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Cerium oxide nanoparticles with antioxidant properties ameliorate strength and prolong life in mouse model of amyotrophic lateral sclerosis

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Abstract

Cerium oxide nanoparticles (CeNPs) neutralize reactive oxygen and nitrogen species. Since oxidative stress plays a role in amyotrophic lateral sclerosis (ALS) in humans and in the SOD1^{G93A} mouse model of ALS, we tested whether administration of CeNPs would improve survival and reduce disease severity in SOD1^{G93A} transgenic mice. Twice a week intravenous treatment of SOD1^{G93A} mice with CeNPs started at the onset of muscle weakness preserved muscle function and increased longevity in males and females. Median survival after the onset of CeNP treatment was 33.0 ± 3.7 days (N = 20), and only 22.0 ± 2.5 days in mice treated with vehicle, control injections (N = 27; $P = 0.022$). Since these citrate–EDTA stabilized CeNPs exhibited catalase and oxidase activity in cell-free systems and in *in vitro* models of ischemic oxidative stress, we hypothesize that antioxidant activity is the protective mechanism prolonging survival in the SOD1^{G93A} mice.

Key words: Oxidative stress; Cerium oxide nanoparticles; Amyotrophic lateral sclerosis

We have been investigating the therapeutic potential of antioxidant, cerium oxide nanoparticles (CeNPs). Cerium oxide nanoparticles are antioxidant on the basis of superoxide dismutase and catalase mimetic activity,[1–3] unlike many other antioxidant therapies (glutathione, ascorbate, vitamin E, etc.), CeNPs are antioxidant on the basis of catalytic activity, and the nanoparticles are not consumed in the redox reaction. The small size of the custom CeNPs (~3 nm diameter; see Supplementary Data for physico-chemical characterization of the CeNPs) gives these nanoparticles unusual access to the central nervous system.[4] These nanoparticles have a relatively long tissue half-life and persist in brain tissue in mice for weeks where they remain biologically active.[4] CeNPs have shown benefit in a variety of disease models in which oxidative stress plays a prominent role: ischemic brain injury,[5–7] Experimental Autoimmune Encephalomyelitis (EAE),[4] heart failure[8] and light-induced damage of the retina.[9]

Given the efficacy of the CeNPs in disease models in which oxidative stress plays a prominent role, we hypothesized that CeNPs might provide similar therapeutic benefit in a murine model of amyotrophic lateral sclerosis (ALS). The majority of patients with ALS have no family history of the disease. However, 5-10% of patients have an inherited form of ALS, and of the familial forms of ALS, approximately 20% are related to inherited mutations of the copper/zinc superoxide dismutase enzyme (SOD1).[10] Transgenic mice that overexpress an SOD1^{G93A} point mutation, which replicates a common mutation in familial ALS, have a toxic gain of function related to

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expression of the mutant SOD1 gene.[11,12] The SOD1^{G93A} mice display many of the biochemical, clinical and pathological features of both familial and sporadic ALS in humans, and the transgenic SOD1^{G93A} animals are often the first model system in which potential ALS therapies are tested.[13,14]

Oxidative stress and oxidative damage have received persistent attention as causes of ALS. First, evidence of excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their attendant damage to DNA, RNA, lipids and proteins are abundant in the SOD1^{G93A} mice.[15–19] Evidence of oxidative and nitrative stress is also detectable in tissue from patients with ALS, though the particular manifestations of oxidative stress detected are variable among studies.[16,17,20–22] Second, antioxidant drugs have shown efficacy in animal studies of ALS,[23–26] and edaravone, a drug approved for ALS in humans, is a redox active agent.[27–29] Furthermore, drugs with anti-inflammatory and antioxidant mechanisms of action emerged as the most promising candidates for further investigation in ALS in a meta-analysis of multiple, preclinical drug trials.[30] Finally, ALS shares many pathological and biochemical features with other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, and evidence of oxidative stress and trials of antioxidant therapies in these diseases also fuels the persistent enthusiasm for antioxidant therapies in ALS.[17,31–33] Antioxidant drugs have not, however, actually been effective in clinical trials in ALS in humans thus far, though this may be changing.[27,28] This failure may reflect more about the pharmacology of particular antioxidants tried rather than the general benefit of antioxidants in the presence of oxidative stress.

There is no genuinely effective treatment for ALS.[10,34] The only widely approved therapy is riluzole, which is expensive and extends life for only two-three months.[35] There have been numerous tantalizingly effective drugs in animal models of ALS, but to date, all of these drugs, except riluzole, have failed in clinical trials in humans.[30,34] As a result, a consensus statement was developed to try to improve the usefulness and predictive power of preclinical, animal studies in ALS.[36] In the current study, we tried to adhere to the consensus guidelines for preclinical testing in ALS, and we tested the hypothesis that treatment of SOD1^{G93A} transgenic mice with CeNPs would ameliorate muscle weakness and prolong survival when CeNP treatment was begun at the onset of motor weakness.

Methods

All protocols involving animals were approved by the St. Lawrence University Institutional Animal Care and Use Committee.

Murine model of ALS

High copy number male transgenic B6SJL-Tg(SOD1*G93A)1Gur/J mice and B6SJLF1/J female mice were purchased from Jackson Laboratories (Bar Harbor, ME) and bred. Offspring were genotyped by Mouse Genotype (Carlsbad, CA) using standard PCR primers, and only heterozygotes of both sexes were included in these studies.

CeNP trial in SOD1^{G93A} mice

The onset of muscle weakness was measured using hanging wire testing. SOD1^{G93A} mice were trained on this task for 6 days when males were 83 days old or females were 100 days old to establish a baseline value. Thereafter, each animal was tested on the hanging wire apparatus and weighed twice per week. The onset of muscle weakness was defined by the occurrence of two consecutive decreases in hanging wire performance, and both hanging wire test times had to be less than the average baseline value. A schematic outline of the experiment is shown in Figure 1.

We used a clinical score algorithm to define the onset of clinical disease (Table 1). Both positive and negative attributes of behaviors associated with specific scores were defined, and the date of clinical disease onset was defined by the first occurrence of a clinical score < 5. The surrogate endpoint for death was the righting reflex, a standard test in ALS.[37] In addition, mice were euthanized by an isoflurane overdose for any of the following reasons: prolapse of the anus/uterus, autosarcophagy, loss of ≥20% of body weight or any clinical score ≤2.

After the onset of muscle weakness, the clinical disease and body weight assessment were conducted daily, and the hanging wire testing was conducted two times per week. Once the clinical score was <5, each mouse was weighed daily, the clinical score was assessed twice per day, and the hanging wire test was performed three times per week. Investigators weighing the animals, performing the behavioral scoring and measuring the hanging wire times were all blinded to the treatment each animal received.

Drug therapy with CeNPs started once muscle weakness was apparent on the hanging wire test. We used custom made CeNPs that were synthesized with equal amounts of ethylenediaminetetraacetic acid (EDTA) and citrate as a surfactant (see Supplementary Data). As each mouse developed muscle weakness, it was assigned randomly to one of the treatment groups, either CeNPs or vehicle, control injections. Randomization was stratified by gender. The treatment group received bi-weekly intravenous (I.V.) tail injections via 30 ga needle of CeNPs (20 mg/kg) diluted in 100 μL vehicle (136 mM NaCl buffered with 10 mM Na–HEPES and titrated to pH 7.4). Control animals received identical, bi-weekly, 100 μL, I.V. tail vein injections with vehicle alone. Tail vein injections were given under brief isoflurane anesthesia.

Biodistribution of cerium in SOD1^{G93A} mice

Mice were euthanized with an isoflurane overdose and transcardially perfused with phosphate buffered saline, and internal organs were removed to analyze tissue-specific cerium content. Specimens were digested in nitric acid and prepared for inductively coupled plasma mass spectrometry (ICP-MS) at the Trace Metal and Analytics Facility at Dartmouth College to assess the level of cerium in individual tissues after CeNP treatment.

Statistical analysis

For ordinal data (clinical scores), Freidman's test for nonparametric, multiple comparisons was used to evaluate main effects, and Dunn's post-hoc test (one-tailed) was used to compare treatment groups to control. Day-by-day comparisons