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Not all boronic acids with a five-membered cycle induce tremor, neuronal damage and decreased dopamine

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ABSTRACT

Several striatal toxins can be used to induce motor disruption. One example is MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), whose toxicity is accepted as a murine model of parkinsonism. Recently, 3-Thienylboronic acid (3TB) was found to produce motor disruption and biased neuronal damage to basal ganglia in mice. The aim of this study was to examine the toxic effects of four boronic acids with a close structural relationship to 3TB (all having a five-membered cycle), as well as boric acid and 3TB. These boron-containing compounds were compared to MPTP regarding brain access, morphological disruption of the CNS, and behavioral manifestations of such disruption. Data was collected through acute toxicity evaluations, motor behavior tests, necropsies, determination of neuronal survival by immunohistochemistry, Raman spectroscopic analysis of brain tissue, and HPLC measurement of dopamine in substantia nigra and striatum tissue. Each compound showed a distinct profile for motor disruption. For example, motor activity was not disrupted by boric acid, but was decreased by two boronic acids (caused by a sedative effect). 3TB, 2-Thienyl and 2-furanyl boronic acid gave rise to shaking behavior. The various manifestations generated by these compounds can be linked, in part, to different levels of dopamine (measured by HPLC) and degrees of neuronal damage in the basal ganglia and cerebellum. Clearly, motor disruption is not induced by all boronic acids with a five-membered cycle as substituent. Possible explanations are given for the diverse chemico-morphological changes and degrees of disruption of the motor system, considering the role of boron and the structure-toxicity relationship.

1. Introduction

Parkinson's disease (PD) is one of many movement disorders that represent a global health problem (Fang et al., 2015). It is important to elucidate the unclear etiology and the mechanisms by which the pathological processes of PD advance. In this sense, the study of chemical agents capable of inducing movement disruption could be instrumental (Jagmag et al., 2016).

Several compounds have been identified as toxins that can lead to motor disruption when administered in animals (Rana et al., 2013; Goldman, 2014; More et al., 2016; Jagmag et al., 2016). For example, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) toxicity is generally accepted as a model of parkinsonism in C57BL6 mice and primates (Sundström et al., 1990; Hare et al., 2013; More et al., 2016) Although knowledge is still incipient about its molecular action mechanisms, MPTP is known to produce basal ganglia damage, diminish dopamine striatal concentration, and generate other lesions linked to parkinsonism (Janson et al., 1992; Hare et al., 2013) Recently, another chemical entity, 3-Thienylboronic acid (3TB), was described as a toxic agent for the central nervous system (CNS). It has been shown to cause motor disruption and biased neuronal damage to basal ganglia in two mouse strains. However, boronic acids with six-membered cycles or without

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cycles do not give rise to such effects (Soriano-Ursúa et al., 2014a; Farfán-García et al., 2016a,b)

Boron-containing compounds (BCCs) have been increasingly studied in recent years due to their possible usefulness in several areas of medicine. In particular, there is interest in boronic acid derivatives as antimicrobial and antineoplastic agents as well as multi-purpose drugs (Das et al., 2013; Soriano-Ursúa et al., 2014b). As with any medicinal compound, BCCs have been evaluated in order to identify chemical moieties able to induce desirable or toxic effects. However, the focus on BCCs in drug design and development has often been limited due to the toxicity often associated with them (Farfán-García et al., 2016a).

The aim of the present study was to explore the toxic profile of six BCCs (Fig. 1): boric acid, 3TB and four boronic acids with high structural homology to 3TB (due to their five-membered cycle as substituent). After their administration to mice, the effects of these six BCCs were compared to each other and to MPTP. We assessed their access to the brain, morphological disruption of the CNS, and the behavioral manifestations of this disruption. These parameters were examined through acute toxicity evaluations, motor behavior tests, necropsies of treated mice, determination of neuronal survival by immunohistochemistry, Raman spectroscopic analysis of brain tissue, and HPLC measurement of dopamine (DA) in substantia nigra (SN) and striatum (ST) tissue.

2. Experimental procedures

2.1. Chemicals and reagents

Boric acid, cyclopentyl boronic acid, 3-Thienylboronic acid, 2-Thienylboronic acid, 3-furanylboronic acid, 2-furanylboronic acid, dimethyl sulfoxide (DMSO), paraformaldehyde, sodium phosphate buffer, ethylenglycol and glycerol were purchased from Sigma-Aldrich® (St. Louis, MO, USA).

2.2. Animals

We used the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for performing experiments. Male C57BL6 mice weighing 22 ± 2 g were obtained from the animal facility of the Escuela Superior de Medicina, Instituto Politécnico Nacional in Mexico City. Mice were free of known viral, bacterial and parasitic pathogens, they were maintained at polystyrene cages in a humidity (50 ± 5%) and temperature (20–25 °C) controlled room, on a light/dark cycle (12:12, lights on at 7 am) and given food and water *ad libitum*. The current protocol was approved by the Ethics Committee and Institutional Animal Care and Use Committee of the Escuela Superior de Medicina, and is in accordance with the guidelines established by the Mexican Secretary of Agriculture and Animal Breeding (NOM-062-Z00-2001, SAGARPA) as well as the Guide for the Care and Use of Laboratory Animals of the National Research Council. Animals were randomly assigned to eight groups of study (n = 8, N = 64, sample size calculations were performed online by Biomath, http://www.biomath.info/power/index.html), being control (Group 1) or administered I.P. with MPTP (Group 2), boric acid (Group 3) or any of five tested boronic acids (Groups 4–8).

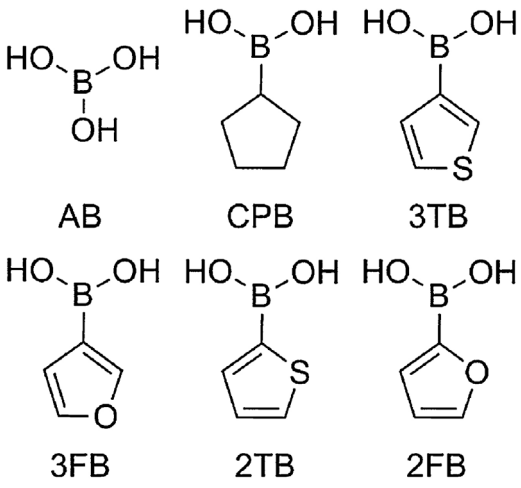

Fig. 1. Chemical structure of boron-containing acids herein tested.

2.3. Evaluation of the motor behavior disruption induced by MPTP and BCCs

We administered MPTP (18 mg/kg) to mice four times (with 2-h intervals) to generate acute motor disruption, as elsewhere (Jackson-Lewis and Przedborski, 2007). Based on previous reports for 3TB (Soriano-Ursúa et al., 2014a; Farfán-García et al., 2016b) and the acute toxicity evaluation carried out by Lorke's method (Table 1), we employed BCCs at a dose of 200 and 400 mg/kg, and DMSO 20% as the vehicle. This strategy enabled a preliminarily exploration of a possible dose-dependent BCC-induced effect on motor behavior. Treated animals (n = 8 per group) were observed 0.5 and 24 h after a single i.p. administration of a compound at a given dose. Motor behavior was assessed by using the open field and slipping tests (with video recording), noting the spontaneous motor activity, coordinated movement and avoidance retention capacity of the animals, as elsewhere (Jackson-Lewis and Przedborski, 2007; Farfán-García et al., 2016b).

Twenty-four hours after the last motor performance test, animals were decapitated. Subsequently, necropsy and macroscopic examination were conducted to identify pathological changes by an established procedure (Elvang-Jensen, 2011). The brains were removed and cryoprotected with FSC 22 (from Surgipath®) or prepared for Raman spectroscopy or immunohistochemical analysis (as described below).

2.4. Raman spectroscopy to detect BCCs in the CNS of mice

To identify BCCs in brain tissue of treated mice, the spectra from solutions of fresh homogenized brain tissue of untreated mice were used as a reference (Soriano-Ursúa et al., 2014a; Farfán-García et al., 2016b). Then for treated mice, a Raman scattering spectrum was obtained from each ultrasonic homogenized sample of the cerebral cortex, ST and SN. All solutions were maintained at ≤20 °C, pH ≈ 7 for Raman spectroscopic studies, which were performed within 8 h after obtaining the sample. For each sample, a 5 μl drop was placed on a quartz slide, which was viewed with a Raman enhanced microscope (Bruker-Senterra system®). The spectra were recorded by using a laser source of 785 nm, 50 mV of power, an integration time of 5s, and an automatic number of acquisitions and 2 co-additions. The resolution was 3 to 5 cm^{-1}, with a spectral range from 1800 to 440 cm^{-1} and an aperture of 50 × 100 μm in a 50× objective. The spectral shape correction and automatic fluorescent rejection mechanisms included in the Senterra system® were used to optimize maximum throughput, detection sensitivity and fluorescence suppression. At least three randomly selected points from each sample were measured, and the spectra were averaged subsequently. Spectra values represent the average of those recorded on samples from three individual mice belonging to a specific group (control or BCC-treated). The average value of the spectra from the control group was taken as a reference and subtracted from each spectrum of tissue from BCC-treated groups (with OPUS® 7.0 software).