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Genetic correlational analysis reveals no association between MPP⁺ and the severity of striatal dopaminergic damage following MPTP treatment in BXD mouse strains

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Abstract

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a pro-neurotoxicant that must be metabolized to 1-methyl-4-phenylpyridinium (MPP⁺) and taken up into striatal dopaminergic neurons to produce neurodegeneration. Recently, we showed wide genetic variability in MPTP-associated neuronal damage in a panel of recombinant inbred mouse strains. Here we examined the amount of MPP⁺ produced in the striatum in the same strains of inbred BXD mice. This allowed us to determine if the differences in the dopaminergic neurotoxicity and associated astrogliosis among the BXD mouse strains were due to differential metabolism of MPTP to MPP⁺. Using the same BXD mouse strains examined previously (Jones et al., 2013) we found that the extent of the striatal damage produced following MPTP treatment is not correlated quantitatively with the production of MPP⁺ in the striatum. Our findings also extend those of others regarding strain differences in MPTP-induced dopaminergic neurotoxicity. Importantly, our finding suggests that additional factors influence the neurodegenerative response other than the presence and amount of the toxicant at the target site.

Keywords

Genetic correlational analysis; BXD recombinant inbred mice

1. Introduction

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a proneurotoxin that is able to enter the brain when given systemically where it is metabolized by astrocytes to produce 1-methyl-4-phenylpyridinium (MPP⁺). MPP⁺ is taken up preferentially by the dopamine (DA)

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transporter resulting in striatal DA terminal degeneration and, at higher dosages, loss of DA neurons in the substantia nigra (SN) (Muthane et al., 1994; O'Callaghan et al., 1990). Treatment of mice with a single low dosage of MPTP serves as an experimental tool for studying factors that may impact striatal dopaminergic nerve terminal degeneration (Jones et al., 2013; Sriram et al., 2004; O'Callaghan et al., 1990, 2014).

Recently, we reported large genetic variation in MPTP-induced dopaminergic neurotoxicity among ten BXD recombinant inbred mouse strains (Jones et al., 2013). Male mice were treated with a single s.c. injection of 12.5 mg/kg MPTP and the striatum was harvested 48 h later (Jones et al., 2013). This dosage regimen damages striatal dopaminergic nerve terminals but does not damage SN. Striatal dopaminergic neurotoxicity was apparent as evidenced by decreases in DA and its metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), as well as in levels of tyrosine hydroxylase (TH) protein, a measure of DA terminal loss. Furthermore, this dopaminergic damage was accompanied by elevation in glial fibrillary acidic protein (GFAP), a hallmark of the astroglial response to underlying neural injury (e.g. see O'Callaghan et al., 2014). Nearly all of these indicators of striatal dopaminergic neurotoxicity were affected in our inbred mouse strains, especially striatal DA content, as measured by HPLC in tissue homogenates and reported in Jones et al. (2013). Because this reduction showed large differences across strains, i.e., between 20 and 90%, the question addressed in this short communication was whether there are differences in metabolism of MPTP to MPP⁺ across the strains. In this work we determined the amount of MPP⁺ produced by each BXD strain following the same single dosage of MPTP used previously (Jones et al., 2013).

2. Materials and methods

Following the protocol of Jones et al. (2013) we obtained male mice from nine of the original ten BXD strains; sufficient numbers of mice from one BXD strain (27) were not available for the determination of MPP⁺ in this paper due to poor reproduction. Mice (n = 5–6 per group) received 12.5 mg/kg MPTP s.c. and were killed 60 min later. The brains were removed and the striatum was dissected free hand and weighed prior to analysis for MPP⁺ by HPLC using a modification of the method of Miller et al. (1998). Briefly, the striatum was sonified in 200 μ l of 5% trichloroacetic acid containing a known amount of the internal standard 1-methyl-3-phenyl pyridinium iodide and the supernatant collected following centrifugation twice at 14,000 g for 10 min. The supernatants were analyzed using a Waters Associates 616 pump, C18 RP column and 474 fluorescence detector (Milford, MA) with a mobile phase of 0.15 M triethanolamine/HCl, 0.1 M acetic acid (17.4 M), pH 2.3 with formic acid, and 9% acetonitrile. Excitation and emission wavelengths of 290 and 370 nm, respectively, were used to detect MPP⁺ with a retention time of ~10 min. Chromatograms were recorded and integrated using Millennium 32 software (Waters Associates, Milford, MA).

We determined the degree of association between the amount of MPP⁺ produced in this study and MPTP-related striatal dopaminergic neurotoxicity as well as the associated astrogliosis reported in the Jones et al. (2013) study. GFAP is an astrogliosis marker (see O'Callaghan et al., 2014; O'Callaghan and Sriram, 2005). The associations were estimated

by Pearson correlation using the BXD strain means for the principal component (PC) analysis-derived composite of MPTP-based reductions in striatal DA, DOPAC, HVA and TH protein from Jones et al. (2013). We also determined the correlation between the GFAP increases induced by MPTP and the degree of dopaminergic neurotoxicity (PCs) as well as MPP⁺ levels.

3. Results

Table 1 presents PC values (z-scores) for the dopaminergic neurotoxicity composite, the striatal MPP⁺ (mg/g tissue) levels determined in this study and the striatal GFAP increases (µg/mg tissue) from Jones et al. (2013). We quantified the MPP⁺ levels in the striatum but not SN as this dosage regimen normally does not damage SN in the C57BL6J. It would be open to question as to whether the BXD strains that show the most damage in the striatum would have SN damage.

Our data show that the association between MPP⁺ levels and the striatal dopamine compromise caused by MPTP treatment is weak and not statistically significant as measured by Pearson's *r*. The association between MPP⁺ and GFAP, a marker of astrocyte activation in response to neural injury, was also weak. However, there was a strong and significant relationship between the degree of dopaminergic neurotoxicity (PC) and GFAP ($r = 0.69$, $p < 0.03$).

4. Discussion

Others have reported strain differences in the response to MPTP in the parental strains of the BXDs (e.g., Hamre et al., 1999). Our observation that the association between MPP⁺ production and a reduction in measures of striatal DA terminal integrity was weak or nonexistent was unexpected. But it should be noted that others also have reported strain differences in MPTP neurotoxicity that were not related to MPP⁺ levels (Boyd et al., 2007). We also found little to no association between MPP⁺ levels and the GFAP response to striatal dopaminergic terminal damage in the BXD strains, i.e., the strains with the most damage did not have the highest levels of MPP⁺. Nonetheless, the MPTP-associated dopaminergic damage (decreased DA, its metabolites and TH protein) was significantly correlated with the increase in GFAP, as expected. It is well-established that MPTP must be converted to MPP⁺ in the brain and its neurotoxicity is assumed to reflect “dose to target” levels of MPP⁺. However, our clear strain differences in dopaminergic neurotoxicity and the lack of association with the MPP⁺ levels indicate that other factors play a role in the degree of damage induced by MPP⁺ in striatal DA nerve terminals. These factors could include but are not limited to a differential susceptibility of the DA terminal to damage or some other aspect of terminal function. The DA transporter (DAT) is responsible for entry of MPP⁺ into the nerve terminal ultimately resulting in sufficient concentration of the toxicant to cause damage. The differential susceptibility of the BXD mice to MPP⁺ could be conferred by strain differences in the DAT levels themselves or in their DA reuptake capabilities. Certainly, a continued investigation into the differential vulnerability of the BXD strains to MPTP is a worthy endeavor as it would help to broaden our understanding of the factors contributing to the susceptibility of the nigral striatal system to neurodegeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Strain means of z-scores for the 1st PC of difference scores between saline and treated (a), following MPTP treatment vs. MPP⁺ concentration (b) and GFAP concentration (c) in the striatum of 10 BXD recombinant inbred mouse strains (taken from Jones et al., 2013). The PC measures included DA, DOPAC, HVA, and TH (all highly correlated). The comparisons clearly show that the degree of neurotoxicity for a given strain (a) is not related to the concentration of MPP⁺ (b) but is related to GFAP concentration (c), an astrocyte marker of injury. Further, MPP⁺ concentration (b) is not related to GFAP concentration (c). All groups in this work and the Jones et al. (2013) contained an n of 5–6 mice.

BXD strain	Mean z score 1st principal component (PC)(a)	Mean ± sem MPP ⁺ mg/g striatum (b)	Mean ± sem increase in GFAP µg/mg striatum (c)
9	-1.75	8.43 ± 1.10	0.44 ± 0.10
27	0.24	NA	0.23 ± 0.05
29	1.53	9.19 ± 1.03	0.08 ± 0.02
32	1.84	8.08 ± 0.90	0.08 ± 0.01
40	-2.73	8.53 ± 0.76	0.31 ± 0.07
48	-1.56	13.36 ± 1.20	0.31 ± 0.03
60	-1.48	16.25 ± 0.95	0.11 ± 0.02
62	1.92	15.95 ± 0.46	0.10 ± 0.06
69	0.06	6.43 ± 0.51	0.22 ± 0.03
84	1.92	6.09 ± 1.27	0.14 ± 0.03

Comparisons: (1) PC (a) vs MPP⁺ (b) $r = -0.15$, $p > 0.30$, one tailed; (2) PC (a) vs GFAP (c) $r = -.69$, $p < 0.025$, one tailed; (3) MPP⁺ (b) vs GFAP (c) $r = -0.23$, $p > 0.25$, one tailed.