Potential mechanisms for low uric acid in Parkinson disease

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Abstract

Several epidemiologic studies have described an association between low serum uric acid (UA) and Parkinson disease (PD). Uric acid is a known antioxidant, and one proposed mechanism of neurodegeneration in PD is oxidative damage of dopamine neurons. However, other complex metabolic pathways may contribute. The purpose of this study is to elucidate potential mechanisms of low serum UA in PD. Subjects who met diagnostic criteria for definite or probable PD ($n = 20$) and controls ($n = 20$) aged 55–80 years were recruited. Twenty-four hour urine samples were collected from all participants, and both uric acid and allantoin were measured and corrected for body mass index (BMI). Urinary metabolites were compared using a twoway ANOVA with diagnosis and sex as the explanatory variables. There were no significant differences between PD and controls for total UA ($p = 0.60$), UA corrected for BMI ($p = 0.37$), or in the interaction of diagnosis and sex on UA ($p = 0.24$). Similarly, there were no significant differences between PD and controls for allantoin ($p = 0.47$), allantoin corrected for BMI ($p = 0.57$), or in the interaction of diagnosis and sex on allantoin ($p = 0.78$). Allantoin/UA ratios also did not significantly differ by diagnosis ($p = 0.99$). Our results imply that low serum UA in PD may be due to an intrinsic mechanism that alters the homeostatic set point for serum UA in PD, and may contribute to relatively lower protection against oxidative damage. These findings provide indirect support for neuroprotection trials aimed at raising serum UA.

Keywords

Uric acid; Allantoin; Oxidative stress; Metabolism; Parkinson disease

Introduction

Idiopathic Parkinson disease (PD) is a neurodegenerative disorder characterized by degeneration of dopamine neurons in the substantia nigra. The mechanisms responsible for

neurodegeneration are not entirely understood, but several studies have pointed to a relationship between PD and uric acid (UA) (Shen et al. 2013). Several studies have shown a decreased risk of developing PD in people with high plasma UA levels, and slower progression in early-stage PD patients with higher UA levels (de Lau et al. 2005; Elbaz and Moisan 2008; Schiess and Oh 2008; Schwarzschild et al. 2008). UA is also lower than normal in the PD substantia nigra and caudate (Church and Ward 1994).

Oxidative stress has been considered as a potential cause of neurodegeneration in PD (Jenner 2003), and UA is believed to be an antioxidant (Andreadou et al. 2009). Therefore, one proposed mechanism for the association of low UA with PD is the lack of adequate antioxidant defenses (Andreadou et al. 2009; Jenner 2003). This concept has encouraged the development of neuro-protective trials in PD that involve increasing UA (Schwarzschild et al. 2014).

While several studies suggest an association between low UA and PD, they do not prove causation. Although it is possible that low UA may be a potential cause of PD, it is equally plausible that the underlying metabolic processes of PD lead to low UA. The oxidative stress implicated in the loss of nigral neurons may consume UA resulting in low levels. In this case, the low UA may be simply a reflection of the progressive disease process, and not necessarily causative (Fig. 1).

Serum UA reflects a balance between endogenous production, external sources, and pathways for elimination (Becker and Roessler 1995; Jinnah and Friedmann 2000). The major source for endogenous production UA comes from intracellular metabolism of purines. Diet provides another source via catabolism of DNA and RNA contained within the cells of meats and vegetables (Gao et al. 2008). The interaction between some food items, such as ethanol and fructose, with purine metabolism causes increased purine turnover and subsequently an increase in UA production. Thus low serum UA could reflect an abnormality of purine metabolism, dietary differences, or an abnormal interaction between diet and purine metabolism.

In humans, UA and its oxidative product, allantoin, are eliminated almost entirely by the kidneys, and they accumulate in the urine as waste products. Unlike serum levels that reflect a rapidly changing equilibrium between inputs and outputs with significant diurnal variation (Devgun and Dhillon 1992) a 24-h urine sample provides a more stable and precise estimate of total daily UA sources and elimination in the body, especially when corrected for body mass index (Puig et al. 2012). Allantoin also provides an index of oxidative damage to UA (Mikami et al. 2000).

Considering the metabolism of UA, there are four possible mechanisms for low serum UA in PD. The first mechanism is low UA production, which would be supported by a low total urinary UA ? allantoin. The second is more rapid renal clearance of UA, which would be reflected by relative high urinary UA ? allantoin in PD. A third possible mechanism is that UA is being directly consumed by an oxidative process, which would be reflected by a disproportionately high urinary allantoin or high allantoin/UA ratio with relatively normal levels of total UA ? allantoin. Finally, low serum UA in PD may be due to serum UA being

homeostatically regulated at a lower level in PD patients compared to controls. This hypothesis would be manifested as normal urinary UA ? allantoin with a normal allantoin/UA ratio. Thus, a 24 h urine sample might reveal if low serum UA in PD is due to deficient UA production, more rapid renal clearance, augmented UA consumption by oxidative damage, or other intrinsic mechanism regulating a lower set-point of UA in PD. In the current studies we compared 24-h urinary UA and allantoin in patients with PD and healthy controls to discriminate among these possibilities.

Methods

Subject selection

This study was approved by the Emory Institutional Review board. Forty individuals between the ages of 55–80 years were recruited from the Emory Movement Disorder Clinic and signed an approved written informed consent form for participating in this study. Demographic and clinical information was gathered including age, ethnicity, body mass index (BMI), level of education, comorbid chronic medical problems, and medications. Subjects with PD were diagnosed at least 3 years prior to enrollment and met diagnostic clinical criteria for definite or probable PD, including responsiveness to levodopa (Gelb et al. 1999). All PD subjects had the severity of disease rated with the 2007 revised Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS UPDRS), and all were taking levodopa (Goetz et al. 2007). The details of their disease course were recorded including duration of illness and any associated complications. Baseline cognitive function was scored using the Montreal Cognitive Assessment (MOCA) (Kandiah et al. 2014). Exclusion criteria included history of gout, nephrolithiasis, renal insufficiency, treatment with UA lowering agents (e.g. diuretics), and smoking. These exclusion criteria were designed to avoid confounding factors known to affect urinary UA levels. The final analyses were conducted with 10 male control subjects, 10 male PD subjects, 10 female control subjects and 10 female PD subjects.

Sample collection and preparation

Urine samples were collected from all 40 participants using a 24-h collection container that held 2 mL of 1 % sodium azide as a preservative. Urinals were gender specific, and instructions were given on how to collect the urine over a 24 h period. Upon receiving a returned sample, all subjects were questioned about their method of collection to ensure a complete sample. Samples were kept at room temperature and processed within 24 h of final collection. A 2L graduated cylinder was used to measure the volume of each sample. The urine samples were heated to 37–40°C and mixed for 30 min to ensure UA was dissolved. Samples were divided into 10 aliquots of 1 mL and stored at −80°C.

Determination of UA

UA was measured in duplicate using the Caymen Uric Acid Assay Kit (Caymen Chemical Company Ann Arbor, MI). This assay uses a florescence-based method to detect UA via uricase-mediated catalysis of UA to allantoin. Urine samples were diluted 1:5–1:10 with an assay buffer comprised of 100 mM Tris–HCl (pH 7.5). UA standards were prepared using varying concentrations of UA stock and assay buffer to create final concentrations ranging

from 0 to 50 μM. A 96-well plate was set up in duplicates with standard UA preparations containing 100 μL of assay buffer, 20 μL of fluorometric detector consisting of 10-acetyl-3,7 dihydroxyphenoxazine and dimethylsulfoxide, and 10 μL of the standard consisting of varying concentrations of UA.

An enzyme mixture was prepared from lyophilized powder of uricase and horseradish peroxidase, reconstituted with 1.2 mL of assay buffer, and then placed on ice. After the plate was set up, 20 μL of the enzyme mixture was added to each well of the reaction mixture for all standards and samples. The plate was incubated for 15 min at room temperature. The plate was then analyzed in a spectrophotometer to measure fluorescence using excitation wavelengths between 530–540 nm and emission wavelengths between 585 and 595 nm. The numerical value obtained after analysis represented the fluorescence for each standard and sample. These fluorescence values for both standards and samples that were measured in duplicate were then averaged. The fluorescence value of the first standard that contained no UA was then subtracted from all other standards and samples. This value was the corrected fluorescence. The corrected fluorescence values of each standard were plotted as a function of the final UA concentration (0–50 μM). The slope and y-intercept was then derived from this standard curve to calculate the UA concentrations (μM) for all samples.

Determination of allantoin

Allantoin measurements were conducted using an assay involving ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS), as previously described (Tolun et al. 2010). The absolute concentrations of allantoin were measured, and then normalized to the urinary creatinine concentration determined using stable isotope dilution-LC–MS/MS, as previously described (Young et al. 2009).

Statistical analysis

The data were analyzed using SPSS Statistics version 21.0 (IBM Corp). Descriptive statistics were calculated as a mean and standard error for each study group. An unpaired t test was used to compare study groups by age, BMI, disease severity by UPDRS, and MOCA score. A two-way analysis of variance (ANOVA) was implemented to determine the main effect and interaction of gender and diagnosis of PD on UA, UA corrected for BMI (UA/BMI), allantoin, and allantoin corrected for BMI (allantoin/BMI). Assumptions prior to performing a two-way ANOVA were tested including testing for normality via Shapiro–Wilk's test, homogeneity of variances, and identifying any significant outliers. Statistical significance was determined using a $p < 0.05$.

Results

Clinical and demographic characteristics for the four groups are shown in Table 1. Ninety-five percent of study participants were Caucasian with two exceptions (1 Asian, 1 Indian). There were no significant differences for age among all groups, and BMI did not significantly differ between PD patients and controls for males or females. Male controls had a higher BMI than female controls ($p = 0.01$). However, a difference was not seen when comparing BMI for all male subjects to all female subjects. PD patients had a mean total

UPDRS score of 30.2. The mean duration of disease for all patients was 7.2 years. Cognitive function was not significantly different between male and female PD patients.

Urinary metabolites were compared using a two-way ANOVA with diagnosis and sex as the explanatory variables. The 24-h total UA was higher in males than females ($F = 10.0$; $p = 0.003$). However there were no statistically significant differences between PD and controls ($F = 0.28$; $p = 0.60$). The interaction of diagnosis and sex was not statistically significant ($F = 0.54$; $p = 0.47$). Similar results were found when correcting UA for BMI (UA/BMI). UA/BMI was higher in males ($F = 6.1$; $p < 0.02$), but there were no statistically significant differences between PD and controls ($F = 0.83$; $p = 0.37$), or when considering the interaction of diagnosis and sex ($F = 1.44$; $p = 0.24$).

Allantoin levels did not significantly differ by diagnosis ($F = 0.53$; $p = 0.47$) or sex ($F = 0.00$; $p = 0.97$). The interaction of diagnosis and sex with allantoin was also not statistically significant ($F = 0.08$; $p = 0.78$). When correcting allantoin for BMI (allantoin/BMI), there were no significant differences for diagnosis ($F = 0.29$; $p = 0.59$) or sex ($F = 0.33$; $p = 0.57$). The interaction of diagnosis and sex with allantoin/BMI was not statistically significant ($F = 0.20$; $p = 0.66$).

The ratio of allantoin to UA (allantoin/UA) did not significantly differ by diagnosis ($F = 0.00$, $p = 0.99$) or sex ($F = 2.23$, $p = 0.14$). In addition, the interaction of diagnosis and sex with allantoin/UA was not statistically significant ($F = 1.51$, $p = 0.23$).

Discussion

Our study aimed to elucidate the potential mechanisms for low serum UA in PD by evaluating 24 h urinary excretion of UA and its oxidized metabolite, allantoin. The results showed that urinary UA and allantoin levels were not significantly different when comparing PD patients with controls, even when correcting for BMI. These findings have important implications regarding prior studies reporting low serum UA in PD and the concept of using drugs that elevate UA as a neuroprotective strategy in PD.

The normal urinary UA and allantoin levels argue against decreased production of UA or abnormally low dietary sources of UA in PD subjects. Our results also argue against rapid renal clearance of UA in PD, a finding consistent with the absence of any apparent relationship between PD and uric acid nephrolithiasis. The normal allantoin and allantoin/UA ratios in the urine also argue against oxidative damage of UA leading to low serum UA levels in PD. Therefore, low UA is unlikely to be a ''byproduct'' of oxidative stress. The relatively normal UA and allantoin levels in PD suggest instead that the low serum UA in PD is due to a lowered homeostatic set point. As a result, serum levels of UA may be chronically low, but urinary levels are normal. Serum UA is regulated by complex mechanisms that involve renal urate transporters and other poorly understood mechanisms, which may contribute to the altered set point for serum UA as opposed to increased elimination or decreased production (Albrecht et al. 2014; Sperling 2006). For example, genetic polymorphisms in the SCL2A9 renal urate transporter affect individual variability in serum urate concentrations (Simon et al. 2014).

Our study has limitations. First, we recruited relatively small numbers of participants. Although urinary UA was not significantly different between PD and control subjects, there was a trend for higher urinary UA in controls, particularly among males. A post hoc power analysis with current sample size of $N = 20$ (males only) allows us to exclude a 30 % difference with 95 % confidence. The sample size needed to exclude a 20 % difference would be $N = 50$ ($p = 0.05$). There was also a trend for higher urinary allantoin in PD patients compared to controls. Although this finding was not statistically significant, a post hoc power analysis with the current sample size of $N = 40$ (both males and females) allows us to exclude a 30 % difference with 95 % confidence. The sample size needed to exclude a 20 % difference in allantoin would be $N = 100$ ($p = 0.05$). However, it is not clear if such small differences in urinary UA or allantoin have biological significance, because differences in UA are usually exaggerated when urine is measured as compared to serum. For example patients with Lesch–Nyhan disease have a metabolic disorder of purine metabolism that results in increased UA production. Average serum UA levels are increased less than twofold, but 24 h urinary UA excretion is increased more than fourfold (Jinnah et al. 2010).

A second potential limitation of the current study is that subjects were not instructed to follow a low purine diet prior to urine collection. Therefore a systematic bias in dietary habits between PD and controls cannot be excluded. However, we aimed to replicate conditions used in prior epidemiological studies showing low serum UA in PD, where subjects were allowed to maintain their usual diets. Additionally, prior epidemiological studies have implied differences in diet are not responsible for low serum UA in PD (Davis et al. 1996). A third potential limitation is that we did not simultaneously measure serum UA to confirm prior epidemiological studies that serum UA is low in PD, and our population may have been unrepresentative of these prior studies. We did not attempt to confirm low serum UA in our PD population because serum differences are small, and a substantially larger sample size would be needed to reproduce the prior epidemiological studies. A fourth potential limitation is that levodopa has been reported to interfere with renal excretion and increase serum UA (Bierer and Quebbemann 1982), and all of the PD patients in this study were taking levodopa. However, these influences of levodopa are small, and prior epidemiological studies have shown low serum UA, not high serum UA in PD. Furthermore, a previous study found an inverse relationship between serum UA and levodopa use, thought to be due to high dopamine turnover and consumption of UA by an increased oxidative load (Andreadou et al. 2009). The final limitation is that the current study was conducted with urine samples as a measure of whole body UA metabolism. Abnormalities restricted to the brain may not be measurable because it provides a relatively small contribution to whole body UA production. To address some of these limitations, future studies may require studying larger groups of PD patients on low-purine diets who are not taking levodopa.

In conclusion, our results imply that low UA in PD may be due to an intrinsic mechanism that alters the homeostatic set point for serum UA in PD. In other words, serum UA is regulated so that it remains at a lower level in PD compared to normal individuals. If this is the case, the chronically lower levels of UA may contribute to relatively lower protection against damage from oxidative stress in PD. These studies provide indirect support for neuroprotection trials aimed at raising serum UA in PD.

Acknowledgments

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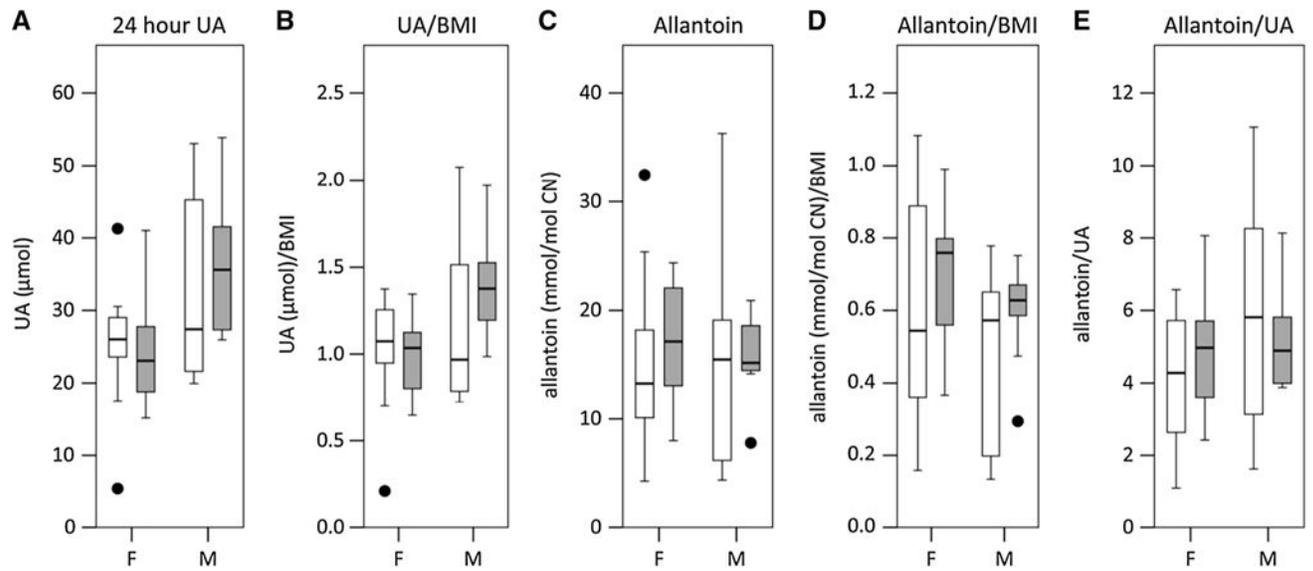
Fig. 1.

Uric acid (UA) and allantoin measures. and **c** show total 24 h measures for patients with Parkinson disease (PD, *gray boxes*) and healthy controls (*white boxes*), while **b** and **d** show the same m easures corrected for body mass index (BMI). Allantoin was measured relative to urinary creatinine (mmol/mol CN). The results are shown as *box-whisker* plots, where the *horizontal line* is the median, the *boxes* depict upper and lower quartiles of data points, *whiskers* depict the entire data range, with outlier points shown as individual *symbols*

Table 1

Demographic and clinical information

	Male control ($N = 10$)	**Male PD ($N = 10$)**	**Female control ($N = 10$)**	**Female PD ($N = 10$)**
Age	66.0 ± 2.3	66.9 ± 1.7	63.5 ± 1.6	66.2 ± 1.7
Race	All Caucasian	9 Caucasian, 1 Asian	All Caucasian	9 Caucasian, 1 Indian
BMI (kg/m^2)	29.2 ± 2.0	26.4 ± 0.9	25.1 ± 1.0	25.3 ± 1.7
UPDRS	NA	33.5 ± 2.8	NA	26.9 ± 2.5
MOCA	NA	26.0 ± 0.9	NA	27.9 ± 0.6
Disease duration	NA	7.8 ± 1.4	NA	6.5 ± 1.4

Results show mean and standard error for age, race, BMI, UPDRS score, MOCA score, and duration of PD

NA not applicable