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Allopurinol reduces levels of urate and dopamine but not dopaminergic neurons in a dual pesticide model of Parkinson's disease

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ABSTRACT

Robust epidemiological data link higher levels of the antioxidant urate to a reduced risk of developing Parkinson's disease (PD) and to a slower rate of its progression. Allopurinol, an inhibitor of xanthine oxidoreductase (XOR), blocks the oxidation of xanthine to urate. The present study sought to determine whether lowering levels of urate using allopurinol results in exacerbated neurotoxicity in a dual pesticide mouse model of PD. Although oral allopurinol reduced serum and striatal urate levels 4-fold and 1.3-fold, respectively, it did not alter the multiple motor deficits induced by chronic (7 week) intermittent (biweekly) exposure to intraperitoneal Paraquat (PQ) plus Maneb (MB). However, striatal dopamine content, which was unaffected after either allopurinol or chronic pesticide exposure alone, was significantly reduced by 22% in mice exposed to the combination. Stereological assessment showed that the numbers of dopaminergic nigral neurons were significantly reduced by 29% and the tyrosine hydroxylase (TH) negative neurons unaffected after PQ+MB treatments. This reduction in TH-positive neurons was not affected by allopurinol treatment. Of note, despite the expectation of exacerbated oxidative damage due to the reduction in urate, protein carbonyl levels, a marker of oxidative damage, were actually reduced in the presence of allopurinol. Overall, allopurinol lowered urate levels but did not exacerbate dopaminergic neuron degeneration, findings suggesting that basal levels of urate in mice do not appreciably protect against oxidative damage and neurotoxicity in the PQ+MB model of PD, and/or that allopurinol produces an antioxidant benefit offsetting its detrimental urate-lowering effect.

1. Introduction

Epidemiological studies have identified both positive and negative risk factors for the incidence of Parkinson's disease (PD). Amongst environmental positive risk factors, pesticide exposures have been linked to an increased risk in developing PD, with Paraquat (PQ) and Maneb (MB) (Costello et al., 2009) among those implicated. Negative risk factors for PD include purine-based compounds, urate and caffeine. In fact, robust epidemiological data have linked higher levels of urate to

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a reduced risk of developing PD (Weisskopf et al., 2007; Chen et al., 2009) and of its progression (Schwarzschild et al., 2008; Ascherio et al., 2009). Urate accounts for most of the antioxidant capacity in human plasma (Yeum et al., 2004); with its antioxidant properties as powerful as those of ascorbic acid (Ames et al., 1981). Urate has been shown to specifically confer protection in cellular (Jones et al., 2000; Duan et al., 2002; Guerreiro et al., 2009) as well as in animal models of disease such as multiple sclerosis (Scott et al., 2002), stroke (Yu et al., 1998) and PD (Wang et al., 2010).

While it has been proposed that higher serum urate levels may be of selective advantage in the evolution of the hominids because of its antioxidant effects; hyperuricemia is associated with multiple diseases in humans and points to the deleterious effects of high concentrations of urate. Current approved pharmacological approaches to lower urate levels in patients with gout rely on allopurinol to reduce urate production (Bieber and Terkeltaub, 2004; Pea, 2005). Allopurinol, an inhibitor of xanthine oxidoreductase (XOR) blocks the successive oxidations of hypoxanthine to xanthine, and xanthine to urate. The enzyme XOR is widely distributed throughout various organs including the liver, heart, lung, brain as well as plasma and can exist in either one of 2 forms, xanthine dehydrogenase (XDH), predominating in healthy tissues or xanthine oxidase (XO) which plays an important role in injured cells and tissues (Harrison, 2002). Both forms are interconvertible with one another, with the XO subtype causing reduction of molecular oxygen leading to generation of reactive oxygen species (Berry and Hare, 2004).

The present study sought to determine whether pharmacologically lowering urate levels in mice using chronic allopurinol treatment alters the pesticide-induced neurotoxic phenotype in an environmental toxin model of PD.

2. Results

2.1. *Allopurinol and not PQ+MB significantly attenuates serum and striatal urate levels*

Serum urate levels of mice exposed to allopurinol in the drinking water were significantly decreased by approximately 4-fold ($p<0.0001$) compared to their unexposed water-drinking counterparts. PQ+MB treatment had no effect (Fig. 1A). Striatal urate levels were found to be significantly reduced ($p=0.0024$) in allopurinol treated mice though only by 1.3-fold compared to non-allopurinol treated mice (Fig. 1B). Measurement of additional purines included in the purine metabolism pathway such as hypoxanthine and xanthine was consistent with those published by Enrico et al. (1997) with no demonstrated differences after allopurinol treatment. The data values (ng/mg tissue units) for striatal hypoxanthine levels for the groups: Tap water–Saline, Tap–PQ+MB, Allopurinol–Saline and Allopurinol–PQ+MB were 0.04±0.005; 0.04±0.004; 0.04±0.004; 0.04±0.002, respectively. For the same groups striatal xanthine levels were 0.10±0.02; 0.12±0.03; 0.11±0.01; 0.08±0.01. The data values (mg/dL units) for serum hypoxanthine levels for the groups: Tap water–Saline, Tap–PQ+MB, Allopurinol–Saline and Allopurinol–PQ+MB were 0.041±0.003; 0.042±0.004; 0.04±0.003; 0.06±0.02, respectively. For the same groups serum xanthine levels were 0.0179±0.0014; 0.014±0.0006; 0.02±0.0010; 0.01±0.001. The lack of a change in the levels of both hypoxanthine and xanthine may be due to the fact that allopurinol increases the conversion of hypoxanthine to inosinic acid and the inhibition of the rate of de novo purine biosynthesis.

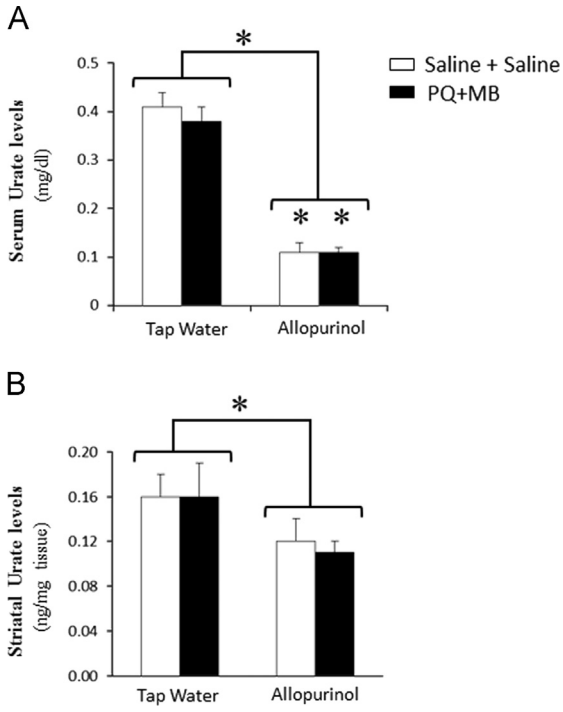

Fig. 1 – Evidence that Allopurinol and not PQ+MB significantly attenuates serum and striatal urate levels in mice. (A) Serum urate level, *$p<0.0001$ vs respective tap water treatment groups; *$p<0.0001$ (combined allopurinol vs combined tap water treatment groups); unpaired *t*-test. (B) Striatal urate level, *$p=0.024$ (comparison between combined allopurinol vs combined tap water treatment groups). Groups are: Tap water [Saline control ($n=8$); PQ+MB ($n=12$)]; Allopurinol [Saline control ($n=8$); PQ+MB ($n=11$)]. Combined treatment groups are: Tap water group ($n=20$); Allopurinol group [Saline control ($n=19$)].

2.2. *Allopurinol does not potentiate PQ+MB-induced motor dysfunction*

The pole test and the beam traversal task were used to detect any motor dysfunction that may reflect toxin-induced dopaminergic deficit. Specifically, MPTP-treated mice have been shown to display slower times in the descent time parameter of the pole test compared to controls, impairments reversed by L-dopa (Matsuura et al., 1997). In addition, Huntington disease knock-in mice displayed significant impairments in the pole test (Hickey et al., 2003), indicating it is a useful test for basal ganglia dysfunction. The beam traversal task is used to specifically assess fine-motor initiation, coordination and postural balance. Hwang et al. (2005) highlighted transgenic mouse models of PD as significantly slower in traversing