Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich’s ataxia

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\section*{Abstract}

There is significant evidence that the pathogenesis of several neurodegenerative diseases, including Parkinson’s disease, Alzheimer’s disease, Friedreich’s ataxia (FRDA), multiple sclerosis and amyotrophic lateral sclerosis, may involve the generation of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) associated with mitochondrial dysfunction. The mitochondrial genome may play an essential role in the pathogenesis of these diseases, and evidence for mitochondria being a site of damage in neurodegenerative disorders is based in part on observed decreases in the respiratory chain complex activities in Parkinson’s, Alzheimer’s, and Huntington’s disease. Such defects in respiratory complex activities, possibly associated with oxidant/antioxidant imbalance, are thought to underlie defects in energy metabolism and induce cellular degeneration.

The precise sequence of events in FRDA pathogenesis is uncertain. The impaired intramitochondrial 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. Recent evidence suggests that frataxin might detoxify ROS via activation of glutathione peroxidase and elevation of thiols, and in addition, that decreased expression of frataxin protein is associated with FRDA. Many approaches have been undertaken to understand FRDA, but the heterogeneity of the etiologic factors makes it difficult to define the clinically most important factor determining the onset and progression of the disease. However, increasing evidence indicates that factors such as oxidative stress and disturbed protein metabolism and their interaction in a vicious cycle are central to FRDA pathogenesis. Brains of FRDA patients undergo many changes, such as disruption of protein synthesis and degradation, classically associated with the heat shock response, which is one form of stress response. Heat shock proteins are proteins serving as molecular chaperones involved in the protection of cells from various forms of stress.

In the central nervous system, heat shock protein (HSP) synthesis is induced not only after hyperthermia, but also following alterations in the intracellular redox environment. The major neurodegenerative diseases, Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Huntington’s disease (HD) and FRDA are all associated with the presence of abnormal proteins. Among the various HSPs, HSP32, also known as heme oxygenase 1 (HO-1), has received considerable attention, as it has been recently demonstrated that HO-1 induction, by generating the vasoactive molecule carbon monoxide and the potent antioxidant...
bilirubin, could represent a protective system potentially active against brain oxidative injury. Given the broad cytoprotective properties of the heat shock response there is now strong interest in discovering and developing pharmacological agents capable of inducing the heat shock response. This may open up new perspectives in medicine, as molecules inducing this defense mechanism appear to be possible candidates for novel cytoprotective strategies. In particular, manipulation of endogenous cellular defense mechanisms, such as the heat shock response, through nutritional antioxidants, pharmacological compounds or gene transduction, may represent an innovative approach to therapeutic intervention in diseases causing tissue damage, such as neurodegeneration.

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1. Introduction

There is increasing evidence for mitochondrial involvement in neurodegenerative diseases including Alzheimer’s and Parkinson’s diseases, ALS, MS and FRDA. A mutation, whether inherited or acquired, leads to impaired electron transport chain (ETC) functioning [1]. Impaired electron transport, in turn, leads to decreased ATP production, increased formation of toxic free radicals, and altered calcium homeostasis. These toxic consequences of ETC dysfunction may sustain further mitochondrial damage, including oxidation of mitochondrial DNA, proteins, and lipids, and opening of the mitochondrial permeability transition pore, an event associated with cell degeneration and death [2]. There is evidence to support that oxidative stress alters the expression of antioxidant enzymes and enhances expression and/or DNA binding of numerous transcription factors, including AP-1, fos, jun, myc, erg-1, SAPK and NFkB [3]. Moreover, it is well known that brain cells are continually challenged by conditions which may cause acute or chronic stress. To adapt to these environmental changes and survive different types of injuries, a network of different responses have evolved which sense and control diverse forms of cellular stress. One of these responses, the heat shock response, has emerged as a fundamental mechanism necessary for cell survival under a variety of unfavorable conditions [4]. In the central nervous system (CNS), heat shock protein (HSP) synthesis is induced not only after hyperthermia, but also following alterations in the intracellular redox environment, exposure to heavy metals, amino acid analogs or cytotoxic drugs [5,6]. While prolonged exposure to conditions of extreme stress is harmful and can lead to cell death, induction of HSP synthesis can result in stress tolerance and cytoprotection in a variety of metabolic disturbances and injuries, including hypoxia, stroke, epilepsy, cell and tissue trauma, neurodegenerative disease and aging [3,7]. This has opened new perspectives in medicine, as molecules activating this defense mechanism appear to be possible candidates for novel cytoprotective strategies. However, although activation of stress tolerance signaling, leading to protective nuclear responses, (such as increased expression of heat shock proteins, antioxidant enzymes and Bcl-2) may be triggered to withstand all the above mentioned pathogenic changes, a vicious cycle of increasing oxidative damage may insidiously develop over a period of years inducing progressive degenerative cell alterations and death [8].

CNS has a large potential oxidative capacity [2] due to the high level of tissue oxygen consumption. However, the ability of the brain to withstand oxidative stress is limited because of: (a) a high content of easily oxidizable substrates, such as polyunsaturated fatty acids and catecholamines; (b) relatively low levels of antioxidants such as glutathione and vitamin E and antioxidant enzymes (such as glutathione peroxidase, catalase and superoxide dismutase); (c) the endogenous generation of reactive oxygen free radicals via several specific reactions; (d) the elevated content of iron in specific areas of the human brain, such as globus pallidus and substantia nigra (SN), while cerebrospinal fluid has very little iron-binding capacity owing to its low content of transferrin; (e) CNS contains non-replicating neuronal cells which, once damaged, may be permanently dysfunctional or committed to programmed cell death (apoptosis). Moreover, it is becoming increasingly clear that the mitochondrial genome may play an essential role in neurodegenerative diseases, such as FRDA [9]. It is generally recognized that, in addition to the nuclear genome, each human cell contains multiple copies of a small double-stranded mitochondrial genome. Mitochondrial DNA disorders present tissue specificity, characterized by the fact that even if a mitochondrial DNA mutation is present in all tissues, only some will be affected and express a pathology. Due to the coexistence in cells of both normal and mutated mtDNA, (a situation termed heteroplasm), the levels of mutation can vary considerably between mitochondria, cells and even tissues. The precise sequence of events in FRDA pathogenesis is uncertain. However, impaired intramitochondrial metabolism associated with increased free iron and the consequent oxidative stress are being considered as a possible pathogenic mechanism. There is now evidence to suggest that frataxin might detoxify ROS via activation of glutathione peroxidase and elevation of thiols [10] and, in addition, that decreased expression of frataxin protein is associated with FRDA [11]. In the present review, we discuss the role of energy thresholds in brain mitochondria and its implications in neurodegeneration. We then review the evidence for the role of oxidative stress in mediating the effects of mitochondrial DNA mutations on brain age-related disorders and, particularly, the oxidative stress...
hypothesis which may underlie the pathogenesis of FRDA. We also discuss new approaches, such as MRI and or MRS for investigating clinical profiles and targeting key mechanisms responsible of this devastating neurodegenerative disease.

1.1. Clinical and genetic features of Friedreich ataxia

Friedreich ataxia is the commonest form of inherited ataxia with a frequency of 1 in 50000 live births. FRDA is an autosomal recessive degenerative disorder characterized by progressive gait and limb ataxia, loss of limb deep tendon reflexes, spasticity and extensor plantar responses [12,13]. Neuropathology in FRDA is characterized by early degeneration of large sensory neurons in the dorsal root ganglia, followed by degeneration of sensory posterior columns, spinal–cerebellar tracts, cortical–spinal motor tracts, dentate nucleus and atrophy of the large sensory fibres in peripheral nerves. Hypertrophic cardiomyopathy is present in large proportion FRDA patients [12,13].

The causative mutation of FRDA is an abnormally expanded GAA triplet repeat in the first intron of the FRDA gene on chromosome 9q13 [14]. Ninety-eight percent of FRDA patients are homozygous for the GAA expansion, the remainder carrying a repeat expansion in one FRDA allele and a point mutation in the other [12,14]. The size of the GAA expansion in FRDA patients ranges from about 100 repeats to 1700 [12,14], normal chromosomes having between 8 and 22 repeats [12]. The expression of a number of symptoms/signs in FRDA is dependent upon the length of the GAA repeat expansion in the smaller allele. In particular, the age at onset correlates negatively [12,15] and the rate of progression of the disease positively with the number of GAA repeats in the smaller allele. The frequency and severity of hypertrophic cardiomyopathy increases with the size of the GAA expansion in the smaller allele [12].

Mutations in the FRDA gene, either GAA expansions or point mutations, result in reduced expression of a protein called frataxin [16] which has been shown to be localized to mitochondria [16–18]. In normal subjects, the highest level of expression of the FRDA gene has been found in the heart and spinal cord, intermediate levels in the cerebellum, liver, skeletal muscle and pancreas and very little in the cerebral cortex [14]. The amount of residual frataxin in lymphoblastoid cell lines from FRDA patients correlates with the GAA expansion size in the smaller allele [16] and likely represents the molecular basis of the relationship between GAA expansion size and phenotypic expression of the disease [12].

1.2. Pathogenic mechanisms

There is significant evidence that the pathogenesis of several neurodegenerative diseases, including Parkinson’s disease, Alzheimer’s disease, Friedreich ataxia, multiple sclerosis and amyotrophic lateral sclerosis, may involve the generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and mitochondrial dysfunction.

Studies using the budding yeast Saccharomyces cerevisiae have provided the first clues to understand the consequences of frataxin loss [17–21]. It has been shown that deletion of the yeast frataxin homolog YFH1 results in a 10-fold increase in iron within the mitochondria along with increased ROS production [17,20]. This leads to loss of mitochondrial function and the appearance of a petite phenotype in nearly all strains that have been examined [11,20,22,23]. Also, an impaired oxidative phosphorylation with severe deficiencies of mitochondrial respiratory chain complexes I and II/III and aconitase activities have been demonstrated in post-mortem cardiac muscle samples from patients with FRDA, associated with reduced levels of mitochondrial DNA and with increased iron deposition in heart, liver and spleen, with a pattern consistent with the mitochondrial location. Aconitase deficiency is suggestive that oxidative stress may induce a self-amplifying cycle of oxidative damage associated with mitochondrial dysfunction, which may also contribute to cellular toxicity and degeneration [24].

Recent evidence suggests that frataxin might detoxify ROS via activation of glutathione peroxidase and elevation of thiols [10]. Transgenic overexpression of human frataxin increases cellular antioxidant defense via activation of glutathione peroxidase and elevation of reduced thiols, thereby reducing the incidence of malignant transformation induced by ROS, as observed by soft agar assays and tumour formation in nude mice [10]. Up-regulation of protein manganese superoxide dismutase (MnSOD) fails to occur in FRDA fibroblasts exposed to iron [25]. This finding, together with the observation of absent activation of the redox-sensitive factor NFkB, suggest that a NFkB-independent pathway that may not require free radical signaling is responsible for the reduced induction of MnSOD [26]. This impairment could constitute both, a novel defence mechanism against iron-mediated oxidative stress in cells with mitochondrial iron overload and, conversely, an alternative source of free radicals that could contribute to the disease pathology.

There is evidence that frataxin acts as a chaperone for Fe(II) and a storage compartment for excess iron [27–38]. This is consistent with the roles played by frataxin in iron export, Fe–S cluster assembly, heme biosynthesis and prevention of oxidative stress. Also, frataxin plays a direct role in the mitochondrial energy activation and oxidative phosphorylation [11]. Several model systems have been developed in an effort to understand the disease [39,40]. In mouse models, deletion of the frataxin gene results in embryonic lethality [40], while its selective inactivation in neuronal and cardiac tissues leads to neurological symptoms and cardiomyopathy associated with mitochondrial iron–sulfur cluster-containing enzyme deficiencies and time-dependent mitochondrial iron accumulation. In contrast, a model expressing 25–35% of wild type frataxin levels by
virtue of a (GAA)$_{230}$ expansion inserted in the first intron of the mouse gene has no obvious phenotype [39].

Cardiac and skeletal muscle bioenergetics was investigated directly in FRDA patients using in vivo $^{31}$P-MRS [41]. Magnetic resonance spectroscopy (MRS) is a non-invasive technique that allows, using clinical MR scanners, the measurement of several compounds in vivo without the use of radioactive tracers. Phosphorus MR spectroscopy ($^{31}$P-MRS) quantifies phosphorus-containing compounds and cytosolic pH. The major compounds detectable are ATP, phosphocreatine (PCr) and inorganic phosphate (Pi). Free (metabolically active) [ADP], the major regulator of the oxidative phosphorylation, can be calculated from the MRS data using the creatine kinase equilibrium expression [42]. Cardiac bioenergetics was assessed in vivo in FRDA patients with and without left ventricular hypertrophy [43]. Cardiac PCr to ATP ratios in the FRDA group as a whole were reduced by about 40%. Cardiac PCr/ATP ratios were significantly reduced compared to controls in both groups of FRDA patients with normal and hypertrophic heart [43]. These findings represented one of the first evidence in humans that cardiac PCr/ATP can be reduced in the absence of either failing contractile function or hypertrophy. In FRDA the hypertrophic process may be compensatory and caused or contributed to by the bioenergetic deficit, which is also known to stimulate myocyte hypertrophy [44]. This hypothesis is supported by the frequent finding of hypertrophic cardiomyopathy in patients with a deficit of oxidative phosphorylation due to mutations of mitochondrial DNA [45].

Two independent $^{31}$P-MRS studies of the calf muscle have shown a reduced rate of mitochondrial ATP synthesis in FRDA patients [46,47]. This is a typical finding in patients with mitochondrial myopathies due to mtDNA mutations. Mitochondrial $V_{\text{max}}$ for ATP production in FRDA patients was also significantly lower than in a group of disease controls with muscular disorders from different causes and with similar maximal motor ability [46], indicating that disability per se did not account for the reduced mitochondrial function in FRDA patients. The same studies also showed that the in vivo deficit of mitochondrial ATP synthesis rate was strongly dependent on the size of the GAA repeats in the smaller allele: the higher the number of GAA repeats the lower the mitochondrial ATP synthesis rate. This is compelling evidence that the GAA expansion is the cause of the mitochondrial deficit and suggests a link between the degree of the mitochondrial respiration deficit and clinical expression of the disease in other tissues. The length of the GAA expansion has been shown to determine the amount of frataxin expressed [16]. Therefore, the residual expression of frataxin probably determines the reduced skeletal muscle mitochondrial ATP production rate we detected in vivo. Consistent with this notion, noninvasive continuous near infrared muscle spectroscopy NIRS, that assesses the delivery and utilization of oxygen in response to exercise, showed in several FRDA patients features related to inadequate oxygen utilization by muscle [48].

2. Energy thresholds in brain mitochondria: implication for neurodegenerative disorders

Human cells contain from a few hundred to more than a thousand mitochondria; each mitochondrion in turn has 2–10 copies of mtDNA, thus, several thousands copies of the mitochondrial genome can be present within a single cell. Importantly, unique to mtDNA is that it is inherited exclusively through the mother, and may exist in many different copies in the oocyte cytoplasm. This implies that no mtDNA recombination occurs at fertilization and only a sequential accumulation of mutations from the maternal lineage account for mtDNA variations. This concept however has been challenged by recent finding showing parental inheritance of mt DNA, albeit rare. Moreover, mtDNA is particularly prone to mutation, being estimated as 10 times greater then nuclear DNA [49], owing to the absence of protective proteins (such as histones) and of a high-efficiency repair system. Thus, mutant and wild-type (normal) mtDNA can coexist within a cell in any proportion and this situation is termed heteroplasmy. It is becoming increasingly clear that the mitochondrial genome may play an essential role in the pathogenesis of neurodegenerative diseases, and evidence for mitochondria being a site of damage in neurodegenerative disorders is based in part on observed decreases in the respiratory chain complex activities in Parkinson’s, Alzheimer’s, and Huntington’s disease. Such defects in respiratory complex activities, possibly associated with oxidant/antioxidant imbalance, are thought to underlie defects in energy metabolism and induce cellular degeneration. That the pathogenesis of these defects is mitochondrial in origin is also supported by the results of experiments with cytoplasmic hybrid, or “cybrid” cells [50], which reproduce the respiratory chain defects present in diseased patients. These cybrids are created by the transfer of mtDNA to clonal neuronal-like cells which have been depleted of their endogenous mtDNA by the application low concentration of ethidium bromide over the long term. Host cells are then polyethylene glycol (PEG)-fused with platelets, containing no nuclear DNA, from a control or diseased patient. This system allows investigators to specifically investigate the role of mtDNA in cellular pathology. The first hint that mitochondria play a role in human disease did not emerge until 1958, when a Swedish patient was identified who had symptoms of severe perspiration, polydipsia, polyphagia, weight loss and weakness [51]. Laboratory studies showed a basal metabolic rate 200% above normal, very low weight (37 kg) and basal temperature reaching 38 °C. Biochemical studies revealed that she had a partially uncoupled respiration which accounted for her generation of excessive heat and high calorie consumption. Although the primary etiologic event
in Luft’s disease remains to be identified, the disease is associated with release of mitochondrial calcium stores, abnormal calcium cycling and sustained stimulation of loosely coupled respiration. The discovery of Luft’s disease cleared the path for fertile investigations, and since the 1960s, over 120 human mitochondrial diseases have been discovered, many of which involve selected populations in the central nervous system consisting of postmitotic, highly energy-dependent cells. Many of these diseases have been associated with specific inherited mitochondrial DNA mutations and respiratory chain deficiencies. Point mutations may involve either the RNA or protein-encoding genes, and rearrangements may take the form of deletions or duplications. In the presence of heteroplasmy there is a critical ratio of mutant to wild-type mitochondrial genomes that is necessary before the disease becomes both biochemically and clinically apparent. As might be expected mtDNA disorders are phenotypically diverse given the ubiquitous presence of mitochondria and the variation in the levels of heteroplasmy in the body. Yet many have predominant neurologic and muscular symptoms, including dementia, seizures, ataxic syndromes, peripheral neuropathies, and progressive myopathy. Postmitotic tissues typically show increased levels of mutant mitochondria due to the inability of these tissues to select against cells containing mutant mtDNA genomes. CNS imaging of patients with mtDNA disorders often reveals moderate degrees of cerebral or cerebellar atrophy that are consistent with neurodegeneration, often comparable with the pathologies associated with the brain in senescence or in dementia [52].

Several mechanisms have been proposed to explain the variability of the phenotypic expression of a mtDNA mutation, such as sporadic mutation or mitotic segregation. However all these hypotheses incorporate a unique feature of mitochondrial genetics and pathologies; that of the heteroplasmic concept of mtDNA mutations. Levels of mutation can vary considerably between mitochondria, cells, and even tissues within the same individual. Consequently, the expression of a mutation in the mtDNA can be thought of as a function of the degree of the heteroplasmy. In general, whether or not a metabolic defect expresses itself as a recognizable clinical disease will depend upon the extent to which it affects the metabolic pathway in question and this can lead to a threshold expression of the disease state. One of the most important features recognized in mitochondrial diseases is the existence of a threshold in the degree of a mitochondrial deficit for the expression of the disease, and these were shown by Wallace [53] to be related to the balance between normal and mutant mtDNA. Accordingly, it has been demonstrated that only 10% of wild type DNA is enough to maintain a normal respiratory rate and also that 80–90% deleted mtDNA must be achieved before complex IV activity is compromised [54,55]. All this represents compelling evidence that there is a threshold in the heteroplasmy of the mutation around 90% before a pathological consequence become manifest. Below this threshold the flux of respiration and of ATP synthesis are at a level which does not compromise normal metabolism. As consequence, there are at least four levels at which threshold effects occur in mitochondrial metabolism, with respect to their possible involvement in the pathogenesis of neurodegeneration. The first is the expression of the heteroplasmy of mtDNA at the level of a given enzymatic step, whereby mitochondria from patients might exhibit a particular ratio of defective DNA compared to normal DNA. The second is the threshold effect observed in the mitochondrial metabolism as a result of a decrease in a given mitochondrial activity. The third may occur in the expression of defective mitochondria with respect to the whole cellular metabolism. The fourth, is the fact that the control coefficient of a given step may vary depending on different types of mitochondria, which leads to the observation that the threshold value for a given complex of the electron transport chain can vary according to the threshold in the energy demand of different tissues. At each level the threshold effect will reinforce the others, as interpreted by the Double threshold hypothesis [56], whose predictions are now beginning to be documented. Data from studies of rat brain mitochondria of non synaptic origin have shown that thresholds exist whereby complex activities need to be reduced by at least 60% before major changes in ATP synthesis and oxygen consumption occur. Interestingly, in synaptic mitochondria, titration of various complexes with specific inhibitors generated threshold curves showing that complex I, III and IV activities had to be decreased 25%, 80% and 70%, respectively, before major changes in rates of oxygen consumption and ATP synthesis were observed [57] (Fig. 1). These results suggest that in mitochondria of synaptic origin complex I activity has a major control of oxidative phoshorylation, such that when a threshold of 25% inhibition is exceeded, energy metabolism is compromised, and reduction in ATP synthesis ensues. Moreover, the same study demonstrated that depletion of glutathione, which has been reported to be a primary event in idiopathic Parkinson’s disease, abolished the threshold for complex I, providing experimental evidence that antioxidant status is critically involved in maintaining energy thresholds in mitochondria [57].

Other data are also consistent with these findings, as it has been shown both in a patient with cytochrome c oxidase deficiency and in an animal model of copper deficiency that more than a 50% deficit in complex IV activity did not affect the respiratory flux [56]. It is possible to explain these findings within the framework of the metabolic control theory [58]. According to this theory, which investigates the effects of infinitesimally small parameter perturbations on the variables of metabolic systems, a crucial stage in the expression of a threshold for a clinical disease is, at molecular level, the impact that a localized defect in a given step has on the global flux of a metabolic network [58]. In this theory an important parameter is the control coefficient which quantitatively expresses the fractional
change in pathway flux of a metabolic network, under steady-state conditions, induced by a fractional change in the individual step under consideration [57]. For the oxidative flux (respiration) in mitochondria it can be determined according to Eq. (1):  

\[ C = \left( \frac{dJ_{O_2}}{d(\text{Inhibitor})} \right) \left( \frac{dV_c}{d(\text{Inhibitor})} \right)^{-1} \]  

where \( C \) is the flux control coefficient of the mitochondrial complex under investigation, \( dV_c/d(\text{Inhibitor}) \) is the rate of change of complex activity (individual step) and \( dJ_{O_2}/d(\text{Inhibitor}) \) is the rate of change of respiration (global flux), at low concentrations of the complex inhibitor. In determining the control coefficients of the various steps of oxidative phosphorylation on respiratory flux with the inhibitory titration method, two very differently shaped curves are observed, for the isolated step and the whole flux. Fig. 1a shows the effect of KCN titration of respiration and complex IV activity in mitochondria. It can be seen that even at 50% cytochrome c oxidase inhibition, there is only 20% inhibition of the whole flux, and 90% of inhibition of the isolated step is required to achieve a significant reduction of the respiration, corresponding to global flux. This is apparent from Fig. 1b, obtained by plotting the inhibition of the respiratory flux as a function of the complex IV activity, given the same KCN concentration. Generation of a threshold curve is evident, and the complex activity must be decreased by 70% before a rapid decline in the rate of respiration occurs. This pattern is a direct consequence of the summation theorem of metabolic control theory [56], which states that the sum of control coefficients of a defined metabolic pathway is equal to 1 with the result that most of the control coefficients are low. Consistent with this notion, a control coefficient of 0.1 for a given complex implies a 10% perturbation in the activity of this complex and can result in an inhibition of respiratory rate by as little as 1%. This implies that in the case of oxidative phosphorylation each single control coefficient is close to zero producing at the beginning a quasi horizontal slope; at very low activity of the step both curves must meet again, due to the fact that the flux becomes zero and the step is completely inactivated [59,60].

3. The mitochondrial theory of aging

Harman in 1972 first proposed that mitochondria may have a central role in the process of aging. According to this theory, free radicals generated through mitochondrial metabolism can act as causative factor of abnormal function and cell death. Mitochondria are the cell’s most significant source of oxidants and in vitro studies have indicated that approximately 1–2% of electron flow through the ETC results in the univalent generation of superoxide [61]. Moreover, various toxins in the environment can injure mitochondrial enzymes, leading to increased generation of free radical that over the life-span would eventually play a major role in aging [62].

Mitochondria make two rather contradictory contributions to cell survival. The classically recognized function is the synthesis of ATP necessary for endergonic reactions, the other is generation of reactive oxygen species which may compromise the long-term survival of cells and constitute a major underlying cause of the aging process. Indeed, these two rather conflicting functions are part of the same process, namely mitochondrial respiration. During aging some of the free radical scavenging systems are decreased [3] so an increased escape of free radicals occurs, targeting membrane lipids and proteins in proximity to the respiratory chain, thereby decreasing the fluidity and increasing the permeability of the inner mitochondrial membrane. Oxidatively damaged proteins are known to increase markedly with age [63–65], including functionally inactive forms of enzyme, as result of free radical-induced damage and consequent DNA damage, which in turn affects the factors responsible for protein oxidation as well as degradation of oxidized proteins [66]. Accumulation of oxidized proteins may result in cross-linking to other proteins which would alter biochemical and physiological function in mitochondria. Toxic roles for oxidized proteins have also been proposed in...
recent literature on Alzheimer’s disease. In particular, the accumulation of aggregated amyloid β-protein in diseased brains as neurofibrillary tangles can occur through oxidative reactions [67]. The content of protein carbonyls in Alzheimer’s brain samples is greater than in age-matched controls [65], and this provides a clear indication of greater accumulation of oxidized proteins in this disease. Brain regions show specific changes in this regard, and protein carbonyl levels correlate well with tangles [68]. Glycation and autioxidative glycosylation products also accumulate in Alzheimer’s brains [69]. Levels of the oxidized nucleotide 8-hydroxy-deoxyguanosine (8-OH-dG), a biomarker of DNA damage, have also been shown to accumulate with aging. In several tissues, including brain and muscle, levels of 8-OH-dG in mtDNA exceed that of nuclear DNA (nDNA) some 16-fold [70], although as yet there have been no studies performed using absolutely pure mtDNA. It has been demonstrated that 8-OH-dG most frequently base pairs with cytosine, but also mispairs with adenine approximately 1% of the time, causing misreading of adjacent residues. Mecocci et al. found that 8-OH-dG significantly correlates with increases in levels of a 7.4-kb deletion in human brain [71]. Ultrastructural changes have been also reported to occur in mitochondria with age. They become larger and less numerous with vacuolization, cristae rupture and accumulation of paracrystalline inclusions. Cardiolipin, an acidic phospholipid that occurs only in mitochondria, has been shown to decrease with age [72,73]. This inner membrane lipid is known to have optimal electrical insulating properties, thereby contributing significantly to the transmembrane potential that drives the formation of ATP via ATP synthase. Indeed, a decrease in membrane potential in mitochondria from older animals has been demonstrated [74]. It has been proposed that accumulation of mtDNA during life is a major cause of age-related disease and this is because of its high mutagenic propensity. The lack of introns and protective histones, limited nucleotide excision and recombination DNA repair mechanisms, location in proximity of the inner mitochondrial membrane which expose it to an enriched free radical milieu, are all factors contributing to a 10-fold higher mutation rate occurring in the mtDNA than in the nDNA. A large body of evidence indicates that mtDNA mutations increase as a function of age reaching the highest levels in brain and muscle. More than 20 different types of deletions have been documented to accumulate in aging human tissues. The first report on an age-related increase in a mtDNA deletion was called the “common deletion” and was found in elderly brain and in Parkinson’s disease [75]. This deletion has been described to occur between 13-bp sequence repeats beginning at nucleotides 8470 and 13447, removing almost a 5-kb region of mtDNA between ATPase 8 and the ND5 genes (Fig. 2). The deletion is thought to occur during replication of the mtDNA, the absent sequence encoding for six essential polypeptides of the respiratory chain and 5 tRNAs. It has been associated with several clinical diseases, such as chronic progressive external opthalmoplegia and Kearns Sayre syndrome. Several age-related disorders have been shown to be linked to higher levels of mtDNA mutations than age-matched controls. In the CNS, Ikebe et al. showed 17 times higher levels of the common deletion in the striatum of patients with Parkinson’s disease compared to age-matched controls. Evidence also exists indicating higher levels of this deletion in patients with Alzheimer’s disease which parallel increased levels in the oxidized nucleotide 8-OH-dG [76]. A major feature of mtDNA disease in humans is the presence of cells with low cytochrome c oxidase activity and evidence exist which indicates that the mechanism for these changes is likely to be clonal expansion of individual mtDNA deletions within single cells [79]. Many studies have also reported the finding of cytochrome c oxidase deficiency at an individual cell level, which is increased with age [78]. Complex IV deficient cells, which occurred only sporadically earlier than the sixth decade of life, were present regularly after this age, with the loss of enzyme activity being always confined to single randomly distrib-
uted cells. Similarly, cytochrome c oxidase-negative neurons have been demonstrated to exist in abundance in the CNS of patients with a mitochondrial disorder [77]. These findings establish the relationship between age-associated accumulation of mtDNA mutations and bioenergy degradation as a key feature of the aging process, at least in tissues predominantly composed by postmitotic cells, such as CNS and skeletal muscle.

4. Mitochondrial damage, reactive nitrogen species, and neurodegenerative disorders

Increasing evidence sustains the hypothesis that mitochondrial energy metabolism underlies the pathogenesis of neurodegenerative diseases. Decreased complex I activity is reported in the substantia nigra of postmortem samples obtained from patients with Parkinson’s disease [80]. Similarly, impaired complex IV activity has been demonstrated in Alzheimer’s disease [81]. Increased free radical-induced oxidative stress has been associated with the development of such disorders [82] and a large body of evidence suggest that NO· may play a central role. Cytokines (INF-γ) which are present in normal brain are elevated in numerous pathological states, including Parkinson’s disease [83], Alzheimer’s disease [84], Multiple sclerosis, ischemia, encephalitis and central viral infections [85]. Accordingly, as cytokines promote the induction of NOS in brain, a possible role for a glial-derived NO· in the pathogenesis of these diseases has been suggested [86,87]. Excessive formation of NO· from glial origin has been evidenced in some study in which NADPH diaphorase (a cytochemical marker of NOS activity) positive glial cells have been identified in the substantia nigra of postmortem brains obtained from individuals with Parkinson’s disease [88]. Loss of nigral GSH is considered an early and crucial event in the pathogenesis of Parkinson’s disease [89] and, as a consequence, decreased peroxynitrite scavenging may also occur. Therefore, such perturbations in thiol homeostasis may constitute the starting point for a vicious cycle leading to excessive ONOO− generation in Parkinson’s disease. Moreover, in support of this, it has been reported that the selective inhibition of nNOS prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism in experimental animals [90].

4.1. Role of NOS and NO in brain pathophysiology

The discovery of the role of NO as a messenger molecule has revolutionised the concept of neuronal communication in the CNS. It is now generally accepted that NO is a major component in signalling transduction pathways controlling smooth muscle tone, platelet aggregation, host response to infection and a wide array of other physiological and pathophysiological processes. Under conditions of excessive formation, NO is emerging as an important mediator of neurotoxicity in a variety of disorders of the nervous system. The enzyme responsible for NO synthesis is the nitric oxide synthase (NOS) family of enzymes, which catalyse the conversion of arginine to citrulline and NO. NOS, localized in the CNS and in the periphery [91], is present in three well characterized isoforms (a) neuronal NOS (nNOS, type I) (b) endothelial NOS (eNOS; type III), and (c) inducible NOS (iNOS, type II). Activation of different isoforms of NOS requires various factors and co-factors. Formation of calcium/calmodulin complexes is a prerequisite before the functional active dimer exhibits NOS activity, which depends also on cofactors such as tetrahydrobiopterin (BH4), FAD, FMN and NADPH [92]. In contrast to nNOS and eNOS, iNOS can bind to calmodulin even at very low concentration of intracellular calcium, thus iNOS can exert its activity in a calcium-independent manner. The levels of iNOS in the CNS are generally fairly low. However, an increased expression of iNOS in astrocytes and microglia occurs following viral infection and trauma [93]. Activation of iNOS requires gene transcription, and the induction can be influenced by endotoxin and cytokines (interleukin-1, interleukin-2, lipopolysaccharide, interferon-γ, tumor necrosis factor). This activation can be blocked by anti-inflammatory drugs (dexamethasone), inhibitory cytokines (interleukin-4, interleukin-10,) prostaglandins (PGA2), tissue growth factors or inhibitors of protein synthesis, e.g., cycloheximide [94].

NO can react with carrier molecules and release oxidized (NO⁺) or reduced (NO−) forms [95]. All these chemical states are found in brain and seem to account for the different controversial effects of NO in CNS. NO reacts with the superoxide anion (O2−) to produce the potent oxidant, peroxynitrite (ONOO−) [96]. The rate of this reaction is three times faster than the rate of superoxide dismutase (SOD) in catalyzing the dismutation of the superoxide anion to hydrogen peroxide. Therefore when present at appropriate concentrations, NO effectively competes with SOD for O2−. Peroxynitrite is a strong oxidant capable of reacting with sulfhydryl groups, such as those of proteins, or directly nitrate aromatic amino acids and possibly affect their participation in signal transduction mechanisms [97]. In addition, peroxynitrite oxidizes lipids [97], proteins [98] and DNA [99]. Thiols are commonly assumed to be a major target for NO [100]. Nitrosothiols with biological relevance have been isolated and characterized, including S-nitrosothiols and the nitrosothiols of serum albumin [101]. The biological role for NO in the S-nitrosylation of many proteins is emerging as an important regulatory system [102]. For instance, the NMDA receptor is inactivated by nitrosylation, hence NO may modulate glutamatergic neurotransmission by this mechanism [103]. NO has been demonstrated to stimulate the auto-ADP ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by reacting with a critical cysteine with resulting binding of NAD to the catalytic cysteine, inhibition of GAPDH activity and depression of glycolysis [104]. Through formation of S-
nitrosothiol-specific enzymes and may play a role in the detoxification of reactive thiols. NO-induced mitochondrial damage causes neurotoxicity via apoptosis and/or necrosis, which are reduced by glutathione in the presence of transition metals. Glutathione is an endogenous antioxidant of great importance. Glutathione (GSH) is required for the maintenance of the thiol redox status of the cell, protection against oxidative damage, detoxification of endogenous and xenogenous reactive metals and electrophiles, storage and transport of cysteine, as well as for protein and DNA synthesis, cell cycle regulation and cell differentiation. Glutathione and glutathione-related enzymes play a key role in protecting the cell against the effects of reactive oxygen species. The key functional element of glutathione is the cysteinyl moiety, which provides the reactive thiol group. Glutathione is the predominant defense against reactive oxygen species (ROS), which are reduced by GSH in the presence of GSH peroxidase. As a result, GSH is oxidized to GSSG, which in turn is rapidly reduced back to GSH by GSSG reductase at the expense of NADPH. The thiol-disulfide redox cycle also aids in maintaining reduced protein and enzyme thiols. Without a process to reduce protein disulfides, vulnerable cysteinyl residues of essential enzymes might remain oxidized, leading to changes in neurotoxicity.
catalytic activity. Glutathione also aids in the storage and transfer of cysteine as well. Cysteine autoxidizes rapidly into cystine producing toxic oxygen radicals. To avoid the toxicity of cystine, most of the nonprotein cysteine is stored in glutathione. In addition to protection against ROS, glutathione is an excellent scavenger of lipid peroxidation products such as HNE and acrolein, both of which have been found to bind proteins inhibiting their activities. Glutathione also reacts with saturated carbon atoms (epoxides), unsaturated carbon atoms (quinones, esters), and aromatic carbon atoms (aryl nitro compounds). This detoxification involves nucleophilic attack by GSH on an electrophilic carbon. This reaction can occur spontaneously, but most often is catalyzed by glutathione S-transferase. Glutathione also forms metal complexes via nonenzymatic reactions. GSH functions in the storage, mobilization and delivery of metal ions between ligands, in the transport of metal across cell membranes, as a source of cysteine for metal binding, and as a reductant in redox reactions involving metals [118]. The sulphhydryl group of the cysteine moiety of GSH has a high affinity for metal ions such as mercury, silver, cadmium, arsenic, lead, gold, zinc, and copper, forming a thermodynamically stable complex that can be eliminated from the body. Glutathione reacts with radicals, and thus, means of increasing glutathione may prove beneficial against oxidative stress. For example, our laboratory has shown that elevated in-vivo glutathione protects brain membranes against oxidative stress associated with hydroxyl free radicals, peroxynitrite, and reactive aldehydic products of lipid peroxidation [4-hydroxy-2-nonenal (HNE) or 2-propenal (acrolein)] [119–123]. HNE and acrolein are increased in AD brain [124,125], and HNE is covalently bound in excess to the glutamate transporter in AD [126]. The latter finding, that could also be induced by addition of Aβ to synaptosomes [127], coupled with the reported loss of glutamine synthetase activity in AD brain [127], suggests that glutamate-stimulated excitotoxic mechanisms could be important in neurodegeneration in AD.

There are evidences of an impairment in vivo of glutathione homeostasis and antioxidant enzymes in patients with Friedreich ataxia, suggesting a relevant role of free radical cytotoxicity in the pathophysiology of the disease. In fact, a reduction of free glutathione levels in the blood of patients with Friedreich ataxia, a total glutathione concentration comparable to the controls and a significant increase of glutathione bound to haemoglobin in erythrocytes have been demonstrated in FRDA patients [128], also associated with a significant elevation in the superoxide dismutase/glutathione peroxidase activity ratio and with an 83% rise of glutathione transferase activity in the blood [129].

6. The heat shock pathway of cell stress tolerance

It is well known that living cells are continually challenged by conditions which cause acute or chronic stress. To adapt to environmental changes and survive different types of injuries, eukaryotic cells have evolved networks of different responses which detect and control diverse forms of stress. One of these responses, known as the heat shock response, has attracted a great deal of attention as a universal fundamental mechanism necessary for cell survival under a wide variety of toxic conditions. In mammalian cells HSP synthesis is induced not only after hyperthermia, but also following alterations in the intra-cellular redox environment, exposure to heavy metals, amino acid analogs or cytotoxic drugs. While prolonged exposure to conditions of extreme stress is harmful and can lead to cell death, induction of HSP synthesis can result in stress tolerance and cytoprotection against stress-induced molecular damage. Furthermore, transient exposure to elevated temperatures has a cross-protective effect against sustained, normally lethal exposures to other pathogenic stimuli. Hence, the heat shock response contributes to establish a cytoprotective state in a variety of metabolic disturbances and injuries, including stroke, epilepsy, cell and tissue trauma, neurodegenerative disease and aging [130–133]. This has opened new perspectives in medicine and pharmacology, as molecules activating this defense mechanism appear as possible candidates for novel cytoprotective strategies [134]. In mammalian cells the induction of the heat shock response requires the activation and translocation to the nucleus of one or more heat shock transcription factors which control the expression of a specific set of genes encoding cytoprotective heat shock proteins. Some of the known HSPs include ubiquitin, HSP10, HSP27, HSP32 (or HO-1), HSP47, HSP60, HSC70, HSP70 (or HSP72), HSP90 and HSP100/105. Most of the proteins are named according to their molecular weight.

HSP70. The 70 kDa family of stress proteins is one of the most extensively studied. Included in this family are HSC70 (heat shock cognate, the constitutive form), HSP70 (the inducible form, also referred to as HSP72), GRP75 (a constitutively expressed glucose-regulated protein found in the endoplasmic reticulum). After a variety of central nervous system (CNS) insults, HSP70 is synthesized at high levels and is present in the cytosol, nucleus and endoplasmic reticulum. Denatured proteins are thought to serve as stimulus for induction. These denatured proteins activate heat shock factors (HSFs) within the cytosol by dissociating other HSPs that are normally bound to HSF [132]. Freed HSF is phosphorylated and forms trimers, which enter the nucleus and bind to heat shock elements (HSE) within the promoters of different heat shock genes leading to transcription and synthesis of HSPs. After heat shock, for instance the synthesis of HSP70 increases to a point where it becomes the most abundant single protein in a cell. Once synthesized, HSP70 binds to denatured proteins in an ATP-dependent manner. The N-terminal end contains an ATP-binding domain, whereas the C-terminal region contains a substrate-binding domain. Heat shock
proteins serve as chaperones that bind to other proteins and regulate their conformation, regulate the protein movement across membranes or through organelles, or regulate the availability of a receptor or activity of an enzyme.

In the nervous system HSPs are induced in a variety of pathological conditions, including cerebral ischemia, neurodegenerative disorders, epilepsy and trauma. Expression of the gene encoding HSPs has been found in various cell populations within the nervous system, including neurons, glia and endothelial cells [135]. HSPs consist of both stress-inducible and constitutive family members. Whether stress proteins are neuroprotective has been the subject of much debate, as it has been speculated that these proteins might be merely an epiphenomenon unrelated to cell survival. Only recently, however, with the availability of transgenic animals and gene transfer, has become possible to overexpress the gene encoding HSP70 to test directly the hypothesis that stress proteins protects cells from injury, and it has been demonstrated that overproduction of HSP70 leads to protection in several different models of nervous system injury [136,137]. Following focal cerebral ischemia, mRNA encoding HSP70 is synthesized in most ischemic cells except in areas of very low blood flow, because of limited ATP levels. HSP70 proteins is produced mainly in endothelial cells, in the core of infarcts in the cells that are most resistant to ischemia, in glial cells at the edges of infarcts and in neurons outside the areas of infarction. It has been suggested that this neuronal expression of HSP70 outside an infarct can be used to define the ischemic penumbra, which means the zone of protein denaturation in the ischemic areas [138]. A number of in vitro studies show that both heat shock and HSP overproduction protect CNS cells against both necrosis and apoptosis. Mild heat shock protects neurons against glutamate-mediated toxicity and protects astrocytes against injury produced by lethal acidosis [139]. Transfection of cultured astrocytes with HSP70 protects them from ischemia or glucose deprivation [140]. HSP70 has been demonstrated to inhibit caspase-3 activation caused by ceramide, and also affect JUN kinase and p38-kinase activation [141]. In addition, HSP70 binds to and modulates the function of BAG-1, the bcl-2 binding protein [142], thus modulating some type of apoptosis-related cell death.

A large body of evidence now suggests a correlation between mechanisms of oxidative and/or nitrosative stress and HSP induction [143]. Current opinion holds also the possibility that the heat shock response can exert its protective effects through inhibition of NFkB signaling pathway [132]. We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of Hsp70 stress proteins. Increase in Hsp70 protein expression was also found after treatment of cells with the NO generating compound sodium nitroprusside (SNP), thus suggesting a role for NO in inducing hsp70 proteins. The molecular mechanisms regulating the NO-induced activation of heat-shock signal seems to involve cellular oxidant/antioxidant balance, mainly represented by the glutathione status and the antioxidant enzymes [5,144,145]. Ubiquitin is one of the smallest HSPs and is expressed throughout brain in response to ischemia. It is involved in targeting and chaperoning of proteins degraded in proteasomes, which include NFkB, cyclins, HSFs, hypoxia-inducible factor, some apoptosis-related proteins, tumor necrosis factor and erythropoietin receptors [146].

HSP27 is synthesized mainly in astrocytes in response to ischemic situations or to kainic acid administration. It chaperones cytoskeletal proteins, such as intermediate filaments, actin or glial fibrillary acidic protein following stress in astrocytes. It also protects against Fas-Apo-1, staurosporine, TNF and etoposide-induced apoptotic cell death as well as H$_2$O$_2$-induced necrosis [147]. HSP47 is synthesized mainly in microglia following cerebral ischemia and subarachnoid hemorrhage [148].

HSP60, glucose-regulated protein 75 (GRP75) and HSP10 chaperone proteins within mitochondria. GRP75 and GRP78, also called oxygen-regulated proteins (ORPs) are produced by low levels of oxygen and glucose. These protects brain cells against ischemia and seizures in vivo, after viral-induced overexpression [149].

HSP32 or heme oxygenase is the rate-limiting enzyme in the production of bilirubin. There are three isoforms of heme oxygenase: HO-1 or inducible isoform, HO-2 or constitutive isoform and the recently discovered HO-3 [149–154]. Heme oxygenase is the rate-limiting enzyme in the production of bilirubin. It catalyzes the degradation of heme in a multistep, energy-requiring system. The reaction catalyzed by HO is the α-specific oxidative cleavage of the heme molecule to form equimolar amounts of biliverdin and carbon monoxide (CO). The iron released by HO-1 is bound by ferritin, perhaps via a HO-1 chaperone function, [155]. The redox environment of cytosol and mitochondria is critical to cellular performance and protection against oxidative stress [156]. Increasing evidence suggests that the HO-1 gene is redox regulated and contains in its promoter region the antioxidant responsive element (ARE), similar to other antioxidant enzymes. Since the expression of heat shock proteins is closely related to that of amyloid precursor protein (APP), heat-shock proteins have been studied in brains of patients with Alzheimer’s disease. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles [157], and HO-1 mRNA was found to be increased in AD neocortex and cerebral vessels [158]. HO-1 increase was not only in association with neurofibrillary tangles, but also co-localized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains [153]. It is conceivable that the dramatic increase in HO-1 in AD may be a direct response to increased free heme associated with neurodegeneration and an attempt to convert the highly harming heme into the antioxidants biliverdin and bilirubin. Heme oxygenase-1 is rapidly upregulated by
oxidative and nitrosative stresses, as well as by glutathione depletion. All these findings have introduced new perspectives in medicine and pharmacology, as molecules activating this defense mechanism appear to be possible candidates for novel cytoprotective strategies [159]. Recently, considerable attention has been focused on identifying dietary and medicinal phytochemicals that can inhibit, retard or reverse the multi-stage pathophysiological events underlying AD pathology [3,159–161]. Spice and herbs contain phenolic substances with potent antioxidative and chemopreventive properties [162]. The active antioxidative principle in *Curcuma longa*, a colouring agent and food additive used in Indian culinary preparations, has been identified as curcumin (diferuloylmethane). Due to the presence in its structure of two electrophilic α, β-unsaturated carbonyl groups which, by virtue of Michael reaction, can react with nucleophiles such as glutathione, curcumin has the potential to inhibit lipid peroxidation and effectively to intercept and neutralize reactive oxygen and NO-based free radicals [163]. This agent is a potent inhibitor of tumor initiation in vivo and possesses antiproliferative activities against tumor cells in vitro [164]. Recent epidemiological studies [165], have raised the possibility that this molecule, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the significantly reduced (4.4-fold) prevalence of AD in India compared to United States. Based on these findings [166] it has been provided compelling evidence that dietary curcumin given to transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brain of this murine model of AD. Furthermore, in a human neuroblastoma cell line it has recently been shown that curcumin inhibits NFkB activation, effectively preventing neuronal cell death, [159]. Remarkably, recent evidence has demonstrated that curcumin is a potent inducer of HO-1 in vascular endothelial cells [7,167]. We have also recently demonstrated in astroglial cells the role of caffeic acid phenylethyl ester (CAPE), an active component of propolis, as a novel HO-1 inducer [162]. The similarity of CAPE to curcumin is striking because CAPE is also a Michael reaction acceptor, endowed with anti-inflammatory, antioxidant and anticancer effects [168]. These agents all appear capable of transcriptionally activating a gene battery that includes antioxidant enzymes and heme oxygenase [169]. Gene induction occurs through the antioxidative responsive element (ARE) [164]. Thus, increased expression of genes regulated by the ARE in cells of the central nervous system may provide protection against oxidative stress.

7. Therapy advances in FRDA

The precise sequence of events in FRDA pathogenesis is uncertain. The impaired intramitochondrial metabolism with increased free iron levels and a defective mitochondrial respiratory chain, resulting in increased free radical generation which will cause oxidative damage may be considered a possible mechanism that compromise cell viability. Evidence of oxidative stress and damage has been identified in other neurodegenerative disorders, including Alzheimer’s disease (AD), Parkinson’s disease (PD) and motor neuron disease (amyotrophic lateral sclerosis) [170]. FRDA offers a unique opportunity to intervene with “neuroprotective” therapy before the disease becomes established. In contrast to PD, where patients have lost >50% of nigral dopaminergic neurons at presentation, and any attempt at neuroprotection must be in the presence of advanced disease and established pathogenetic mechanisms, FRDA patients can be diagnosed by genetic analysis either presymptomatically or early in the course of their disease.

The excessive free radical production and deficit of oxidative phosphorylation shown in FRDA suggests that the mitochondrial respiration deficit may be amenable to treatment with antioxidants [171]. Three FRDA patients were treated for 4 to 9 months with idebenone (5 mg/kg/daily), a short chain quinone analogue which acts as a free-radical scavenger [172]. Idebenone administration resulted in a reduction in septal thickness ranging from 31% to 36% and of the left ventricle posterior wall from 8% to 20%. These results were then confirmed by the same group that showed a reduction in left ventricular mass equal or more than 20% in 17 out of 38 FRDA patients [173], and by two more recent idebenone trials [174,175]. Idebenone administration (5 mg/kg/daily) also resulted in decreased markers of oxidative stress in FRDA patients [176].

The effect of another antioxidant treatment, Coenzyme Q10, 400 mg/day plus Vitamin E, 2100 IU/day, on in vivo cardiac and calf muscle energy metabolism, left ventricle hypertrophy (LVH) and ataxia has been evaluated in ten FRDA patients [177] After 6 months of therapy cardiac PCr to ATP ratio increased by more than 50% in the patients as a group [177]. Skeletal muscle mitochondrial *V*max for ATP production, after 6 months of CoQ10 and vitamin E treatment, increased by 34% in the patients’ group, being unchanged in only two patients. Echocardiography showed unchanged interventricular septum and posterior wall thickness in patients with and without LVH. [177]. FRDA patients, assessed neurologically using the semi-quantitative International Cooperative Ataxia Rating Scale (ICARS), showed lack of progression of their neurological deficits after 6 months of therapy [177]. The follow up of the same patients after 4 years of CoQ10 and vitamin E demonstrated a sustained improvement in cardiac and skeletal muscle energy metabolism associated with lack of progression of both neurological and echocardiographic signs [178,179].

Antioxidants targeted to mitochondria appear a promising approach to effectively slow disease progression. To validate this hypothesis, the efficacy of mitochondria-targeted and untargeted antioxidants derived from coenzyme Q10 and from vitamin E at preventing cell death due to endogenous oxidative stress has been recently investigated
in cultured fibroblasts from FRDA patients in which glutathione synthesis have been blocked. The mitochondria-targeted antioxidant MitoQ revealed to be several hundred-fold more potent than the untargeted analog idebenone. The mitochondria-targeted antioxidant MitoVit E was 350-fold more potent than the water soluble analog Trolox [180]. This is the first demonstration that mitochondria-targeted antioxidants prevent cell death caused by endogenous oxidative damage. Targeted antioxidants may have therapeutic potential in FRDA and in other disorders involving mitochondrial oxidative damage.

The potential role of iron chelators 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) analogues, as agents to remove mitochondrial iron deposits have been recently under investigation [181]. These ligands have been specifically designed to enter and target mitochondrial iron pools, which is a property lacking in desferrioxamine, the only chelator in widespread clinical use. This latter drug may not have any beneficial effect in FA patients, probably because of its hydrophilicity that prevents mitochondrial access. Indeed, standard chelation regimens will probably not work in FA, as these patients do not exhibit gross iron-loading. Considering that there is no effective treatment for FA, it is essential that the therapeutic potential of iron chelators focuses on the mitochondrial iron pools as primary target. Given the physiopathological mechanisms responsible for FRDA, selenium administration could represent another therapy strategy. As is known, selenium deficiency is harmful to the heart in that may cause a fatal dilated congestive cardiomyopathy in animals (white muscle disease) and in man (Keshan disease). Both of these syndromes are selenium-responsive. A deficiency of the micronutrient has also been reported in patients with Friedreich ataxia and there are histological similarities between Friedreich’s cardiomyopathy and Keshan disease. A low selenium status results in reduced selenium-dependent glutathione peroxidase activity. This essential antioxidant enzyme protects membranes from oxidative insults. As iron-induced mitochondrial oxidative damage is central to the pathology of Friedreich ataxia and, in addition, some studies suggest a link between frataxin expression, glutathione peroxidase (GPX) activity and oxidative stress, the administration of selenium supplements could normalize the antioxidant activity of myocardial glutathione peroxidase and slow the progression of the life-shortening cardiomyopathy associated with this disease [182]. Hence, the ability of small-molecule GPX mimetics, such as ebselen, monoselenide or diselenide are currently under investigation, although further studies are needed to address the toxicity of GPX mimetics in humans before human FRDA trials can be considered. Finally, new technologies are emerging particularly those based on high-throughput screening which can speed up the process of drug development via generation of enormous compound libraries in a limited time through automated organic synthesis (combinatorial chemistry). This approach can be used in the biological screening of compounds that have potential in the treatment of FRDA [183].

8. Conclusions

Since the discovery of the genetic basis of FRDA only a few years ago, the progress made in our understanding of the pathogenic mechanisms underlying FRDA has been remarkable. Although the precise function of frataxin still remains to be defined, FRDA has clearly been identified as a nuclear encoded mitochondrial disorder. Our and others’ pilot studies have indicated the potential effect of antioxidant therapy in this condition [183–185] have now a robust background for designing larger randomised trials which will confirm whether an early diagnosis of FRDA can be exploited to initiate antioxidant treatment and prevent the progression of this disorder.

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