Review article

Oxidative stress as a cofactor in spinocerebellar ataxia type 2

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Spinocerebellar ataxia type 2 (SCA2) is a redox-sensitive neurodegenerative disease affecting the cerebellum, fibre connections in the cerebellum, the peripheral nervous system, and extracerebellar central pathways. Currently, Cuba has the highest reported global rate for this disease. The aim of this review article is to summarize and discuss the current knowledge about evidence of oxidative stress during SCA2. Recent reports have suggested that ataxin 2 and other related factors contribute to the redox imbalance in this disease. It is important to recognize and clarify the molecular mechanisms associated with the redox imbalance to consider ataxias innovative approaches to counteract oxidative stress-induced tissue damage, through alternative therapeutic or nutritional intervention in SCA2 and related diseases.

Keywords: Oxidative stress, Ataxine 2, Neurodegenerative, Spinocerebellar ataxia, Redox markers

General aspects of the ataxias

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically, pathologically and genetically heterogeneous group of neurodegenerative disorders characterized by the degeneration of the cerebellum and its afferent and efferent connections. There are three major subtypes of ADCAs. Degeneration of the pontomedullar systems, pyramidal tracts, basal ganglia, cerebral cortex, and peripheral nerves is classified as ADCA I; degeneration of the retina as ADCA II; and degeneration of the cerebellum as ADCA III.[1,2] The most common dominantly inherited autosomal ataxia, ADCA I, includes many spinocerebellar ataxia (SCA) subtypes. The majority of these SCA subtypes are caused by pathological CAG trinucleotide repeat expansion in the coding region of mutated genes, such as SCA1, SCA2, SCA3/MJD, SCA 6, SCA7, and SCA17, and dentatorubral-pallidoluysian atrophy (DRPLA), Huntington disease, and spinal and bulbar muscular atrophy (SBMA).[1] Although many of the mutated genes in the SCAs play a clear role in gene expression and dendritic signalling, the existence of additional pathways also plays a role, and indicates the complexity of this phenotype. Among 32 SCA subtypes, SCA2 is one of the three that are most frequent worldwide together with SCA3 and SCA6.[3] In general, SCA disorders show clinical features that overlap with other neurodegenerative diseases and between subtypes. An unequivocal diagnosis of the subtype requires molecular genetic studies. Since the discovery of the ataxia disorders, to date extensive research has been performed in interdisciplinary fields including neuroepidemiology, molecular genetics, neurophysiology, pathological anatomy, neuroimaging, neuropsychology, and neuropsychiatry. Important molecular evidence has been emerging; however, the related knowledge has not been enough to avoid considering SCA and other ataxias as orphans of disease. This review is focused on the main features, molecular aspects, and the evidences of oxidative stress related to SCA2. We also discuss the oxidative stress hypothesis which may underlie the pathogenesis of SCA2 and the possible beneficial effect of counteracting intervention, based on the clinical interventions results in SCA2 and other similar disorders.

Epidemiology of SCA2

The collective worldwide prevalence of ataxia is estimated to be about five to seven cases per 100 000

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inhabitants, although higher figures have been reported in particular populations because of founder effects such as SCA3 in Portugal and SCA2 in Cuba. Epidemiological studies of hereditary ataxias have mainly been performed in isolated geographical regions in families not large enough for linkage analysis. Recent molecular analyses of genes SCA1-3, SCA6, SCA17, and DRPLA through an epidemiological coordinated survey in the whole Cuban population identified 753 patients with SCA and 7173 asymptomatic relatives, belonging to 200 families in Cuba. It is estimated that nearly 87% of SCA patients (578) affected by SCA2, were distributed in 12 of the 16 provinces of the country with a national prevalence rate of 657 cases per 100 000 inhabitants. The highest concentration of SCA2 families in the world is in Holguín, Cuba, where the prevalence rate is already 182.75 per 100 000 inhabitants, with 1893 pre-symptomatic carriers and patients. Within this region, the most affected area is Baguanos, with a rate of 715 cases per 100 000 inhabitants. Despite the dissemination of Cuban families around the island the high prevalence of the SCA2 mutation in Holguín differs from other regions of the country, most likely due to a founder effect.[2,3]

Large families with SCA2 diagnostic and clinical features have been found in India, Martinique, Australia, Tunisia, Germany, Italy, Mexico, and Poland, though with a lower incidence and prevalence compared to Cuba.

The existence of a pre-symptomatic and prenatal diagnostic programme worldwide also encourages the scientific community to explore and develop therapeutic procedures to modify the course and severity of the disease.[4,5]

SCA2 features

The ataxia is a neurodegenerative disease that affects not only the cerebellum and its fibre connections, but also the peripheral nervous system and extracerebellar central pathways. Some of these abnormalities may appear during pre-symptomatic stages.[5] Some inherited ataxias, such as ataxia with vitamin E deficiency, are caused by defects in small-molecule antioxidants and could be treated by supplying the defective molecule.[6,7] However, most ataxias have complex disease-specific causes that must be better understood to enable effective treatments.

Clinically, SCA2 is characterized by a progressive gait and limb ataxia, cerebellar dysarthria, dysmetria, dysdiadochokinesia, tremor, slowing of horizontal saccadic eye movements, and voluntary eye movements with reduced amplitude.[4,8–11] Patients with ataxia often have impaired coordination, dexterity or gait in the absence of significant muscular weakness, defining the presence of peripheral neuropathy.[5] Other clinical features include abnormal swallowing, autonomic abnormalities (urinary dysfunction, hypohydrosis, constipation, and sexual dysfunction), sleep disturbances, and cognitive disorders. Some patients exhibit retinitis pigmentosa and myoclonus epilepsy and others may develop Parkinsonian signs.[9,10]

The gene associated with SCA2 is located on the long arm of chromosome 12 (12q23-24.1) and encodes a cytoplasmatic protein (ataxin 2) found in many tissues and neurons.[12–14] In addition to its role in RNA metabolism, recent studies have begun to shed light on additional functions of ataxin 2 in the cytoplasm. This protein is predominantly cytoplasmic and associates with endophilin A1/A3 at the endoplasmic reticulum and plasma membrane and may be involved in endocytosis.[15]

The peripheral lesion of nerves stays mainly at the axonal level but with signs of demyelination, and is interpreted as a secondary axonal disorder. The reduction in amplitude of the sensory potential is more evident in patients with longer duration of the disease, suggesting a progressive increase in the number of affected fibres.[4,16,17] Motor and sensory nerve conduction measurements in a large sample of SCA2 gene carriers, over a 20-year study, indicate progressive changes in electrophysiological abnormalities that were suggestive of three electrophysiological stages of this disease.[3]

First stage: preclinical sensory axonal neuropathy

During this stage, electrophysiological abnormalities appear before clinical disease onset (SCA2 stage 0). These alterations consist of a decrease of sensory potential amplitudes without definitive clinical manifestations of peripheral neuropathy and could be classified as the earliest subclinical alterations in SCA2. The results of normal motor nerve conduction studies suggest the sparing of the motor nerve fibres during pre-symptomatic stages.

Second stage: sensory axonal neuropathy

This stage corresponds to the initial clinical manifestations of the cerebellar syndrome. It is characterized by increased sensory electrophysiological abnormalities and the emergence of other more variable sensory alterations, such as latency increases and reduced nerve conduction.

Third stage: sensory-motor neuropathy

This stage is characterized by a mixed (sensory and motor) peripheral neuropathy with decreased motor potential amplitudes and accentuated sensory involvement.

SCA2 commonly starts in adulthood with the predominant age of 30 years old. Genetic anticipation of onset related to age is observed in a high percentage (80%) of patients. Progression of disease is related to

limitation of validity and confining the patients to a wheelchair and later to the bed where they can die approximately 15–20 years after the initial symptoms.

Oxidative stress and neurotoxicity in ataxias

The central nervous system (CNS) has a potential oxidative capacity due to the high level of tissue oxygen consumption. The CNS is poorly able to withstand oxidative stress because of the presence of (1) a high content of easily oxidizable substrates; (2) relatively low levels of the primary and secondary antioxidants; (3) reactive oxygen species generation by several endogenous systems; (4) elevated iron concentration in specific areas of the human brain (gobus pallidus and substantia nigra); and (5) non-replicative neuronal cells. Once the CNS is damaged, it is permanently dysfunctional and the cells undergo apoptosis.[18,19]

A role for oxidative stress has been suggested in the pathogenesis of different neurodegenerative complications, including some kinds of ataxias. The cause of disease is diverse. Detailed genetic and family studies emphasize the heterogeneity. They have identified that ataxias can be inherited via an autosomal recessive or dominantly inherited pattern. In all cases, the distinct repeated triplet (CAG, GAA, etc.) expansion on genes located on different chromosomes plays a major role. The expansion and/or point mutation result in reduced expression of specific proteins encoded by mutated genes.[20,21] The relationship between ataxia and oxidative stress depends on molecular, *in vitro* and animal studies. Nevertheless, evidence from human biomarker studies is contradictory.

Molecular mechanisms

The pathogenic effects of SCAs depend on the wild-type function of the protein and the cellular context of the mutations. Previous studies have shown that the accumulation of mutated or damaged proteins, wrongly processed variants, or misfolded proteins and their aggregates is associated with the pathogenic effects of SCAs.[22]

In the case of SCA2 the underlying mutation consists of the expansion of a translated trinulecotide CAG repeat within the first exon of the ATXN2 gene. In the case of pathological conditions, the alleles have more than 32 CAG repeats. The most common size of this is 37 triplets (72%) but the length of repeats can be as high as 60 units. A significant increase in probability of the age of onset is related to this augmentation. Other unidentified genetic factors and unknown environmental aspects could also influence this augmentation.

The mutated product may lead to both aberrant splicing of important genes affecting neuronal function and survival, and reduced functions of these transcriptional complexes within the cell. The direct or indirect protein interaction of different ataxins products suggests a high degree of the pathway convergence.[23]

Recent studies have led to the identification of some common pathways to the ataxias, consisting of the dysfunction of gene expression, synaptic transmission, and other intracellular signalling pathways.[21]

Ataxin 2 interacts with poly(A)-binding protein 1 and can assemble into polyribosomes, suggesting that ataxins play a role in RNA metabolism.[22] In addition, the complex formed is important for functional regulation. The complex interacts with many cytosolic and nuclear proteins. Previous reports suggest that ataxin 2 may play a role in regulating cellular mRNA turnover.

Downstream of these events, metal-catalyzed oxidation, as a common final pathway of injury, leading to oxidative stress has been implicated.[24] In contrast, exogenous zinc (Zn) exerts neuroprotective actions through antagonism of NMDA receptors in the cerebellum, the brain area most affected by type SCA2. Previous reports show that increased Zn concentrations counteract the prooxidant response in type SCA2, suggesting that the diminished Zn concentration found in spinocerebal fluids of terminal patients could be related to the course of oxidative reactions in the onset and/or clinical outcome of the disease.[24–27] Reduced levels of iron and copper also were found in serum. Zn depletion may be related to environmental deficiency and unknown metabolism associated with CAG repeats.

Presumably, a similar pathogenic pathway is involved in SCAs, as each of the expanded CAG repeats encodes polyglutamine and the pathogenic threshold for disease is roughly the same at around 40 copies of the repeat in most of the different subtypes. It is assumed that the common toxic gain of action mechanisms for the polyglutamine-containing protein is aggregation and deposition of misfolded proteins in recognized stress granules or permanent presence in different tissues including membranes. This may lead to neuronal dysfunction and eventually to cell death. Cellular and molecular pathways implicated in the SCAs are diverse. In SCA2 aggregation, activation of apoptosis/caspases, autophagy, calcium and dopaminergic signalling, endoplasmic reticulum signalling, gene transcription, heat shock pathway, mitochondrial dysfunction, and synaptic neurotransmission deficits (glutamate, GABA) are involved. Some of these molecular mechanisms generate reactive species that in turn are messengers or mediators of the related process. Reactive species seem to lead to dark degeneration of Purkinje cells and cerebellar dysfunction, implicating impaired mitochondrial proteolysis as a pathway in cerebellar neurodegeneration.[28]

Until now, two transgenic mouse models for ataxin 2 have been generated. The first employed a Purkinje

cell-specific promoter to drive transgene expression of human ataxin 2 with an expansion to Q58 expressed by a progressive motor deficit associated with the loss of the Purkinje cell dendritic arbor and finally of Purkinje cell numbers. In a later mouse model, a fragment of the SCA2 promoter was used to drive a more ubiquitous expression of the human transgene with Q75, and once again causing a motor deficit and neurodegeneration.[29,30,31] These models, and others developed *in vitro*, have helped characterize ataxin 2. They have shown that ataxin 2 contains an RNA-binding Lsm domain characterized by a conserved sequence motif consisting of two short segments. These are known as Sm1 and Sm2, which are separated by a variable linker. Lsm domain proteins are involved in a variety of essential RNA processing events including RNA modification, pre-mRNA splicing, mRNA decapping and degradation, and some of them are also important components of spliceosomal small nuclear ribonucleoprotein complexes.[31] Interestingly, ataxin 2 interacts with ataxin 2 binding protein 1, whose RNA-binding *Caenorhabditis elegans* homologue, fox-1, regulates tissue-specific alternative splicing. Deciphering the mechanisms by which ataxin 2 regulates alternative splicing should provide insights into the deregulated pathways during disease progression, which in turn may lead to the identification of potential therapeutic targets.

Post mortem studies of SCA2 patients have traditionally made a diagnosis of olivo-ponto-cerebellar atrophy or Menzel type ataxia.

Elevated lactate levels in SCA2 cerebella were observed by proton magnetic resonance spectroscopic imaging, suggesting alteration of glycolysis and mitochondrial function.[32,33,36]

During mitochondrial dysfunction, superoxide production is markedly increased as a consequence of the electrochemical potential difference generated by the elevation of the proton gradient across the inner mitochondrial membrane. Previous reports concerning SCA2 and oxidative lipid damage, data on reactive species generation increase are contradictory. Some investigators found similar malondialdehyde concentrations in ataxia patients and healthy controls, but a significantly different hydroperoxide concentration was found.[34–36]

A marked decrease of the total antioxidant capacity as well as an increase in malondialdehyde concentration in serum, with significant differences with respect to healthy subjects, were found in SCA2 Cubans patients.[36,37]

In recent reports, neurochemical alterations in SCA2 patients was analyzed: Oz and colleagues, for instance, compared diverse biomarker levels in the main cerebellar (vermis and cerebellar hemispheres) and brainstem (pons) tissues with other SCA phenotypes such as SCA 6 and MSA-C in comparison with age-matched controls.[34,35] The investigators found high glutathione concentrations only in the vermis of SCA2 patients. They did not find differences in lactate concentrations in SCA2. The reason for this discrepancy could be attributed to the different magnetic resonance spectroscopic methods used.[12]

Other recessive and dominant inherited ataxia phenotypes such as Friedriech ataxia (FA) and ataxia telangiectasia (AT) are clearly associated with oxidative stress. Oxidative stress is involved in the pathophysiological pathways of these diseases. Frataxin, a mutated protein related to FA, is localized to the mitochondria. Its defect impairs mitochondrial iron handling and respiratory chain function, contributing to oxidative stress. This phenotype exhibits similarities to ataxia with vitamin E deficiency.[20] On the basis of these principles, a therapeutic approach counteracting damage by free radical and respiratory chain activation may be attempted. A recent uncontrolled open-labelled 4-year pilot study of patients taking CoQ10 and vitamin E demonstrated a sustained improvement in cardiac and skeletal muscle energy metabolism and suggested a possible stabilization or a diminished decline in certain neurological and echocardiographic symptoms.[38] An another study, three FA patients have been treated for 4–9 months with idebenone – a free radical scavenger – resulting in myocardial hypertrophy reduction, but no improvement in neurological symptoms.[39] This was confirmed by two recent trials that also resulted in decreases in markers of oxidative damage.[40–42]

In AT, the altered gene code seems to be related to a protein kinase involved in the regulation of the cellular response to double strand breaks and in other processes that maintain cellular homeostasis.[43,44] *In vivo* evidence is contradictory about redox abnormalities in AT patients but several reports point to protective effects of low-molecular-weight antioxidants in the AT phenotype.[45–50]

Considering previous reports, an oral zinc sulphate supplementation (50 mg/day) for 6 months in 36 SCA2 Cuban patients was conduced to evaluate the effects and safety of this intervention. Randomized, double-blind, placebo-controlled trials were developed, measuring the Zn levels in cerebrospinal fluid (CSF) and serum, ataxia score, oxidative stress indices, routine clinical diagnostic tests and saccadic eye movements. The treated group showed, with respect to the placebo group: a significant increase of the Zn levels in CSF and serum, a mild decrease in the ataxia scale subscore for gait, posture, stance, and dysdiadochocinesia, a reduction of lipid oxidative damage in serum and a reduction of saccadic latency.[50–52] All subjects of the study reached the normal serum Zn concentration of the Cuban

population (≈9.8 μmol/l). For CSF, all subjects modified significantly ($P < 0.05$) the Zn levels but only 30% of them reached the normal values defined for the Cuban population (>0.12 mg/l). The study was carried out simultaneously with a programme including neurorehabilitation therapy and there were no significant changes in the blood parameters. Some adverse reactions were reported, classified as mild events; only two of them related to the treatment. Thus, the Zn supplementation protocols are mandatory to reduce the Zn deficiencies in CSF of SCA2 patients, with positive but limited effects as an enhancer of the neurorehabilitation on the cerebellum function. The findings also suggested that Zn deficiencies are not associated with malabsorption. In the treated group, the patients with the highest Zn concentration had less lipid peroxidation at the end of the study, which supports its protective effects.[50,51]

At the present, there are no meaningful pharmacologic treatments for SCA2. Palliative treatment is available to support physiological needs and treatment of swallowing, balance, and bladder issues.[52]

Conclusions

An efficient clinical diagnostic assessment of the redox balance could be a way to supervise the degenerative damage associated with oxidative metabolism imbalance in SCA2. Despite the diversity of ataxias, oxidative damage may be a common factor in the pathogenesis of these disorders, suggesting that antioxidants may be of potential benefit in these currently incurable conditions. However, the efficacy of such strategies is limited, particularly when the protective agent does not cross the blood–brain barrier or does not arrive at intracellular compartments at effective concentrations. Further studies to evaluate and improve the efficacy of redox interventions in SCA2 are encouraged.

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