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Original Contribution

Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by motor neuron degeneration that ultimately results in progressive paralysis and death. Growing evidence indicates that mitochondrial dysfunction and oxidative stress contribute to motor neuron degeneration in ALS. To further explore the hypothesis that mitochondrial dysfunction and nitroxidative stress contribute to disease pathogenesis at the in vivo level, we assessed whether the mitochondria-targeted antioxidant [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl]triphenylphosphonium methane sulfonate (MitoQ) can modify disease progression in the SOD1^{G93A} mouse model of ALS. To do this, we administered MitoQ (500 μM) in the drinking water of SOD1^{G93A} mice from a time when early symptoms of neurodegeneration become evident at 90 days of age until death. This regime is a clinically plausible scenario and could be more easily translated to patients as this corresponds to initiating treatment of patients after they are first diagnosed with ALS. MitoQ was detected in all tested tissues by liquid chromatography/mass spectrometry after 20 days of administration. MitoQ treatment slowed the decline of mitochondrial function, in both the spinal cord and the quadriceps muscle, as measured by high-resolution respirometry. Importantly, nitroxidative markers and pathological signs in the spinal cord of MitoQ-treated animals were markedly reduced and neuromuscular junctions were recovered associated with a significant increase in hindlimb strength. Finally, MitoQ treatment significantly prolonged the life span of SOD1^{G93A} mice. Our results support a role for mitochondrial nitroxidative damage and dysfunction in the pathogenesis of ALS and suggest that mitochondria-targeted antioxidants may be of pharmacological use for ALS treatment.

ALS[1] is a fatal and devastating neurodegenerative disease that affects motor neurons, with no effective therapy available, leading to death within 3–5 years of diagnosis [1]. Mitochondrial dysfunction and oxidative stress in both the neurons and the surrounding glia have been implicated as a key part of the pathogenic process [2]. Reactive oxygen and nitrogen species are required for the execution of motor neuron apoptosis under various experimental conditions [3–5]. In addition, reactive astrocytes represent another source of nitric oxide and oxidants that in turn contributes to motor neuron apoptosis [5–7], suggesting that oxidative stress is involved in astrocyte-mediated motor neuron loss. As mitochondria are both the main producers and the targets of reactive species, targeting mitochondrial oxidative damage may be clinically useful in ALS.

Many antioxidant approaches have shown beneficial effects on animal models of ALS but have not been effective in human ALS

Abbreviations: ALS, amyotrophic lateral sclerosis; SOD1^{G93A}, ALS-linked superoxide dismutase 1 bearing the G93A mutation; MitoQ, [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl]triphenylphosphonium; TPP, triphenylphosphonium; decylTPP, decyltriphenylphosphonium cation; LC/ESI–MS/MS, liquid chromatography/electrospray ionization–mass spectrometry; MRM, multiple-reaction monitoring; d_{15}-MitoQ, deuterated MitoQ; IS, internal standard; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; UCR, uncoupling control ratio; TBS, Tris-buffered saline; PBS, phosphate-buffered saline; BSA, bovine serum albumin; GFAP, glial fibrillary acidic protein; HO-1, heme oxygenase-1; HNE, 4-hydroxynonenal–protein adduct; EDL, extensor digitorum longus muscle; TMR–BgTx, tetramethylrhodamine-conjugated α-bungarotoxin; non-Tg, nontransgenic; ARE, antioxidant-response element; RONS, reactive oxygen and nitrogen species

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patients [8]. In particular, two molecules that stimulate mitochondrial function and prolonged survival in ALS mice, coenzyme Q10 and creatine, have been tested in phase II clinical trials and failed to demonstrate efficacy against disease progression [9,10]. These two studies, despite measuring elevated plasma levels of the compounds and assessing their ability to cross the blood–brain barrier, did not address the elevated free radical production and oxidative damage at the mitochondrial level. This is relevant because mitochondria constitute a main source of free radical production. An emerging pharmacological approach to treating mitochondrial disorders is to use mitochondria-targeted antioxidants that concentrate specifically within mitochondria in vivo to selectively decrease mitochondrial oxidative damage [11–13]. The most widely used mitochondria-targeted antioxidant to date is MitoQ, which comprises a triphenylphosphonium (TPP) functionality conjugated to an antioxidant ubiquinone moiety [14]. MitoQ is accumulated within mitochondria in vivo in response to the large mitochondrial membrane potential (negative inside) and there protects mitochondria from oxidative damage. Mitochondria are reported to be a key intracellular site for the generation of reactive species in a wide range of pathologies, and consequently MitoQ has been shown to mitigate oxidative damage in various disease animal models [15], including Alzheimer [16,17] and Parkinson [18] diseases.

The most widely used animal model of ALS is the transgenic mouse expressing a familial ALS-linked SOD1 mutation, which generally develops progressive motor neuron degeneration resembling many aspects of ALS in human patients [19,20]. Studies in SOD1 mutant mice have demonstrated "non-cell autonomous" effects of the mutations, highlighting the important role of glia in motor neuron support [21,22]. Disease onset seems to be dependent on the expression of mutant SOD1 in motor neurons, and the expression of mutant SOD1 in glial cells modulates the rate of degeneration. Interestingly, the toxicity of mutant SOD1 in cultured motor neurons [23] and astrocytes [24] was prevented by their treatment with low concentrations (nM) of MitoQ. Together, these results suggest that MitoQ is a promising candidate for ALS treatment. To further support this hypothesis we tested its ability to affect disease progression in the $SOD1^{G93A}$ mouse model of ALS. To do this we administered MitoQ orally to ALS mice, starting at 90 days of age, when symptoms are already evident [25]. This regime mimics the clinical situation more closely, as this corresponds to initiation of therapy after first diagnosis and would indicate if this therapy may be translatable to the clinic. We then analyzed the effects of MitoQ on nitroxidative stress markers and key features of disease progression: survival, motor performance, neuronal loss, astrocytosis, and neuromuscular plaque decline. This approach may represent a novel mitochondria-targeted therapy for ALS.

Materials and methods

Materials

All reagents were obtained from Sigma Chemical Co (St. Louis, MO, USA) unless otherwise specified. MitoQ as MS010 (an ~20% w/w mixture of MitoQ and β-cyclodextrin) and decyltriphenylphosphonium bromide (decylTPP) were obtained from Antipodean Pharmaceuticals, Inc. Deuterated MitoQ (d_{15}-MitoQ) was synthesized as described previously [17].

Ethics statement

Procedures using laboratory animals were in accordance with the *International Guiding Principles for Biomedical Research Involving Animals,* as issued by the Council for the International Organizations of Medical Sciences, and were approved by Institutional Animal Committee Resolution No. 66 (Exp. 071140-001465-10), Comisión Honoraria de Experimentación Animal de la Universidad de la República (http://www.chea.udelar.edu.uy).

Animals

Transgenic ALS mice carrying the G93A mutated human SOD1, strain B6SJL-TgN(SOD1-G93A)1Gur [19], were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The strain was maintained by breeding hemizygous male carriers to B6SJLF1 female hybrids. The offspring were genotyped as previously described [24]. Transgenic mice hemizygous for the SOD1 G93A transgene and non-Tg littermates were used for analysis. The mice were housed under controlled conditions with free access to food and drinking water.

MitoQ treatment trial

Male and female transgenic mice and nontransgenic littermates were divided randomly into the following groups: (A) transgenic and nontransgenic mice that received MitoQ, (B) transgenic and nontransgenic mice that received equal concentrations of decylTPP bromide to determine if the TPP cation had any nonspecific effects, and (C) transgenic and nontransgenic control groups that received regular drinking water. MitoQ and decylTPP were administered as β-cyclodextrin complexes (Antipodean Pharmaceuticals) [26] at a 500 μM concentration of MitoQ or decylTPP in the drinking water supplied ad libitum, with fresh solutions made twice a week. In a previous study, this concentration yielded a daily dose of 3.2 μmol MitoQ/day/mouse or 95–138 μmol MitoQ/day/kg, showed no toxicity, and had no significant effect on animal body weight or food and liquid consumption [27]. Treatment was administered from 90 days of age (early symptomatic stage [19]) until death. Animals were observed weekly for onset of disease symptoms, as well as progression to death. Disease onset was scored as the first observation of abnormal gait or overt hindlimb weakness. End stage of the disease was scored as complete paralysis of both hindlimbs and the inability of the animal to right itself within 20 s of being placed on its side. When this condition was met, the mice were euthanized to limit animal suffering.

Grip strength measurements

Motor function was tested with a grip strength meter (San Diego Instruments, San Diego, CA, USA). Tests were performed by allowing the animal to grasp the platform with both hindlimbs, followed by pulling the animal until it released the platform. The force measurement was recorded twice a week from week 6 (baseline) until death in five separate trials.

Quantification of tissue MitoQ content

Groups of mice were treated as detailed above for 20 days and brain, heart, liver, and quadriceps muscle samples were dissected, weighed, snap-frozen, and stored at −80 °C. Protein concentrations were measured in tissue homogenates by Bradford assay, using BSA as protein standard. Quantification of MitoQ was performed by liquid chromatography coupled to electrospray ionization/mass spectrometry on an ion trap mass spectrometer (Qtrap2000; Applied Biosystems-MDS Sciex) [28]. Electrospray ionization of MitoQ in positive mode generated a *m/z* signal of 583.5 and this ion was fragmented to obtain a *m/z* 441.3, so using multiple-reaction monitoring (MRM) mode a quantification protocol can be set following the transition 583.5/441.3 and 598.3/456.3 for d_{15}-MitoQ simultaneously [29]. MitoQ was measured