NeuroToxicology 44 (2014) 110–113

Contents lists available at ScienceDirect

NeuroToxicology

Blood harmane (1-methyl-9H-pyrido[3,4-*b*]indole) concentration in dystonia cases vs. controls

Elan D. Louis [a,b,c,d,*], Pam Factor-Litvak [d], Monika Michalec [a], Wendy Jiang [e], Wei Zheng [e]

[a] GH Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[b] Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[c] Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA
[d] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA
[e] Purdue University School of Health Sciences, West Lafayette, IN, USA

ARTICLE INFO

Article history:
Received 16 May 2014
Accepted 16 June 2014
Available online 23 June 2014

Keywords:
Harmane
Neurotoxin
Dystonia
Neurological disease
Tremor

ABSTRACT

Background: Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) (HA) is a potent neurotoxin that has been linked to two neurological diseases, essential tremor and Parkinson's disease. Blood harmane concentrations [HA] are elevated in patients with both diseases. An important question is whether HA is specifically linked with these diseases or alternatively, is a non-specific marker of neurological illness.
Objectives: We assessed whether blood [HA] was elevated in patients with a third neurological disease, dystonia, comparing them to controls.
Methods: Blood [HA] was quantified by high performance liquid chromatography. Subjects comprised 104 dystonia cases and 107 controls.
Results: Mean log blood [HA] in dystonia cases was similar to that of controls (0.41 ± 0.51 g^{-10}/ml vs. 0.38 ± 0.61 g^{-10}/ml, $t = 0.42$, $p = 0.68$). In unadjusted and adjusted logistic regression analyses, log blood [HA] was not associated with the outcome (diagnosis of dystonia vs. control): odds ratio $(OR)_{unadjusted} = 1.11$, 95% confidence interval (CI) = 0.69–1.79, $p = 0.68$; $OR_{adjusted} = 1.07$, 95% CI = 0.58–1.97, $p = 0.84$.
Conclusions: In contrast to the elevated blood [HA] that has been reported in patients with essential tremor and Parkinson's disease, our data demonstrate that blood [HA] was similar in patients with dystonia and controls. These findings provide the first support for the notion that an elevated blood [HA] is not a broad feature of neurological disease, and may be a specific feature of certain tremor disorders.

1. Introduction

β-Carboline alkaloids are a group of neurotoxins that have a variety of neurological effects, including tremor (Louis, 2008). Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) (HA) is among the most potent of the β-carboline alkaloids. Harmane is produced endogenously, but it is also present in the diet, especially in animal tissue, and also to a lesser extent in many vegetables; exogenous exposure is thought to be the main source of bodily harmane (Pfau and Skog, 2004). In several studies, we demonstrated that blood harmane concentration ([HA]) was elevated in patients with neurological diseases characterized by tremor, including essential tremor (ET) patients compared with controls (Louis et al., 2002, 2008, 2013) and Parkinson's disease (PD) patients compared to controls (Louis et al., 2014), suggesting that harmane is an environmental risk factor for these diseases. These recent studies raise the important but as yet unaddressed question – is elevated blood HA specifically linked with these diseases, or rather, is it merely a global marker of neurological illness?

Dystonia, like ET and PD, is a neurological disease characterized by involuntary movements. Although tremor may occur as a clinical feature of dystonia, the predominant feature is sustained muscle contractions and twisting movements. In 2009, we made the decision to assess blood [HA] in patients with dystonia, with the a priori hypothesis that, in contrast to ET and PD, blood [HA] would not be elevated in patients with dystonia. We now have a sufficient number of subjects to report our results.

* Corresponding author at: Unit 198, Neurological Institute, 710 West 168th Street, New York, NY, 10032, USA. Tel.: +1 212 305 9194; fax: +1 212 305 1304.
E-mail addresses: EDL2@columbia.edu (E.D. Louis), prf1@cumc.columbia.edu (P. Factor-Litvak), mg3360@cumc.columbia.edu (M. Michalec), jiangw@purdue.edu (W. Jiang), wzheng@purdue.edu (W. Zheng).

http://dx.doi.org/10.1016/j.neuro.2014.06.010
0161-813X/

2. Materials and methods

2.1. Participants

Dystonia cases were enrolled in a study of the environmental epidemiology of ET and other neurological disorders at Columbia-University Medical Center (CUMC) (Louis et al., 2008, 2014). Recruitment began in 2009 and continued to present (May 2014). By design, dystonia cases were identified from a computerized billing database at the Center for Parkinson's Disease and Other Movement Disorders at the Neurological Institute of New York, CUMC; selection was based on date of appointment, with more recent cases being selected first. All cases had received a diagnosis of dystonia from their treating neurologist at the Institute and lived within two hours driving distance of CUMC. One of the authors (E.D.L.) reviewed the office records of all selected dystonia patients; patients with diagnoses or physical signs consistent with PD, ET, spinocerebellar ataxia, or other movement disorders were excluded, and the diagnosis of dystonia was confirmed using published diagnostic criteria (Fahn, 1988). The location of dystonia (writer's cramp, torticollis, blepharospasm, combination or other locations) was extracted from the chart review.

Control subjects were recruited for the same study during an overlapping time period. Controls were identified using random digit telephone dialing within a defined set of telephone area codes that were represented by neurological cases (e.g., 212, 201, 203, 516, 718, and 914) within the New York Metropolitan area. There was one group of controls for all neurological disease cases (ET, PD, and dystonia). Patients with ET and PD are generally older than those with dystonia. Controls were frequency-matched to ET and PD cases based on current age (5 year intervals); the ratio of controls to dystonia cases was ~1:1.

The CUMC Internal Review Board approved of all study procedures; written informed consent was obtained upon enrollment.

2.2. Clinical evaluation

All cases and controls were evaluated in person by a trained tester who administered clinical questionnaires. Most evaluations were performed in the late morning or early afternoon, making fasting blood [HA] impractical. Data suggest that plasma [HA] do not change significantly during the day (Rommelspacher et al., 1991). Thus, in one study (Rommelspacher et al., 1991), human subjects ingested food or ethanol, and plasma [HA] were measured hourly for eight hours; the concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (i.e., 3 week) study period (Rommelspacher et al., 1991). Additionally, our data show that log blood [HA] is not correlated with the time latency since last food consumption (Louis et al., 2011).

The tester collected demographic and clinical information, including duration of symptoms. A screening questionnaire included the question, "Do you have shaking or tremor that you can't control?" Current smoking status was assessed, and all current medications were recorded and then collapsed into 19 medication classes (e.g., neuroleptic medications, cardiac medications, etc.).

Weight and height were assessed and body mass index was calculated (weight in kg divided by the square of height in meters).

2.3. Blood [HA]

After phlebotomy, blood [HA] was quantified, blinded to demographic, clinical and diagnostic information, by a well-established high performance liquid chromatography method (Dr. Zheng, Purdue University) used in our previous studies (Zheng et al., 2000; Louis et al., 2002, 2008).

2.4. Statistical analyses

Chi-square (χ^2) tests were used to analyze proportions, and the Student's t tests were used to examine group differences in continuous variables. Pearson's correlation coefficients were used to assess correlations between continuous variables; when variables were not normally distributed, Spearman's correlation coefficient was used.

The empirical distribution of harmane was positively skewed. Using a one-sample Kolmogorov–Smirnov test, we tested whether [HA] was normally distributed and it was not (Kolmogorov–Smirnov test, $z = 5.56$, $p < 0.001$). Therefore, [HA] were logarithmically transformed, and after this transformation it was normally distributed. Case–control differences in log blood [HA] were assessed using the Student's t tests.

To assess the null hypothesis that blood [HA] was not a predictor of diagnostic group (dystonia vs. control), logistic regression analysis was performed using diagnostic group as the outcome, and log blood [HA] as the primary independent variable. We considered a number of potential confounders (age in years, gender, race, years of education, body mass index, current cigarette smoker, and medications [19 classes]) and included these in the adjusted logistic regression analyses if they were associated with either dystonia or blood [HA] in this dataset or if prior studies suggested a relationship. Analysis of variance (ANOVA) was used to

Table 1
Demographic and clinical characteristics of dystonia cases vs. controls.

Characteristic	Dystonia cases ($N = 104$)	Controls ($N = 107$)	Significance
Age in years	61.7 ± 11.6	70.9 ± 8.3	$t = 6.61$, $p < 0.001$
Female gender	69 (66.3)	65 (60.7)	$\chi^2 = 0.71$, $p = 0.40$
Non-Hispanic white race	83 (79.8)	93 (86.9)	$\chi^2 = 1.93$, $p = 0.17$
Education in years	15.7 ± 3.2	15.9 ± 2.5	$t = 0.57$, $p = 0.57$
Body mass index kg/m^2	25.5 ± 4.6	26.1 ± 5.0	$t = 0.79$, $p = 0.43$
Current cigarette smoker	9 (8.7)	5 (4.7)	$\chi^2 = 1.35$, $p = 0.25$
Duration of dystonia symptoms in years	20.3 ± 15.1	Not applicable	Not applicable
Location of dystonia		Not applicable	Not applicable
Torticollis	56 (53.9)		
Blepharospasm	21 (20.2)		
Writer's cramp	9 (8.7)		
Combination or Other locations	18 (17.3)		
Patient complained of tremor	30 (28.9)	Not applicable	Not applicable

Values are mean ± standard deviation or numbers (percentages).