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β-Estradiol-3-benzoate confers neuroprotection in Parkinson MPP^+ rat model through inhibition of lipid peroxidation

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ABSTRACT

Estradiol (E2), in addition to its known hormone function, is a neuroactive steroid that has shown neuroprotective profile in several models of neurological diseases. The present study explores the antioxidant effect of β-estradiol-3-benzoate (EB) on the neurotoxicity elicited by MPP^+ in rat striatum. Male Wistar rats, that were gonadectomized 30 days prior to EB, were given 100 μg EB per rat every 48 h for 11 days and animals were infused with MPP^+ via intrastriatal at day six after beginning EB treatment. EB treatment completely prevented the fall in dopamine caused by MPP^+, such result was related with decreased lipid peroxidation, a marker of oxidative stress; diminished number of ipsilateral-to-lesion turns and increased signal of the dopamine-synthesizing enzyme Tyrosin Hydroxylase in *substantia nigra*. The protection elicited by EB was not related to Mn or Cu-Zn superoxide dismutase enzymatic activities or glutathione modulation since none of these parameters were influenced by EB at the times assayed. Whereas, increased expression of PON2 as a result of EB treatment was observed, this phenomenon could be one of the mechanism by which the steroid conferred protection to dopaminergic cells against MPP^+ injury.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder. This neurological disease has a significant world prevalence, being the second most important, affecting 1–2% of the population above 65 [1]. Current epidemiological datasets consistently suggest that men are at greater risk for developing PD than women [2] by approximately 3:2 ratio [3]. In relation to these epidemiological data, it has been suggested that estrogen influences the symptoms associated to the onset and severity of the disease [4].

The principal event underlying the characteristic motor disturbances of PD patients is unequivocally the loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc), that physiologically innervates caudate and putamen nuclei [5]. Consequently, an important dopamine (DA) deficit accompanied with decreased Tyrosine Hydroxylase (TH) enzymatic activity are observed in the nigrostriatal pathway. TH is the rate-limiting enzyme for catecholamine synthesis, including DA. It is also considered as an important marker of dopaminergic neurons integrity in the SNpc [6].

The neurodegeneration observed in PD is closely related with oxidative stress and it has been proposed as one major factor leading to cell death. In this regard, excessive reactive oxygen species, nitric oxide increased production, and lipid peroxidation have been pointed to initiate a neurotoxic cascade of events [7] that progresses to cell death. Brain is rich in polyunsaturated fatty acid and consumes high rate of oxygen by mitochondrial metabolism, making it prone to generate reactive oxygen and nitrogen species and lipid peroxides through the oxidative attack to those membrane polyunsaturated lipids. The lipid peroxidation once present, is a self-propagating event leading cells to a state of vulnerability [8].

To counteract the influence of oxygen reactive species, there are endogenous anti-oxidant systems relying mainly in the function of

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superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes. However, it has been reported that the activities of these enzymes are decreased in PD [9]. Another less explored antioxidant system is comprised by paraoxonases, which are a family of highly conserved calcium dependent proteins, PON1, PON2 and PON3. All of them possess lactonase activity and can hydrolyze lipid peroxides, but only PON1 shows esterase activity [10]. In spite of their structural similarity, these proteins differ in location. PON1 and PON3 are found in circulating blood, associated with high-density lipoprotein (HDL). While, PON2 is ubiquitous and intracellular, mainly located in endoplasmic reticulum, mitochondria and peri-nuclear region [11]. In the brain, PON2 has been detected in SNpc, nucleus accumbens and striatum, areas related to PD [12]. PON2 levels in brain tissue from female mice are higher than those from males, possibly due to the effect of estrogens [13]. E2 has been shown to cause a time- and concentration-dependent increase in PON2 protein levels in striatal astrocytes from male mice; 12–24 h of exposure with 200 nM E2 increased the levels of PON2 in astrocytes, a result of transcriptional activation of PON2 gene, mediated by α-estrogen receptor activation [14].

Historically, PD clinical trials have mainly focused on preserving patients' motor function. The replacement of DA has been the cornerstone treatment through the use of Levodopa (L-DOPA) [15]. Nevertheless, in the long-term, this treatment causes incapacitating adverse effects and worsens the disease symptoms, and it is only recommended for 2–5 years [16]. The L-DOPA treatment has been related to oxidative stress that would further aggravate the disease [12]. A different therapeutic scope that focuses on targets based to counteract the characteristic mitochondrial disruption and oxidative stress has been proposed [17]. Numerous studies have demonstrated that E2 promotes neuron survival and shows a neuroprotective profile in several models of neurological diseases such as Stroke, Alzheimer's, Schizophrenia and Parkinson's [18]. Steroids are bio-synthesized from cholesterol in different tissues including the brain. Specifically, E2 can be produced from circulating testosterone or cholesterol by neurons and astrocytes. It is well known that E2 is involved in regulating neural development, synaptic plasticity and cell survival. Therefore, it has been linked with protection in neurodegenerative diseases including PD. However, the mechanism by which E2 protects dopaminergic neurons in PD it is not clear [19]. Tripanichkul and co-workers [20] demonstrated that E2 enabled neuronal protection in SNpc in MPTP-treated male C57BL/6 mice by preserving TH immunoreactivity over 1.7 times against the injury caused by the toxic insult. In another study, this estrogen also prevented the DA decrease caused by MPTP in mice [21].

The actions of steroids can be observed at several levels, for example it has been observed that E2 protected dopaminergic neurons *in vitro* of the MPP^+ toxicity by suppressing AP-1 activity which was elevated in the process of neuronal death [22].

The objective of the present work was to explore, as a first approach, the effects of BE on the main endogenous antioxidant systems in a rat model of PD.

2. Methods

2.1. Animals

We used Male Wistar rats weighing 200 g at the beginning of the experiment. Animals were housed in a 12 h light-dark cycle room, with constant temperature (23 °C), with access to food and water *ad libitum*. Animals were treated humanely to minimize discomfort in accordance with the ethical principles and regulations specified by the Animal Care and Use Committee of the National Institute of Neurology and Neurosurgery and the standards of the National Institutes of Health of Mexico.

2.2. Treatments

Since the testes are the main producers of testosterone in males and considering that a part of the testosterone is transformed to E2 by action of the aromatase enzyme. All rats used in this project were gonadectomized to eliminate this endogenous concentration of E2 and thus only evaluate the effect of BE that was administered. Thirty days after gonadectomized, they were randomly assigned into four experimental groups of 6–7 animals each (control, group C; EB treatment, group EB; MPP^+ injury, group M; EB treated and MPP^+ injured, group EB/M). Animals were treated with a subcutaneous injection of 100 μg EB (Sigma-Aldrich, E8515) or saline every 48 h for 11 days. On day 6 of EB treatment, rats were infused with MPP^+ (15 μg/8 μL) by a stereotaxic injection into the right striatum at coordinates 0.5 mm posterior, −3.0 mm lateral and −4.5 mm ventral to the bregma, according to Paxinos and Watson [23], as described by Rubio-Osornio and coworkers [24]. Animals of groups C and EB underwent stereotaxical infusion with saline solution. Before surgery, animals were anesthetized with Ketamine/Xylazine (100/10 mg/kg).

Determination of the different variables represents different events after MPP^+ injury. Therefore, the apomorphine-induced behavioral test, the TH-ir determination and PON2 expression were assessed 6 days after MPP^+ administration. DA determination was done at day 7 after MPP^+, 24 h after circling behavior evaluation (in order to reduce the number of animals used). These variables were assessed in the times specified based on the damage caused by MPP^+ on dopaminergic cells, since they are the consequence of such damage, and they are commonly explored days after acute MPP^+ injury [25]. The SOD and GPx enzymatic activities, as well as GSH, GSSG and lipid peroxidation were determined at earlier times because these variables are related to MPP^+ oxidative mechanism of damage and the times used represent peaks of activity reported in other studies [26] [27].

2.3. Circling behavior

Six days after MPP^+ striatal injection, rats received a subcutaneous apomorphine dose (1 mg/kg), a D1 and D2 dopamine receptor agonist, dissolved in ascorbic acid/saline. Five minutes later, the total number of complete rotations was recorded for one hour [27].

2.4. Determination of striatal dopamine

Twenty-four hours after the circling behavior test, striatal tissue was extracted. The rats' right striatum was homogenized in 10 volumes of perchloric acid/sodium metabisulfite solution (1 M, 0.1% w/v) and centrifuged at 10,000 × *g* for 10 min at 4 °C. The concentration of DA was measured in the supernatants by the use of a high-performance liquid chromatography (HPLC) system (LC 250, Perkin Elmer) with electrochemical detection (CC4, BAS), using a catecholamine analytical column (100 mm × 4.8 mm with 3 μm of particle size). The mobile phase was a phosphate buffer (50 mM, pH 3.2) prepared with 0.2 mM sodium octyl sulfate, 0.1 mM EDTA and 15% (v/v) methanol. Concentrations were calculated using a linear calibration curve, constructed with catecholamine standards [28].

2.5. Tyrosine hydroxylase immunostaining

Six days after stereotaxic surgery, rats were perfused with 10% formaldehyde for 30 min. Brains were extracted and preserved one week by immersion in the same solution. Then, brains were cut in coronal sections in the line 6 and 12 of a brain matrix stainless steel, these sections corresponded to striatum and SNpc respectively according to Paxinos and Watson [23]. After paraffin embedded, series of ten tissue slices (5 μm) were obtained and immunostained as described. Sections were deparaffinized by washing in xylene twice. Afterwards, all sections were hydrated with decreasing alcohol concentrations