostasis. Its hallmarks are distinct morphological alterations such as nuclear condensation, cell shrinkage, and bleb formation, and the absence of inflammatory responses of the affected tissue. Deranged apoptosis plays a major role in diseases such as cancer, acquired immune deficiency syndrome (AIDS), autoimmune diseases, and neurodegeneration (reviewed in [25]). The program for apoptotic cell death appears to be present constitutively in virtually all mammalian cells and can be activated by a variety of extra- and intracellular signals.

Mediators of Apoptosis

Several conditions, molecules, or organelles such as oxidative stress, Ca^{2+}, proteases, nucleases, or mitochondria are considered participants of apoptosis, but at present it is not always clear whether they are required for or are the consequence of apoptosis [21].

There is ample evidence that apoptosis is accompanied by oxidative stress (reviewed in [7]). A valuable tool used to elucidate its importance in apoptosis is the protooncogen *bcl-2*, which stimulates an antioxidative response in cells and prevents apoptosis. The importance of oxidative stress as a cause for apoptosis was questioned because cells can undergo *bcl-2*-inhibitable apoptosis also under very low oxygen tensions, which supposedly precludes formation of reactive oxygen species (ROS). However, 10 nM molecular oxygen still reacts efficiently *in vivo*, as revealed in bioluminescene studies with bacteria, making the case against ROS involvement in apoptosis weaker.

Also the requirement of Ca^{2+} for apoptosis is controversial (see [5]). Early reports suggested that a rise of the intracellular Ca^{2+} leads to apoptosis *via* endonuclease activation, and more recent work indicated that apoptosis is accompanied by shifts of Ca^{2+} between various intracellular pools. It is worth noting that cellular Ca^{2+} handling and ROS production are related. Thus, increased mitochondrial Ca^{2+} release followed by re-uptake driven by the mitochondrial membrane potential ($\Delta\psi$) (Ca^{2+} "cycling") stimulates ROS production.

Presently the role of proteases in apoptosis receives much attention (reviewed in [16]). Gene analysis in *C. elegans* identified a key pro-apoptotic gene, *ced-3*, which encodes a putative cysteine protease that is related to the mammalian protease interleukin-1β-converting enzyme. Related proteases cleave poly(ADPribose) polymerase, which results in the activation of a Ca^{2+}/Mg^{2+}-dependent endonuclease implicated in internucleosomal DNA cleavage characteristic for apoptosis.

ATP as Control Element of Apoptosis

There is general agreement that apoptosis is an active, energy-requiring process. We have proposed that the cellular ATP level is an important determinant for cell death, either by apoptosis or necrosis [21]. This hypothesis is supported by circumstantial evidence, is consistent with most available data, has a correlary in aging, and is amenable to direct experimental testing. We have argued that a cell stays alive as long as a certain ATP level is maintained. When ATP falls below this level apoptosis ensues provided enough ATP is still available for energy-requiring apoptotic processes such as enzymatic hydrolysis of macromolecules, nuclear condensation and bleb formation. Only when there is a severe drop in cellular ATP controlled cell death ceases and ushers in necrosis.

A decreased cellular ATP level is characteristic for cell death, but there is no systematic investigation whether the decrease is the cause or the consequence of cell death. However, it has been shown with six human and murine leukemia/lymphoma cell lines [24] that the expression of the *bcl-2* gene and the cellular energy status are highly correlated, and that both parameters are inversely related to the sensitivity for glucocorticoid-induced apoptosis. An ADP/ATP ratio of about 0.2 was the critical discriminator between survival and apoptosis in all cell types. Also, acute inhibition of the mitochondrial respiratory chain induces apoptosis in cells carrying normal mitochondria, but not in ρ° cells (cells lacking mitochondrial DNA (mtDNA)), which maintain their ATP levels by glycolysis. This was taken as support for the argument that active mitochondria are required for apoptosis, and it was suggested that cells with impaired mitochondrial energy metabolism reach an energy threshold which triggers apoptosis. It was similarly argued that aging and age-related diseases are caused by a fall of the energy charge below a threshold (reviewed in [27, 28]).

Mitochondrial Reactive Oxygen, Decline of OXPHOS, Myopathies, and Cell Death

ROS such as superoxide radical, hydrogen peroxide, and hydroxyl radical are physiological reactants (reviewed in [8]). When produced in excessive amounts, or when antioxidative defense systems (scavengers such as vitamins C and E, glutathione, coenzyme Q_{10} (CoQ); enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase) are weakened ROS damage lipids, nucleic acids, and proteins, and participate in the development of pathological states. Examples are acute and chronic inflammation, artherosclerosis, hypoxia/reperfusion injury, and some forms of cancer [9].

Mitochondria are the richest cellular source of ROS. Several sites of the mitochondrial respiratory chain constitutively generate them. The "redox cycler" alloxan, rotenone, methylphenylpyridinium, tetrachloro-dibenzo-*p*-dioxin, elevated Ca^{2+}, or tumor necrosis factor-α stimulate their production by mitochondria, as does hypoxia/reperfusion (reviewed in [17]). ROS directly damage mitochondrial lipids, nucleic acids, and proteins (see [20]). ROS also cause the formation of the mutagen 8-hydroxyguanine and strand breaks in mtDNA, thus indirectly alter the properties of mitochondrially-encoded polypeptides, which are part of the oxidative phophorylation (OXPHOS) machinery. OXPHOS defects thereby initiate a self-perpetuating downward spiral involving electron transport inhibition, reduced energy output and increased ROS production, further damage and greater OXPHOS inhibition. This vicious cycle can be broken *in vivo* by antioxidants [1].

The idea that defective mitochondria relate to a disease was first proposed by Luft et al. [15]. Since then, various myopathies with heterogeneous clinical features have been correlated with distinctive changes in the number and morphology of muscle mitochondria seen in ragged-red fibers (RRF) detected with Gomori trichrome staining [26]. Several years ago the study of mutated mtDNA ushered in a new epoch in the understanding of mitochondrial myopathies (reviewed in [27, 28]) since it was found that myoclonic epilepsy and RRF (MERRF), neurogenic muscle weakness, ataxia and retinitis pigmentosa, and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) are caused by mtDNA point mutations. Large deletions of mtDNA are now implicated in chronic external ophthalmoplegia (CEOP) syndrome including Kearns-Sayre (KS) syndrome, whereas MERRF and MELAS are caused by single base substitution in mitochondrial transfer RNA^{Lys} and transfer RNA^{Leu}. It is highly likely that myopathies are at least in part due to mitochondrial ROS production [29]. Indeed, antioxidative intervention at the mitochondrial level has been reported to combat muscle disorders caused by defects in OXPHOS (see the following paragraph).

Attempts have been made for metabolic therapies of diseases related to mitochondrial dysfunction, but they are isolated efforts, and their efficacy was not confirmed in controlled studies (reviewed in [23]). CoQ, an electron carrier in the inner mitochondrial membrane, is an outstanding antioxidant and may stabilize the respiratory chain components. Protection of peroxidation-sensitive cardiolipin is particularly important in preserving mitochondrial functioning since this lipid facilitates the interaction between cytochrome c and cytochrome oxidase. CoQ had beneficial effects in KS/CEOP syndromes and in MELAS. Succinate, an electron donor to the mitochondrial respiratory chain, decreased stroke-like episodes in a MELAS patient. Vitamin K_1 (phylloquinone) and vitamin K_3 (menadione) can transfer electrons to cytochrome c by an electron bypass from CoQ. Also vitamin C alone or together with menadione can reduce cytochrome c. A combination of the latter two reductants improved the bioenergetic capacity of skeletal muscle with a severe defect in complex III of the respiratory chain, as shown by *in vivo* ^{31}P-NMR. Phylloquinone improved retinal cone function in CEOP, and thiamine, a cofactor of pyruvate dehydrogenase, improved plasma lactate and pyruvate levels in a KS/CEOP patient. Medication with riboflavin, a

precursor of flavin adenine nucleotides, raised the exercise capacity in a patient with complex I dysfunction.

The Case of Azidothymidine (AZT)

Azidothymidine (AZT) is one of the most common drugs used in people infected with human immune deficiency virus (HIV). AZT's use is limited by the development of myopathies [12] which present themselves at the cellular level as RRF indistinguishable from "classical" myopathy RRF and as swollen mitochondria. AZT inhibits the retroviral reverse transcriptase of HIV-1, but also the mtDNA polymerase γ [14]. As a consequence, muscle mtDNA is depleted in AIDS patients with AZT-induced myopathy [2], and mitochondrial functions are impaired [13]. In an animal model of AZT toxicity [10] AZT fed to mice for four weeks at a dose of 1/10th given to AIDS patients causes massive ROS-dependent oxidation of mtDNA. These findings offer an insight into the role of altered mtDNA in some heritable and AZT-induced mitochondrial myopathies. The data suggest that mitochondrial myopathies are basically due to an overproduction of ROS and/or an overwhelmed antioxidative defense.

β-Amyloid in Alzheimer's Disease: Studies in Brain and Neurons

Among the most common neurologic diseases are neurodegenerative ones, such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. There is now substantial experimental evidence that oxidative stress is causally involved in the etiology of neurodegenerative diseases. The evidence for AD is outlined below (original references relevant to this section can be found in [6]).

Patients suffering from AD have increased levels of lipid peroxidation in the brain. Similarly, various regions of the brain harbour a higher level of protein oxidation and a decreased activity of glutamine synthetase compared to age-matched controls. Glutamine synthetase is critical for the survival of neurons because it removes the potentially neurotoxic substances glutamate and ammonia. At the same time it is especially vulnerable to oxidative damage. SOD and catalase are found in 15 to 25% of neurofibrillary tangles and 50% of senile plaques, which may be a response to oxidative stress. Glucose-6-phosphate dehydrogenase, which supplies reducing equivalents in the form of NADPH, is increased in AD brains. AD is associated with reduction of cytochrome oxidase activity in four cortical regions, which results in increased production of hydrogen peroxide. Mutations in mtDNA have also been found in AD patients, where oxidative damage to nuclear and particularly to mtDNA is increased (in mtDNA threefold, and highly significantly). It is highly likely that impaired mitochondrial function contributes to the pathogenesis of AD. Oxidative reactions may facilitate β-amyloid peptide (βAP) aggregation and tau polymerization, hallmarks of AD: Amyloid precursor protein (APP) fragments aggregate in the presence of metal-catalyzed oxidation systems, and oxidation of tau protein facilitates its dimerization and polymerization into filaments. This effect is inhibited by free radical scavengers. The toxicity of APP fragments in PC12 cells is inhibited by vitamin E and propyl gallate, another antioxidant. βAP itself generates free radicals [11]. It stimulates cells to produce cytotoxic hydrogen peroxide, and clones resistant to βAP are also resistant to hydrogen peroxide. Another link between oxidative damage and AD is the observation that oxidation of apolipoprotein E4 increases its binding to amyloid.

Conflicting results concerning enzymatic antioxidant systems in AD have been reported (for review, see [6]). Mitochondrial SOD is slightly increased in the hippocampus, and cytosolic SOD slightly increased in the caudate. However, also decreased SOD activity is found in the frontal cortex, hippocampus, and cerebellum. In association cortex and hippocampus from control subjects, high levels of cytosolic SOD are seen by immunostaining in large pyramidal neurons, which are susceptible to degeneration in AD.

βAP is a direct source of oxygen radicals and stimulates an enzyme, presumably a plasma membrane-bound oxidase, to produce hydrogen peroxide (see above). On the basis of these and other findings a unifying hypothesis was proposed to integrate the neurotoxic properties of βAP into the framework of disparate biochemical aberrations observed in AD [11]: Perturbation of proteolytic or other regulatory pathways promotes cleavage of APP and release of βAPs into the extraneuronal space. There they may fragment to form smaller, toxic oligopeptide radicals, which damage membranes and key protective enzymes. This would result in neurodegeneration, possibly expedited by a disturbed Ca^{2+} homeostasis. This model is consistent with the slow onset of AD: Younger persons have greater antioxidant capacities and withstand oxidative stress; aging, coupled to environmental insults or genetic defects that depress antioxidant status, could exacerbate consequences of βAP radicalization. While this hypothesis is consistent with many extant AD data, it can only be considered an initial attempt to understand a novel and complex chemistry. According to Hensley and coworkers [11] this "underscores the need for further research into the mechanism of βAP aggregation and neurotoxicity".

β-Amyloid in Alzheimer's Disease: Studies in Cultured Human Muscle

Inclusion-body myositis (IBM) is the only human disease in which APP and βAP accumulate excessively outside the brain. The hallmark of IBM are mtDNA deletions and vacuolated muscle fibers, where APP epitopes are concentrated (reviewed in [3]). Vacuolated muscle fibers show a staining pattern very similar to that found in AD brain, and contain helical filaments comprising hyperphosphorylated tau protein. It was recently reported [4] that transfer of APP into differentiated cultured human muscle fibers results in

decreased cytochrome oxidase activity and severe structural mitochondrial abnormalities. The cause of the abnormalities is presently not known and the possible relationship to oxidative stress has not yet been explored. This experimental model of AD promises to be very useful for the study of mitochondrial abnormalities due to APP overexpression in brain and muscle cells.

Abbreviations

AD, Alzheimer's disease; AIDS, acquired immune deficiency syndrome; APP, amyloid precursor protein; AZT, azidothymidine; βAP, β-amyloid peptide; CoQ, coenzyme Q_{10}; CEOP, chronic external ophthalmoplegia; HIV, human immune deficiency virus; IBM, inclusion-body myositis; KS, Kearns-Sayre syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; $\Delta\psi$, mitochondrial membrane potential; mtDNA, mitochondrial DNA; MERRF, myoclonic epilepsy and RRF; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; RRF, ragged-red fibers; SOD, superoxide dismutase.

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