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Targeting urate to reduce oxidative stress in Parkinson disease

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Abstract

Oxidative stress has been implicated as a core contributor to the initiation and progression of multiple neurological diseases. Genetic and environmental factors can produce oxidative stress through mitochondrial dysfunction leading to the degeneration of dopaminergic and other neurons underlying Parkinson disease (PD). Although clinical trials of antioxidants have thus far failed to demonstrate slowed progression of PD, oxidative stress remains a compelling target. Rather than prompting abandonment of antioxidant strategies, these failures have raised the bar for justifying drug and dosing selections and for improving study designs to test for disease modification by antioxidants. Urate, the main antioxidant found in plasma as well as the end product of purine metabolism in humans, has emerged as a promising potential neuroprotectant with advantages that distinguish it from previously tested antioxidant agents. Uniquely, higher urate levels in plasma or cerebrospinal fluid (CSF) have been linked to both a lower risk of developing PD and to a slower rate of its subsequent progression in numerous large prospective epidemiological and clinical cohorts. Laboratory evidence that urate confers neuroprotection in cellular and animal models of PD, possibly via the Nrf2 antioxidant response pathway, further strengthened its candidacy for rapid clinical translation. An early phase trial of the urate precursor inosine demonstrated its capacity to safely produce well tolerated, long-term elevation of plasma and CSF urate in early PD, supporting a phase 3 trial now underway to determine whether oral inosine dosed to elevate urate to concentrations predictive of favorable prognosis in PD slows clinical decline in people recently diagnosed, dopamine transporter-deficient PD.

Keywords

Parkinson disease; urate; uric acid; antioxidant; epidemiology; biomarker; antioxidant; nrf2 activator neuroprotection; mitochondrial dysfunction; oxidative stress

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Introduction

Parkinson disease (PD) is the second most common neurodegenerative disease and has an incidence of 8 to 18 per 100,000 person-years (1). Its prevalence is age-related, affecting more than 1% of those older than 60 years and up to 4% of those older than 80 years (1). Although the phenotype of PD as traditionally defined is relatively homogeneous, its genotype and putative environmental influences are increasingly viewed as heterogeneous with the underlying etiology and pathogenesis likely multifactorial in nature. Over the past few decades significant advances have been made in our understanding of PD including greater awareness of environmental, genetic and stochastic factors contributing to the development and progression of PD. Biochemical and cellular abnormalities have been identified in the brains of people with PD including mitochondrial dysfunction, free radical-mediated damage, excitotoxicity and inflammatory changes with oxidative stress proposed to play a critical role. Oxidative stress may represent a common pathway following the convergence of genetic and environmental etiologic factors.

In the first half of our paper we will review oxidative stress in the pathogenesis of PD and evidence to support antioxidant therapies. In the second half of the paper we will focus on evidence that urate elevation strategies targeting oxidative stress pathways may lead to neuroprotective therapy in PD.

Oxidative stress

Oxidative stress has been implicated as a core contributor to the initiation and progression of multiple neurological diseases including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), PD, multiple sclerosis (MS) and stroke (2, 3, 4, 5, 6, 7). The brain is particularly susceptible to oxidative stress for many reasons. First and foremost, although it comprises only 2% of total body weight the brain consumes 20% of the body's total oxygen supply, with a significant portion of that oxygen subsequently being converted into reactive oxygen species (ROS) (7, 8, 9). Secondly, in the brain there are specific neurochemical reactions, like dopamine oxidation, which create large amounts of reactive oxygen or nitrogen species (ROS, RNS). Thirdly, the brain has large amounts of lipids (e.g., myelin), which are sensitive to oxidative stress. Lastly, with aging there is increased deposition of metal ions in the brain, which are catalysts in the production of ROS and RNS (9, 10).

Oxidative stress occurs when there is an imbalanced redox state due to either excessive oxidants (including ROS and RNS) or dysfunction of the antioxidant system. ROS and RNS are generated in both neurons and glia by the enzymes, monoamine oxidase, NADPH oxidase, nitric oxide synthase and other flavoenzymes. They are generated by infiltrating monocytes, macrophages and activated microglia (11). The electron transport chain is the major contributor of ROS at the mitochondrial level (12). Impaired cellular antioxidant defense also predisposes to oxidative stress, resulting in an inflammatory state, contributing to neurodegeneration. This is seen with reduction in the activities of enzymes superoxide dismutase, catalase and glutathione/glutathione peroxidase or decreased α -tocopherol and urate (a.k.a. uric acid) levels (13).

Oxidative stress in PD

In PD mitochondrial dysfunction, oxidative stress and pro-oxidant environmental toxins have been postulated to play a role in the pathogenesis of the disease (14, 15). Oxidative stress occurs with protein misfolding and aggregation, mitochondrial dysfunction, cell cycle reactivation, apoptosis and excitotoxicity, all of which are observed in PD (16, 17). Evidence of oxidative stress has been found in post-mortem studies of people with PD with decreased levels of antioxidant enzyme activity including glutathione peroxidase and catalase; reduced levels of glutathione (GSH); increased iron; increased activity of superoxide dismutase and decreased activity of complex I of the mitochondrial respiratory chain (18–20). The reduction in the antioxidant defense system may result in increased lipoxygenase activity, a branch of the arachidonate cascade. In PD, selective loss of GSH in the substantia nigra (reduced by 40% compared to control subjects) has been seen (21). This loss of GSH is thought to be one of the earliest biochemical changes in PD (22–25) and was not found in other parts of the brain (21). A reduction in mitochondrial complex I activity was also demonstrated in PD compared to controls (26–29).

In post-mortem brains of PD patients, lipid peroxidation products, cholesterol lipid hydroperoxides and malondialdehyde are 10-fold higher when compared with other brain regions and age-matched controls (30). There is also damage to protein in PD through nitration with increased amounts of nitrotyrosine (3-NT), which have been identified in peripheral polymorphonuclear cells in Parkinson's patients and in their brains in Lewy bodies, the pathological hallmark of the disease (31, 32). Protein carbonyls are also found throughout the brain in PD compared to controls (33). Also, the presence of toxic products of oxidative lipid damage, such as 4-hydroxynonenal, impairs cell viability by interacting with various proteins (18). Lastly, oxidized adducts of nucleic acid (DNA and RNA) are also increased in the central nervous system (CNS) of PD patients, with elevated levels of 8-OHG and 8-OHdG in the substantia nigra (SN) and cerebrospinal fluid (CSF) (34–37).

Magnetic resonance spectroscopy (MRS) in PD has identified high-energy phosphate levels in the temporoparietal region (38) and occipital lobe (39), and increased cerebral lactate (40) consistent with oxidative stress and a shift to anaerobic metabolism in vivo. Using specific spectral editing techniques such as MEdscher-GArwood-PRESS (MEGA-PRESS) one can also measure GSH and vitamin C levels in the human brain (41). A small, preliminary study measured GSH by MRS in PD with no evidence of altered levels (42). Reduced glutathione levels are found in the human brain, particularly in the hippocampus and frontal cortices of patients with Alzheimer disease (43). Magnetic resonance imaging (MRI) studies showed a rise in iron concentrations in the SN in PD patients (44).

Positron emission tomography (PET) using [^{62}Cu] diacetyl-bis (N4-methylthiosemicarbazone) ([^{62}Cu] ATSM) as a radiotracer can identify an intracellular over-reductive state (45). This PET tracer accumulates in the brain regions in an over-reductive state through the reduction of Cu (II) to Cu (I) (46). In patients with PD, deposition of [^{62}Cu] ATSM has been observed in the striatum, suggesting a regional over-reductive state secondary to mitochondrial dysfunction (47).

Dopaminergic neurons in the SN are particularly susceptible to oxidative injury in PD. This differs from the selective neuronal vulnerability seen in other neurodegenerative conditions, in which oxidative stress is also postulated to have a pathogenic role. For example, in AD the entorhinal cortex and the hippocampus CA1 region are most affected. Interestingly, even within the dopaminergic neurons of the SN, some areas are spared in PD, with the ventral tier rather than the dorsal layers of the nigra seen to be most affected (48). Dopaminergic neurons are thought to be affected in PD due to their high intrinsic basal oxidative stress from both the enzymatic and non-enzymatic metabolism and autooxidation of dopamine, including through semiquinone formation and its polymerization (18, 49–51). The degradation of dopamine by monoamine oxidase can produce 6-hydroxydopamine and hydrogen peroxide, which create increased amounts of oxidized GSH impairing the antioxidant system (51). Hydrogen peroxide, in the presence of high iron levels in the SN, is also converted by Fenton reactions to the highly toxic hydroxyl radical, another free radical resulting in further oxidative damage (52, 53). 6-hydroxydopamine is polymerized to neuromelanin, which is also demonstrated to induce apoptosis in these cells (15). In PD, dopamine oxidation is increased due to early compensatory changes in dopamine turnover secondary to nigral cell degeneration (54).

In addition to dopamine oxidation, the dopaminergic neurons in the SN pars compacta are known to experience increased oxidative stress from their autonomous pacemaker mechanism, which utilizes L-type calcium channels resulting in intracellular calcium oscillations. These channels control the repeated and persistent entry of calcium into dopaminergic cells and require ATP-dependent pumps to restore the calcium concentration. Interestingly, these calcium channels are also seen in other areas of the brain which are selectively involved in PD such as the dorsal motor nucleus of the vagus, the serotonergic neurons of the raphe nuclei, and neurons of the locus coeruleus. The functioning of these channels result in high levels of oxidative stress in mitochondria (55).

Mitochondrial dysfunction in PD

The relatively recent discovery of familial PD cases and their genetic associations with mitochondrial function support the role of mitochondrial dysfunction leading to oxidative stress. In addition, mitochondrial electron transport chain NADH dehydrogenase (complex I) deficiency has been demonstrated in the SN and prefrontal cortex of subjects with PD compared to controls (27, 29, 56). Subsequent studies also found complex I deficiency in the platelets, lymphocytes and skeletal muscle tissues of patients with PD, although this was not consistently seen in skeletal muscle (28, 57). Complex I deficiency is thought to render cells more vulnerable to Bax-induced apoptosis by reducing ATP production and generating free radicals, which cumulatively contribute to the demise, or at least dysfunction, of cells during the disease process (58).

Mitochondrial proteins and familial PD

Alpha-synuclein, *Parkin*, *PINK1*, *DJ-1* and *LRRK2*, all products of genes whose mutations can cause familial PD, are proteins involved in mitochondrial function (59). Mutations in *Parkin*, also known as *PARK2*, are the most common cause of autosomal recessive PD in

people younger than 45 years (60). *Parkin* is transcribed ubiquitously and is located in the mitochondria, endoplasmic reticulum, synaptic vesicles and golgi apparatus (61, 62). It is an E3 ubiquitin ligase which is involved in the ubiquitin proteasomal pathway (63). In the setting of excitotoxicity-mediated damage it can become impaired due to S-nitrosylation and result in the accumulation of its substrates – Pael-R leading to cell death (64). *PARK2* knockout mouse models have decreased mitochondrial respiratory chain function and reduced antioxidant proteins (65). In people with *PARK2* mutations they have decreased complex I and IV activities in peripheral blood (66).

PINK1-PTEN-induced putative kinase 1 gene mutations cause autosomal recessive PD due to homozygous mutations resulting in loss of function of the gene. They typically lead to onset of parkinsonism before 40 years of age (67). The *PINK1* gene encodes a serine/threonine kinase which is rapidly imported into mitochondria when there is loss of membrane potential (68). Like the *Parkin* gene, it is also involved in mitochondrial quality control through mitophagy (68). *PINK1* knockout flies have increased susceptibility to paraquat and rotenone. *PINK1* or *Parkin* knockout flies or mice have synaptic dysfunction that correlates with progressive loss of mitochondrial function and increased oxidative stress in the striatum with age (68). They both appear to participate in the same pathogenetic linear pathway. *PINK1* is upstream of *Parkin* in their pathophysiological cascade, which allows rescue of *PINK1*-related abnormalities by overexpression of *Parkin* (69).

DJ-1 gene (*PARK7*) mutations also are a cause of autosomal recessive young-onset PD (70). The gene encodes a 23kDa protein, which is present throughout the body and is located in the intermembrane space and matrix of the mitochondria (71). It acts as an oxidative sensor which relocates to the outer mitochondrial membrane during oxidative stress (72). It forms a nuclear complex with RNA-binding and DNA-binding proteins, and thereby regulates gene transcription and prevents apoptotic cell death by alpha-synuclein or oxidative stress (59). Further support for this mechanism is seen in cells with deleted or silenced *DJ-1*, which are sensitized to oxidative stress, in contrast to cells with overexpressed *DJ-1*, which are protected (73, 74).

Alpha-synuclein gene (*PARK1* or *SNCA*) mutations are a cause of autosomal dominant PD. Its protein product is present on the inner mitochondrial membrane and presynaptic nerve terminals of human brains. Overexpression of mutant alpha-synuclein and phosphorylation of the serine residue at position 129 may contribute to oxidative stress (75). Toxicity is seen with wild-type alpha-synuclein in cell models and patients with familial PD with multiplications of gene encoding alpha-synuclein (76). Higher amounts of alpha-synuclein are seen in the mitochondria of PD subjects compared to the controls (68). Its presence reduces mitochondrial complex I activity and increases the production of ROS and calcium (68).

Leucine-rich repeat kinase 2 (*LRRK2*) gene mutations produce an autosomal dominant form of PD found in 5–8% of individuals with a first-degree relative with PD and 0.4–1.6% of patients with sporadic PD (59). G2019S is the most common mutation of *LRRK2*. *LRRK2* is found in the cytoplasmic membrane and the microsomal and outer mitochondrial

membrane (68). Mutations in this gene result in mitochondrial fragmentation and reduced mitochondrial fusion resulting in oxidative stress, as well as increased kinase activity (68).

Oxidative stress and toxins

The wide range of dopaminergic neuron-targeting toxins used to model the degeneration of the nigrostriatal pathway in PD highlight its vulnerability to free radical attack. MPTP/MPP + administration disrupts mitochondrial respiratory chain function resulting in enhanced superoxide formation, in turn resulting in reduced mitochondrial membrane potential and apoptosis (18). 6-OHDA also causes dopaminergic cell degeneration through free-radical mediated mechanisms (77). Several pesticides (e.g., rotenone and paraquat) employed in more recent toxin models of PD similarly rely on disruption of mitochondrial respiration and on oxidative stress to produce dopaminergic cell death (78).

Both in vivo and in vitro studies have raised the possibility that L-dopa treatment adds to the oxidative load in PD by free radical-mediated mechanisms or potentiating selective toxins (79–81). However other in vitro studies have shown contradicting results, with L-dopa causing neuroprotective effects by either inducing neurotrophic factors or increasing glutathione levels (82, 83). Animal and human models have not shown oxidative damage or nigral cell degeneration with L-dopa use (84, 85).

Antioxidants

In the human body there are both endogenous and exogenous antioxidant agents. Endogenous antioxidants include enzymes and non-enzymatic molecules. Among the enzymes are superoxide dismutase (SOD), glutathione peroxidase/reductase (GPx/GR), catalase and peroxiredoxin. The non-enzymatic molecules include urate, glutathione, pyruvate, amino acids, transferrin and ferritin. There are also precursors of antioxidants (e.g. N-acetyl cysteine and inosine) and cofactors of antioxidants (e.g. selenium and coenzyme Q10). Lastly, there are also nutritional antioxidants including those that are water-soluble (ascorbate and other polyphenols) and lipid soluble (α -tocopherol, carotenoids, quinones, some polyphenols) (10).

Epidemiology of antioxidants other than urate in PD

Case-control studies investigating antioxidants other than urate (see below) in PD have reported largely inconsistent results. SOD activity has been measured in PD patients with variable results (86–89). Marttila et al. showed increased levels of SOD in the SN and basal ganglia in PD patients without any alteration in the activities of catalase, GPx, and GR compared to age-matched controls (90). On the contrary, another study of 11 patients found a small reduction (19%) in the activity of GPx in the SN in patients with PD (91).

Although reduced levels of antioxidants vitamin A, C and E and reduced activities of SOD and glutathione reductase have been reported in AD (92, 93), most early studies of vitamin A, C and E and beta-carotene found no association with PD (94–98). Abbott et al. found lower plasma levels of vitamins E and A in PD cases than in controls (99). Hellenbrand et al. found no association with vitamin E or beta-carotene intake but found that PD risk was

inversely related to vitamin C intake (p for trend 0.04; OR for highest quartile compared to lowest 0.60, 95% CI 0.33–1.09) (100). A population-based case-control study conducted in the state of Washington found no associations for vitamin C, E, A or beta-carotene intake in the initial study or in an extended dataset (102).

A few prospective epidemiological studies have also investigated antioxidants. No association between intake of vitamin E and PD was found in the Honolulu-Asia Aging Study (103). In contrast, an inverse association with vitamin E intake (OR 0.3, 95% CI 0.1–0.9 for the highest vs. the lowest tertile, P for trend 0.03), but no association between PD and vitamin C or beta-carotene intake, was found in the Rotterdam study (101). In the Leisure World Cohort Study, in California, an increased risk of PD with dietary vitamin C and A intake was found in univariate analysis, but results were not significant in multivariate analysis (104). In the Singapore Chinese Health Study, an inverse relationship was found between total vitamin E intake and PD risk (RR 0.64, 95% CI 0.39–1.05 for highest vs. lowest quartile, p for trend 0.03), but there was no association with intake of vitamins C or A (105). Lastly, no association between intakes of total vitamin E, vitamin C or beta-carotenoids and PD was found in the Nurses' Health Study and the Health Professionals Follow-up Study (106, 107). However, vitamin E from food alone was inversely associated with PD risk (RR 0.68, 95% CI 0.49–0.93 for highest vs. lowest quintile of dietary vitamin E intake) (108).

Clinical studies on antioxidant use

Prompted by the data cited above, there have now been multiple studies investigating the effect of antioxidant administration in PD. Vitamin E, creatine and coenzyme Q10 have been the most commonly and thoroughly trial-tested antioxidants in PD. Vitamin E (α -tocopherol), a major scavenger of lipid peroxidation in the brain, was studied in the Deprenyl and Tocopherol Antioxidant Therapy of Parkinsonism study (DATATOP study), a randomized clinical trial of early PD, with no beneficial effect seen from α -tocopherol (2,000 IU/day) in either symptom control or slowing disease progression (109). Vitamin E has been included in subsequent studies as a supplement in both coenzyme Q10 and its placebo (110, 111).

Coenzyme Q10, which transfers electrons from complexes I and II to complex III in the mitochondrial respiratory chain, was evaluated in several multicenter, randomized, placebo-controlled, double-blind trials. Subjects with early PD given 1200 mg per day showed some evidence for slowed functional decline compared to placebo with marginal statistical significance (111) prompting further evaluation in a phase 3 trial. However, the QE3 trial did not demonstrate long-term benefit of coenzyme Q10 at either of two doses (1200 and 2400 mg/day) taken over 16 months in 600 patients with early PD (110).

Creatine monohydrate, a nutritional supplement which enhances cellular energy function and has antioxidant properties, and minocycline, an antibiotic with anti-inflammatory and anti-apoptotic properties, were studied in a phase 2 trial on subjects with early PD, diagnosed within 5 years. Subjects were given creatinine (10 grams/day), minocycline (200 mg/day) or placebo in 1:1:1 ratio for 12 months. This study showed that these drugs were

safe, well tolerated and did not interfere with symptomatic therapy. An ambitious phase 3 trial was then carried out with 1,741 PD subjects randomized to receive placebo versus creatine (10 grams/day) for five years. This study was discontinued early as a planned interim analysis showed no benefit of creatine compared to placebo based on a composite long-term measure of clinical decline (112–115).

Both GSH and N-acetylcysteine (a GSH precursor) have been tested in small preliminary clinical studies in PD. In an open-label, single-group pilot study, Sechi et al. 600 mg of intravenous GSH twice daily for 30 days to nine patients in the early stages of PD, and reported a reduction in clinical disability by 42% lasting 2 to 4 months (116). N-acetylcysteine given orally or intravenously has been shown to increase GSH levels in the blood, brain and cerebrospinal fluid (117, 118).

Despite a large, compelling body of evidence supporting the contribution of oxidative stress to PD pathogenesis, agents with antioxidant properties have thus far failed to show benefit in slowing disease progression. It is unclear whether the lack of clinical translation of antioxidant promise in PD is due to antioxidant selection, clinical trial design (dose selection, target engagement, outcome measures, etc.) or to the oxidative stress hypothesis itself. Urate has emerged as an antioxidant with several *a priori* advantages over previously pursued antioxidant targets and has recently advanced to phase 3 efficacy testing. The remainder of the review will focus on urate as a candidate disease-modifying therapy in PD based on its antioxidant and neuroprotective properties and convergent epidemiological and clinical data linking it to reduced risk and progression of PD.

Urate

Urate, the anionic form of uric acid (pKa 5.4) (119), predominates at physiological plasma and CSF pH (120). It is present intracellularly and in all body fluids. It is synthesized by the xanthine oxidoreductase enzyme (a.k.a. xanthine oxidase), which successively oxidizes hypoxanthine to xanthine and then to urate (figure 1). Urate is the enzymatic end product of purine metabolism in humans and higher primates, unlike all other mammals in which urate is metabolized further to allantoin by the enzyme urate oxidase (a.k.a. uricase) (121). Over the past 10 to 15 million years during the Miocene epoch with hominid evolution, the urate oxidase gene (*UOx*) appears to have undergone several spontaneous independent mutations leading to incomplete transcription of *UOx* and thus the absence of its enzyme product in humans and apes, leading in turn to higher urate levels (121).

There are multiple theories as to why the uricase enzyme disappeared during primate evolution and why this was advantageous to humans. Humans and other ‘higher’ primates, which include apes and certain New World monkeys, have higher urate levels (>2 mg/dl) compared with other mammals (122). Having higher levels of urate may be beneficial as it is an antioxidant and has been posited to act as a defense against aging and cancer by preventing oxidative damage by reactive oxygen species which is of increasing importance with increased lifespan (123). In addition, higher urate may have been essential in human ancestors for a suspected hypertensive effect when low-salt diets may have been common and resulted in symptomatic hypotension otherwise (124). Interestingly, the first hypothesis

proposed to explain an evolutionary advantage for higher urate in humans was that it might have played a critical role as “catalyzer of mental development”. It was posed because urate has a similar purine-based structure to the cerebral stimulant caffeine (1,3,7-trimethyl-2, 6-dioxy-purine), which can enhance cognition, alertness and motivation in humans (125).

Evolutionary increases in circulating urate have been attributed not only to *UOx* disruption but also to mutations in urate transporter genes accounting for the human kidney’s capacity to resorb 90% of filtered urate, thus contributing to our higher urate levels. This development of an efficient reabsorption mechanism is critical for high plasma urate levels as humans who do not actively reabsorb urate from the kidney have abnormally low urate levels (0.2–1.8 mg/dl) (126). Lastly, with evolution from prosimians to monkeys, ascorbate synthesis was lost and ascorbate was replaced by urate as the main antioxidant in plasma. This replacement may be advantageous as ascorbate as an antioxidant is limited by its propensity to oxidize, generating oxygen radicals and mutagens (127). However, urate can also act like a pro-oxidant at higher concentrations or under other conditions (128, 129).

Nonetheless, there are established adverse effects of higher urate levels attributable to crystal deposition, primarily gouty arthritis and uric acid urolithiasis. Hyperuricemia is also associated with hypertension, cardiovascular disease including myocardial infarction and congestive heart failure, along with stroke, renal disease and metabolic syndrome (130–134).

Urate properties

Urate accounts for 60% of total plasma antioxidant capacity and may serve as one of our major endogenous defenses against oxidative and nitrosative damage (135). It has been demonstrated to scavenge singlet oxygen, hydroxyl radicals, hydroxyl peroxide and peroxynitrite (123, 136). By scavenging peroxynitrite it produces a nitrated urate derivative, which acts as a vasoactive nitric oxide donor and prevents free radical-mediated DNA damage (137–139). It interacts with and stabilizes other antioxidant systems including superoxide dismutase (SOD), ascorbate, and tetrahydrobiopterin. By preventing the degradation of SOD, urate allows clearance of superoxide from the cell, thereby preventing its reaction with nitric oxide and reducing the formation of peroxynitrite (140, 141). Urate has also been shown to prevent peroxynitrite from nitrating the tyrosine residues of proteins, which prevents inactivation of cellular enzymes and modification of the cytoskeleton (140).

Urate has metal-complexing properties too, allowing it to bind with iron and copper ions (52). It chelates iron by forming stable complexes with Fe^{3+} , and thus blocking iron-dependent oxidation reactions, diminishing the oxidizing potential of Fe^{3+} and preventing ascorbate oxidation (52). Low urate concentration may reduce iron-chelating capacity and expose dopaminergic neurons to iron toxicity (142, 143). Both urate and ribosylurate protect against copper ion-induced peroxidation of arachidonate, linolenate and erythrocyte membrane lipids (144–146). Urate has also been shown to inhibit ascorbate oxidation by forming urate-metal ion complexes and thus preventing transition metal ions from interacting with ascorbate (147). EDTA and DTPA, which are metal ion-chelating agents, also inhibit ascorbate oxidation (51).

Despite the evidence (see below) of urate's role in neuroprotection there have been other studies questioning its role. In humans there has been mixed evidence of its antioxidant action under pathophysiological conditions (148, 149).

Urate levels in PD brain

Church and Ward in 1994 (150) examined postmortem caudate and substantia nigra samples from PD patients and controls (n=4 for each group) and found that urate levels were lower in the SN ($p<0.05$) and caudate ($p<0.1$) of PD patients compared with controls, consistent with others and our subsequent findings in a larger post-mortem series (151, 152). They also observed a higher dopamine oxidation rate constant in PD compared to the control striatum homogenates, supporting a "pro-oxidative stress" state in PD. By adding urate they were able to reduce the dopamine oxidation rate in PD nigral homogenates. By adding urate oxidase to control homogenates they demonstrated an increase in the dopamine oxidation rate. Their study increased interest in studying urate in PD along with its role in oxidative stress and the hypothesis that urate could reduce or prevent oxidative damage (150).

Determinants of urate levels

Urate levels are ten times higher in humans than most other mammals due to the absence of the urate oxidase enzyme (153). The levels of urate in blood are dependent on a balance between the uricogenic dietary factors (including purine intake), the metabolism of purines to urate, its reabsorption and excretion by kidneys and its excretion by the intestines (154). Higher levels of serum urate are seen in men compared to women (126) with urate's reference range in clinical practices being for example, 3.4–7.2 mg/dl for men and 2.4–6.1 mg/dl for women (155), apparently due to renal estrogen effects (156). Its concentration is consistently lower in CSF (30 $\mu\text{mol/l}$), approximately ten times lower than its peripheral concentration in blood (157, 158). This CSF concentration is dependent on blood urate and integrity of the blood-CSF barrier. CSF urate concentrations also have a strong caudo-rostral gradient from the lumbar space, with lumbar region values approximately 50% higher than those arising at the cisterna magna (brainstem) level (159, 160).

Genetic determinants of PD

Urate levels are also in part heritable with the estimate of between-person variation due to inherited genetic factors ranging from 25 to 70% (161). Rare mutations in purine metabolism or transport genes can alter urate levels. For example, Lesch–Nyhan syndrome caused by deficiency in the enzyme hypoxanthine phosphoribosyl-transferase results in elevated urate levels (162), whereas mutations in the major urate transporter genes *SLC2A9* and *URAT1* can lead to marked hypouricemia (163, 164). The product of the *SLC2A9* is *GLUT9*, a facilitative glucose transporter expressed in the kidneys and brain. It is one of the main urate transporters determining serum urate levels and has been implicated in PD pathogenesis. The *SLC2A9* gene is also the strongest inherited determinant of serum urate in humans (165). Allelic variants among the single nucleotide polymorphisms identified in *SLC2A9* have been linked to lower serum urate, an earlier age for PD onset and faster clinical decline among those with early PD (166, 167). Interestingly, a recent preliminary

finding of lower PD incidence and prevalence in the native Maori population of New Zealand (approximately half the rates of New Zealanders of European descent) (168) may be explained by the significantly greater urate levels among Maori, which precede adulthood (169) and have been attributed to *SLC2A9* gene variants (170).

Dietary determinants of PD

Meat, alcohol and fructose increase blood urate levels with a 1.0 mg/dl reduction in urate achieved by strict avoidance of dietary purines (171). Dairy products have been shown to reduce urate levels (172, 173). Prospective epidemiological studies have studied men on a urate-elevating diet or with gout and found that they had a reduced likelihood of PD, supporting further the PD-urate link. Gao et al. used data from a subpopulation of the Health Professionals Follow-up Study in order to create a diet composite index, which included dietary factors associated with higher urate levels. They investigated whether particular diets could predict a reduced risk of developing PD. Their dietary urate index included lower dairy protein, higher alcohol and other factors that correlated with higher plasma urate levels. The subpopulation was followed for 14 years with 248 cases of PD documented among 47,406 men. Gao et al. found that a higher dietary urate index was associated with a lower risk of PD (relative risk 0.47 between top quintile vs. bottom, p-value of the trend = 0.0008). Analysis was adjusted for age, smoking, caffeine intake and other potential confounders (174).

Dairy protein, which lowers serum urate, has also been studied in regards to its risk of PD. In the Honolulu Heart Program milk was linked to a reduced risk of PD among 7,504 men (175). During 30 years of follow-up, there was a 2.3-fold lower risk of PD (95% CI: 1.3–4.1) among those who consumed no milk compared with those in the highest intake group (175). This association between dairy products and PD was also seen in another prospectively study by the American Cancer Society's Cancer Prevention Study II Nutrition Cohort which followed a cohort of 57,689 men and 73,175 women of which 250 men and 138 women developed PD during the study (176). Those men and women who consumed higher amounts of dairy products, in particular milk, were found to have a higher risk of developing PD (RR comparing the highest versus the lowest intake categories was 1.5 [95 percent CI: 1.0–2.3, p for trend=0.07] for total dairy and 1.7 [95 percent CI: 1.2–2.5, p for trend=0.03] for milk) (176). A meta-analysis of all prospective studies confirmed a moderately elevated risk of PD among persons with higher dairy product consumption. The combined RRs between extreme intake categories were 1.6 (95% CI: 1.3–2.0, p<0.0001) for men and women combined (176), findings that were recently substantiated (177). Although the higher concentration of pesticides and other toxins in milk and other dairy products has been pointed out as a potential explanation for the dairy link to PD, the inverse link between urate levels and dairy consumption has emerged as a similarly compelling hypothesis.

Meat and alcohol also cause elevated urate levels and may therefore also be linked to a reduced PD risk. A prospective nested-case control study in rural China, surveyed 16,488 subjects and analyzed 85 PD cases and 340 controls. They found that more meat consumption was inversely associated with developing PD (178). A similar inverse association was seen in a Swedish case-control questionnaire study between meat

consumption (broiled or smoked) and PD risk (179). While wine, liquor and alcohol in general have inconsistently or modestly been associated to a lower likelihood of PD (180), beer, which is rich in purines and can increase urate (181, 182), has a stronger link to a reduced risk of PD in some (183, 184) but not all studies (185).

Urate and PD risk

Numerous case-control studies and prospective epidemiological studies have investigated the association between urate levels and PD. They have generally reported lower urate levels among men who have PD or who will later develop it, with less consistent results among women. In addition to the few post-mortem studies with small sample sizes suggesting lower levels of urate in brains of PD patients compared to controls (150–152), others have typically measured urate concentrations in biofluids, particularly plasma and serum (which are effectively indistinguishable with respect to urate concentration). Case-control studies have consistently shown lower urate in subjects with PD compared to controls. Qin et al. compared bilirubin and urate levels in 425 PD patients and 460 controls and found lower urate levels in PD compared to controls ($p<0.0001$) (186). Sakuta et al. evaluated serum urate levels in 100 patients with PD, 42 patients with MSA, 30 patients with PSP, and 100 controls. They found reduced urate levels in male patients with PD, MSA and PSP compared to controls ($p<0.05$). This relationship was not observed in female patients (187). Pan et al. compared 160 Chinese patients with Parkinson disease and vascular parkinsonism and found that urate levels were lower in PD compared to vascular parkinsonism (188). Another study investigating levels of urate in 40 patients with PD and 29 controls found lower levels of serum urate in patients with PD compared to controls ($p\ 0.03$) (189). However, Ahlskog et al. found no difference in the levels of urate between PD cases and controls (190). The differences in results may be due to the inclusion of confounding factors, such as medication, BMI, gender and nutrition (120).

Multiple prospective epidemiological studies have similarly reported an inverse association between urate level and subsequent development of PD. In contrast, to cross-sectional case-control studies these longitudinal cohort studies can assess true risk of developing PD, and because its incidence is determined after urate levels are measured, they avoid the reverse causation bias that can confound the interpretation of case-control studies. The Honolulu Heart Program, which followed 7,968 men of Japanese or Okinawan ancestry for 30 years, found an inverse trend between urate levels and development of PD. They reported a 40% reduction in the incidence of idiopathic PD if baseline serum urate concentrations were higher than the median (rate ratio 0.6, 95% CI 0.4–1.0 vs. below the median) (175).

The largest prospective study in men to date was conducted by Weisskopf et al. at the Harvard School of Public Health, who followed 18,018 men who had blood samples taken between 1993 and 1995 in the Health Professionals Follow-up Study. 84 subjects were diagnosed with PD between the time of blood collection and 2002. They reported a lower mean plasma urate concentration in the PD cases compared to controls ($p<0.01$) and that men in the top quartile for plasma urate had a 55% lower risk of developing PD than men in the bottom quartile (rate ratio 0.43 with 95% CI 0.18–1.02). This decrease in risk was even greater in those with blood collected at least 4 years before diagnosis (rate ratio 0.17, 95%

CI 0.04–0.69 for highest vs. lowest quartile of serum urate), suggesting that urate level was not a consequence of dietary, behavioral, or medical treatment changes early in the course of the disease. Analyses were adjusted for age, smoking and caffeine intake (107).

The Rotterdam study, a prospective study of 4,695 men and women aged 55 years and older, reported 68 new cases of PD within 9.4 years of follow-up. They also found a lower PD risk with increasing baseline serum urate (hazard ratio: 0.71; 95% CI: 0.51–0.98, p-value for the trend = 0.04). Their results were adjusted for sex, age and smoking (191).

The Atherosclerosis Risk in Communities (ARIC) study, which enrolled 15,792 participants with a majority of women aged 45 to 64 years between 1987 and 1989, identified 95 new cases of PD over a 20-year follow-up. They also found an association between urate and PD occurrence with an odds ratio of 0.4 (95% CI: 0.2–0.8) for PD occurrence between the highest and lowest quartiles of baseline plasma urate. Analysis was adjusted for age, sex and race (192). Stratified analysis demonstrated that lower urate was a PD risk factor in men and in Caucasians, while suggesting it was also a PD predictor in women and in African-Americans of the ARIC cohort.

A 2007 meta-analysis of these three available prospective studies on urate and PD risk showed that patients with higher plasma urate concentration had a lower risk of PD, with RR of 0.80 (95% CI 0.71–0.90), a 20% reduction in the pooled rate ratio of PD for each standard deviation, 1.3 mg/dL increase in blood urate concentration ($p < 0.0001$) (107). A more recent meta-analysis of cohort studies of urate and PD incidence in these and three additional qualifying studies substantiated the lower serum urate as a PD risk factor, particularly in men (193).

A community-based cross-sectional survey which had 69,000 subjects, found that participants with higher urate levels had lower odds of reporting PD on treatment compared to those with lower urate levels, indicating an association between higher urate levels and lower PD prevalence (194).

Gender and urate in PD

For men, lower serum urate concentration has consistently been shown to be a risk factor for PD, reproducible in multiple studies, as above. However, this inverse association has been inconsistently observed or weaker in women. A prospective female-only nested case-control study within the Nurses' Health Study between 1990 and 2004, demonstrated no significant difference in PD risk across quartiles of baseline plasma urate (p-value 0.17) (106). It remains unknown whether this finding represents a true gender difference in the underlying disease biology. Alternatively, the reduced risk observed among those with higher serum urate (in the 6–8 mg/dL range) (107) may be similar in men and women but less apparent in women, who on average have substantially lower concentrations than men (e.g., with means of 4.8 and 6.4 mg/dL) (199).

Gout and PD

Studies have investigated the association between gout, a marker for elevated serum urate concentration, and the risk of developing PD. The General Practice Research Database, UK, which is a computerized dataset from GP practices containing information on 3 million Britons, identified 1,052 PD cases and 6,634 controls. They found that those patients with gout had a lower risk of developing PD (OR 0.69, 95% CI 0.48–0.99), with the strongest associations among non-smokers and individuals above 60 years (196). This association was significant among men (OR: 0.60; 95% CI: 0.40–0.91), but not present among women (OR: 1.26; 95% CI: 0.57–2.81; p value: 0.11). They also found that anti-gout medications were associated with a lower risk of PD (OR: 0.57; 95% CI: 0.19–1.70) (196). In a population cohort, health insurance records in British Columbia, USA were reviewed and they also found that individuals with gout had a lower PD risk (RR 0.70, 95% CI 0.59–0.83) (197). However, other studies did not find an inverse link between gout and PD (198, 199).

Urate as a prognostic marker in PD

The discovery of urate as an inverse risk factor for PD in healthy populations raised the possibility that higher urate levels may also serve as a favorable prognostic marker among people diagnosed with PD. In collaboration with the Parkinson Study Group we investigated this question using two clinical trials, DATATOP and PRECEPT (200, 201). These two studies collectively enrolled over 1,600 early cases of PD.

In PRECEPT (Parkinson Research Experiment on CEP-1347 Trial), the original investigators' aim was to determine whether the anti-apoptosis drug CEP-1347 could slow the clinical progression of PD. The study comprised 804 subjects with early PD who were not on dopaminergic therapy at enrollment (200, 202). After all patients were followed an average of 21.4 months, the study was stopped early based on a determination at a planned interim analysis that continuing would be futile. At that point 493 patients had reached the original trial's primary endpoint of developing disability that required dopamine treatment. CEP-1347 failed to show efficacy in slowing PD progression. Serum urate concentration was measured at the start of the trial to monitor the safety of the drug. Our group found that a higher urate level at baseline was associated with slower clinical progression with the hazard ratio declining with increasing serum urate (P for trend <0.0001). Subjects in the top quintile of urate reached the endpoint at only half the rate of subjects in the bottom quintile (HR: 0.51; 95% CI: 0.37–0.72; p-value for the trend <0.001). This association was stronger in men (HR: 0.39; 95% CI: 0.26–0.60; p-value for the trend <0.001) than in women (HR: 0.77; 95% CI: 0.39–1.50), for whom the trend was not statistically significant (p = 0.3). Baseline urate levels also predicted favorable outcomes using the secondary clinical (Unified PD Rating Scale [UPDRS]) and radiographic (dopamine transporter brain scan) outcomes collected in the PRECEPT study. For the latter, loss of striatal dopamine transporter binding sites was estimated as the change in striatal uptake of [¹²³I] β-CIT, a cocaine analog that binds the transporter, over the course of the trial. Increasing serum urate concentration was predictive of slower rates of losing [¹²³I] β-CIT binding sites in striatum (p-value for the trend = 0.002). Again, the relationship was robust in men (p = 0.001) but not significant in women (p = 0.43), despite a similar trend (200).

In DATATOP (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism), a placebo-controlled, randomized, double-blinded, clinical trial which was designed to test the hypothesis that treatment of patients with early PD with monoamine oxidase type-B inhibitor deprenyl (selegiline) 10 mg/d and/or α -tocopherol (vitamin E) 2000 IU/d will slow the progression of disease and disability requiring dopaminergic therapy (primary end point) (203). Of the 800 US and Canadian subjects with early, untreated PD who enrolled in DATATOP, 774 had available values of baseline serum urate, which was originally measured as a routine safety laboratory. 50% of these patients progressed to the primary end point during the 2 years of follow-up. Our group found a similar association as observed in PRECEPT with higher serum urate associated with slower clinical progression of PD. The adjusted hazard ratio for highest versus lowest quintile of serum urate concentration progressing to the development of disability warranting dopaminergic therapy was 0.64; 95% CI: 0.44–0.94; p for trend=0.002. The risk of PD progression was reduced by 18% for each 1.5 mg/dL increase in serum urate concentration.

In addition to serum, CSF was collected from DATATOP subjects. Some twenty years later, frozen samples of baseline CSF available from 713 patients were analyzed for their urate concentration. Although CSF urate levels on average were an order of magnitude lower than serum urate, individuals' CSF and serum urate correlated well in DATATOP ($r=0.73$; $p<0.0001$). Like urate in serum, its CSF concentration was a predictor of a slower rate of clinical progression. The hazard ratio for progressing to the need for dopaminergic therapy in the highest versus lowest quintile of CSF urate concentration was 0.65; 95% CI: 0.54 to 0.96; $p=0.009$ (201).

PRECEPT and DATATOP studies both showed that higher serum urate concentrations were associated with slower clinical progression, defined by development of disability warranting dopaminergic therapy (primary endpoint) and rate of UPDRS score worsening (secondary endpoint) (200, 201). There was no statistically significant interaction between serum urate and sex for either the DATATOP or PRECEPT cohorts (200, 201).

The weaker relationship in women may reflect the fact that there were fewer women than men with PD with a 1:2 ratio of women to men in both the DATATOP and PRECEPT cohorts (200, 201). Also, as previously noted, women on average have considerably lower blood urate levels. In both the DATATOP and PRECEPT studies, women accounted for approximately 75% of the first (lowest) quintiles of serum urate but only 14% or less of the fifth (highest) quintiles, in which progression was most reduced.

Interestingly, exploratory analyses in DATATOP also suggested an interaction between urate levels and vitamin E (but not deprenyl) treatment. Stratification by vitamin E assignment showed that urate was strongly associated with progression rate among those not taking vitamin E but was not significantly predictive in those assigned to this antioxidant vitamin. Conversely, among those subjects in the lowest quintile of serum urate at baseline, randomization to vitamin E appeared to slow progression of PD based on the primary endpoint or rate of change in UPDRS ($p<0.01$; without post-hoc analysis corrections), raising the possibility that those with the lowest levels of endogenous antioxidants, like urate, may represent responders to exogenous antioxidant treatment.

Sun et al. studied serum urate concentration in their cohort of Chinese patients and found a similar association between urate and clinical progression, which they defined by Hoehn and Yahr stages (204). Moccia et al.'s group also reported that higher serum urate concentrations predicted a slower rate of worsening for non-motor symptoms in PD (205).

Together, the above studies provide substantial evidence for urate as a molecular marker of both the risk of PD and its rate of clinical progression. We will now discuss the neuroprotective potential of urate in PD including published data on urate elevation in cellular and animal models of PD and in clinical trials for early PD.

Urate in Cellular and animal models of PD

As clinical and epidemiological data have increasingly substantiated urate as a predictive and prognostic biomarker in PD, the possibility that urate may mediate as well as mark favorable outcomes has prompted investigation of urate's neuroprotective actions in PD models. The first preclinical studies of urate and inosine (a precursor of urate) (figure 1) as potential neuroprotective strategies were carried out in cellular and animal models of other neurological diseases including multiple sclerosis, experimental autoimmune encephalomyelitis, stroke and spinal cord injury.

In mouse models of multiple sclerosis and experimental autoimmune encephalomyelitis, urate or its precursor inosine were demonstrated to delay the onset of disease while inhibiting peroxynitrite-mediated oxidation, likely preventing CNS inflammation and tissue damage in the mice (160, 206, 207, 208). Urate treatment in rat models of stroke also showed that urate treatment preceding or administered simultaneously with stroke onset protected against cortical or striatal damage by reducing the final infarct volume, reducing ischemia-induced tyrosine nitration, inducing axonal rewiring and improving behavioral performance (209, 210, 211, 212). Urate administration similarly showed benefit in spinal cord injury models by protecting against glutamate toxicity and secondary damage including lipid peroxidation, activation of poly(ADP-ribose) polymerase and general tissue damage (213, 214). Intraperitoneal urate therapy given to mice resulted in increased glutathione production in the hippocampal CA1 pyramidal neurons in slice culture, protecting these cells from oxidative stress (215).

Urate's neuroprotective properties have also been demonstrated in both cellular and rodent models of PD. Urate administration prevented spontaneous degeneration of cultured nigral neurons and dopaminergic cell death induced by oxidative and mitochondrial toxins. Jones et al. and Zhu et al. demonstrated in PC12 cells that urate protected against 6-OHDA toxicity and in parallel reduced malondialdehyde, 8-hydroxy-deoxyguanosine (8-OHdG) and lactate dehydrogenase formation and release while inducing SOD activity and increasing glutathione levels, in turn blocking oxidative stress and reducing dopamine-induced apoptosis (216, 217). In a rotenone toxicity model of PD, co-treating with urate was shown to prevent membrane depolarization and dopaminergic cell death by homocysteine plus rotenone or iron (142). Urate treatment has also been found to protect primary cultures of ventral mesencephalon dopaminergic neurons, possibly by reducing intracellular reactive oxygen species thereby preventing their spontaneous degeneration in vitro (218).

In an in vivo rodent model of PD, urate reduced 6-OHDA toxicity (217). In a complementary genetic approach to modeling urate elevation in humans, our group found that mice with a urate oxidase gene (*UOx*) knockout had elevated brain urate levels, were resistant to 6-OHDA toxicity on dopaminergic neurons and had preserved functional performance. Conversely, transgenic overexpression of *UOx* led to lower urate levels in the brain along with enhanced susceptibility to unilateral 6-OHDA resulting in more dopamine depletion, more extensive neuron loss, and exacerbated asymmetry of movement (219).

Interestingly, increased levels of urate have also been seen in MPTP rat models and after the administration of MPP+, 6-OHDA or iron chloride. This phenomenon is thought to reflect either an increase in the oxidative metabolism rate or a compensatory protective mechanism after these insults to prevent dopaminergic cell death, which older animals are unable to mount after injury (220, 221, 222).

In addition to its direct antioxidant effect, urate also appears to work indirectly in conferring neuroprotection. Urate's indirect action may actually be more pathophysiologically relevant as studies have shown that without astrocytes present in culture, urate concentrations need to be 100-fold higher (158, 223) or 200 μ M and above, whereas CNS concentrations of endogenous urate are typically 10- to 100-fold lower (201, 219). In the presence of astrocytes, lower levels of urate are required (201, 219). Du et al's group demonstrated this in a cell culture model of spinal cord injury. They observed that urate required astrocytes for neuroprotection and that urate acted upon astroglial cells to increase the protein levels of EAAT-1, a glutamate transporter, which protected spinal cord neurons from glutamate-induced toxicity (213). In pure neuronal cultures from the same tissue, urate did not protect against glutamate toxicity (213).

We subsequently found that the urate's protective effect on dopaminergic nigral neurons in culture was enhanced in the presence of astrocytes (224), and showed that isolated astrocytes treated with urate produced a soluble neuroprotective factor, which was insensitive to urate oxidase (223). Further characterization identified glutathione as the astrocytic factor induced by urate that likely accounts for its neuroprotective effects (225).

Urate's non-cell autonomous neuroprotective effects also appear to be mediated by nuclear factor E2 (erythroid-derived 2)-related factor 2 (Nrf2) signaling in astrocytes. Nrf2 is a regulator of cellular defense against oxidative stress (158). It is a transcription factor that controls the expression of antioxidant response element (ARE)-containing genes such as heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1, glutathione-S-transferases and other glutathione synthesizing enzymes (226), with subsequent increase in glutathione levels (158). Impaired Nrf2 signaling results in abnormal redox homeostasis. Zhang et al. reported on the role of Nrf2 in association with urate for neuroprotection in 6-OHDA- and hydrogen peroxide-induced dopaminergic cell death (158). In 6-OHDA and MPTP models of PD, Nrf2 knockout mice show increased vulnerability to the toxins and reduced glutathione concentration in contrast to the wild type mice, suggesting that the Nrf2/ARE pathway acts as an endogenous defense against neurotoxicity (227). Urate-induced Nrf2 accumulation in cells by inhibiting its ubiquitination and degradation, promoting Nrf2 translocation into nuclei, where it transactivates the transcription and translation of Nrf2

target genes including c-GCLC and HO-1, which are oxidation-related genes involved with redox homeostasis (228). However, urate does not modulate Nrf2 mRNA and Keap1 protein levels, nor does it disrupt Nrf2-Keap1 association (158).

Inosine, a nucleoside and purine precursor of urate (figure 1), has also been shown to have trophic protective effects on neurons and astrocytes after they are subjected to hypoxia (229) or glucose-oxygen deprivation (230). Inosine has been demonstrated to induce axonal growth by upregulating the expression of axonal growth-associated genes following neuronal insult in vivo and in vitro (231, 232). Its neuroprotective effects have also been suggested to occur by a receptor-independent mechanism, like its direct activation of the cytoplasmic protein kinase Mst3b as seen in rodents after a stroke or traumatic brain injury (233).

Extracellular inosine is also known to act via the G-protein coupled receptors of its aminated purine precursor adenosine, which are known to modulate cell death. In fact, adenosine A1, A2A and A3 receptors have been identified in studies to act as a mediator of inosine effects in the setting of inflammatory or ischemic injury (212, 234–236). Inosine was found to reduce ischemic brain injury in rats, likely via an adenosine A3 receptor-dependent pathway (212). In addition, our lab found that inosine could prevent oxidative stress induced-cell death in dopaminergic MES 23.5 cells cultured with astrocytes, independent of increased intracellular urate levels (237). However, because systemically administered inosine is rapidly metabolized to urate peripherally (238), it is likely that any neuroprotective effects of oral inosine are mediated by its metabolites.

Urate elevation therapy

The feasibility of elevating urate in humans as a therapeutic strategy was first systematically tested in multiple sclerosis (MS). Although intravenous administration of urate was found to be well tolerated and capable of significantly increasing serum total antioxidant capacity (239), Spitsin et al. found oral urate to have poor bioavailability (240). They went on to show that by contrast, oral administration of its precursor inosine increased both serum and likely CSF urate levels in MS patients (240). Though several MS trials of urate-elevating inosine did not consistently demonstrate slowed clinical progression, these studies established the feasibility of chronic urate elevation as a neurotherapeutic strategy and identified nephrolithiasis as the most common adverse effect and safety concern (241–244).

Whereas intravenous administration of urate is not a practical strategy for long-term urate elevation in neurodegenerative disease, it is being applied to effectively achieve a transient elevation of urate as a candidate protectant against acute neurotoxic insults. Specifically, when infused together with a thrombolytic agent during ischemic stroke, urate has shown potential benefit for long-term outcomes along with reduction in biomarkers associated with oxidative stress and neuroprotection (245–247).

For PD, the multiple convergent lines of laboratory, clinical and epidemiological evidence prompted us to pursue long-term urate elevation in a phase 2 clinical trial of oral inosine. The Safety of Urate Elevation in PD (SURE-PD) trial was a safety-focused, randomized,

double-blinded, placebo-controlled, dose-ranging trial of oral inosine. Its objectives were to determine the safety, tolerability and ability to raise urate of inosine taken daily by mouth for up to two years in early PD (248). It also sought to assess its suitability and potential design features for a disease-modification trial. SURE-PD enrolled 75 early PD subjects who were randomized at their baseline visits in a 1:1:1 distribution to three treatment groups: 1) placebo, 2) inosine titrated to mildly elevate serum urate (to 6.1 – 7.0 mg/dL), and 3) inosine titrated to moderately elevate serum urate (to 7.1 – 8.0 mg/dL). They found that treatment with inosine for up to 24 months was clinically safe and well tolerated at doses that elevated serum urate concentrations from a mean of 4.5 mg/dL to 6–7 and 7–8 mg/dL in the two dosing regimens. It provided proof-of-principle of the ability of oral inosine to raise urate to concentrations in CSF (>0.50 mg/dL) and serum (>6.0 mg/dL) that are predictive of slower disease progression in prior studies. In a secondary analysis we also noted modest trends towards slower PD progression rates with increasing urate, although the study was not powered to assess efficacy. Interestingly, they also found that treatment with inosine appeared to prevent slight worsening of depressive symptoms during the trial, which if confirmed in future studies, could strengthen the long-standing theory of enhanced motivation as one of the reasons for urate elevation during human evolution (249–252).

Regarding side-effects, though there were no gout-like episodes, 6% of subjects who were randomized to active drug developed a kidney stone. Despite theoretical concerns, inosine showed no evidence of adverse effect on blood pressure, body mass index, serum glucose and cholesterol or electrocardiographic parameters (248).

Currently a phase 3 study, SURE-PD3 trial; NCT02642393, is enrolling early PD patients within 3 years of diagnosis and not on dopaminergic medications. It is a multicenter, randomized, double-blind, placebo-controlled, phase 3 trial of placebo versus inosine. Its main objective is to determine whether oral inosine dosed to moderately elevate serum urate (from 5.7 mg/dL to 7.1–8.0 mg/dL) over 2 years slows clinical decline in early PD (253). Clinical decline will be assessed as change in the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS).

Urate and genetic PD

Associations between the multiple causative genes underlying familial PD and mitochondrial pathophysiology provide supportive evidence that oxidative stress secondary to mitochondrial dysfunction may contribute to genetic forms of PD. Alpha-synuclein, *Parkin*, *PINK1*, *DJ-1* and *LRRK2* mutations, are all associated with familial PD and are all proteins linked to mitochondrial function (254). In a study investigating idiopathic PD, *LRRK2 PD* and *LRRK2* controls, Johansen et al. found that urate was lower in both idiopathic and *LRRK2 PD* compared to controls (18% decrease, $p<0.01$). They also found a 10% decrease in urate levels in asymptomatic *LRRK2* mutation carriers, compared to controls, although the latter difference was not significant and further studies with larger numbers of asymptomatic *LRRK2* mutation carriers are warranted (255).

Urate and other neurological conditions

Given the common pathogenic mechanism of oxidative stress across neurodegeneration, urate has been considered as marker or modulator of other neurodegenerative and acutely neurotoxic disorders. Lower urate levels have been implicated in Lewy body disease more broadly (256), Alzheimer's disease and mild cognitive impairment (257–259). In a study investigating urate levels in 95 patients with Lewy body disorders (55 non-demented PD patients, PDND; 20 PD patients with dementia, PDD; and 20 Diffuse Lewy body patients) and 76 controls, the authors found lower serum urate in patients with Lewy body disorders compared to controls. They also saw higher CSF urate in PD patients without dementia (median 0.7 mg/dl) compared to patients with Lewy body disorders and dementia (0.4 mg/dl; $p = 0.03$) (256). A prospective population-based cohort study following 1,724 participants who were dementia free at 11 years, found that after correcting for cardiovascular risk factors, higher urate was associated with reduced risk of dementia (HR 0.89; 95% CI 0.80–0.99, per standard deviation increase in urate). Higher serum urate at baseline was also associated with better cognitive function later in life for all cognitive domains evaluated (257). A study that measured antioxidants including urate in AD, mild cognitive impairment and controls found lower levels of urate in patients with mild cognitive impairment and AD (258).

Reduced urate has also been associated with Huntington's disease (HD) (260), and amyotrophic lateral sclerosis (ALS) (261–265). Higher serum urate has been associated with slower progression of HD (260) and prolonged survival of ALS (263).

Recent or ongoing trials have investigated urate elevation for neuroprotection in patients with ALS. There was a pilot, open-label, phase 1 study of urate elevation with inosine in ALS (NCT02288091) that demonstrated the feasibility of using inosine to elevate urate levels in patients with ALS (264). A follow-up, multi-center, biomarker-driven, placebo-controlled phase 2 study (SURE-ALS2) is scheduled to begin enrollment in 2017.

Urate therapy in stroke showed encouraging clinical results following the Safety and Efficacy of Uric Acid in Patients with Acute Stroke (URICO-ICTUS) study, a phase 2b/3 randomized, double-blind trial of intravenous urate administration, within a few hours of stroke onset (246, 247). Although a greater proportion of excellent outcomes 3 months after the stroke was not demonstrated for the primary analysis (adjusted risk ratio of 1.23 for urate *vs* placebo; $p=0.099$), secondary analysis of this study indicated that the addition of urate treatment to thrombolysis in acute ischemic stroke improved these clinical outcomes in women ($p=0.036$). The finding mirrored that of a preliminary gender-stratified secondary analysis of the phase 2 study of urate-elevating inosine in PD in SURE-PD, raising the possibility that women, who have lower urate levels than men, might have more to gain by urate-elevating therapy.

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Purine metabolism in humans

Figure 1. Purine Metabolism in Humans: Urate as enzymatic end product due to urate oxidase gene (*UOx*) mutations during hominoid evolution

The catabolism of purines such as adenosine ends with urate in humans and apes (hominoids) due to multiple unique exonic mutations (red bar in circle) in the *UOx* gene (see text for references). The resulting loss of functional *UOx* and increase in urate concentrations distinguish hominoids from other mammals, in which *UOx* metabolizes urate disrupting its purine ring structure and generating allantoin. Although the putative evolutionary advantages of increased urate remain speculative, its established antioxidant properties have suggested health benefits including neuroprotective actions. However, the higher concentrations of urate in humans approach the limits of its solubility, occasionally

exceeding them, leading to crystal deposition disorders of uric acid kidney stones and gouty arthritis.

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