Development of a fullerene-based synthetic superoxide dismutase mimetic (C_3) for treatment of Central Nervous System (CNS) diseases

Objective: TBP seeks to develop a novel small-molecule catalytic superoxide dismutase (SOD) mimetic as treatment for nervous system disorders, with an initial focus on Parkinson's disease, and the goal of bringing this compound to Phase I clinical testing.

Background and Rationale:

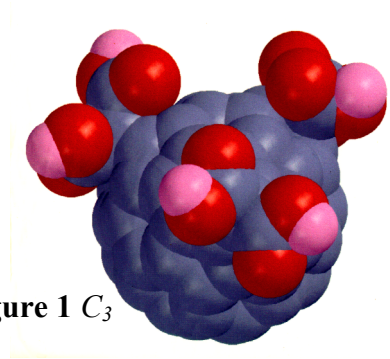

Figure 1 C_3

A large body of literature suggests that reactive oxygen species (ROS) contribute to tissue dysfunction and injury across a broad range of diseases, including diabetes, cancer transformation and metastases, ischemia and the most important CNS disorders, including Alzheimer's disease, Parkinson's disease, and ALS. In animal models of many of these diseases, genetic manipulation of antioxidant systems or treatment with antioxidant compounds has been shown to slow or prevent disease, but translation of these findings to efficacy in humans has been surprisingly difficult. It has been proposed that this is due to limited tissue uptake, metabolism and inactivation of antioxidant molecules, and relatively low potency of most antioxidant compounds that have been tested. The specific rationale for the development of an alternative, fullerene-based synthetic SOD mimetic enzyme for CNS applications is based on 12 years of published and ongoing work on the C_3 compound (described more fully below), which shows that it can effectively replace the natural superoxide dismutase (SOD) enzyme in vivo, and that it is an effective neuroprotective agent in animal models of CNS disease states; it is neuroprotective in standard, established mouse models of familial ALS, Parkinson's disease, and schizophrenia, among others. Our current plan to move C_3 into clinical testing in ALS patients is based on our previously published data showing neuroprotective efficacy in animal models of ALS, and our recent data from an NIH-funded study of extended systemic C_3 administration to Macaque fascicularis monkeys, which demonstrated that, on a broad range of parameters, including blood studies, EKGs, and complete autopsies, C_3 lacks toxicity. We have also recently reported that C_3 is highly effective in reducing superoxide produced by NADPH oxidase, the respiratory burst oxidase (Behrens, Dugan *et al.*, ***Science***, 2007), and finding that is highly relevant to ALS because of the recent report that blocking NADPH oxidase in ALS mice dramatically extends the lifespan of these mice. C_3, however, does not share the increased risk of infection that is found with NADPH oxidase inhibitors.

Description of the technology:

The lead compound, C_3, is a water soluble derivative of buckminsterfullerene (C60) (Figure 1), in which three malonic acid groups are attached symmetrically to one hemisphere of the sixty-carbon C60 sphere. This symmetric attachment harnesses the unique metal-like characteristic of the C60 molecule[1] to produce a highly effective synthetic enzyme which carries out catalytic decomposition (dismutation) of superoxide in a manner similar to the naturally-occurring enzyme, superoxide dismutase (SOD), producing stoichiometrically water and oxygen[2], a mechanism that has recently been independently confirmed[3,4]. C_3 has cellular uptake and distribution into key cellular compartments, including mitochondria[2], and can act as a replacement for mitochondrial SOD activity in vivo (see Ali below). The C_3 compound has a plasma half-life of 8 hours, has renal and

hepatic clearance, has significant uptake into brain, and does not undergo appreciable metabolism (please see detailed discussion of pharmacokinetic studies described below).

and

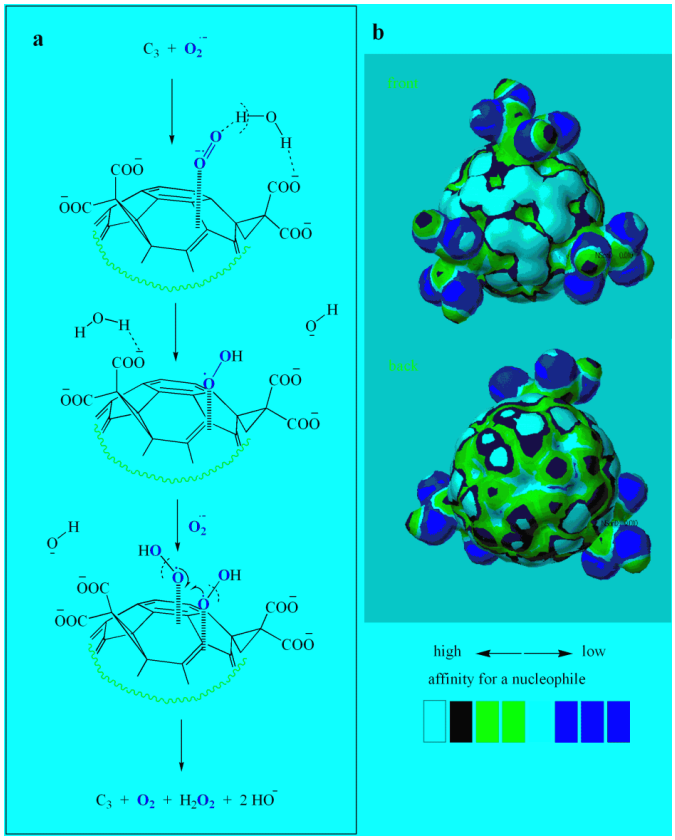

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Figure 2 Mechanism of superoxide dismutation by *C3*. **a**, Schematic representation of the catalytic interaction of C_3 and superoxide. Chemical bonds colored in red are associated with electron deficient areas predicted through semi-empirical quantum calculations (compare with **b**). Incoming superoxide ions and oxygen atoms derived from them are colored in purple to facilitate visualizing the suggested mechanism. Broken lines represent hydrogen bonding between oxygen and hydrogen atoms and hyphenated lines are used to represent electrostatic attraction between negatively charged oxygen atoms and electron-deficient areas on the C_{60} moiety. In the proposed mechanism, C_3 suggested to electrostatically drive superoxide anions towards electron deficient areas on its surface until a second $O_2^{\cdot -}$ arrives to undergo dismutation with the help of protons from carboxyl groups and/or surrounding water molecules. **b**, Map of the electron density reflecting nucleophilic superdelocalizability. On the resulting isosurface, locations that are susceptible to attack by superoxide are designated by a color scale showing decreasing susceptibility, in the order white through cyan (legend).

Animal models of CNS disease in which C_3 has been tested. Of note, all animal studies have been carried out as randomized, "placebo"-controlled (dilute food coloring to match the C_3 solution), double-blind studies:

In Vivo Studies (published)

- Amyotrophic Lateral Sclerosis (ALS) - G93A SOD1 FALS mouse (*PNAS*)
- Focal cerebral ischemia-mouse, rat (*Pharmacol. Cereb. Ischemia)*
- Schizophrenia-mouse (*Science*)
- Aging (increased lifespan, prevented memory deficits) - aging mice (*Neurobiol. Aging; Aging cell*)
- Parkinson's disease models:
    - Mouse - MPTP (*Parkinsonism Relat. Disord.*)
    - Rat – 6OHDA (*Parkinsonism Relat. Disord.*)

In Vivo Studies (unpublished/ongoing)

- Parkinson's disease models: macaque fascicularis monkeys - unilateral MPTP (study complete)
- Age-induced degeneration of nigral dopaminergic neurons (study complete)
- Neurofibromatosis – *Nf1-/+* mice (study complete; preservation of spatial learning by C_3 in the Barne's maze)

Key points of published data in *in vivo* models:

Key findings in Dugan et al., *PNAS.* 94:9434-9 (1997). PMID: 9256500

- Intraperitoneal administration of C_3 (or color-matched control solution) was carried out in the Gurney FALS mouse model. C_3 treated FALS mice had a 15% delay in motor deterioration and death. This was the first report of an effective treatment in these mice. This study with C_3 has since been replicated and published.

Key findings in Ali et al., *Free Rad. Biol. Med.* (2004).

- C_3 effectively removes superoxide radical with the rate constant $k_{C3} = 2.2 (\pm 0.1) \times 10^6\ M^{-1}s^{-1}$ (pH 7.4), which is similar to values reported previously for a number of manganese-based superoxide dismutase mimetics.
- C_3 acts as a catalyst rather than a stiochiometric free radical-scavenger in its reaction with superoxide.
- Entry of C_3 into the mitochondria was confirmed by confocal microscopy.
- Injection of C_3 daily into neonatal Sod2-/- mice which lack expression of mitochondrial manganese superoxide dismutase (MnSOD), increased their lifespan by 300%. This mouse, developed by Charlie Epstein, UCSF, is a standard model to confirm *in vivo* efficacy of putative SOD mimetics.

Key findings in Behrens et al., *Science* (2007).

- Administration of C_3 in an established mouse model of schizophrenia (repeated ketamine injection) rescued hippocampal and cortical inhibitory interneurons known to be lost in schizophrenic patients.

- C_3 treatment also significantly decreased superoxide production from NADPH oxidase, which is highly induced in brain by ketamine exposure, but is also increased in several other CNS disease states, including Alzhiemer's disease.

Key findings in Quick et al., *Neurobiol.Aging* (2006).

- Chronic oral administration of a low dose (1 mg/kg/day) of C_3 to mice prevented age-related decline in working memory, as documented by Morris water maze testing.
- C_3-treated animals (wild-type C57BL6 mice) also had a 12% increase in mean and maximal lifespan, and on autopsy, had a lower incidence of cancer, renal pathology, and cataracts.
- Mitochondria isolated from brain and muscle of old C_3-treated mice had better respiratory function and decreased superoxide production than control mice.

References describing *in vivo* testing of the C_3 compound:

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3. Witte, P. et al. Water solubility, antioxidant activity and cytochrome C binding of four families of exohedral adducts of C60 and C70. *Org Biomol Chem* **5**, 3599-613 (2007).
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