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Peripheral levels of angiotensins are associated with depressive symptoms in Parkinson's disease

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ABSTRACT

Background: The pathogenesis of PD remains elusive. The renin-angiotensin-system (RAS) has recently been implicated in the degeneration of dopaminergic neurons. This study aimed to compare plasma levels of components of the RAS of individuals with PD with controls. We also investigated the association between these circulating markers and motor, depressive and cognitive parameters.
Methods: Thirty PD patients and twenty controls were subjected to clinical evaluation, including cognitive and depressive symptoms assessment. Plasma levels of Angiotensin (Ang) I, Ang II, Ang- (1–7), angiotensin-converting enzyme (ACE) and ACE2 were measured by Enzyme-Linked Immunosorbent Assay (ELISA).
Results: PD patients presented lower plasma levels of Ang I, Ang II and Ang- (1–7) than control individuals. Among PD patients, lower circulating levels of angiotensins were associated with increased severity of depressive symptoms.
Conclusions: This is the first study showing that peripheral levels of RAS components are changed in PD and associated with depressive symptoms.

1. Introduction

Parkinson's disease (PD) is characterized by progressive loss of dopaminergic neurons in the substantia nigra *pars compacta* (SNpc) and the accumulation of abnormal aggregates of α-synuclein (called Lewy bodies) in the remaining neurons. As a result, patients present typical motor symptoms, *i.e.*, bradykinesia, resting tremor, rigidity and postural instability. Of note, PD clinical signs are evident only when about 50% of nigral neurons are lost [31]. Although the selective damage to neurons in the SNpc with severe obliteration of the nigro-striatal pathway is considered the most important hallmark of PD, other brain areas are also affected. For example, Lewy bodies can be observed in peripheral autonomic nervous system, glossopharyngeal and vagal nerves, anterior olfactory nucleus, amygdala and cortex. Changes in non-motor areas might account for several non-motor symptoms in PD [4].

Despite these well-described pathological features, the cause of neurodegeneration in PD is still a matter of debate. Oxidative stress, mitochondrial dysfunction and inflammation might play a role in the mechanisms underlying neuronal death in PD (for a review, [26]). Recently, a new component has emerged in the pathophysiology of PD: the involvement of the brain renin-angiotensin system (RAS) in dopaminergic neurons death [20].

Although the RAS is classically regarded as a system that controls water and sodium homeostasis, and blood pressure, a range of evidence points to the role of this system in different cerebral functions such as motor control, behavior and emotions (for review, see [27]). It is currently assumed that there is a local brain RAS. Therefore, some studies have investigated the RAS role in central nervous system disorders, such as stroke, Alzheimer's disease, depression and, notably, PD. Most of studies focused on the Angiotensin (Ang) II effects. Ang II is thought to enhance dopaminergic cell death *via* microglial activation and NADPH-derived reactive oxygen species through AT_1 receptors [7,13,29].

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Despite available data pointing to the involvement of the RAS in the pathophysiology of PD, no study to date has evaluated the circulating levels of RAS components in PD. Therefore, this study aimed to evaluate plasma levels of components belonging to the classical and counter-regulatory pathways of the RAS in PD patients in comparison with controls. We hypothesized that circulating levels of Ang I, Ang II, Ang-(1–7), angiotensin-converting enzyme (ACE) and ACE2 are different in PD patients in comparison with controls and that these changes are associated with clinical symptoms of PD.

2. Patients & methods

2.1. Subjects

This study included 30 patients with PD diagnosed according to the United Kingdom PD Brain Bank criteria [15], and a group of 20 controls matched by age, gender, body mass index (BMI) and educational level. Patients were recruited from the outpatient movement disorders clinic of the 'Santa Casa de Belo Horizonte' Hospital, Belo Horizonte, Brazil. Controls were recruited from the local community. Participants were excluded if they had undergone previous neurosurgery or if they had any other neurological disorder and/or cognitive decline and infectious or autoimmune diseases in activity in the previous four weeks. In addition, individuals who had used anti-inflammatories or antibiotics in the four weeks prior to the study were also excluded. All subjects provided written informed consent before admission to the study. The Research Ethics Committee of the *Universidade Federal de Minas Gerais*, Brazil approved this study.

2.2. Clinical evaluation

All patients were evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS) [9]. The modified Hoehn and Yahr staging scale (HY) was used to establish the stage of PD [14]. The modified Schwab and England activities of daily living (ADL) scale (S&E) was used to assess daily routine of PD patients [9].

All individuals were subjected to cognitive examination which included the Mini-Mental State Examination (MMSE) [10] adapted for the Brazilian elderly population [6] and the Frontal Assessment Battery (FAB) [2,8]. MMSE is a brief test for cognitive screening, comprising items from different domains including orientation, attention, memory, and language. Since impairment in executive functions is the most common cognitive deficit in PD, the FAB was also applied [8]. In addition, all participants were evaluated using the Beck's Depression Inventory (BDI), a self-rating instrument for depressive symptoms [3]. BDI has been validated as a tool for depression screening and diagnosis in PD [32,34].

2.3. Assessment of plasma levels of proteins related to the RAS

Ten milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin (Vacuplast, Huangyn, China) on the same day of the clinical assessment. Blood was collected in non-fasting state. In order to rule out any confounding factor caused by circadian rhythm, all samples were collected at the same time of the day (between 14 and 16 h). Whole blood samples collected were used for plasma obtaining within 2 h of having been drawn. These samples were centrifuged at 3000*g* for 10 min, 4 °C, twice. Plasma was collected and stored at −70 °C until assayed. Samples were then thawed and plasma levels of Ang I, Ang II, Ang- (1–7), ACE and ACE2 were measured by Enzyme-Linked Immunosorbent Assay (ELISA), according to the procedures supplied by the manufacturer (MyBioSource, San Diego, CA, USA). All kits applied the sandwich ELISA technique, except for ACE measurement whose kit applied the competitive ELISA method. Concentrations were expressed as pg/mL. The sensitivity of the assays was 1.0 pg/mL for ACE and ACE2; 3.9 pg/mL for Ang I; 2.0 pg/mL for Ang- (1–7); and 18.75 pg/mL for Ang II. Experiments were performed blinded regarding groups (*i.e.* PD patients and controls).

2.4. Statistical analysis

Association between dichotomous variables was assessed with the Fisher's exact test. All variables were tested for Gaussian distribution by the Kolmogorov-Smirnov normality test. Comparisons between patients and controls were made by Mann–Whitney or Student's *t*-tests, according to the non-Gaussian or Gaussian distribution of the variables. Spearman's correlation analyses were performed to examine the relationship between clinical variables and plasma levels of Ang I, Ang II, Ang- (1–7), ACE and ACE2. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, California, USA).

3. Results

3.1. Sociodemographic and clinical data

Demographic and clinical data of PD patients and controls are shown in Table 1. PD patients and controls did not differ regarding gender, age, BMI and formal educational level. Taken into account the low educational level of our sample, both groups presented normal performance on cognitive tests. PD patients presented slightly worse performance (7%) in the MMSE in comparison with controls, but they presented similar performance in the FAB and its subtests. When analyzing depressive symptoms, BDI scores were over 3-fold higher in PD patients compared with controls, showing higher frequency and/or severity of depressive symptoms in PD patients in comparison with controls.

3.2. Plasma levels of RAS components

As shown in Fig. 1, PD patients exhibited lower plasma levels of Ang I, Ang II and Ang- (1–7) than controls, while ACE and ACE2 levels did not differ between groups. Among PD patients, lower Ang I, Ang II and Ang-(1–7) levels were associated with increased severity of depressive symptoms, as evaluated by the BDI [Fig. 2; rho = −0.508, −0.486 and −0.445; $p = 0.004$, 0.006 and 0.014 for Ang I, Ang II and Ang-(1–7), respectively]. No associations were found in controls.

4. Discussion

In this study, we sought to investigate the involvement of the RAS in the pathophysiology of PD by identifying and characterizing RAS components and their correlation to symptoms severity in PD patients. To the best of our knowledge, this is the first study to investigate circulating levels of RAS components in PD. We showed that PD patients present reduced plasma levels of angiotensins when compared with age and gender-matched controls. Moreover, lower circulating levels of Ang I, Ang II and Ang- (1–7) were associated with increased severity of depressive symptoms in PD patients.

There is growing evidence indicating a role for RAS in the pathophysiology of PD. *In vitro* and *in vivo* studies using animal models of PD showed that the dopamine release in the striatum can be modulated by the RAS, mainly through angiotensin type 1 (AT_1) receptor, evidencing the interaction between the RAS and the central dopaminergic system [5,16,23]. Corroborating these data, AT1 receptor antagonists displayed neuroprotective effects in animal models of PD [12,28]. ACE inhibitors were also tested in animal models of PD, resulting in increase in dopamine striatal levels [17] and/or decrease of neuronal death [19,25]. Accordingly, a nationwide cohort study conducted with patients with hypertension in Taiwan found that cumulative doses of AT_1 receptor antagonists and ACE inhibitors were associated with decreased