Cerium Oxide Nanoparticles Protect Against MPTP-Induced Dopaminergic Neurodegeneration In A Mouse Model For Parkinson's Disease

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ABSTRACT

Cerium oxide nanoparticles (CeONP) are regenerative free radical scavengers that protect against oxidative stress. Previous work from this lab has shown that CeONP extend cell (mixed brain, neuronal, endothelial) and organism (*Drosophila*) longevity, protect against oxidative stress and free radical production, and are effective in tissue culture models of neurodegenerative disorders. Given that Parkinson's Disease (PD) is strongly associated with oxidative stress, and that CeONP act as free radical scavengers, it was hypothesized that CeONP may be a promising disease-modifying therapy for treatment of PD. Here, we demonstrate for the first time, that CeONP preserve striatal dopamine and protect dopaminergic neurons in the substantia nigra, in the MPTP-mouse model of Parkinson's Disease. This work demonstrates the potential for CeONP as a novel nanopharmaceutical for the treatment of neurodegenerative disorders.

Keywords: nanomedicine, cerium oxide nanoparticles, neurodegenerative disease, antioxidants, Parkinson's Disease

INTRODUCTION

Nanomedicine has the potential to advance medical treatment through construction of materials with enhanced catalytic capabilities, taking advantage of quantum effects predominating at the nano scale. In this study, we investigate the efficacy of cerium oxide nanoparticles (CeONP) as a novel nanopharmaceutical for treatment of Parkinson's Disease (PD).

One of the hallmarks of PD is loss of dopaminergic neurons in the substantia nigra (SN) and striatum, resulting in the associated motor deficits. Evidence links oxidative stress, free radical production, and mitochondrial dysfunction as upstream initiating events leading to PD, occurring years before clinical symptoms are evident (1). Consequently, reduction in oxidative stress and protection of mitochondria constitute important targets in therapeutic intervention. However to date, traditional pharmaceutical antioxidants have met with only limited success in treatment (1, 2). Currently available antioxidants are consumed after a single interaction with a free radical, limiting their ability to effectively scavenge high radical concentrations at sites of oxidative stress, unless a continuous dose is provided. The present studies target oxidative stress and mitochondrial dysfunction with a novel regenerative antioxidant - CeONP - and seek to further develop CeONP as a therapeutic for modifying the outcome of PD.

Cerium is a rare earth element of the lanthanide series, with multiple valence states - either +3 (fully reduced) or +4 (fully oxidized), and numerous excited sub-states (3). In the oxide form, the lattice structure affords even more redox capacity, due to electron "holes" or oxygen defects (4). Creation and annihilation of oxygen vacancies and alterations in cerium valence, impart exceptional redox activity to CeONP, which is further enhanced by the increased surface area and quantum lattice alterations that occur at the nano-scale. Further, CeONP are unique in that there is a high hydrogen and oxygen-absorbing capacity on the surface, providing for ease of reaction with H_2,O_2, or H_2O and their associated radical species. In the materials industry, CeONP are utilized to prevent oxidation, remove carbon monoxide, and reduce nitrogen oxide emissions. If one compares the reactions of CeONP as utilized in materials applications, they are strikingly similar to the actions of antioxidants in the biological context.

What we know thus far about CeONP at the biological level has been reviewed by our group (5). CeONP dramatically preserves the lifespan of mixed organotypic cultures of brain cells and pure neuronal cultures, while preserving normal calcium signaling during the extended lifespan of the cultures. Further, CeONP protect neurons and other cell types from free radical challenge, as reported by our group and others (6-12). Additionally, CeONP protected traumatically injured neurons from calcium dysregulation and cell death, and decreased inflammatory functions in microglia (7, 12, 13). Notably, the antioxidant effects of CeONP were produced by a single 10nM dose (avg particle size 10 nm) delivered once, on day 10 in vitro. CeONP were incorporated into the cytoplasm and mitochondria (14) and were retained within the cells for the culture lifespans. *In vivo*, we found that CeONP induce dramatic extension of median and maximal lifespan in Drosophila and preserve motor function with aging (7, 15). Preliminary toxicology studies in the rat found that intravenous delivery of 90-150 nMols accumulated predominantly in the most oxidative organs of the body – brain, heart, and lung; increasing total tissue cerium 2-3 fold (but remained in the ppt-ppb range), as measured by ICP-MS (14). These tissue cerium concentrations were retained for at least 6 months post-injection, with no overt toxicological effects noted. In summary, data thus far supports the potential for CeONP as an effective biological free radical scavenger.

However antioxidant functions may not be the sole mechanism of action of CeONP. CeONP have a high propensity to absorb and release both protons and electrons, a property conveyed by the oxide lattice structure and its surrounding hydration shell. In this vein, they may serve as electron or proton carriers. Such properties are consistent with elements of the electron transport chain, suggesting that CeONP may also affect mitochondrial oxidative phosphorylation. In recent studies, we have found that CeONP localize, in part, to mitochondria, and decrease cellular death and dysfunction associated with rotenone-induced inhibition of complex I of mitochondria (16). Given that PD is strongly associated with oxidative stress and mitochondrial dysfunction, and that CeONP are potent and regenerative radical scavengers which preserve mitochondrial function, we hypothesize that CeONP are a promising disease-modifying therapy for treatment of PD.

METHODS

For these studies, 2 ½ month old male C57Bl/6 mice were treated with CeONP, average size 10 nm (Nanophase, Romeoville, Illinois) via 3 tail vein injections, one day apart for a total dose of 0.05, 0.5. 5.0 and 50 μg/g. Controls received saline. Nanoparticle suspensions were bath-sonicated for 5 minutes prior to injection.

Five days after the CeONP injections, mice received four tail vein injections of 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP, 20 mg/kg) every two hours for one day, a routinely used model for PD. Controls received saline injections. All mice were sacrificed 7 days after the last MPTP injection. In half the animals, striata were removed, extracted, and dopamine and DOPAC were measured via HPLC. The remaining areas of the brain and other organs, were collected for cerium biodistribution analysis. In the second half, mice were transcardially perfused and fixed with phosphate buffered formalin. Brains were removed, cryoprotected, and stereotaxically sectioned into 50 μm slices. To determine neuronal numbers in the SN, sections were stained for , tyrosine hydroxylase (TH) with Nissl counterstain. Five sections through the substantia nigra (SN) were counted for TH+, Nissl+ staining in each mouse by an observer blinded to group identity.

RESULTS

As shown in Fig. 1 (left graph) CeONP at the 0.5 and 5 μg/g dose increased striatal dopamine content by 29 and 45% respectively. MPTP induced an 89% decline in striatal dopamine, indicating striatal damage and neuronal loss. The decline in striatal dopamine was inhibited, in part, by CeONP over the dose range of 0.05-5 μg/g, with 0.5 μg/g being the most effective dose (70% preservation of striatal dopamine as compared to CeONP controls). Note that at this dose, striatal dopamine in MPTP treated animals was preserved to the level of controls that had not received MPTP. Similar results were observed the dopamine metabolite, DOPAC.

In the substantia nigra, CeONP significantly increased the numbers of TH+ neurons at the 0.05 and 0.5 μg/g doses, as compared to untreated controls (Fig. 2, left group of bars). MPTP induced a 52% decline in TH+ neurons in the SN, indicating loss of dopaminergic neurons. However, CeONP-treated animals maintained numbers of TH+ neurons similar to animals not exposed to MPTP, demonstrating the neuroprotective abilities of CeONP. Importantly, the doses of CeONP at which neuroprotection was observed were low, resulting in a brain cerium concentration of 380 PPB, which is about a doubling of the basal levels of cerium in the brain.

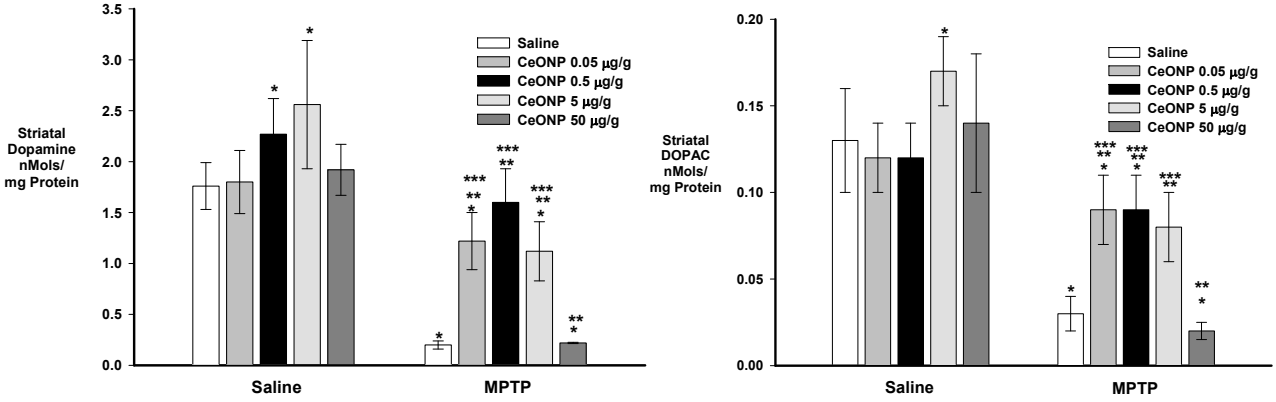

Figure 1. CeONP Inhibit the MPTP-Induced Decline in Striatal Dopamine and DOPAC
* Sig. from saline control, p<0.01; ** Sig. from CeONP control of same dose group, p<0.01; *** Sig. from MPTP, p<0.01; All groups contained 6-8 animals.

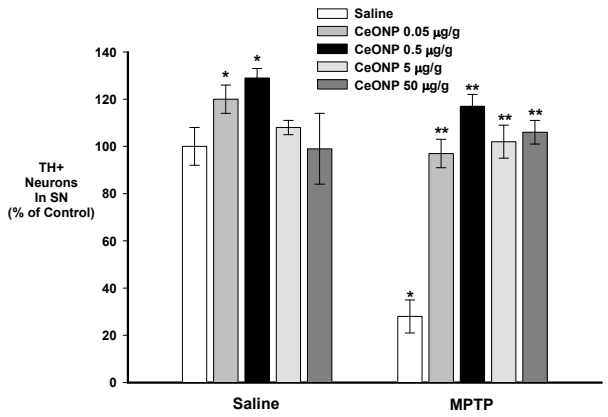

Figure 2. CeONP Preserve TH+ Neurons in the SN After MPTP Challenge
*Sig. from saline control, p<0.01
**Sig. from MPTP, P<0.01
All groups contained 6-8 animals

DISCUSSION

We currently lack effective treatment and protection mechanisms for PD and other neurodegenerative disorders associated with oxidative stress. Here, we demonstrate for the first time, that very low doses of CeONP protects against PD in a mammalian model.

CeONP increase striatal dopamine and protect against the MPTP-induced decline in striatal dopamine. CeONP increased the basal levels of striatal dopamine at the 0.5 and 5 µg/g doses, by 29 and 45% respectively, as compared to controls treated with saline. These results suggest that CeONP may have a beneficial effect on increasing dopaminergic activity in this area of the brain. Over the same dose range, CeONP preserved striatal dopamine after MPTP challenge, by 50-70%, as compared to mice treated with the same dose of CeONP, without MPTP challenge. Importantly, striatal dopamine in MPTP-challenged mice treated with 0.5 µg CeONP were not significantly different from untreated controls. These results suggest that pretreatment with CeONP is neuroprotective in the striatum, after MPTP challenge.

The dose-response for maintenance of striatal dopamine after MPTP treatment appears to be bell shaped, similar to results we have observed in tissue culture and Drosophila studies (5-9). In these studies, once the dose achieved a certain maximum level of benefit, neuroprotective effects declined. In tissue culture, the dose at which positive effects were abrogated was 1 µM. In the present studies, we see a similar drop-off in protective effects at the 50 µg dose, at which no maintenance of striatal dopamine was observed after MPTP treatment. We initially thought that delivery may have been compromised at the high (50 µg) dose, given that CeONP is delivered as a nanoparticulate suspension. However two pieces of data suggest that this is not the case. First, neuroprotective effects at the 50 µg dose were observed in the SN, suggesting that this dose did have some neuroprotective capacity. Second, biodistribution studies showed that the 50 µg dose produced the highest levels of brain cerium, suggesting that delivery was not compromised. However the biodistribution studies do demonstrate increased tissue cerium levels in liver, kidney, and spleen after the 50 µg dose, which did not occur at lower doses. Although low doses of CeONP have been found to be anti-inflammatory, the alteration in tissue distribution suggests that immune cells may begin to play a role in phagocytic elimination at the high dose, resulting in distribution changes.

CeONP Increase TH+ Neurons in the SN and Preserve TH+ Neurons After MPTP Challenge.

Consistent with the increase in striatal dopamine observed in CeONP-treated mice, CeONP also increased basal TH+ neuronal staining in the SN. These increases were small, but significant. Taken together with the finding that CeONP increase basal striatal dopamine, it is tempting to speculate that CeONP may induce formation of new dopaminergic neurons in these systems. However substantiation of this hypothesis will require further study.

MPTP administration substantially decreased TH+ neurons in the SN. However as shown in Fig. 2, this decline was completely blocked by all doses of CeONP examined. CeONP-treated animals showed SN TH+ neuronal numbers similar to non-MPTP controls. Interestingly, no dose-response was observed with CeONP and all doses afforded protection from MPTP in the SN. This is particularly interesting with respect to the high (50 µg) dose. At this dose, no preservation of striatal dopamine was observed after MPTP treatment, yet SN neurons appear to be protected. Several reports have noted such differences between the SN and striatum, and this may be due to the fact that different aspects of cell demise are at work in both areas (17).

Biodistribution and Toxicity

When utilizing nanoparticles as drug treatments, one must be mindful that they are unlike traditional pharmaceutical agents used to date. CeONP are classified as ceramics by nature, and they are not metabolized (i.e. the cerium- oxide bonds are not subject to biological alteration). Our prior work has shown that once distributed to tissues or cells, they are retained. In vivo, ICP-MS studies have shown that a single dose that increases tissue cerium by 50-75%, retains its original tissue distribution for up to 6 months post administration (longer time points have not been examined). In cell culture and Drosophila studies, this imparts a long duration of antioxidant and neuroprotective activity, and can counteract multiple insults over an extended time period.

All doses of CeONP were well-tolerated by mice. No behavioral or physiological alterations were noted after CeONP injections. No tail vein necrosis or alterations were noted at injection sites. These results are consistent with our

prior observations, along with observation of other groups, which report little to no CeONP toxicity at the doses used here (5, 10, 11). Although the literature contains some reports of cellular toxicity in vitro, the doses utilized were 1000 – 10,000 fold higher than doses utilized in the present studies.

Our biodistribution studies show similar cerium distribution as we have previously reported in the rat. Further, these studies demonstrate that neuroprotection in the MPTP model, is observed at brain levels in the PPB range, which represent an approximate doubling of tissue cerium. It is interesting to note that low doses, in the 0.5-5 μg/g range, distribute primarily to the most oxidative organs in the body; brain, heart and lung. However once dosing proceeded to the higher range of 50 μg/g, significant accumulation of cerium was noted in liver, kidney and spleen. These results suggest possible alterations in distribution at higher doses, and possible participation of the immune system in elimination of excess particulate nanoparticles in the system.

SUMMARY

To summarize, we have demonstrated that pretreatment with CeONP:

- Increases striatal dopamine content and TH+ neuronal number in the SN
- Preserves striatal dopamine and DOPAC in mice challenged with MPTP
- Preserves TH+ neurons in the SN of mice challenged with MPTP

Taken together, these results strongly support a potential role for CeONP as a nanopharmaceutical that may halt or slow the progression of PD. The next logical step in the progression of these studies is to determine whether CeONP are effective neuroprotectants when given after MPTP challenge, and is the subject experiments currently in progress.

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REFERENCES

1. A. Reynolds, C. Lauri, R. Lee-Mosely, et al., Oxidative stress and the pathogenesis of neurodegenerative disorders. Int. Rev. Neurobiol. 82, 297-325, 2007.
2. R. Pahwa, Understanding Parkinson's Disease: an update on current diagnostic and treatment strategies. J. Am. Med. Dir. Assoc. 7, 4-10, 2006.
3. C.H. Evans, Biochemistry of the Lanthanides, Plenum Press, NY, 1990.
4. V.T. Davis, Measurement of the electron affinity of cerium. Phys. Rev. Lett. 88, 1-4,.
5. Rzigalinski, B.A., et al., Radical nanomedicine. Nanomedicine 1, 399-412, 2006.
6. D. Bailey, L. Chow, S. Merchant, S.C.Kuiry, .S. Patil, S. Seal, and B.A. Rzigalinski, Cerium oxide nanoparticles extend cell longevity and act as free radical scavengers. Nature Biotechnol. 14, 112, 2003.
7. . Singh, N., C.A. Cohen, and B.A.Rzigalinski, Cerium oxide nanoparticles are neuroprotective for free radical injury and enhance neuronal longevity. Proc. NY Acad. Sci. 1122, 219-230, 2007.
8. N. Singh and B.A. Rzigalinski, Antioxidant Properties of Cerium Oxide Nanoparticles. Free Rad. Biol. Med., 114, 2006.
9.. A. Clark, E. Strawn, C. Cohen, and B.A. Rzigalinski, Cerium oxide nanoparticles provide superior antioxidant activity and neuroprotection as compared to vitamin E, n-acetyl cysteine, and melatonin. Soc. For Neuroscience Abstracts, 2006.
10. D. Schubert, et al. Cerium and yttrium oxide nanoparticles are neuroprotective. Bio.. Biophys. Res. Comm. 342, 86-91, 2006.
11. J. Chen, Nanoceria particles prevent ROI-induced blindness. Adv. Exptl. Biol. Med. 613, 53-59, 2008.
12. R Fry, A. Ellison, and B.A. Rzigalinski, Engineered oxide nanoparticles protect against neuronal damage associated with in vitro trauma. J. Neurotrauma 20, 1054, 2003.
13. P.J.Callaghan, J. Colon, S. Merchant, S., and B.A. Rzigalinski, Deleterious effects of microglia activated by in vitro trauma are blocked by engineered oxide nanoparticles. J. Neurotrauma 20, 1053, 2003.
14. B.A. Rzigalinski, et al., Biological nanoparticles for cell engineering – A radical concept. In Nanotechnologies for the Life Sciences, C. Kumar, editor, Wiley & Sons, NY 2003.
15. C.A. Cohen, et al., CeO2 Nanoparticles Extend Lifespan of Drosophila Melanogaster and protect from Oxidative Stress, Free. Rad. Biol. & Med., 123, 2006.
16. B.A. Rzigalinski, K. Meehan, M.D. Whiting, C.E. Dillon, K.Hockey, and M. Brewer, Antioxidant Nanoparticles, in Nanomedicine in Health & Disease, in press, 2011.
17. L. Aron, P. Klein, T.T. Pham, et al., Pro-survival role for Parkinson's associated gene DJ-1 revealed in trophically impaired dopaminergic neurons. PLos Biol. 8, 1-15, 2010.