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Parkinsonism and Related Disorders 7 (2001) 243–246

Parkinsonism & Related Disorders

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Fullerene-based antioxidants and neurodegenerative disorders

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Abstract

Water-soluble derivatives of buckminsterfullerene (C_{60}) derivatives are a unique class of compounds with potent antioxidant properties. Studies on one class of these compounds, the malonic acid C_{60} derivatives (carboxyfullerenes), indicated that they are capable of eliminating both superoxide anion and H_2O_2, and were effective inhibitors of lipid peroxidation, as well. Carboxyfullerenes demonstrated robust neuroprotection against excitotoxic, apoptotic and metabolic insults in cortical cell cultures. They were also capable of rescuing mesencephalic dopaminergic neurons from both MPP^+ and 6-hydroxydopamine-induced degeneration. Although there is limited in vivo data on these compounds to date, we have previously reported that systemic administration of the C_3 carboxyfullerene isomer delayed motor deterioration and death in a mouse model of familial amyotrophic lateral sclerosis (FALS). Ongoing studies in other animal models of CNS disease states suggest that these novel antioxidants are potential neuroprotective agents for other neurodegenerative disorders, including Parkinson's disease.

Keywords: Parkinson's disease; Antioxidants; Superoxide; Excitotoxicity; Apoptosis

Evidence from studies in cell culture and animal models of disease suggest that superoxide ($O_2^{-\cdot}$) and hydroxyl ($^{\cdot}OH$) radicals, and the non-radical molecules H_2O_2 and hypochlorous acid — are major contributors to oxidative injury in mammals. In addition, nitric oxide ($NO^{\cdot}$), a biologically generated free radical gas with limited intrinsic reactivity, can combine with $O_2^{-\cdot}$ to generate the reactive oxidant, peroxynitrite ($ONOO^-$). These reactive oxygen species may then cause oxidative damage to cellular components, such as peroxidation of cell membrane lipids, oxidation and fragmentation of DNA, inactivation of transport proteins, and inhibition of energy production by mitochondria. Furthermore, while nitric oxide and hypochlorous acid appear to be generated by a restricted number of cell types, $O_2^{-\cdot}$ and H_2O_2 are ubiquitous "by-products" of many biological processes, including energy metabolism and macromolecule synthesis.

Free radical injury has been specifically implicated in the pathogenesis of a number of neurological insults, including trauma, ischemia and neurodegenerative disorders. Furthermore, the contribution of oxidative damage to neurological injury is believed to be especially prominent for a number of reasons, including the reliance of the brain on aerobic metabolism, its rich content of unsaturated fatty acids — targets of lipid peroxidation — and its limited ability to regenerate or replace damaged tissue. Evidence accumulated over more than three decades supports the idea that free radical-mediated injury may occur during acute insults, such as stroke, head trauma, and spinal cord injury. More recently, a link between free radicals and neurodegenerative conditions, such as Parkinson's disease and Alzheimer's dementia has emerged. In certain disease processes, e.g. hypoxia-ischemia, oxidative tissue injury appears to be a primary injury mechanism. However, recent work has suggested that other injury cascades, such as excitotoxicity, loss of growth factor support, and impaired energy

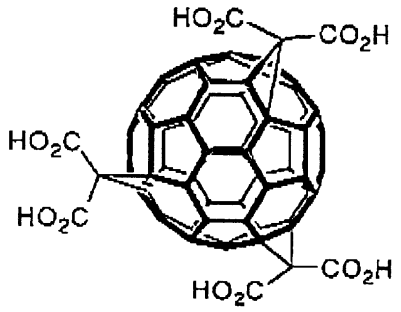

Fig. 1. Structure of the C_3 tris malonic acid C_{60} derivative, showing the paired carboxylic acid groups attached to the three cyclopropane carbons on the C_{60} molecule. The extensive double bond system of the fullerene moiety was omitted for the sake of clarity.

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1353-8020/01/$ - see front matter
PII: S1353-8020(00)00064-X

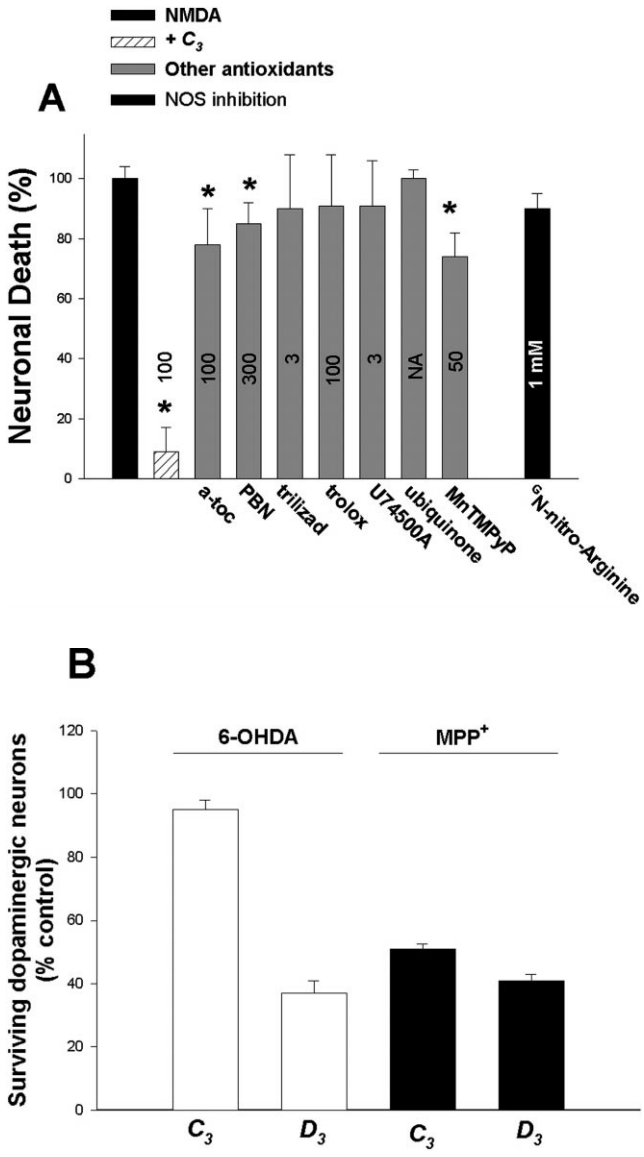

Fig. 2. Comparison of neuroprotection by various antioxidants and a NOS inhibitor versus the C_3 compound against NMDA toxicity (A). Cortical cultures were exposed to NMDA (200 μM) for 10 min in the presence of the listed compounds. ^{G}N-Arginine was applied 4 h before application of NMDA, and re-applied during the NMDA exposure. All other compounds were co-applied with NMDA, and all drugs including ^{G}N-Arginine were washed out after the 10 min exposure to NMDA. Cell death was assessed 24 h later by assaying LDH release, and by examining the neurons by phase-contrast microscopy. Values are mean ±SEM, $n = 12$–16, $^{*} < 0.05$ by ANOVA and Student–Neuman–Keuls. Concentrations on the bars reflect the most effective concentration of each drug (in μM), determined from dose–response curves. Carboxyfullerene isomers C_3 and D_3 differ in neuroprotection against 6-OHDA and MPP^{+} injury in cultured mesencephalic dopaminergic neurons (B). Cultures were exposed to 6-OHDA or MPP^{+} as described in Ref. [7] in the presence of C_3 or D_3 (75 μM). The percent of remaining dopaminergic neurons is shown. Values are mean ±SEM.

metabolism, which have been implicated in CNS injury, may also involve oxidative processes as common downstream mediators of cell death.

In light of the multiple injury cascades to which free radicals contribute, antioxidant strategies, including the development of small molecule free radical scavengers, remain attractive therapeutic targets. We have previously reported that water-soluble derivatives of the fullerene C_{60} molecule are excellent antioxidants which possess a broad spectrum of neuroprotective abilities [2,3]. Recently, we have focused on further characterizing mechanisms underlying the free radical scavenging properties of this novel class of antioxidants, and have continued to test them as therapeutic agents in several cell culture and animal models of CNS injury, including Parkinson's disease. Early work suggests that water-soluble C_{60} compounds are promising candidates for further evaluation as therapy for Parkinson's disease.

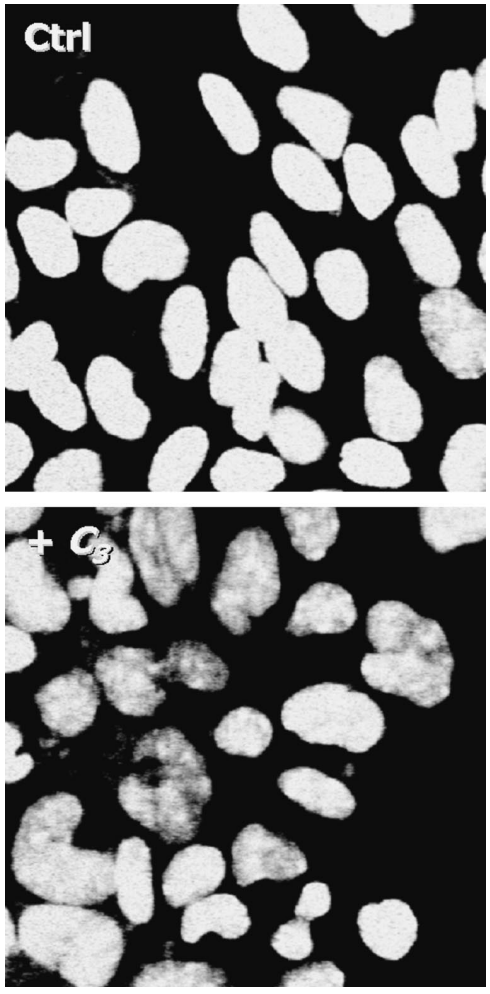

Fig. 3. C_3 decreases mitochondrial superoxide anion production by cortical astrocytes. Cultured astrocytes were loaded with dihydroethidium (DHE), then treated with vehicle (H_2O) or C_3 for 1 h. DHE is oxidized to ethidium, which is detected as increasing nuclear fluorescence in the astrocyte monolayer. C_3 decreases basal superoxide production by astrocytes, which derives primarily from the mitochondrial electron transport chain (Dugan, unpublished). Cultures were imaged on a Noran Odessey confocal microscope using Ex λ 488 nm, Em $\lambda > 590$ nm.

1. Results and discussion

We have focused on defining the free radical neurobiology

Table 1
Protective effects of C_{60} derivatives in biological model systems

	Compound	System	Injury condition	Results	References
In vitro	Carboxyfullerene C_3, D_3 Fullerenols	Cortical neuronal cultures	Excitotoxicity: NMDA and AMPA	60–90% ↓ in death	[2], Fig. 2A [3]
	C_3	Same	Apoptosis induced serum by deprivation	50% ↓ in death	[2]
	Fullerenols				[3]
	C_3	Same	$A\beta_{1-42}$ toxicity	Complete protection	[3]
	C_3	Same	Oxygen–glucose deprivation	80% ↓ in death	[3]
	C_3	Same	Apoptosis following NMDA receptor blockade	50% ↓ in death	Kim-Han and Dugan, unpublished
	C_3	Mesencephalic dopaminergic neuronal cultures	MPP^+	40% ↓ in death	[7], Fig. 2B
	C_3	Same	6-hydroxydopamine	Complete protection	[7], Fig. 2B
	C_3	Cerebellar granule neuronal cultures	Apoptosis induced by NGF withdrawal	Partial protection	[1]
	C_3 and D_3	Hepatoma cells	TGFβ-induced death	Partial protection	[5]
	C_3	Epithelial cells	Radiation	Partial protection	[10]
In vivo	C_3	FALS mice (with SOD1 G93A mutation)	Progressive motor deterioration/ death produced by overexpression of mutant protein	Improved motor performance, 9–12 day increased survival	[2,4]
	C_3	Rats	6-OHDA intrastriatal lesioning (systemic C_3)	Preserved dopaminergic terminals and behavior	[8]
	C_3	Rats	Iron-induced striatal dopamine depletion (intrastriatal C_3)	Partial preservation of striatal dopamine	[6]

of one type of C_{60} derivative, the tris malonic acid C_{60} adducts (carboxyfullerenes) [2]. The structure of the C_3 isomer, which has been most extensively studied by our laboratory and others, is shown in Fig. 1. The placement of the malonic acid head-groups on one hemisphere of the fullerene sphere conveys substantial lipophilicity to this compound, while the malonic acid groups provide water-solubility (up to 100 mM as the sodium salt). Using pure carboxyfullerene isomers and a plate reader assay for $O_2^{-\cdot}$ developed for these studies [9], we have investigated which species of free radicals are eliminated by these compounds. We have found that C_3 eliminates both superoxide and H_2O_2 at concentrations in the micromolar range. This coordinated ability to eliminate both superoxide and H_2O_2 by one molecule is a highly desirable feature, since excess H_2O_2 generated by superoxide dismutase-like activity in the absence of sufficient catalase (or glutathione peroxidase) can be detrimental by enhancing H_2O_2-mediated injury. In addition, C_3 blocks iron-induced lipid peroxidation in vitro [4] and in vivo [6]. Although C_3 inhibits certain isoforms of nitric oxide synthase [11], we have found it to be very unreactive with $NO^{\cdot}$ itself (Dugan and Lin, unpublished). We have also evaluated the ability of C_3 to eliminate superoxide generation in intact cells. Using confocal microscopy and the superoxide-sensitive fluorescent compound, dihydroethidium, we have determined that C_3 can reduce basal mitochondrial production of superoxide in cortical astrocytes (Fig. 3) and neurons (not shown). Thus, carboxyfullerenes demonstrate an attractive spectrum of antioxidant capabilities, which can be demonstrated to translate to intact cells.

In comparing the neuroprotective efficacy of C_3 against several other benchmark antioxidants and against NOS inhibitors in cultured cortical neurons, we found that C_3 afforded greater protection against acute NMDA receptor-mediated neurotoxicity [2] than these other agents (Fig. 2A). We believe this reflects not only its range of antioxidant properties, but other features of the C_3 molecule, including its amphipathic nature. The C_3 isomer also rescued cultured mesencephalic dopaminergic neurons from degeneration after exposure to MPP^+ or to 6-hydroxydopamine (6-OHDA) [7]. Another tris malonic acid C_{60} isomer (D_3) was also protective against 6-OHDA injury and MPP^+-mediated death (Fig. 2B), but to a lesser extent than C_3. This supports other data which suggests that the malonic acid isomers possess differing antioxidant properties as well as different levels of neuroprotective efficacy. The structural basis of these differences is one area of current investigation in our lab. A number of studies in other cell culture and in vivo models of injury have been performed using carboxyfullerenes, polyhydroxyfullerenes, and a limited number of other C_{60} derivatives. Table 1 is a current list of most of these studies. Taken together, these studies indicate that this class of compounds provides effective neuroprotection against excitotoxic cell death, apoptosis initiated by several different types of triggers (growth factor deprivation, NMDA receptor blockade in immature neurons), and metabolic insults (oxygen–glucose deprivation) in cultured cells.

Table 1 also lists in vivo studies which have begun to determine how well neuroprotection observed in cell cultures translates to the intact organism. Initial studies in two models of Parkinson's disease (PD) have been relatively promising. One study used intrastriatal injection of iron to produce striatal injury [6] and found that C_3, when co-injected with the iron, reduce dopamine depletion. A high content of iron in the basal ganglia is believed to

contribute to oxidative damage during PD, and to the vulnerability of this region in PD. C_3 may bind to iron, so the ability of C_3 injected to reduce injury in this model may reflect its ability to interact directly with iron, or its ability to decrease lipid peroxidation. A second study, in which C_3 was delivered systemically for 1 month to rats made Parkinsonian by intrastriatal injection of 6-OHDA indicated that dopaminergic terminals and behavior were significantly improved by C_3 treatment [8]. While data on the in vivo efficacy of C_{60} derivatives is extremely limited so far, recent in vivo studies are promising. Future studies will determine whether C_{60} compounds will, indeed, be useful therapy for acute or chronic diseases of the nervous system.

Acknowledgements

This work was supported by NS 37688 (LLD).

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