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CSF xanthine, homovanillic acid, and their ratio as biomarkers of Parkinson's disease

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Abstract

Diminished nigrostriatal dopaminergic neurotransmission is a biochemical hallmark of Parkinson's disease. Despite this, a reliable trait biomarker of sporadic Parkinson's disease has not emerged from measurements of cerebrospinal fluid dopamine metabolites. Previous studies have highlighted strong neurochemical relationships between dopamine and various purine compounds. In this study, we analyzed cerebrospinal fluid concentrations of homovanillic acid (the major catabolite of dopamine) and the purine compound xanthine for a comparison of 217 unmedicated Parkinson's disease subjects and 26 healthy controls. These compounds were highly correlated for both the Parkinson's disease subjects ($r=0.68$) and for controls ($r=0.73$; both groups, $p<0.001$). While neither homovanillic acid nor xanthine concentrations differentiated Parkinson's disease from controls, their ratio did. For controls, the mean [xanthine]/[homovanillic acid] quotient was 13.1±5.5 as compared to the Parkinson's disease value of 17.4±6.7 at an initial lumbar CSF collection ($p=0.0017$), and 19.7±8.7 ($p<0.001$) at a second CSF collection up to 24 months later. The [xanthine]/[homovanillic acid] ratio in the Parkinson's disease subjects differed as a function of disease severity, as measured by the sum of Unified Parkinson's Disease Rating Scale Activities of Daily Living and Motor Exam ratings. The [xanthine]/[homovanillic acid] ratio also increased between the first and second CSF collections, suggesting that this quotient provides both

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a *state* and *trait* biomarker of Parkinson's disease. These observations add to other neurochemical evidence that links purine metabolism to Parkinson's disease.

Keywords

Parkinson's disease; Biomarker; Dopamine; Homovanillic acid; Xanthine; Cerebrospinal fluid

1. Introduction

Based just on clinical history and examination, experienced clinicians can discern the clinical features of Parkinson's disease (PD) with a high degree of sensitivity and specificity (Hughes et al., 1992). Nonetheless, there is a continuing need for enhanced diagnostic capabilities, especially at the earliest stages of this disorder. Even when Parkinsonian signs and symptoms are relatively mild, the pathological impact of the disease is already advanced due to extensive loss of dopamine-synthesizing neurons in the substantia nigra pars compacta (SNpc) (Hornykiewicz and Kish, 1986). Biomarkers that can detect PD at very early or even pre-clinical stages are needed if effective neuroprotective strategies are to be utilized. Beyond its value as a diagnostic tool, a biomarker for PD is likely to offer insights into its pathophysiology.

Researchers have explored a diversity of clinical and laboratory tests in efforts to differentiate PD patients from a healthy population. Among these are transcranial sonography (Vlaar et al., 2009) and other applications of neuroimaging (Ravina et al., 2005; Vaillancourt et al., 2009). While radiotracer studies using positron or single photon emission computed tomography can demonstrate SNpc neuronal dropout through measurements of dopaminergic nerve terminal decline, these methods are impractical for screening purposes or for detecting the earliest stages of PD (Scherfler et al., 2007). Other evaluations yielding distinctive but non-specific changes in PD include testing of olfactory function (Verbaan et al., 2008), cardiac sympathetic innervation (Fujishiro et al., 2008), motor performance (de Frias et al., 2007), eye movements (Rivaud-Péchoux et al., 2007), and various motor reflexes and evoked responses (Meigal et al., 2009). Extensive biochemical analysis of cerebrospinal fluid (CSF) and blood has been conducted for dopamine metabolites, α-synuclein, and other CSF constituents offering diagnostic potential (Antoniades and Barker, 2008; Bogdanov et al., 2008; LeWitt and Galloway, 1990; Michell et al., 2008; Zhang et al., 2008). Though the search for PD biomarkers has led to some promising candidates, none has provided a reliable diagnostic test.

The CNS metabolism of purine compounds has garnered attention in PD research because of strong associations found between serumurate concentration and the risk for developing this disorder (Schlesinger and Schlesinger, 2008). Furthermore, both serum and CSF urate concentrations are inversely correlated with the rate of PD progression (Ascherio et al., 2009). These findings have been interpreted as evidence for a possible neuroprotective effect conferred by urate. As a strong antioxidant, urate in the PD patient might add to defenses against a disease mechanism acting through oxidative stress (Moore et al., 2005). On the other hand, the relationships observed between PD and systemic urate concentration might

reflect an alteration of purine metabolism (especially that of adenosine) on the basis of its interplay with striatal dopamine neurotransmission (Stone et al., 1989). Adenosine receptors are involved in modulating striatal dopamine release (Jin et al., 1993; Okada et al., 1996). Other pharmacological implications of dopamine–adenosine relationships have been demonstrated by clinical trials showing enhanced anti-Parkinsonian effect of levodopa with co-administration of a selective adenosine receptor antagonist (LeWitt et al., 2008). Besides adenosine, other purine compounds also interact with dopamine metabolism (Loeffler et al., 1998, 2000). Understanding the particular link between dopamine neurotransmission and purines has been challenging because the latter compounds are abundant throughout the CNS and serve in a variety of roles (involving nucleic acids, energy transfer, and cellular signaling).

The relationship between striatal dopaminergic neurotransmission and purine metabolism led us to investigate for a PD biomarker associated with these neurochemical systems. Of particular interest has been xanthine (XAN), the second-to-last intermediate formed before the purine end-product in man, urate (Fig. 1). Toghi et al. (1993) reported that CSF XAN concentration was decreased by 19% in 11 PD subjects as compared to 14 controls. We sought to confirm these observations and hypothesized that indexing CSF XAN concentration to that of the dopamine metabolite homovanillic acid (HVA) might be informative as a PD biomarker, since high correlation between concentrations of these CSF constituents has been reported (Niklasson et al., 1983).

2. Results

2.1. Comparisons of PD subjects and controls

Demographic and clinical information for the PD subjects are listed in Table 1. The healthy control group consisted of 13 males and 13 females, with a mean age (±S.D.) of 40.6±11.8 years (range: 21–63 years). Measurements of CSF HVA concentration in both the control and PD subjects were comparable to values previously reported (Ballenger et al., 1980; LeWitt and Galloway, 1990), as were measurements of CSF XAN concentration (Amorini et al., 2009; Degrell and Niklasson, 1988; Eells and Spector, 1983; Kurčacka et al., 1996; Niklasson et al., 1983; Stover et al., 1997; Toghi et al., 1993). CSF concentrations (nM) of XAN and HVA were highly correlated for both the healthy control ($r=0.73$) and for the PD subjects ($r=0.68$ for the initial CSF collection; for both controls and PD, $p<0.001$). The quotients of the molar concentrations of XAN divided by HVA were significantly increased over control values for the first and second CSF collections from PD subjects (Table 2 and Fig. 2). In the second (but not the first) CSF collection from PD subjects, there was a significant reduction (19%) from control values in mean HVA concentration ($p=0.046$). Our CSF XAN findings also differed from a report of a 19% reduction of XAN concentration in PD specimens (Toghi et al., 1993) in that we found an 18% increase over control values ($p=0.032$). The methods of CSF collection differed between the two studies are one possible explanation for the differences between the two studies with respect to CSF XAN concentration.

Since the mean age of the control group was younger than that of the PD subjects, we analyzed CSF results from younger sub-sets of PD subjects in our cohort to investigate

whether the observed differences in HVA and XAN concentrations and their ratio might be confounded by an effect of age. We assembled a group comprising 29 PD subjects whose ages at time of CSF collection were ≤50 years (Table 3a) and another sub-set of 58 subjects whose ages were ≤55 years (Table 3b). These sub-sets provided a comparison of PD subjects closer in age to the control group. For each of the younger PD subject groups, the [XAN]/[HVA] ratios measured in the second CSF collection were increased significantly versus controls (and as also was the result for the first CSF collection in the ≤55 year-old group). On this basis, we concluded that age alone couldn't account for the observed differences in [XAN]/[HVA] ratios between PD subjects and controls.

2.2. CSF changes from 1st to 2nd collections

In Table 4a, we assessed the effect of time on the CSF HVA and XAN changes observed from the first to second CSF collections. For this analysis, we restricted the sample set to just those subjects whose experimental treatment regimen in the DATATOP study was either placebo or α-tocopherol. By excluding subjects receiving the monoamine oxidase-B inhibitor selegiline, our intent was to avoid the potentially confounding neuropharmacological effects of this drug on HVA concentration. Selegiline increases CNS dopamine concentration by slowing its catabolism and inhibiting synaptic dopamine re-uptake (Ebadi et al., 2002). For subjects not receiving selegiline, there was no significant change in HVA concentration as compared to controls (and confirming other research findings that CSF HVA content is not a useful indicator for the progressive loss of dopaminergic SNpc neurons in PD). For those subjects receiving selegiline, this monoamine oxidase-B inhibitor would be expected to diminish CSF HVA concentration. As expected, our data showed less formation of HVA in the second CSF collection for the selegiline-treated subjects (Table 4b), as compared to subjects not receiving this drug (Table 4a). Selegiline treatment also was associated with a change in mean CSF XAN concentration over time that was not observed between CSF collections for PD subjects who were not receiving this drug. In the DATATOP study, the 2nd CSF collection was carried out after variable (staggered) periods of drug washout (Parkinson Study Group, 1993, 1995). For most subjects, this was 4 weeks, although some had the specimen collection 1 day after the drug was stopped while others waited as long as 6 weeks. Despite these varying intervals, continuing MAO-B inhibition from selegiline was expected to persist through 4 weeks and possibly for longer (Fowler et al., 1994).

For the 217 PD subjects whose data was analyzed in the report, a mean of 15.8±7.9 months elapsed between first and second CSF collections; UPDRS Part II (Activities of Daily Living) increased, on average, by 5.2 points and UPDRS Part III (Motor Exam) worsened by a mean of 11.4 points. During this time (Tables 4a and 4b), the [XAN]/[HVA] ratio increased for the non-selegiline subjects by a mean of 1.2 ($p=0.01$) and for the selegiline-treated subject, by 3.6 ($p<0.001$).

2.3. CSF findings and correlations to clinical data

To explore further questions about the utility of the [XAN]/[HVA] ratio as a biomarker of PD, the CSF data was analyzed in additional sub-divisions of the total PD group. The [XAN]/[HVA] ratio did not differentiate between the 21 subjects with a slower pace of PD

progression (e.g., PD symptomatology that evolved for ≥2 years prior to CSF collection) compared with 24 subjects with more rapid worsening (defined as the onset of PD symptoms beginning ≤1 year before and reaching Hoehn and Yahr stage of at least 2.5 by the time of the second CSF collection): 19.3±6.0 versus 16.8±8.1; p=0.247. These conditions of slower and more rapid progression of PD were explored previously in an analysis of the entire DATATOP study population (Jankovic et al., 1990). For the 7 subjects whose age of PD onset was ≤40 years as compared to those ≥70 years (40 subjects), their mean [XAN]/[HVA] ratio also did not differ significantly: 14.8±7.9 versus 16.5±5.7, p = 0.544. However, subjects with the later onset of PD had increased mean CSF XAN concentration as compared to the subjects with earlier PD onset: 2855.9±929.8 versus 2010.4±293.0 nM (p<0.001). We also examined a sub-group of 33 PD subjects who, at the time of the first CSF collection, had a total of combined UPDRS Part II and Part III scores ≥40, for comparison to 72 subjects whose combined total scores were < 20 (Table 5). For the group with lower UPDRS scores, the mean [XAN]/[HVA] ratio was significantly increased (p = 0.02). Taken together, characteristics of the [XAN]/[HVA] ratio suggest that it has components of both a state and trait marker of PD.

We explored other pertinent questions by exploring possible relationships between the CSF findings and additional measures of Parkinsonism. The Purdue Pegboard Task was scored by the number of pegs correctly placed over 30 s by the right and the left hands (Hietanen et al., 1987). The scores for this test did not show significant correlation with mean XAN or HVA concentrations, or with the [XAN]/[HVA] ratio. The UPDRS Part II (Activities of Daily Living) composite score also did not correlate with these measurements. However, a small inverse relationship was found between UPDRS Part III (Motor Exam) and the [XAN]/[HVA] ratio, with higher values of the UPDRS Part III score being associated with lower [XAN]/[HVA] ratio, and vice versa (−0.148, p= 0.029). An analysis of Schwab and England Activities of Daily Living ratings (carried out by the investigators) revealed that PD subjects assessed to have ≥90% of normal function had greater [XAN]/[HVA] ratios than subjects judged to have ≤85% of normal function (18.0±6.7 versus 15.7±6.7, p = 0.033).

In previous studies, an improved prognosis for disability and PD progression has been linked to the presence (or clinical prominence) of resting tremor (Birkmayer et al., 1979). For this reason, we selected a subset of PD subjects with at least one UPDRS rating of resting tremor that was “1” (mild) or greater by either history or examination. CSF findings from the 33 subjects lacking resting tremor, as compared to the 175 subjects with a rating of ≥1 for resting tremor, showed no differences with regard to mean XAN or HVA concentration or to the [XAN]/[HVA] ratio. Using a paradigm previously used for investigation of the DATATOP cohort (Jankovic et al., 1990), subjects were categorized as either PIGD-predominant or tremor-predominant. Comparing the 92 PIGD subjects to the 94 tremor-predominant subjects, a statistically significant difference emerged for only one item in the CSF profile: the lower mean HVA concentration found in the tremor-predominant subjects (156.9±97.8 nM versus 187.7±115.8 nM, p=0.05). Though these and others of the pre-specified statistical analyses of this data have not been corrected for multiple observations, these exploratory results are of interest because of their clinical relevance.

3. Discussion

Although neurodegeneration in PD arises in several brain regions (Braak and Del Tredici, 2008), the midbrain lesion responsible for motor impairment has been a major focus for biomarker discovery. The loss of SNpc neurons projecting to the striatum leads to markedly reduced tissue concentrations of dopamine and its metabolite HVA (Hornykiewicz and Kish, 1986). Turnover of dopamine in caudate and putamen makes a major contribution to HVA measured in CSF (Ballenger et al., 1980). Although there are several reports that a reduced CSF HVA concentration is correlated to disease severity and can distinguish PD subjects from healthy controls (Chase, 1980; LeWitt and Galloway, 1990), our measurements of CSF HVA concentration did not confirm these observations.

In this exploratory study, indexing the CSF concentrations of XAN to HVA permitted the PD subject group to be distinguished from healthy controls, and so this ratio can be regarded as a trait marker of PD. A trait marker is an all-or-none feature or a surrogate disease indicator (whether or not its presence precedes the onset of the disease). In contrast, a state marker offers a gradation of changes in correlation to the extent of the disease (for example, its duration or severity). While [XAN]/[HVA] also provided some state marker characteristics (for example, correlation to UPDRS scores), the most robust effect of this ratio appears to be its differentiation of PD and control groups. We recognize that the considerable overlap in [XAN]/[HVA] ratio means that this measurement does not provide a diagnostic test for individual subjects. Also, specimens from subjects with “Parkinson-plus” disorders were not available for study, and so we cannot comment on the relative specificity of an altered [XAN]/[HVA] ratio for the PD subjects (whose diagnosis was based on clinical criteria alone). Although we used established assay methods for studying in duplicate a large number of samples, it has not been able to conduct test–retest reliability assessments to verify further the validity of our results.

Another limitation of this study is the older mean age of the PD subjects versus controls, differing by approximately 2 decades. Despite this, we attempted to assess the effect of age by evaluating subsets of younger PD subjects. These subset analyses indicated that the increased [XAN]/[HVA] ratio in PD was not an artifact of older age. Younger age-of-onset Parkinsonism is associated with increased likelihood of having the LRRK2 gene or another genetically-determined form of Parkinsonism (Hardy, 2010) rather than sporadic PD; hence, any comparison of age-matched controls to younger PD subjects might be confounded by the additional factor of hereditary Parkinsonism subjects intermingled with idiopathic PD. Finally, in the analysis of data, we recognize that the statistical correlations have not been corrected for multiple comparisons. However, beyond the primary analysis presented in Table 2, the other statistical testing can be regarded as exploratory exercises in an effort to guide future investigations of CSF and other specimens for biomarkers related to dopamine and purine metabolites.

Our exploratory data suggests that the information from measuring CSF [XAN]/[HVA] ratios might provide a biochemical clue for PD. This would be especially interesting if similar changes are not found with other Parkinsonian syndromes that also involve degeneration of dopaminergic SNpc neurons (such as multiple system atrophy and

progressive supranuclear palsy). The specificity of an altered [XAN]/[HVA] ratio in PD and "Parkinson-plus" disorders will be explored in an upcoming clinical investigation. Other questions to be explored include investigation of whether an increased altered [XAN]/[HVA] ratio might help in the detection of PD at a pre-symptomatic stage, and whether the biochemical changes leading to the altered ratio are present systemically in blood or other specimens.

The high correlation we observed between XAN and HVA is consistent with known interactions between the metabolism of dopamine and purines (Loeffler et al., 1998; Xie et al., 2007). Like HVA, whose CSF concentration rises in sequential aliquots collected from the lumbar region (LeWitt et al., 1992), the CSF concentration of XAN increases with sampling of more rostral specimens (Niklasson and Ågren, 1984). XAN does not diffuse across the blood–brain barrier and its concentration in CSF is approximately 5-fold greater than in plasma (Niklasson et al., 1988). Taken together, these observations support a hypothesis that XAN measured in CSF is, like HVA, representative of its brain metabolism.

While HVA is solely the end product of dopamine turnover, XAN arises from several sources whose catabolism converges on a common pathway (Fig. 1). Its production and clearance are subject to several physiological influences. For example, tissue and CSF concentrations of XAN can be enhanced by physiological stresses including hypoxia, ischemia, glutamate-mediated excitotoxicity, and various CNS inflammatory disorders (such as infection and multiple sclerosis) (Amorini et al., 2009; Stover et al., 1997). Adenosine and adenosine-5′-triphosphate (ATP), which are released from glia and neurons in neurotransmitter and neuromodulatory roles (Burnstock, 2008), also contribute to the creation of XAN. Within a single category of physiological stress such as hypoxia, increased XAN production arises from several origins, including the nucleosides adenosine, inosine, and guanosine, the cyclic nucleotides, and phosphate-bound nucleotide energy transfer molecules. Once formed, XAN is not converted irreversibly to urate since some of it can be recycled into a purine salvage pathway that starts with the synthesis of inosine monophosphate.

Perhaps the best clue to understanding [XAN]/[HVA] ratio as a PD biomarker comes from the high correlation between mean CSF concentrations of XAN and HVA. The loss of dopaminergic SNpc neurons may be responsible for altering concentrations of both compounds. In the PD brain, putamen and caudate nucleus specimens demonstrate an increased ratio of HVA relative to dopamine concentrations, as compared to controls (Hornykiewicz and Kish, 1986). These findings support an enhanced rate of dopamine synthesis within striatal nerve terminals that originate from the remaining SNpc neurons (Zigmond et al., 2002). Since the CNS turnover of dopamine appears to be closely linked to that of striatal purine metabolism (Jin et al., 1993; Loeffler et al., 1998; Okada et al., 1996), the altered CSF [XAN]/[HVA] ratio in PD may result from upregulated dopamine synthesis in the surviving dopaminergic nerve terminals. Another possible explanation for increased XAN concentration in PD CSF is that it reflects chronic impairment of mitochondrial function known to occur in PD as a result of a reduction in Complex 1 electron transport chain activity (Büeler, 2009). The consequences of this metabolic defect include impaired generation of cellular energy and increased mitochondrial production of oxyradicals

(Arduíno et al., 2010). Oxidative stress leads to enhanced release and degradation of adenosine and ATP, which results in increased formation of XAN (Amorini et al., 2009; Cristofori et al., 2005; Stover et al., 1997). A more thorough understanding of disease-specific implications for the [XAN]/[HVA] ratio will require further study of how the purine pool is generated and regulated in the brain.

4. Experimental procedures

4.1. Subjects

We studied CSF specimens from PD subjects participating in a controlled clinical trial of possible neuroprotective treatments, "*Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP)*" (Parkinson Study Group, 1989a,b, 1993). Enrolled subjects, whose age ranged between 30 and 79 years, were affected by PD for ≤5 years and manifested relatively mild Parkinsonian symptomatology. They were selected by PD specialists based on distinctive features of the disorder and the absence of evidence for other neurodegenerative conditions or secondary causes of Parkinsonism. Among exclusion criteria were clues for other CNS disorders, prior brain surgery, and clinically significant depression or cognitive impairment. During the clinical trial, subjects could not receive anti-Parkinsonian drugs or other CNS-active medication. Anticholinergics or amantadine, if previously used, had been discontinued at least 6 weeks earlier, and any prior use of levodopa or a dopaminergic agonist ceased at least 3 months before starting the DATATOP study. After initial assessments, DATATOP subjects were randomized to treatment regimens of placebo or regimens of α-tocopherol or selegiline (deprenyl) to test for neuroprotective effects (Parkinson Study Group, 1989a,b). Other details of study methodology have been published (Parkinson Study Group, 1989a,b, 1993, 1995).

At the start of the clinical trial, each subject provided informed consent and underwent a detailed neurological history and examination. Similar assessments were carried out at the end of the study along with reconsideration of the PD diagnostic impression. The same enrolling PD specialists recorded their level of confidence as to whether, for each subject, the initial diagnosis of PD was likely to be correct at the end of the study. For the results reported here, we utilized specimens only from DATATOP study participants whose retrospective assessment was judged to be ≥90% probability of a correct PD diagnosis.

Participants in DATATOP underwent standardized research lumbar punctures for CSF collection after entering the study (but before study medications were started), and on a second occasion. The second procedure was carried out when subjects reached the study endpoint (defined as the need for starting PD symptomatic therapy such as levodopa), or else 24 months after the first CSF collection. Before the second CSF collection, the study medication had been washed out for varying periods of time and no antiparkinsonian medication had been administered (Parkinson Study Group, 1995). Besides testing for clinical evidence of neuroprotection, an additional goal of the DATATOP study was to measure CSF concentrations of the dopamine metabolite HVA as a possible correlate of disease progression and neuroprotective intervention (Parkinson Study Group, 1995). CSF specimens were also analyzed for other dopamine metabolites (LeWitt et al., 1992) and the DATATOP specimen collection continues to be available for further study.

The DATATOP clinical trial enrolled subjects from September 3, 1987 to November 15, 1988. During the same period, additional CSF specimens were obtained from 26 healthy control subjects (who were either family members of DATATOP participants or recruited from community advertising). The control subjects provided IRB-approved informed consent and underwent a CSF collection procedure identical to that used in the DATATOP study. The control subjects met the same exclusion criteria noted above for DATATOP subjects; in all instances, their neurological and psychiatric histories and examinations were normal, and they were not receiving drugs active in the CNS.

4.2. CSF specimen collection

The CSF collection protocol for the DATATOP and control subjects involved overnight bed rest for at least 8 h. The lumbar puncture was carried out in the decubitus position between 6 and 10 AM. CSF was collected sequentially in measured aliquots according to an established protocol. By means of this methodology, a linearly increasing caudal–rostral HVA concentration gradient was established (LeWitt et al., 1992). No preservatives were added to specimens, which were placed into tight-seal tubes and immediately placed on ice before freezing at −70°C. For the CSF studies reported here, pooled aliquots collected from the 18th–20th ml were used.

Of 800 subjects enrolled in the DATATOP study (Parkinson Study Group, 1989b; 1993), 525 underwent CSF collection on 2 occasions (Parkinson Study Group, 1995). The numbers of DATATOP and control specimens in the studies to be reported here were dictated by available funding. The 217 subjects whose specimens were studied were chosen randomly from the available pool of 467 DATATOP subjects with the retrospective confirmatory diagnosis of highly probable PD. Because of difficulties encountered in recruiting control subjects, the age of this population, although overlapping that of the DATATOP study participants, had a mean value that was approximately 2 decades lower.

4.3. Assay methods

The PD and control CSF specimens were assayed by high-performance liquid chromatography (HPLC) within two years after freezer storage of the specimens. Measurements were conducted in duplicate with unprocessed 50 μl aliquots, thawed immediately before assay and injected into a reverse-phase C18 HPLC column. Assay equipment was a 16-channel electrode array system (ESA CEAS Model 55–1650, Chelmsford, Massachusetts, USA) that made near-simultaneous recordings from 16 coulometric electrodes in a series that ranged, in 60 mV increments, from 0 to 900 mV. Compounds were analyzed from their retention times and from the multichannel configurations of potentials at which they oxidized through the coulometric electrodes. For identification of targeted compounds, each was referenced to retention times and multichannel “fingerprinting” profiles developed with authentic chemical standards, including those for XAN and HVA. Further technical details of these study methods have been published (Matson et al., 1984, 1987; Ogawa et al., 1992).

4.4. Clinical ratings

In addition to extensive demographic information, DATATOP study participants underwent evaluation of Parkinsonian features by PD specialists trained to use the Unified PD Rating Scale (UPDRS) (Lang and Fahn, 1989). Other assessments included the Hoehn and Yahr staging, the Schwab and England Activities of Daily Living rating (an evaluation of overall disability) (Schwab and England, 1969) and the Purdue Pegboard Task (a timed test of bimanual dexterity) (Hietanen et al., 1987). In the current study, we examined UPDRS data from 2 subsections, Part II (Activities of Daily Living— the sum of UPDRS scores on items 5–17) and Part III (Motor Exam — the sum of scores on UPDRS items 18–31). Demographic and UPDRS data were also used to categorize several clinical profiles of Parkinsonism, similar to those previously investigated for other analyses of the study database (Jankovic et al., 1990):

- Subjects with a young age of PD onset (at ≤40 years), for comparison to subjects≥70 years at onset
- Subjects with a slower progression of Parkinsonism (defined by symptom duration of at least 4 years before DATATOP study enrollment), for comparison to subjects defined as having more rapid advance of Parkinsonism (duration<1 year and progressing to a rating of 2.5 on a modified Hoehn and Yahr rating scale, which was defined as exhibiting bilateral Parkinsonian symptomatology and pull test findings resulting in ≥3 steps of retropulsion) (Parkinson Study Group, 1989a)
- Subjects with total baseline UPDRS Part II plus Part III scores of ≥40, for comparison to subjects whose total was <20
- Subjects lacking UPDRS ratings of tremor in Part II (by history) or in Part III (by examination), for comparison to subjects with at least one tremor rating ≥1 in either Part II or Part III (or both).

As described by Jankovic et al. (1990), a tremor score was calculated from the mean of 9 UPDRS items: right and left arm tremor (by history, from Part II), and tremor at rest, during action, and with postural maintenance in the limbs and face (by examination, from Part III). A postural instability and gait disturbance (PIGD) score was calculated from the mean of five UPDRS items: falling, freezing of gait, and walking difficulty (by history, from Part II), and instability of gait and walking difficulty (by examination, from Part III). The tremor-predominant group was defined as those subjects whose tremor score divided by PIGD score was ≥1.5, while a PIGD-predominant group was defined as subjects for whom the ratio was <1.5.

4.5. Statistical methods

Using a pre-specified analytic plan, descriptive statistics such as means and standard deviations were computed for the specified groups of subjects. Comparisons between groups of subjects were done using two-sample t-tests. When comparing baseline and final CSF measurements within PD subjects randomized to the non-selegiline treatment groups (i.e., placebo or α-tocopherol), paired t-tests were done. Pearson's correlation coefficients were computed to assess the relationship between the CSF concentration data and the various

clinical and functional measures. All testing was done at the 0.05 level, and in these exploratory studies, no adjustments were made for multiple comparisons.

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Abbreviations

CSF	cerebrospinal fluid
DATATOP	Deprenyl and Tocopherol Anti-oxidative Therapy of Parkinsonism
HPLC	high-performance liquid chromatography
HVA	homovanillic acid
PD	Parkinson's disease
PIGD	postural instability and gait disturbance
UPDRS	Unified Parkinson's Disease Rating Scale
XAN	xanthine

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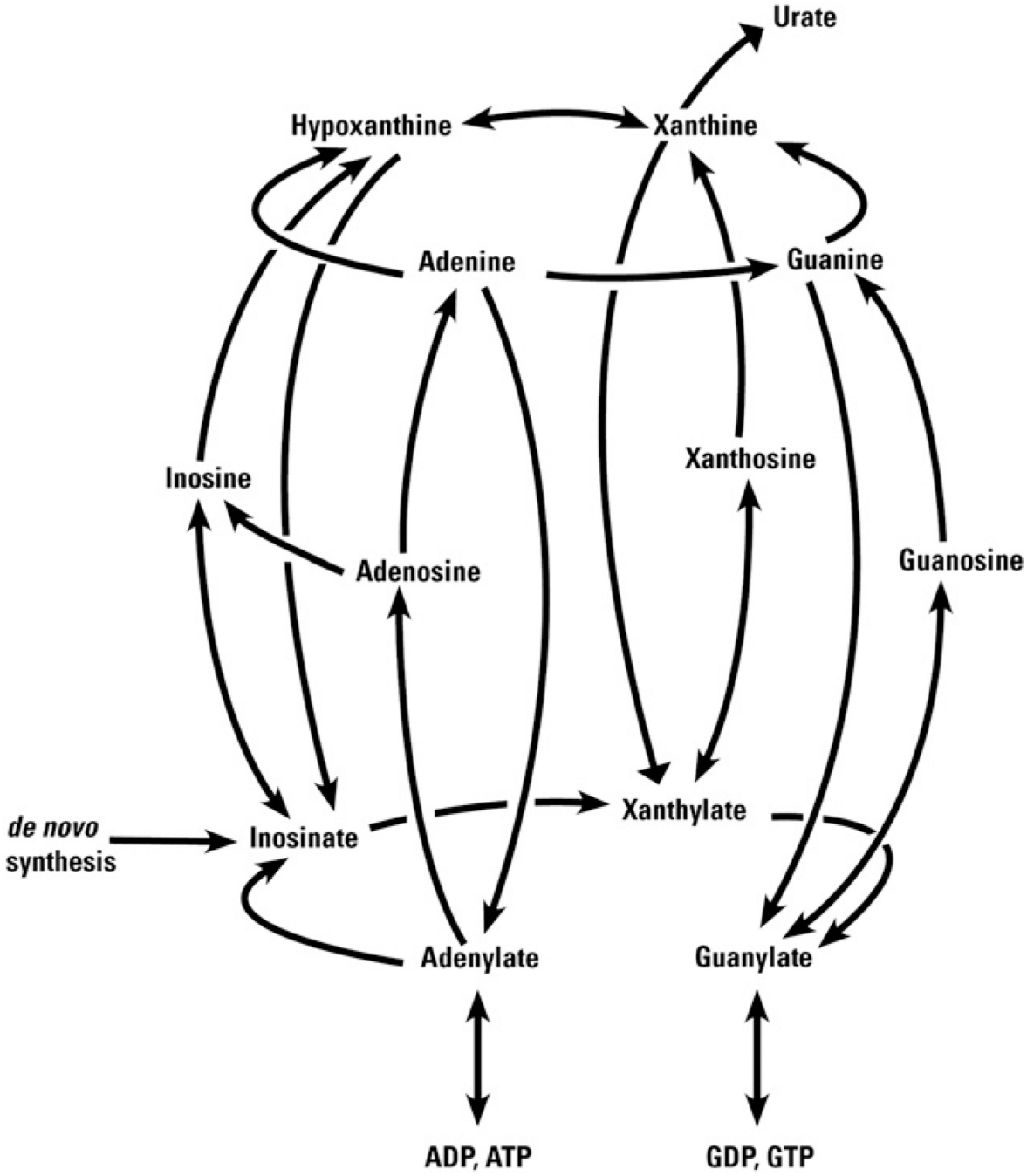

Fig. 1.

Schematic pathways of purine metabolism. Inter-relationships of purine metabolism are shown, illustrating that xanthine is the last intermediate before urate (the end-product of purine degradation in man).

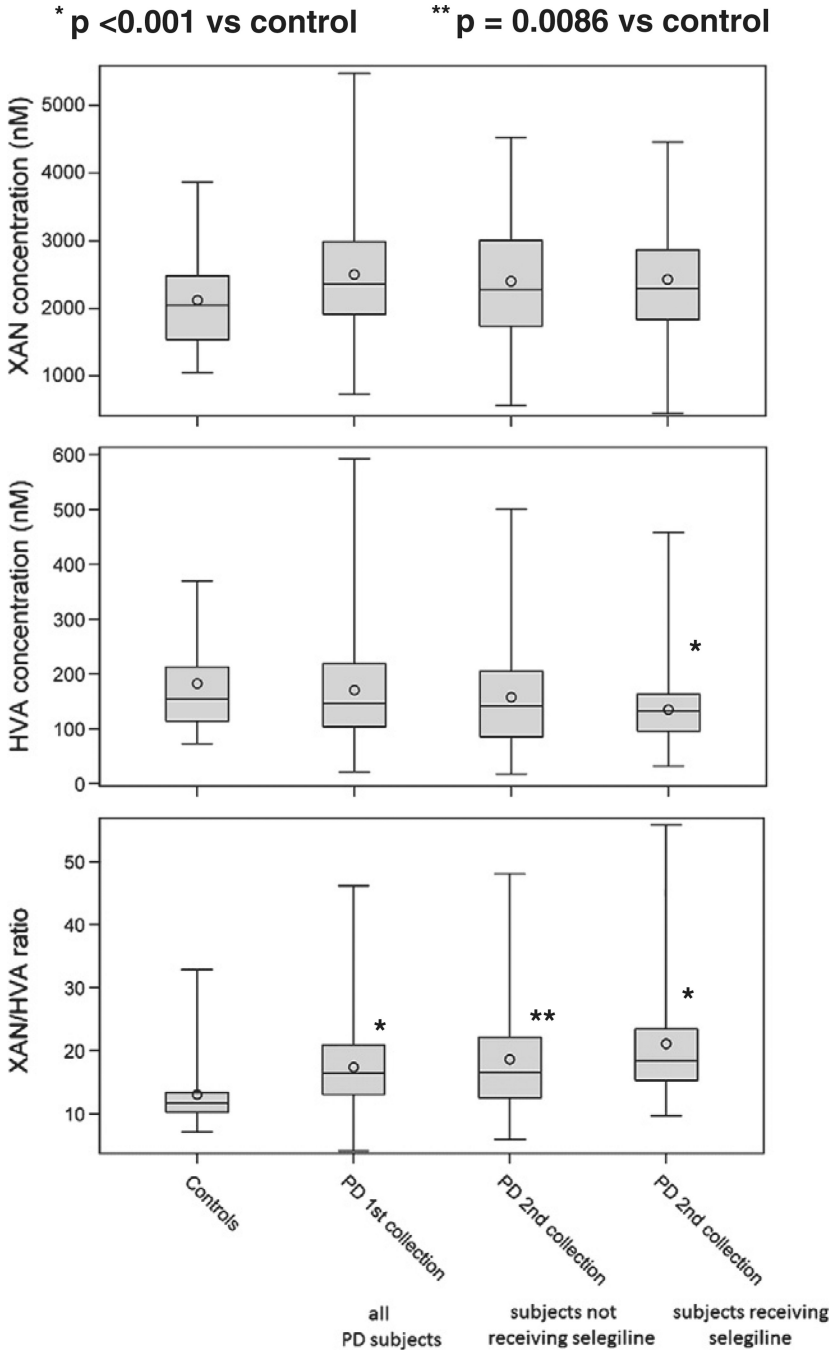

Fig. 2.

Cerebrospinal fluid concentrations of xanthine, homovanillic acid, and their ratio in Parkinson's disease subjects and controls. Box-and-whisker diagrams for control values and changes found for Parkinson's disease (PD) subjects between the first and second cerebrospinal fluid (CSF) collections. CSF xanthine (XAN) and homovanillic acid (HVA) nanomolar concentrations and their ratios in healthy control and PD subjects (see Sections 2.1 and 2.2). The first CSF collection for the PD subjects was carried out in an unmedicated state. Data from the second CSF collection is sub-divided into subjects who either received

or else did not receive selegiline (see Tables 4a and 4b). The shaded boxes indicate boundaries of the upper and lower quartiles of the data, while the upper and lower horizontal bars in each graph (“whiskers”) indicate the range of data. Circles in shaded boxes represent mean values, and the horizontal lines near the middle of each box indicate median values.

Table 1

Demographics and clinical features of Parkinson's disease (PD) subjects studied for their CSF concentrations of homovanillic acid and xanthine.

Subjects with ≥90% certainty (retrospective diagnosis) of PD	**Male: 149 (69%)**
	Female: 68 (31%)
Mean age (years ± S.D.); range: 34–79 years	61.0 ±8.8
Duration of Parkinsonian symptoms before 1st CSF collection (months±S.D.)	10.7 ±10.6
Duration between 1st and 2nd CSF collections (months±S.D.)	15.8±7.9
Mean Unified Parkinson's Disease Rating Scale composite scores at time of 1st CSF collection (mean±S.D.)	
•Part 1 (Mental): total	1.2±1.2
•Part 2 (Activities of Daily Living): total	8.1±3.6
•Part 3 (Motor Exam): total	18.8 ±9.6
Hoehn and Yahr ratings (number of subjects in each category)	Stage 1: 64
	Stage 1.5: 30
	Stage 2: 90
	Stage 2.5: 33
Schwab-England Activities of Daily Living rating (%; mean score±S.D.)	90±6.7
Purdue Pegboard Task score (pegs placed in 30 s; mean±S.D.)	19 ±4.2

Table 2

CSF results of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio in Parkinson's disease (PD) subjects compared to healthy control subjects.

CSF constituent (nM) and ratio	PD (n=217)		Control (n=26)		p-value
	Mean	S.D.	Mean	S.D.	
First CSF collection					
XAN	2508.5	875.0	2125.6	711.0	0.032
HVA	170.7	102.7	182.9	90.2	0.565
[XAN]/[HVA]	17.4	6.7	13.1	5.5	0.0016
Second CSF collection					
XAN	2421.7	811.2	2125.6	711.0	0.076
HVA	147.8	83.6	182.9	90.2	0.046
[XAN]/[HVA]	19.7	8.7	13.1	5.5	<0.001

Table 3

a – CSF results of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio as a function of age: Parkinson's disease (PD) subjects≤50 years old compared with healthy control subjects.

CSF constituent (nM) and ratio	PD (n=29)		Control (n=26)		p-value
	Mean	S.D.	Mean	S.D.	
First CSF collection					
XAN	2156.4	528.9	2125.6	711.0	0.855
HVA	170.4	122.1	182.9	90.2	0.672
[XAN]/[HVA]	16.1	6.2	13.1	5.5	0.061
Second CSF collection					
XAN	2233.2	643.4	2125.6	711.0	0.558
HVA	133.5	57.5	182.9	90.2	0.021
[XAN]/[HVA]	18.3	6.6	13.1	5.5	0.002

b – CSF results of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio as a function of age: Parkinson's disease (PD) subjects≤55 years old compared with healthy control subjects.

CSF constituent (nM) and ratio	PD (n=58)		Control (n=26)		p-value
	Mean	S.D.	Mean	S.D.	
First CSF collection					
XAN	2191.2	719.8	2125.6	711.0	0.699
HVA	157.3	117.0	182.9	90.2	0.324
[XAN]/[HVA]	17.7	7.5	13.1	5.5	0.0061
Second CSF collection					
XAN	2181.6	731.0	2125.6	711.0	0.744
HVA	130.1	84.2	182.9	90.2	0.011
[XAN]/[HVA]	20.2	8.2	13.1	5.5	<0.001

Table 4

a – CSF results of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio from the first and second CSF collections for Parkinson's disease subjects who did not receive selegiline (n=118).

CSF constituent (nM) and ratio	First collection		Second collection		Difference		p-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
XAN	2398.2	825.9	2414.3	848.8	−16.1	810.4	0.829
HVA	163.7	97.4	158.2	91.6	5.5	75.3	0.428
[XAN]/[HVA]	17.4	6.7	18.7	8.4	−1.2	4.9	0.0086

b –CSF results of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio from the first and second CSF collections for Parkinson's disease (PD) subjects who received selegiline (n=99).

CSF constituent (nM) and Ratio	First collection		Second collection		Difference		p-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
XAN	2633.5	921.6	2437.0	765.6	196.5	745.0	0.010
HVA	179.0	108.8	135.5	71.4	43.4	85.3	<0.001
[XAN]/[HVA]	17.4	6.8	21.1	9.0	−3.6	6.3	<0.001

Table 5

CSF findings of xanthine (XAN) and homovanillic acid (HVA) concentrations and their ratio at the first CSF collection in Parkinson's disease subjects with UPDRS Part II + III scores≥40, compared with those with total scores<20.

CSF constituent (nM) and ratio	Score≥40 (n=33)		Score<20 (n=72)		p-value
	Mean	S.D.	Mean	S.D.	
XAN	2378.3	956.6	2504.6	783.3	0.476
HVA	182.7	112.9	161.8	96.1	0.331
[XAN]/[HVA]	15.1	5.6	18.4	7.3	0.021