Evidence of Oxidant Damage in Huntington's Disease: Translational Strategies Using Antioxidants

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Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder characterized by progressive motor dysfunction, emotional disturbances, dementia, and weight loss. It is caused by an expanded trinucleotide CAG repeat in the gene coding for the protein, huntingtin. Although no one specific interaction of mutant huntingtin has been suggested to be the pathologic trigger, a large body of evidence suggests that, in both the human condition and in HD mice, oxidative stress may play a role in the pathogenesis of HD. Increased levels of oxidative damage products, including protein nitration, lipid peroxidation, DNA oxidation, and exacerbated lipofuscin accumulation, occur in HD. Strong evidence exists for early oxidative stress in HD, coupled with mitochondrial dysfunction, each exacerbating the other and leading to an energy deficit. If oxidative damage plays a role in HD, then therapeutic strategies that reduce reactive oxygen species may ameliorate the neurodegenerative process. Two such strategies, using coenzyme Q_{10} and creatine, have been proposed. Although each agent has had limited efficacy in HD patients, the optimal therapeutic dose may have been underestimated. High-dose coenzyme Q_{10} and creatine are safe and tolerable in HD patients and are currently under investigation. In addition, there are parallels in reducing markers of oxidative stress in both HD mice and HD patients after treatment. It is likely that high-dose coenzyme Q_{10}, creatine, or both agents, will represent a cornerstone defense in ameliorating the progression of HD.

Key words: **oxidative stress; creatine; coenzyme Q_{10}**

Introduction

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an expanded trinucleotide CAG repeat in the gene coding for the protein huntingtin.[1] An inherited autosomal dominant disorder of the central nervous system, HD affects roughly 250,000 Americans and is characterized by cognitive and memory impairments, weight loss, and prominent choreic motor abnormalities.[2–4] Once manifest disease occurs, the duration of HD is approximately 15–20 years,[5] with the disease symptoms becoming increasingly disabling prior to death.[3] Although the disorder had previously been reported, the initial detailed description of HD was made by George Huntington, a medical practitioner of Pomeroy, Ohio, in 1872.[6] He gave a detailed account of the disease based on the descriptions taken by his father and grandfather from their practice in East Hampton, Long Island, NY. Those patients could be traced to a few individuals who had emigrated from a small village in Suffolk, England, in 1630. Although neuropathologists had previously described HD as a chronic encephalitis, Jelgersma first described the characteristic neuropathologic alterations within the basal ganglia.[7] HD leaves a characteristic pathologic signature on the affected brain. The most striking neuropathologic changes are found within the

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neostriatum, in which gross atrophy of the caudate nucleus and putamen occurs, accompanied by marked neuronal loss and astrogliosis. The extent of the topographic evolution of the striatal neuropathology provides a basis for grading the severity of HD pathology into five grades of increasing pathologic severity.[8] The grades closely correlate with the extent of the clinical disability.

A dorso-ventral progression of neuronal death occurs, with the dorsal striatum affected earliest. General cell stains disclose a regional pattern of striatal degeneration with relative sparing of the ventromedial striatum and the nucleus accumbens. The destructive process, however, is not equally expressed in all striatal neurons. There is a selective pattern of neuronal vulnerability.[9] Consistent with this finding, an extensive enzyme- and immunehistochemical examination of the cell types in the striatum of patients with HD has shown that the medium-sized spiny striatal neurons are disproportionately affected early and most severely, whereas large and medium-sized aspiny neurons are relatively spared.[10–18] Despite the severity of neuronal loss, a striking persistence of somatostatin/neuropeptide Y/NADPH-diaphorase neurons occurs in both the caudate nucleus and putamen of HD patients.[13,14] The density of these neurons is increased 4- to 5-fold, reflecting the combined effects of both neuronal sparing and tissue shrinkage.

A significant advance in understanding the pathogenesis of HD was made with the identification of the genetic mutation. The HD gene, first cloned in 1993, codes for a large, highly conserved protein, huntingtin (Htt).[1] Evidence suggests that Htt is involved in fast axonal transport,[19,20] enhancing vesicular transport of brain-derived neurotrophic factor along microtubules.[21] In individuals with HD, a polymorphic trinucleotide repeat sequence (CAG), near the 5′ end of the gene, is expanded beyond the normal repeat range (17–29), leading to translation of an expanded polyglutamine (polyQ) sequence in the Htt protein.[1] In HD patients, more than 38 repeats occur. Once expanded into the pathogenic range, an inverse relationship exists between the CAG repeat number and the age of onset, with a higher repeat number associated with a younger age of onset. Although expression of Htt is observed throughout the brain—within the nucleus, cytoplasm, axons, and dendrites of neurons[22–24]—Htt expression appears elevated in medium spiny neurons.[25] Such differential Htt expression within the striatum may underlie the vulnerability of medium spiny neurons in HD.

Processing of the mutant Htt (mHtt) protein releases a persistent N-terminal fragment containing the expanded polyQ sequence. This fragment forms aggregates with itself and other proteins, observed in the cytoplasm and nucleus, and is believed to confer toxicity via a gain-of-function mechanism.[23] Interestingly, nuclear aggregates have been observed in interneuron populations resistant to HD-induced neurodegeneration,[24] suggesting that soluble mHtt is the primary arbiter of neurodegeneration. Despite great progress in the past 15 years since the discovery of the genetic mutation in HD, a direct causative pathway from the gene mutation to neuronal dysfunction and death has not yet been established. Although the exact cause of neuronal death in HD remains unknown, it has been postulated that the abnormal aggregation of mHtt may cause toxic effects in neurons, triggering a cascade of pathogenic mechanisms associated with oxidative stress, mitochondrial alterations, transcriptional dysfunction, apoptosis, bioenergetic defects, and subsequent excitotoxicity. It is not unreasonable to propose that the HD mutation may increase reactive oxygen species, resulting in a primary defect associated with oxidative stress. As such, a significant body of evidence from studies in both HD patients and experimental models of HD supports a role for oxidative stress and attendant mitochondrial dysfunction in mediating the neuronal degeneration observed in HD.[26]

Oxidative Stress in Huntington's Disease

In disease-free neurons, the generation of reactive oxygen species is a normal by-product of cellular respiration, mediated by mitochondria. Accumulation of reactive oxygen species in neurons and subsequent oxidative stress is blocked by free radical scavengers, such as glutathione and superoxide dismutase, preventing subsequent damage.[27–30] In HD, the generation of reactive oxygen species and the resulting oxidative stress is thought to play a central role in the neurodegeneration observed.[31–35] Multiple lines of evidence implicate oxidative stress in the etiology of neuronal death in HD.[34] Studies of the postmortem human HD brain show increased levels of oxidative damage. These include increased cytoplasmic lipofuscin, DNA strand breaks, and the accumulation of oxidative markers in DNA bases, along with other cellular macromolecules associated with protein nitration and lipid oxidative damage.

Lipofuscin is an aging wear-and-tear pigment and is the product of unsaturated fatty acid peroxidation.[36] Lipofuscin increases at a greater rate under oxidative stress.[37] The abnormal accumulation of lipofuscin has been reported in HD patients.[38–40] A marked increase in lipofuscin within both cortical and striatal neurons occurs in patients with HD, with little or no presence of lipofuscin in spared NADPH-diaphorase neurons in the caudate nucleus.[39] Mutant proteins are processed through lysosomes and, as such, the lipofuscin accumulation has been suggested to impair lysosomal function, resulting in selective neuronal damage.

DNA strand breaks are related to free radical damage.[41] We and other researchers have shown that DNA fragmentation is increased in HD patients and correlates with CAG repeat length.[39,42–44] Using *in situ* end-labeling, which identifies DNA fragmentation in apoptotic or necrotic nuclei, we showed significant increases in DNA fragmentation in human HD striatal and cortical neurons compared with levels of DNA fragmentation in age-matched control brains.[39] In addition, mitochondrial DNA may be more susceptible than nuclear DNA to fragmentation because less *in situ* end-labeling is detected within cell nuclei.

The oxidation of either nuclear or mitochondrial DNA results in the formation of the metabolite 8-hydroxy-2′-deoxyguanosine (OH^8dG) and is a direct result of free radical activity.[32,43,45] Significant increases in OH^8dG levels from nuclear DNA occur in the caudate nucleus in postmortem tissue from HD patients[32] as well as in mitochondrial DNA from the parietal cortex.[45] In addition, OH^8dG levels are markedly elevated in serum from HD patients[46] and, as such, provide a peripheral biomarker as an indicator of therapeutic response. These findings are consistent with elevations of OH^8dG levels that occur in other neurodegenerative diseases in which oxidative damage has been implicated as a pathogenic mechanism.[47,48] Other investigators, however, have not observed changes in nuclear DNA in HD patients.[49]

In parallel, additional markers of oxidative damage, including heme oxygenase (an inducible isoform that occurs in response to oxidative stress), 3-nitrotyrosine (a marker for peroxynitrite-mediated protein nitration), and malondialdehyde (a marker for oxidative damage to lipids), are elevated in human HD striatum and cortex compared with age-matched control brain specimens.[39,50] The extent and intensity of these markers mirror the dorsal-ventral pattern of progressive neuronal loss in the neostriatum, with increased immunoreactive expression in the dorsal striatum compared with the less severely affected ventral striatum. Consonant with the immunohistochemical data, analysis of colorimetric assays in HD patients show significant increases in malondialdehyde and 4-hydroxynonenal brain levels, almost 8-fold greater than in control subjects.[51]

Oxidative Stress in Experimental Models of Huntington's Disease

Although cell culture models may provide insight into huntingtin-mediated oxidative stress, many of the oxidative alterations observed in human HD are recapitulated in neurotoxin and genetic murine models of HD, making these models ideal for the study of pathogenesis and therapeutic potential. Mitochondrial toxins, such as 3-nitropropionic acid (3-NP) or malonate, can produce a neuropathology in animals that is remarkably similar to that observed in human patients with HD.[52–54] Oxidative stress plays a role in 3-NP-induced neurotoxicity.[55] Brain levels of hydroxyl free radicals were significantly increased after systemic administration of 3-NP, and the striatal lesions were attenuated by the free radical spin traps, alpha-phenyl-n-tert-butyl-nitrone and n-tert-butyl-alpha-(2-sulfophenyl)-nitrone. Elevated striatal brain levels of OH^8dG and 3-nitrotyrosine are also present after 3-NP administration, further suggesting that oxidative free radical damage is associated with the striatal damage. In the R6/2 transgenic model of HD, which expresses the N-terminal fragment of mHtt containing the CAG repeat[56] and shows striatal neuronal loss,[57] a significant increase in brain and urinary OH^8dG levels has been found,[57,58] arguing that oxidative stress may be the consequence of mHtt expression. In addition, these mice show increases in lipid peroxidation that worsen with disease progression.[59] Malondialdehyde, 4-hydroxynonenal, and the isoprostane, 8-iso-prostaglandin, are all elevated in R6/2 mice at disease onset and increase with disease progression;[59] immunostaining for inducible nitric oxide synthase and nitrotyrosine also increases with disease progression.[60] In the less fulminant R6/1 transgenic HD mice, a progressive increase in striatal lipid peroxidation parallels the progression of the neuropathologic phenotype.[61] Additional evidence of alterations in oxidative stress come from studies in CAG140 full-length knock-in mice.[62] In contrast to segment models of HD, mice containing the full-length huntingtin gene provide the best possible molecular genetic comparison to human HD. We have evidence that OH^8dG urine and brain levels are significantly elevated in the CAG140 mice. As in human HD, the mouse data provide a direct link between mHtt expression, metabolic dysfunction, and the generation of reactive oxygen species and oxidative stress.

Additional evidence that supports the concept of oxidative stress in HD arises from recent data demonstrating mHtt-induced repression of peroxisome proliferators–activated receptor-coactivator 1α (PGC-1α) *in vitro*.[63] PGC-1α is a transcriptional coactivator regulating a number of genes and metabolic processes that protect against reactive oxygen species, among other functions. Reduced levels of PGC-1α result in striatal neurodegeneration and motor abnormalities in HD mice, along with increased sensitivity to oxidative stressors. Delivery of lentiviral-mediated PGC-1α expression into the striatum of R6/2 mice significantly improved the pathologic phenotype.

Oxidative Damage and Mitochondrial Dysfunction

The primary source of reactive oxygen species in neurons is mitochondria and, as such, mitochondrial dysfunction in HD is intimately associated with oxidative stress. In patients with HD, a reduction in striatal glucose use precedes tissue loss.[64] This is coupled with a significant decrease in the activity of several mitochondrial complexes.[32,60,65,66] In addition, lactate levels are elevated in the striatum and cortex in the HD brain.[67,68] Recently, evidence of an aberrant association between mHtt and elements of the cellular metabolic machinery has been reported, demonstrating a direct mHtt–mitochondrial interaction through the expanded CAG repeat.[69,70] This interaction results in altered mitochondrial calcium buffering, leading to mitochondrial dysfunction and an increase in free radical generation. Whether excessive free radicals and oxidative stress result

in mitochondrial dysfunction or mitochondrial alterations cause an increase in reactive oxygen species is not yet clear. It is certain, however, that these pathologic processes are intertwined and each likely exacerbates the other.

Therapeutic Strategies Ameliorating Oxidative Stress

Given the importance of oxidative stress in HD, several experimental preclinical antioxidant strategies have been employed in HD mice, some with promising parallels in human clinical trials, in supporting antioxidant approaches to treat HD.

It has been suggested that the free radical scavenger, melatonin, may play a therapeutic role in neurodegenerative diseases. Melatonin has significant antioxidant properties, scavenging hydroxyl, carbonate, reactive nitrogen species, and other organic radicals.[71] Melatonin has demonstrated significant neuroprotection in an *in vivo* model of neurodegeneration.[72] In the kainic acid rodent model of neurodegeneration, melatonin significantly reduced DNA damage and improved neuronal survival. In another *in vivo* study using the 3-NP model of HD, a significant increase in lipid peroxidation and protein carbonyls coincided with increased superoxide dismutase activity within the striatum. Treatment with melatonin significantly reduced lipid peroxidation, protein carbonyl formation, and superoxide dismutase activity in the rodent striatum,[73] demonstrating the potential benefit of melatonin for the treatment of HD.

Lipoic acid was first identified as a pyruvate oxidation factor and is an essential cofactor for many enzyme complexes.[74] Endogenous in mitochondria as the cofactor for pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, it is an effective antioxidant and has been used to treat disease associated with impaired energy.[75,76] It is reduced to dihydrolipoate by mitochondrial α-keto acid dehydrogenase complexes, at the site of significant free radical generation. In both the R6/2 and N171-82Q transgenic models of HD, dietary supplementation with lipoic acid resulted in significant extension of survival, while also delaying the weight loss typically observed in N171-82Q mice.[77]

Similar neuroprotective effects are also demonstrated by the antioxidant selenium, an essential element required by glutathione peroxidase to form the active enzyme.[78] It has been reported that selenium dose-dependently reduces lipid peroxidation within the striatum of rodents treated with quinolinic acid, an N-methyl-D-aspartate antagonist that results in striatal neurodegeneration.[79] Additional data demonstrated a significant improvement in striatal γ-amino butyric acid concentrations in these rodents compared with untreated control rodents. This was concurrent with significant improvements in behavior, including a reduction in ipsilateral turning, an effect commonly observed after unilateral striatal lesion. Importantly, selenium treatment improved striatal neuronal morphology, significantly reducing the overall number of damaged neurons following quinolinic acid administration. These results show evidence for the neuroprotective potential of selenium, providing a rationale for possible clinical trials in HD patients.

Two additional antioxidant compounds, BN82451 and pyruvate, have also demonstrated potential benefit in treating HD. Although pyruvate plays a major role in glycolysis, it also possesses significant antioxidant capacity. When pyruvate is incubated with hydrogen peroxide, a significant degradation of hydrogen peroxide occurs via a decarboxylation reaction.[80] BN82451 is a brain-permeable compound that that has multitargeting neuroprotective effects that include the inhibition of lipid peroxidation.[81] Using a quinolinic acid striatal lesion model of HD, treatment with pyruvate dose-dependently protected against striatal neurodegeneration.[82] Although lower doses provided no protection, higher doses (> 500 mg/kg, i.p.) provided significant neuroprotection, reducing striatal lesion area

relative to control. Interestingly, treatment with dichloroacetate, which stimulates pyruvate dehydrogenase, improved the HD phenotype of both R6/2 and N171-82Q mice,[83] suggesting that, in addition to its antioxidant capacity, pyruvate may also promote neuroprotection by inducing energetic potential. Similarly, administration of BN82451 in R6/2 mice resulted in a significant extension of survival compared with untreated control mice.[84] A significant improvement in motor function also occurred, as assessed by rotarod performance, concomitant with improvements in gross morphology, striatal volume, and striatal neuronal areas, compared with control mice. Finally, a significant decrease in the number of ubiquitin-positive aggregates was found in BN82451-treated R6/2 mice compared with untreated R6/2 mice. The marked behavioral and neuropathologic improvement observed after treatment with BN82451 clearly demonstrates the preclinical potential of this antioxidant and provides evidence for its potential use in clinical trials.

Metalloporphyrins, metal-containing catalytic antioxidants, have also emerged as a novel class of potential therapeutic agents that scavenge a wide range of reactive oxygen species. These have been used in amyotrophic lateral sclerosis models of neurodegenerative disease.[85,86] A manganese porphyrin has been reported to significantly reduce cell death in an *in vitro* model of HD. [87]

Although the antioxidant compounds discussed above have demonstrated preclinical potential, none has been investigated in human clinical trials to date. For several other antioxidant compounds, however, both preclinical animal studies and human clinical trials provide data regarding their potential therapeutic benefit in HD. Among these compounds, ascorbic acid and α-tocopherol, also known as vitamins C and E, respectively, have demonstrated antioxidant potential. Ascorbic acid is a potent antioxidant obtained exogenously, which oxidizes readily to dehydroxyascorbic acid in the presence of reactive oxygen species. Similarly, α-tocopherol is also a potent antioxidant obtained exogenously. When exposed to free radicals, α-tocopherol can donate a hydrogen ion to neutralize the free radical. The effects of ascorbic acid and α-tocopherol were assessed in an *in vitro* chemical model of neurodegeneration. Treating cultured rodent cortical neurons with glutamate resulted in significant neurodegeneration, which was completely rescued with ascorbic acid co-treatment.[88] Similar results were obtained with α-tocopherol. Using a neuronal cell-based assay, glutamate-induced neuronal death was significantly attenuated in a dose-dependent manner by α-tocopherol.[89]

Successful preliminary demonstration of α-tocopherol to limit oxidative damage and neurodegeneration has led to clinical assessment of its potential in human HD.[90] A year-long placebo-controlled, double-blind study was carried out in patients with mild to moderate HD symptoms. Assessment of cognitive status using Mini-Mental Status scores revealed a significantly lower score in the α-tocopherol group compared with the placebo group, although serum α-tocopherol levels were elevated in the α-tocopherol group compared with control patients. However, comparisons of Quantified Neurological Examinations (QNE) scores below 45 demonstrated an improvement over baseline in the α-tocopherol group, showing significant improvements over placebo-treated subjects. Although α-tocopherol had no effect on neurologic or neuropsychiatric symptoms in the treatment group overall, *post hoc* analysis showed a significant effect on neurologic symptoms in HD patients early in the course of the disease. Peyser and colleagues[90] concluded that α-tocopherol therapy may slow the rate of motor decline early in the course of HD.

The antioxidant idebenone may also be of benefit in the treatment of HD. Idebenone, a benzoquinone derivative, possesses potent antioxidant capacity and readily penetrates the brain. In a neuronal cell-based assay, glutamate administration induced significant neuronal death. Treatment with idebenone in this *in vitro* model resulted in complete neuroprotection

in a dose-dependent manner.[89] These results were expanded in an *in vivo* trial using chemical models of striatal neurodegeneration. Intrastriatal injection of kainic acid into the rat striatum resulted in a marked reduction in the presynaptic striatal marker glutamic acid decarboxylase (GAD). Treatment with idebenone resulted in a significant, nearly complete restoration of GAD immunoreactivity.[91] Interestingly, idebenone had no effect on decreases of GAD immunoreactivity using quinolinic acid in these studies.

As a result of the positive *in vitro* and *in vivo* studies, a double-blind, placebo-controlled trial of idebenone in 92 HD patients was performed.[92] One of the primary outcome measures included performance on the QNE. Sample groups were further delineated to allow stratification of patients scoring ≤ 45 or ≥ 46 on the QNE. Given that idebenone had been previously used in clinical contexts, no adverse events associated with idebenone treatment were expected or observed. Treatment with idebenone had no effect on QNE status when compared with placebo controls. The lack of effect persisted even when analyses were restricted to individuals with a baseline QNE ≤ 45. Although this trial was sufficiently powered to detect a large drug effect comparable to a complete cessation of disease progression, more subtle beneficial effects of idebenone may have been present and undetected. Given that the sample size required to detect a slowing of disease progression by roughly 25% would require over 1000 patients, the trial was underpowered. A multicenter trial to determine the potential therapeutic benefit of idebenone to treat HD is still warranted.

Perhaps more promising is the antioxidant creatine, a guanidine compound that is produced endogenously and also obtained from the diet.[93] Creatine is a naturally occurring compound that has been reported to act as an antioxidant, scavenging reactive oxygen species.[94] In addition to its antioxidant capacity, creatine also buffers intracellular energy reserves through its intermediate, phosphocreatine (PCr); stabilizes intracellular calcium; and inhibits activation of the mitochondrial transition pore.[95] It may also ameliorate the effects of other pathophysiologic mechanisms associated with HD by providing the necessary energy reserve for cellular homeostasis. In neurons, creatine exists as either free substrate or PCr, shuttling between sites of energy production and sites of energy consumption, where a phosphoryl group from PCr is transferred to adenosine diphosphate in a creatine kinase–mediated reaction, creating adenosine 5′-triphosphate (ATP).[96] In human HD, the PCr shuttle system is altered, with a significant shift in the ratio of PCr to phosphate.[68]

In assessing the therapeutic potential of creatine to treat HD, several preclinical animal studies have provided evidence of the neuroprotective benefit of creatine in multiple models of HD.[97–104] Creatine supplementation significantly reduces striatal lesion volumes in the neurotoxin models of 3-NP and malonate.[103,105] Genetic mouse models of HD have been used extensively to assess potential neuroprotective therapies. We have shown in R6/2 mice that creatine significantly improves survival and motor performance, ameliorates brain and striatal atrophy, and reduces striatal mHtt aggregation in a dose-dependent manner. In addition, oral creatine administration increased brain creatine levels. The neuroprotective benefit of creatine in HD mice has been confirmed in N171-82Q transgenic mice.[97] The efficacy of creatine at different stages of the R6/2 phenotype has also been examined by initiating creatine administration well after manifest disease appears in the R6/2 mice at 6, 8, and 10 weeks of age.[99] These time points are analogous to early-, middle-, and end-stage disease in human HD. There was a significant extension in survival in the 6- and 8-week start groups, as well as improved motor performance, body weight, and neuropathology. In addition, we have evidence showing that both brain and urine OH^8dG levels are ameliorated by creatine supplementation in both R6/2 mice and the full-length CAG140 mice.

Although several clinical trials of creatine in HD have been conducted, none has been sufficiently powered to detect a significant slowing of progression or improvement in clinical measures. Creatine, 3–5 g/day, has been shown to be safe and well tolerated in HD patients.[106] Another trial using 5 g/day creatine for 1 year found no differences in measures of strength, neurologic status, or cognitive status.[107] In a 1-year, open-label pilot study, creatine (10 g/day) administered for 12 months was safe and tolerable and resulted in increased creatine brain concentrations.[108] The United Huntington Disease Rating Scale scores were unchanged after 12 months, suggesting that creatine (10 g/day) may be effective in stabilizing disease progression. In a multicenter double-blind, placebo-controlled study using 8 g/day of creatine in HD patients, serum levels of creatine were increased up to 15-fold, brain levels of creatine were significantly increased by 7.2%, and N acetyl aspartate (NAA) levels were improved (a biomarker of neuroprotection).[46] In addition, serum OH^8dG levels were significantly reduced by creatine treatment. The OH^8dG findings are the first instance of parallel efficacy using a common peripheral biomarker in the administration of a therapeutic agent in HD mice and human patients.

None of these studies was sufficiently powered to be informative about whether creatine slows the clinical progression of HD; however, they do attest to its safety and tolerability as well as its favorable effects on serum and brain levels of creatine and biomarkers of HD pathology. Although the optimal dose of creatine is not yet certain, it is possible that the dose of creatine supplementation in the above studies may have been underestimated. Human Equivalent Dose extrapolation measurements derived from body surface area criteria in animals may not accurately predict the maximum recommended safe dose in neurologic disorders. This is evident in human trials in which human-equivalent dosing of antioxidant and bioenergetic agents (e.g., coenzyme Q_{10}[CoQ_{10}]) comparable to that given to mice, although considered safe and tolerable, have not demonstrated significant efficacy in HD patients. The strengths of the HD mouse models are in their ability to provide parallel pathophysiologic targets that are present in HD patients, in their potential as sensitive predictors for therapeutic intervention, and their promise in the development of novel drug agents. Drug trials in mice confirm therapeutic direction; however, because of dissimilarities in the pharmacokinetics of mice and humans, the challenge is in determining which dose might be of value in patients. As such, a much higher dose may be feasible for humans. In this regard, a dose escalation study up to 40 g/day has been initiated to determine whether there is a maximally tolerated dose in HD and whether there are doses at which serum and brain levels of creatine are maximized. Previous research has been consistent in finding that creatine, overall, does not have a significant deleterious effect in humans (reviewed in[93]). Although caution is warranted in association with high-dose, long-term creatine administration, prolonged high-dose use of creatine in the sports community has been ongoing for 10–15 years and, as such, the absence of reported adverse events is important to note.

A preliminary report has examined the effects of high-dose creatine administration in two genetic models of HD.[109] High-dose creatine administration was well tolerated by R6/2 mice and CAG140 mice, with administered creatine doses 5 times that of previously successful preclinical dosing strategies.[100] We demonstrated significant improvement in R6/2 survival and motor performance beyond that previously reported at lower doses. Additional analyses revealed significant improvements in striatal neuropathology, with attendant reductions in both mHtt aggregation and OH^8dG brain and serum levels. Similar results were observed in CAG140 mice in which we found a significant creatine-mediated improvement in the behavioral phenotype, including a reduction in mHtt aggregation. We also found a significant increase in striatal ATP levels and an associated reduction in OH^8dG levels. These

results clearly demonstrate the clinical potential for high-dose creatine in treating human HD.

The antioxidant compound CoQ_{10} has also demonstrated preclinical efficacy in murine models of HD.[110–114] CoQ_{10}, ubiquinone, is a lipid-soluble benzoquinone which, when reduced to ubiquinol, possesses significant antioxidant potential. In addition, CoQ_{10} can induce increases in vitamin E,[26] furthering its antioxidant capacity. Located in the inner mitochondrial membrane, CoQ_{10} is essential for complex I and II electron transfer activities during oxidative phosphorylation,[115] playing a vital role in ATP production. Treatment with CoQ_{10} has also been demonstrated to significantly increase levels of mitochondrial CoQ_{10} in the brain.[116]

In initial experiments using the mitochondrial toxin malonate, treatment with CoQ_{10} resulted in dose-dependent neuroprotection, with a significant CoQ_{10}-mediated reduction in striatal lesion volume.[110] Expanding on these results, we and other investigators have conducted preclinical therapeutic trials using CoQ_{10} in mouse models of HD showing drug efficacy.[111] We found a significant CoQ_{10}-mediated extension in survival, along with a delay in weight loss and in the decline of motor performance. CoQ_{10} administration also significantly attenuated brain weight loss, gross brain atrophy, ventricular enlargement, and striatal neuron atrophy typically observed in R6/2 mice.

Based on favorable preclinical CoQ_{10} efficacy, several human safety and tolerability trials have been conducted using CoQ_{10}.[68,117,118] In each trial, CoQ_{10} was found to be safe and tolerable. Treatment with CoQ_{10} resulted in significant decreases in cortical lactate,[68] demonstrating improved metabolic potential. A multicenter clinical trial (CARE-HD) of CoQ_{10} (600 mg/day) found a trend toward slowing in total functional capacity decline over 30 months, a significant slowing in decline on the independence scale, and a significant beneficial effect on measures of cognitive function, including Stroop color-naming and word-reading tasks.[118] Because the single target dose did not significantly affect the specified primary outcome of the trial, however, it remains unclear whether a higher CoQ_{10} dose would provide greater efficacy in HD patients. A number of studies in other neurodegenerative diseases suggest that a higher CoQ_{10} dose is possible. A double-blind, randomized, controlled trial in Parkinson's disease patients, using CoQ_{10} at 1200 mg/day, slowed the rate of deterioration in the Unified Parkinson's Disease Rating score.[119] Follow-up studies in both Parkinson's disease and amyotrophic lateral sclerosis patients have demonstrated safe and tolerable doses up to 3000 mg/day.[120,121] To this end, we recently completed a high-dose trial using CoQ_{10} in R6/2 mice,[122] with doses at 10 times those previously reported.[110,111] High-dose CoQ_{10} treatment resulted in significant survival extension in R6/2 mice beyond that previously reported, with significant improvements in motor performance. High-dose CoQ_{10} treatment in R6/2 mice also improved the neuropathologic sequela, along with marked reductions in striatal mHtt aggregation. Coupled with a significant improvement in brain ATP levels and a reduction in brain OH^8dG levels, these results clearly demonstrate the benefit of high-dose CoQ_{10} in the treatment of HD mice and provide a rationale for further clinical trials in HD patients.

Although significant evidence from studies in HD patients and from experimental models of HD suggests that oxidative stress plays a role in the pathogenesis of HD, the etiology of this pathologic event is not yet clear. It is evident, however, that antioxidant therapy is effective as a therapeutic strategy, and that either high-dose creatine or CoQ_{10} may represent the first line of defense in ameliorating the progression of HD.

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Conflicts of Interest

The authors declare no conflicts of interest.

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