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Chelation therapy of manganese intoxication with para-aminosalicylic acid (PAS) in Sprague–Dawley rats

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ABSTRACT

Para-aminosalicylic acid (PAS), an FDA-approved anti-tuberculosis drug, has been used successfully in the treatment of severe manganese (Mn)-induced Parkinsonism in humans [Jiang Y-M, Mo X-A, Du FQ, Fu X, Zhu X-Y, Gao H-Y, et al. Effective treatment of manganese-induced occupational Parkinsonism with p-aminosalicylic acid: a case of 17-year follow-up study. J Occup Environ Med 2006;48:644–9]. This study was conducted to explore the capability of PAS in reducing Mn concentrations in body fluids and tissues of Mn-exposed animals. Sprague–Dawley rats received daily intraperitoneally (i.p.) injections of 6 mg Mn/kg, 5 days/week for 4 weeks, followed by a daily subcutaneously (s.c.) dose of PAS (100 and 200 mg/kg as the PAS-L and PAS-H group, respectively) for another 2, 3 or 6 weeks. Mn exposure significantly increased the concentrations of Mn in plasma, red blood cells (RBC), cerebrospinal fluid (CSF), brain and soft tissues. Following PAS-H treatment for 3 weeks, Mn levels in liver, heart, spleen and pancreas were significantly reduced by 25–33%, while 3 weeks of PAS-L treatment did not show any effect. Further therapy with PAS-H for 6 weeks reduced Mn levels in striatum, thalamus, choroid plexus, hippocampus and frontal cortex by 16–29% ($p < 0.05$). Mn exposure greatly increased iron (Fe) and copper (Cu) concentrations in CSF, brain and liver. Treatment with PAS-H restored Fe and Cu levels comparable with control. These data suggest that PAS likely acts as a chelating agent to mobilize and remove tissue Mn. A high-dose and prolonged PAS treatment appears necessary for its therapeutic effectiveness.

1. Introduction

Occupational exposure to manganese (Mn) has been linked to the majority of the reported cases of Mn intoxication. Neurotoxicity due to inhalation exposure to airborne Mn has been reported in miners, smelters, welders, as well as workers in dry-cell battery factories (Bowler et al., 2006; Chandra et al., 1981; Couper, 1837; Huang et al., 1989; Myers et al., 2003; Ono et al., 2002). Patients who suffered from Mn intoxication, namely manganism, display an extrapyramidal syndrome in a pattern similar to, but not identical to idiopathic Parkinson's disease, including tremor, bradykinesia and gait difficulties. Patients can also display neuropsychological difficulties that include memory loss, apathy, and even psychosis (Aschner et al., 2007; Crossgrove and Zheng, 2004). Patients with severe manganism have difficulties in coping with daily life. While an increasing and immediate demand exists for an effective therapy for Mn-induced neurological impairment, a viable treatment has yet to be discovered.

Clinically, levodopa has been used to treat extrapyramidal syndromes, but with limited benefits (Huang et al., 1993; Lee, 2000; Mena et al., 1970; Rosenstock et al., 1971). In a more rigorous designed clinical trial, Koller et al. (2004) found that treatment with levodopa among parkinsonian welders did not lead to a significant beneficial effect. The strategy to remove the body burden of Mn to normal levels has also been tested in manganism patients. Chelation therapy with ethylene-diamine-tetraacetic acid (EDTA) has shown in some cases to produce promising clinical results (Hernandez et al., 2006), while in other cases it increases Mn elimination in urine but does not improve clinical syndromes (Calne et al., 1994; Cook et al., 1974; Crossgrove and Zheng, 2004; Ono et al., 2002). Thus, a search for other chelating agents for Mn intoxication has become necessary.

Para-aminosalicylic acid (PAS, 4-amino-2-hydroxybenzoic acid, 4-aminosalicylic acid, CAS #89-57-6, MW 153.14), also nicknamed PASER, Paramycin, or Parasal, has been used as an anti-tuberculosis drug since the early 1950s. The therapeutic benefit is believed to be due to its inhibitory effect on folic acid synthesis and therefore the synthesis of the cell wall of the tuberculosis mycobacterium, the primary bacterium causing tuberculosis (PDR, 2000; Rengarajan

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et al., 2004). The chemical structure of PAS is comprised of carboxyl, hydroxyl and amine groups, which provide promising chelating moieties for metals. Ky et al. (1992) first reported two successful clinical cases using PAS for treatment of chronic severe Mn poisoning. This group subsequently conducted a 17-year follow-up study on one of the patients and found that the PAS therapy led to a promising long-term prognosis (Jiang et al., 2006). Combined with 86 other cases effectively treated with PAS in the literature, this evidence suggests that PAS may be a promising therapy for manganism. However, the exact mechanism of drug action (i.e., by chelation, anti-inflammation, or both) remains unknown. Thus, one of the major purposes of this study was to investigate the effectiveness of PAS in reducing the body burden of Mn.

Upon exposure, Mn accumulates in brain regions, including the basal ganglia structures, and to a lesser extent, the caudate nucleus and putamen (Calne et al., 1994; Reaney et al., 2006; Roels et al., 1997; Yamada et al., 1986). T1-weighted magnetic resonance images (MRI) of patients with Parkinson-like symptoms exhibits high signal densities in the basal ganglia attributed to Mn, especially the globus pallidus (Jiang et al., 2007; Kim, 2006; Nagatomo et al., 1999). Mn exposure is known to alter iron (Fe) homeostasis in the cerebrospinal fluid (CSF) and brain tissues (Li et al., 2005, 2006; Zheng et al., 1999). An increased Fe concentration in the CSF is believed to be the result of Mn interference with Fe transport by the blood–CSF barrier at the choroid plexus (Wang et al., 2008a,b). Early studies by Lai et al. (1999) also suggest an increased Cu level in the striatum of rats exposed to Mn in drinking water. In light of these studies, we were interested in investigating whether PAS treatment would restore the altered Fe and Cu status to normal physiological levels.

The aims of this study were (1) to investigate whether PAS treatment reduced Mn levels in selected brain regions and organs of rats subchronically exposed to Mn, (2) to study the time-dose response of PAS treatment in reducing Mn tissue levels, (3) to verify the alteration of Fe and Cu in body fluids and brain tissues following Mn exposure, and (4) to investigate whether PAS restored tissue Fe and Cu to the normal levels.

2. Materials and methods

2.1. Materials

Chemicals were obtained from the following sources: manganese chloride ($MnCl_2$) from Fisher scientific (Pittsburgh, PA); para-aminosalicylic acid (PAS) from Sigma (St Louis, MO); nitric acid form Mallinckrodt (Hazelwood, MO); atomic spectrophotometry standard solutions for Mn (as Mn nitrate in 3% nitric acid), iron (Fe, in 3% nitric acid) and copper (Cu, in 3% nitric acid) from Ricca Chemical Company (Fenton MO). All reagents were of analytical grade, HPLC grade or the best available pharmaceutical grade.

2.2. Animals

Male Sprague–Dawley rats were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). At the onset of the study, the rats were 7–8 weeks old, weighing 220 ± 10 g (mean ± S.D.). Upon arrival, the rats were housed in a temperature-controlled, 12/12 light/dark room, and acclimated for 1 week prior to experimentation. They were allowed to have free access to pelleted Purina semi-purified rat chow (Purina Mills Test Diet 5755C) purchased from Purina Mills (Richmond, IN) and the distilled, deionized water. Purina Mills Diet 5755C contains 0.60% calcium, 0.57% phosphorus, 0.40% potassium, 0.07% magnesium, 0.21% sodium, 60 ppm Fe, 20 ppm Zn, 65 ppm Mn, 15 ppm Cu, 3.2 ppm cobalt, 0.6 ppm iodine, 3.0 ppm chromium, and 0.2 ppm selenium. The diet is highly consistent in its composition and has been used in our past studies on chronic lead exposure (Zhao et al., 1998; Zheng et al., 1996). The study was conducted in compliance with standard animal use practices and was approved by Institutional Committee on Animal Uses at Purdue University.

2.3. Mn and PAS administrations and sample collection

There were eight rats in each study group. Both $MnCl_2$ and PAS sodium salt were dissolved in sterile saline each day prior to administration. The study was designed as follows: rats in the Mn-only group received i.p. injections of 6 mg Mn/kg (6 mg Mn/mL) once daily, between 9:00 a.m. and 10:00 a.m., 5 days/week for 4 weeks; they were then injected with saline subcutaneously once daily for 2, 3 or 6 weeks until tissue dissection (designated as the Mn-only group).

Rats in PAS treatment groups received the same daily i.p. injections of 6 mg Mn/kg as those in the Mn-only group. Following 4 weeks of subchronic Mn exposure, exposure ceased and the animals were treated with PAS subcutaneously at dose of 100 mg/kg (designated as PAS-low-dose group or PAS-L) or 200 mg/kg (PAS-high-dose group or PAS-H) once daily, between 9:00 a.m. and 10:00 a.m., 5 days/week for additional 2, 3, or 6 weeks. The animals were then subjected to tissue dissection and subsequent metal analyses. Two control groups were designated as follows: rats in the saline control group received the daily i.p. injections of saline at a volume equivalent to the Mn-only group throughout the experiment (designated as the control group). Another group of rats received the same saline injections as those in the control group for 4 weeks, followed by subcutaneous injection of 200 mg/kg of PAS for 2, 3, or 6 weeks (designated as the PAS-only group).

The dose regimen for Mn exposure was chosen because it was known to be associated with a significant increase of Mn concentration in brain tissues and altered biochemical parameters in rats (Seth et al., 1977, 1981; Zheng et al., 1998, 1999). Subcutaneous injection of PAS was chosen in order to avoid the in situ chemical interaction between PAS and Mn at the same dosing site.

Twenty-four hours after the last injection, rats were anesthetized with ketamine/xylazine (75:10 mg/kg, 1 mg/kg i.p.). CSF samples were obtained through a 26-gauge needle inserted between the protuberance and the spine of the atlas, and were free of the blood. Blood samples were collected from the inferior vena cava into a 2 mL heparinized syringes. Following standing in room temperature for at least half an hour, the blood was centrifuged at 3400 × *g* for 10 min at which point the plasma was transferred to an Eppendorf tube. The pellets were then washed with saline for three times. The red blood cells (RBC) were obtained; an aliquot of the RBC was used for determining the hemoglobin concentration; and the rest was for metal analyses. All CSF, plasma and RBC samples were stored at −20 °C prior to analysis.

Rat brains were dissected from the skull and the choroid plexus was collected from lateral and third ventricles. Various brain regions, i.e., striatum, hippocampus, motor cortex, and cerebellum and thalamus, were dissected as we previously described (Zheng et al., 1998; Li et al., 2006). The major organs, i.e., heart, kidney, liver, testis, pancreas and spleen, were also dissected. All collected tissues were stored at −80 °C prior to analysis.

2.4. Samples digestion and atomic absorption spectrophotometry (AAS) analysis

All brain tissues and organ samples were thawed at room temperature and a 200 mg tissue sample was weighed in a 10 mL-MARSXpress microwave digestion vessel (CEM Corp., Matthews, NC). An aliquot of 2 mL ultrapure nitric acid was added into the vessel. The tightly capped vessels were placed in a model MARSXpress Microwave Reaction System (CEM Corp.) and digested