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Preventive impacts of PAS-Na on the slow growth and activated inflammatory responses in Mn-exposed rats

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ABSTRACT

Background: Sodium para-aminosalicylic acid (PAS-Na), an anti-tuberculosis drug, has been demonstrated its function in facilitating the Mn elimination in manganism patients and Mn-exposed models in vivo and improving the symptoms of Mn poisoning. But whether it can improve the growth retardation and inflammatory responses induced by Mn have not been reported.
Objectives: This study was designed to investigate the preventive effects of PAS-Na on the development of retardation and inflammatory responses in Mn-exposed rats.
Methods: Male Sprague Dawley (SD) rats (8 weeks old, weighing 180 ± 20 g) were randomly divided into normal control group and Mn-exposed group in the 4 weeks experiment observation and normal control group, Mn-exposed group, PAS-Na preventive group and PAS-Na control group in the 8 weeks experiment observation. The Mn-exposed group received an intraperitoneal injection (i.p.) of 15 mg/kg $MnCl_2$ and the normal control group i.p. physiological Saline in the same volume once a day for 4 or 8 weeks, 5 days per week. The PAS-Na preventive group i.p. 15 mg/kg $MnCl_2$ along with back subcutaneous (s.c.) injection of 240 mg/kg PAS-Na once a day for 8 weeks, 5 days per week. PAS-Na control group received s.c. injection of 240 mg/kg PAS-Na along with i.p. injection of saline once daily. The body weight was determined once a week until the end of the experiment. The manganese contents in the blood were detected by graphite furnace atomic absorption spectrometry. The inflammatory factor levels (TNF-α, IL-1β, IL-6, and PGE2) in the blood were detected by using enzyme-linked immunosorbent assay (Elisa) and each organ taking from rats were weighed and recorded.
Results: Mn exposure significantly suppressed the growth in rats and increased heart, liver, spleen and kidney coefficients as compared with the control group. The whole blood Mn level and serum levels of IL-1β, IL-6, PGE2, and TNF-α in sub-chronic Mn-exposure group were markedly higher than those in the control group. However, preventive treatment with PAS-Na obviously reduced the whole blood Mn level, the spleen and liver coefficients of the Mn-exposed rats. And serum levels of IL-1β and TNF-α were significantly reduced by 33.9% and 14.7% respectively in PAS-Na prevention group.
Conclusions: PAS-Na could improve the growth retardation and alleviate inflammatory responses in Mn-exposed rats.

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1. Introduction

Many neurodegenerative disorders have been associated with altered metal homeostasis, emphasizing the importance of a balanced metal homeostasis for normal brain function [1,2]. Analyses of metal neurotoxicity have revealed Huntington's disease (HD) and other neurodegenerative disorders share similar pathophysiological mechanisms [1,3,4]. Both protein aggregation and oxidative stress have been implicated in the toxicity associated with HD and metal overexposure [5,6]. An appropriate amount of manganese (Mn) is required to maintain normal physiological function [7]. Despite its essentiality, excessive Mn accumulated in the brain can cause manganism which usually occur in various occupational and non-occupational circumstances [8]. Manganism is characterized as extrapyramidal motor disorder which shows similar symptoms with idiopathic Parkinson's disease (IPD), e.g., postural instability, rigidity, speech disturbance, fatigue and cognitive dissonance [8]. Some researchers suggested that Mn poisoning could affect the rats' growth and development [9]. And epidemiological studies show that either Mn level in umbilical cord blood (UCB) or in mother whole blood (MWB) had a quadratic curvilinear relationship with birth size. Previous study indicated that the reduction of food intake might be responsible for slow weight gain in rats following Mn exposure [10]. However, the weight between the Mn-treated and control group remains significantly different even with accurate monitor of the food intake [9]. This result is thought to be caused by Mn poisoning, which may reduce the dopamine levels and inhibit its activity [11,12]. Abnormal dopamine signaling was found to modulate the hypothalamo-hypophyseal-gonadal axis in Japanese quail [13]. However, whether the abnormal dopamine signal affects the secretion of growth hormone from the pituitary gland leading to growth and developmental disorders and the associated underlying mechanism remain unclear.

Several recent studies show that the neurotoxic effect of Mn is associated with genetic polymorphism. Specifically, the polymorphism of ATP13 A2 (PARK9) and Slc39a14 as well as the knockout of NOS2 or P73 can significantly modify the susceptibility of Mn-induced neurotoxicity [14–17]. Several mechanisms associated with Mn-induced neurotoxicity have been reported including oxidative stress via inhibiting glutathione (GSH) Synthesis and mitochondrial damage [18], damages in learning and memory abilities via disrupting the interaction between dopamine D1 receptor and N-methyl-D-aspartate receptor (NMDAR) [19], as well as neurotoxic iron accumulation by suppressing the expression of Amyloid Precursor Protein (APP) and H-Ferritin [20]. In addition, increasing evidences have indicated that Mn can cause autophagy dysfunction in glial cells by activating the pyrin domain containing 3 (NLR family, NLRP3)-caspase-1 inflammasome pathway or α-synuclein oligomerization, which can lead to neuroinflammation [21,22]. However, to date, limited studies have been performed to evaluate the systemic inflammatory responses induced by Mn outside the brain.

Clinically, levodopa is a common treatment for IPD to increase the catecholamine synthesis thereby to restore the neuronal function, but it is not effective for Mn-induced neurotoxicity [23,24]. In the early clinical trial, Jiang [25] and Mena [26] found that patients' treatment with levodopa could relieve symptoms of manganism, but soon relapsed. Taurine, a free sulphur-containing amino acids, can reduce the level of glutamic acid (Glu) by activating glutamate decarboxylase and prevents mitochondrial membrane permeabilization and swelling, improves the spatial learning and memory ability on Mn-exposed rats, but it has not been reported to reduce the content of Mn in the brain [27–29]. Additionally, there are many other drugs or biochemical substances used to treat Mn-exposed animals or cells, including valproic acid, heme oxygenase-1, leucine rich repeat kinase 2 (LPPK2) inhibitors and melatonin [30–33], but few clinical reports. In clinics, combination of EDTA and iron supplements has been used for the treatment of hereditary hypermanganesemia, which is because of SLC30A10, a key player in Mn transporter in humans, occurred homozygous mutation [34–36]. After more than one year of treatment with EDTA and iron supplements, symptoms of patients showed improvements such as hypermanganemia, dystonia and T1-hyperintense signals of brain MRI [34,35]. The effectiveness of EDTA in this patients are believed to be due to its chelation which may increase Mn excretion in urine and decrease Mn level in blood [25,34,35]. Nevertheless, both in vitro and in vivo studies found that iron supplementation could mitigate manganism via the ASK1-JNK signaling axis or antioxidant etc [37,38]. Besides, the symptoms of environmental manganism patients had only slightly improved after EDTA treatment, and recurred soon as the drug was discontinued [24,25]. Such results are considered to be due to its four highly water soluble carboxyl groups, which prevent the molecule from effectively crossing the blood-brain barrier observed in the previous radioisotope tracking monitored study [39]. Surprisingly, sodium para-aminosalicylic acid (PAS-Na), an anti-tuberculosis drug, has been demonstrated to promote the elimination of Mn in manganism patients and Mn-exposed animals [25,40,41]. Besides, PAS-Na would improve the symptoms of manganism patient, such as headache, weariness, speech disorders and dystonia [25,40]. Additionally, our current data clearly supported a beneficial effect of PAS over EDTA for its ability to reduce Mn in the CSF, choroid plexus as well as other brain regions and systemic organs [25,42]. Moreover, PAS and its major metabolite, N-acetylated metabolite (AcPAS), can cross the blood brain barrier and increase the elimination of Mn from the brain [43]. Interestingly, PAS has anti-inflammatory effects on Mn-induced neurotoxicity via decreasing the PGE2 levels [44]. And it also can inhibit Mn-induced DNA damage [45]. However, whether PAS-Na has effects on Mn-induced inflammatory responses in peripheral blood remains unclear. Therefore, we designed this study to investigate the development of retardation and inflammatory response on rats following Mn exposure and the intervention effects of PAS-Na treatment.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague Dawley (SD) rats (8 weeks old, weighing 180 ± 20 g) were purchased from Experimental Animal Center of Guangxi Medical University (SCXKG2014-0002). Rats were housed in a temperature-controlled, 45–65% humidity and 12 h/12 h light/dark room with food and water ad libitum. Measure the rats' bodyweight once a week until the end of the experiment. All animal care and treatments were performed in strict compliance with the principles and guidelines of the National Institutes of Health Guide (NIH) for the care and use of laboratory animals. All of the experimental protocols used in this study were evaluated and approved by the Animal Use and Care Committee of Guangxi Medical University, Nanning, China (Ethical approval No.201412001).

After acclimated for 1 week prior to experimentation, animals were randomly divided into sub-acute and sub-chronic toxicological experiments. For the sub-acute Mn exposure, 40 male SD rats were randomly divided into control and Mn-exposed groups with 20 rats in each group. The Mn-exposed group received intraperitoneal (i.p.) injection of 15 mg/kg Mn chloride tetra-hydrate ($MnCl_2.4H_2O$) once a day, 5 days per week for 4 consecutive weeks; while the control group received physiological saline in the same volume. The dose of poisoning regimen was based on ours previous study that Mn levels in liver, spleen, cerebral cortex et al. were significantly increased [46].

For the PAS-Na intervention, 80 male SD rats were randomly divided into four groups with 20 rats in each group, including the control, Mn-exposed, PAS-Na intervention and PAS-Na control groups. The Mn-exposed group received i.p. injection of 15 mg/kg $MnCl_2$, while the control group received i.p. injection of saline in the same volume once a day for 8 weeks, 5 days per week. PAS-Na prevention group received i.p. injection of 15 mg/kg $MnCl_2$ along with back subcutaneous (s.c.)

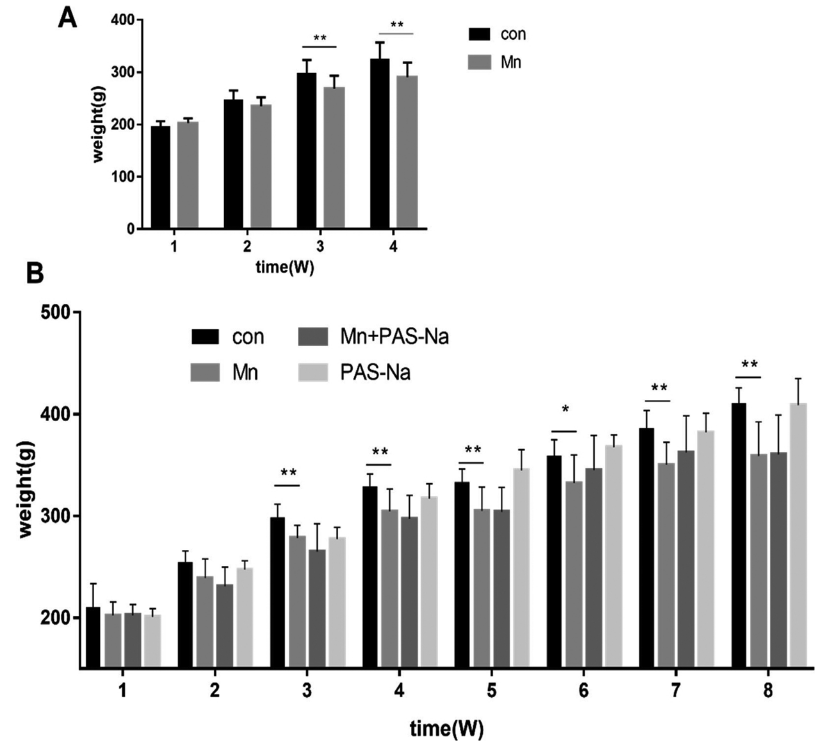

Fig. 1. Preventive effects of PAS-Na on the Mn-induced decrease in rats' body weight.
Data represent mean ± S.D., n = 20 per group. $^{*}p < 0.05$, $^{**}P < 0.01$, when compared with the control group.

injection of 240 mg/kg PAS-Na. PAS-Na control group received s.c. injection of 240 mg/kg PAS-Na along with i.p. injection of saline once daily, 5 days per week, for 8 consecutive weeks. The dose of intervention based on our previous study which manifested that sodium p-aminosalicylic acid (PAS-Na, 240 mg/kg) would inhibit sub-chronic Mn-induced neuroinflammtion in rats by modulating MAPK and COX2 [47].

2.2. Sample collection

At the end of the experiment, animals were anesthetized by using 3.5% chloral hydrate (1 ml/100 g body weight, i.p.). After anesthesia, whole blood samples were collected from the abdominal aorta and divided into two tubes, one was for the determination of Mn level in the whole blood which was collected into anticoagulant vacuum tubes, and the other of whole blood sample was for serum isolation to measure levels of inflammatory factors, which was collected into non-anticoagulant vacuum tubes, and then kept at room temperature for about 30 min to clot and centrifuged within 30 min at 2500 rpm for 10 min to extract serum. All samples were stored at -80 ℃ before analysis. The organ tissues including heart, liver, spleen, kidney and testes were rapidly excised on a petri-dish placed on ice and the attached external vessels and connective tissue were removed carefully. The organs were washed with cold saline. After blotting dry with filter paper, tissues were weighed. At last, the organ coefficients were calculated as: organ coefficient = (tissue weight/bodyweight) × 100%.

2.3. Determination of Mn levels in the whole blood

All samples were handled with special care by using trace element-free materials to avoid metal element contamination. Blood was collected in an anticoagulant (EDTA) tube from the abdominal aorta. Mn level in the whole blood was determined by flameless atomic absorption spectrophotometry (Shimadzu AA-6800, Japan) equipped with Zeeman background corrector, an auto-sampler, graphite furnace and high-density graphite tube (Shimadzu, Japan) as described previously [48]. Analyses were carried out at wavelength of 279.5 nm with 2.0 nm slit width and 10 mA current. 40ul samples were deproteinated with matrix modifier (240ul 1.4% nitric acid containing 0.2 mg/L palladium chloride, 320ul 1% Triton X-100) mixed in vortex mixer. After 20 min at room temperature, the mixtures were centrifuged at 4000 rpm for 5 min. Each sample was analyzed twice and the difference in readings was less than 10%. Accuracy ranged from 95% to 107%. The results were shown as μg/L Mn.

2.4. Determination of inflammatory factor levels in serum using enzyme-linked immunosorbent assay (ELISA)

The serum inflammatory factors including IL-1β, IL-6, PGE2, TNF-α were measured using commercial ELISA kits (eBioscience, USA). All serum samples thawed at room temperature.

2.5. Statistical analysis

The data are presented as mean ± S.D. and analyzed using the software package SPSS version16.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to determine statistical significance between the Mn-treatment and control groups. Statistical significances among multi-group comparisons were tested by one-way ANOVA, followed by Dunnett's multiple comparison tests. Prior to ANOVA, variance homogeneity test was conducted to determine the homogeneity of the data. Pearson correlation was used to determine the relationship between Mn levels in the whole blood and inflammatory factor levels in the serum. Differences with a p values equal to or less than 0.05 was considered statistically significant.

3. Results

3.1. Preventive effects of PAS-Na on the growth and development of Mn-exposed rats

3.1.1. Preventive effects of PAS-Na on Mn-induced body weight reduction

Prior to the exposure, there was no significant difference in body weights between groups. As shown in Fig. 1, the body weight of rats in both group increased in a time-dependent manner. Three weeks post the Mn exposure, the body weight gain of Mn-exposed rats was significantly decreased as compared with that of Controls, and this trend persisted for the rest exposure duration ($p < 0.05$, Fig. 1B). Preventive treatment with PAS-Na did not improve the Mn-induced decrease in the body weight gain ($p > 0.05$, Fig. 1B). No significant difference was found in the body weight gain between the control and PAS-Na control groups ($p > 0.05$, Fig.1B).

3.1.2. Preventive effects of PAS-Na on the organ coefficient of Mn-exposed rats

Following the 4-week Mn exposure, the spleen and kidney coefficients of Mn-exposed animals were significantly higher than those of controls ($P < 0.01$, Fig. 2A). However, sub-acute toxicological experiment had no significant changes in heart, liver and testis coefficients ($P > 0.05$, Fig. 2A). Sub-chronic Mn-exposure group, heart, liver, spleen and kidney coefficients were significantly increased as compared with those of the control group ($P < 0.01$, Fig. 2B). Preventive treatment with PAS-Na for 8 weeks, the spleen and liver coefficients were significantly reduced by 12.2% ($P < 0.05$) and 7.1% ($P > 0.05$) as compared those of Mn-exposed rats without PAS-Na treatment (Fig. 2B). PAS-Na preventive treatment did not appear to improve Mn-induced alterations in heart, kidney and testis coefficients ($p > 0.05$, Fig. 2B). No significant differences were found in all organ coefficients between the control and the PAS-Na control groups ($p > 0.05$, Fig. 2B).

3.2. Preventive treatment with PAS-Na reduced the whole blood Mn concentration of Mn-exposed rats

Following sub-acute and sub-chronic Mn exposure, Mn concentrations in whole blood of Mn-exposed groups were markedly increased by 322.43% and 378.92%, respectively, as compared to the corresponding control group ($P < 0.01$, Fig. 3A and B). In contrast, the whole blood Mn concentration was significantly reduced in PAS-Na prevention group, compared with Mn-exposed group ($P < 0.05$, Fig. 3B). No statistical difference was found in the whole blood Mn concentrations between the control and the PAS control groups ($p > 0.05$, Fig. 3B).

3.3. Interventional treatment with PAS-Na attenuated the Mn-induced increase levels of inflammatory cytokines in serum of Mn-exposed rats

Although the sub-acute Mn exposure for 4 weeks did not affect the PGE2 level in serum, the levels of IL-1β, IL-6 and TNF-α in the serum of the Mn-exposed rats were robustly higher than those of controls ($p < 0.05$, Fig. 4A–D). In sub-chronic Mn-exposure group, IL-1β, IL-6, TNF-α and PGE2 levels in serum were markedly increased as compared with those of the control group ($P < 0.01$, Fig. 4E, F and $P < 0.05$, Fig. 4G, H). In contrast, the serum levels of IL-1β and TNF-α were reduced by 33.9% and 14.7% respectively in PAS-Na prevention group ($p < 0.05$, Fig. 4E and H), but has no positive effects on the serum levels of IL-6 and PGE2 ($p > 0.05$, Fig. 4F and G). No statistical differences were detected in the serum levels of inflammatory cytokines between the control and the PAS-control groups ($p > 0.05$, Fig. 4E–H).

3.4. Pairwise correlations between whole blood Mn level and serum levels of inflammatory cytokines

The relationships between whole blood Mn level and inflammatory cytokines are shown in Table 1. After the 4-week exposure to Mn, the whole blood Mn level was positively correlated with the levels of IL-1β ($r = 0.983$) and TNF-α ($r = 0.864$) in serum ($p < 0.05$, Table 1). However, no correlations were detected between whole blood Mn level and the level of IL-6 ($r = 0.542$, $p = 0.267$) and PGE2 ($r = 0.604$, $p = 0.204$). After the exposure to Mn for 8 weeks, the whole blood Mn

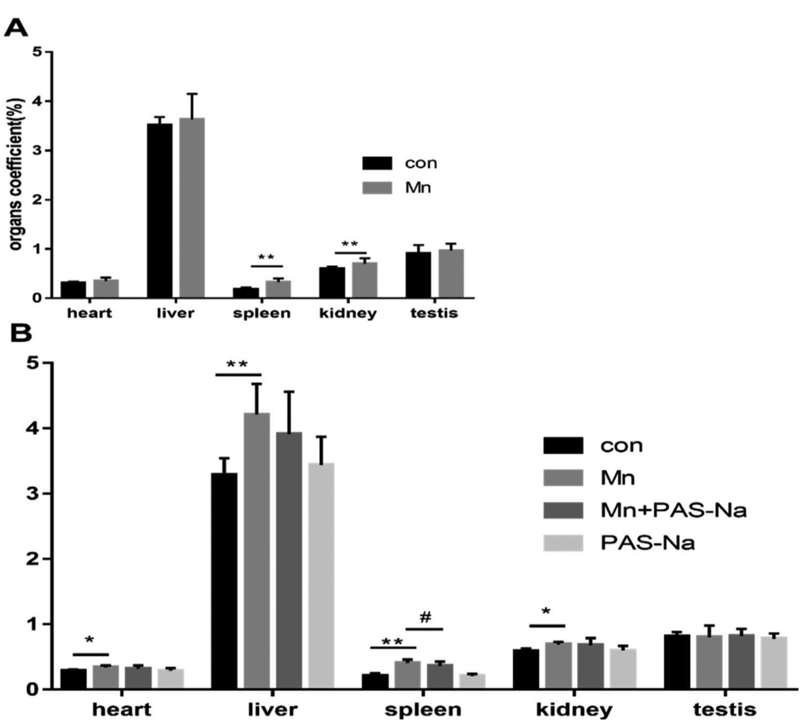

Fig. 2. PAS-Na ameliorate the organ coefficient (%) of Mn-exposed rats.
Note: The organ coefficient was calculated after sub-acute Mn exposure for 4 weeks (A) and sub-chronic Mn exposure for 8 weeks (B). Data represent mean ± S.D., n = 20 per group. **$P < 0.01$, as compared with the corresponding control group. # $P < 0.05$, as compared with the Mn-exposed group.

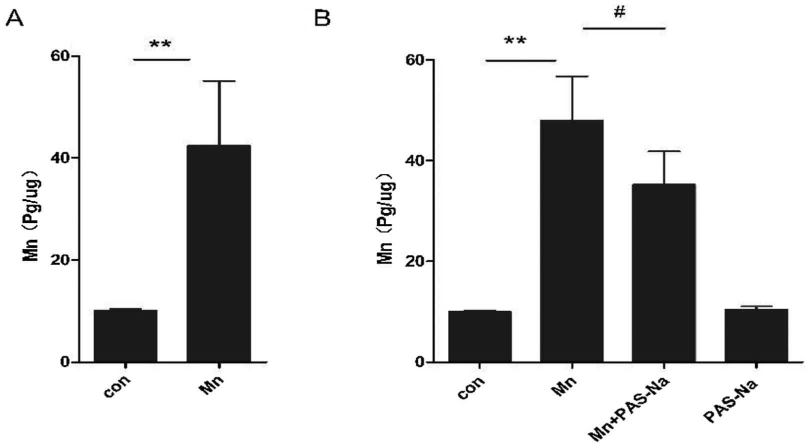

Fig. 3. Preventive treatment with PAS-Na reduced the whole blood Mn concentration of Mn-exposed rats.
Note: Whole blood Mn concentrations were determined after sub-acute Mn exposure for 4 weeks (A) and sub-chronic Mn exposure for 8 weeks (B). Data represent mean ± S.D., n = 20 per group. **: $P < 0.01$, as compared with the corresponding control group. #: $p < 0.05$, as compared with the Mn-exposed group.

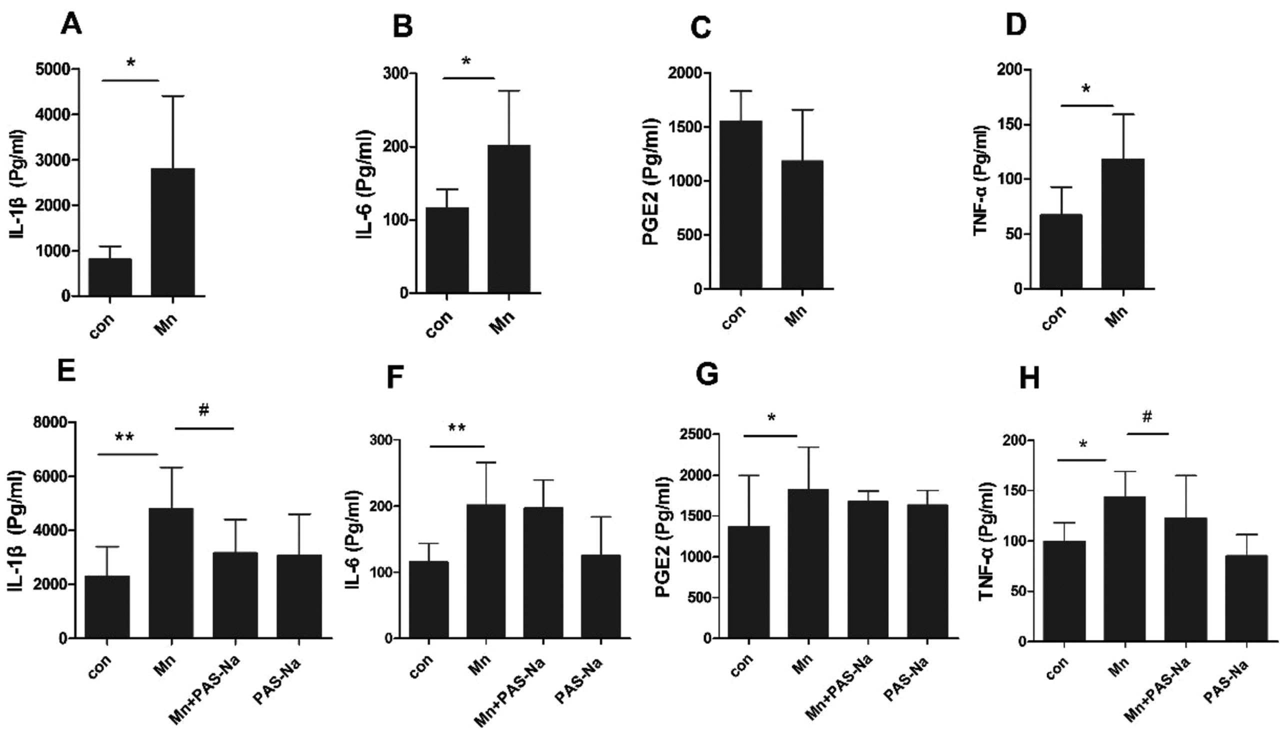

Fig. 4. Preventive treatment with PAS-Na attenuated the Mn-induced increases in levels of inflammatory cytokines in serum.
Note: IL-1β, IL-6, TNF-α, PGE2 levels in serum were determined after sub-acute Mn exposure for 4 weeks(A to D) and sub-chronic Mn exposure for 8 weeks respectively(E to H).Data represent mean ± S.D., n = 20 per group. *$P < 0.05$, ** $P < 0.01$ as compared with the corresponding control group. #$P < 0.05$, as compared with the Mn-exposed group.

Table 1
Pairwise correlations between whole blood Mn level and levels of inflammatory cytokines in serum.

Inflammatory cytokines	Correlation coefficient (r)	p value
Sub-acute Mn exposure period		
IL-1β	0.983	0.000**
IL-6	0.542	0.267
PGE2	0.604	0.204
TNF-α	0.864	0.027*
Sub-chronic Mn exposure period		
IL-1β	0.834	0.039*
IL-6	0.635	0.176
PGE2	0.897	0.015*
TNF-α	0.912	0.011*

Note:
* Correlations were considered significant at $P < 0.05$.

level was positively correlated with the levels of IL-1β (r = 0.834), PGE2 (r = 0.897) and TNF-α (r = 0.912) in serum ($p < 0.05$, Table 1). However, there was no correlation between the whole blood Mn level and IL-6 level (r = 0.635, p = 0.176).

4. Discussion

Mn is an essential nutrient for maintaining normal development [7]. However, excessive Mn accumulation in the brain may cause a variety of metabolic disorders and dysfunctions [8,49]. For example, excess accumulation of Mn in brain would alter dopamine, glutamic acid, γ-aminobutyric acid homeostasis, which maybe one of the causes for growth retardation. Besides, it would produce a large number of damage-associated molecular patterns (DAMPs) through oxidative stress or autophagy disorders, which can lead to inflammatory response and apoptosis [11,47,49,50]. Therefore, we designed this study to explore the therapeutic effect of PAS-Na on Mn-induced growth retardation and inflammatory response in rats. Previous study shown that Mn could be transferred to the central nervous system and soft organs from the blood circulation during the long-term exposure [46]. Conversely, Mn can be continuously released into the blood from the deposited tissues even after the Mn exposure ceased [3,51]. In addition, epidemiological studies have shown that the serum Mn level of Mn-exposed workers was significantly higher than those of the control group [52,53]. Thus, blood Mn level has been used as a typical biomarker of Mn exposure in occupational and environmental studies of workers, resident and children. In our study, sub-acute and sub-chronic Mn exposure increased blood Mn levels in the Mn-exposed groups' rats proved that the rat model of Mn exposure was constructed successfully.

The toxic endpoints of toxicants on organs can be evaluated as the pathological changes and indicators of growth and development [54]. A previous epidemiological study demonstrated that there were U-shaped relationships between maternal blood Mn concentrations, birth weight and head circumference [55]. Moreover, a cohort study in Costa Rica reported that there was a positive linear association between hair Mn concentrations and infant chest circumference [56]. Besides, a large number of in vivo studies has shown that excessive Mn exposure could cause toxic effects on soft organs. For example, excessive Mn exposure can induce apoptosis and inflammatory responses in the chicken testes via increasing the oxidative stress and activating NF-kB/iNOS-COX-2 signaling pathway [57,58], and cause mouse skin ulceration [59]. Additionally, transmission electron microscope results from two recent studies showed that chronic Mn exposure induced myocardial mitochondria superfine lesions, myocardial fiber damage and heart organ coefficient changes [60,61]. Two recently published epidemiologic studies found that environmental Mn exposure could result in oxidative impairment and kidney dysfunction and adverse effects on visuoperception and visual memory in children [62,63]. Our results clearly showed that the body weight gain of Mn-treated rats were much slower than that of control animals, and Mn exposure markedly increased the organ coefficients in spleen, kidney, heart, and liver.

Accumulating evidence have demonstrated that neuroinflammation has a close relationship with neurodegenerative diseases and contributes Mn-induced neurotoxicity [64,65]. A previous non-human primate study showed that Mn exposure up-regulated the expressions of inflammation related genes and increased the number of astrocytes [66]. Additionally, the combination treatment of Mn and LPS can strengthen the inflammatory responses induced by LPS alone [67,68]. Macrophages, microglia and astrocytes, and immunity-associated cells can recognize DAMPs through molecular-associated pattern recognition receptors (PRRs), and subsequently induce the expression of inflammation-related genes via multiple signaling pathways leading to the ultimate goal of clearing cellular debris and restoring the tissue integrity and function [69–71]. Over exposure to Mn serves as an external stimuli that can damage the mitochondria and induce the generation of reactive oxygen species (ROS) leading to dopamine self-oxidation, biological macromolecule changes, mitochondrial membrane damage, abnormal ATP production [31,69,72]. Moreover, high level of Mn is also found to destroy the hydrogen bond and induce DNA mutations by reducing the DNA stability [73,74]. Nevertheless, ROS and damaged gene fragments act as DAMPs to activate immune cells, which can trigger the production and release of inflammatory factors [71]. Our ELISA data demonstrated that Mn exposure significantly induced the levels of IL-1β, IL-6 and TNF-α. Thereby, we speculate that inflammatory responses may have impacts on the growth and development of rats which needs further validation.

The capability of PAS crossing the blood-CSF barrier allows these drug molecules to gain access to Mn ions accumulated in the brain. PAS-Na has a short blood half-life (t1/2) of 2–3 h [41]. Our previous study used in vivo methods to quantify the regional pharmacokinetics of PAS and AcPAS in the rat brain and our data showed that both compounds possessed the ability to enter the brain parenchyma with AcPAS having a much higher brain concentration and longer t1/2 than PAS [43]. Hence, sufficient doses of PAS-Na is required to maintain a high sustainable blood level of PAS-Na to interact with or bind to Mn deposited in the brain to facilitate the excretion of Mn. A 17-year follow-up clinical case study conducted by this laboratory showed that after received the 3-month intravenous infusion of PAS-NA at the dose of 4–8 g/day, a female patient with manganism showed significant improvements and she was further confirmed to be manganism symptoms-free 17 years later post the PAS-Na treatment [25]. However, results from the current study showed that the interventional treatment of PAS-Na during the 8-week of Mn exposure didn't show significant impact on restoring the suppressed growth and development induced by Mn. The possible reason might be that the treatment duration for PAS-Na wasn't long enough for the appearance of improvements. Furthermore, PAS has been confirmed that it can markedly reduce Mn levels in some organs of Mn-exposed animals. Zheng and colleagues found that PAS could effectively reduce Mn and restore Fe and Cu concentrations to the physiological levels in body fluids and brain tissues [41]. A recent published study from this laboratory explored the effects of Mn treatment on the distribution of other divalent metals and the protection effects of PAS-Na, specifically, rats exposed to Mn along with or without PAS-Na treatment and our findings indicated that PAS-Na could reinstate the disrupted balance of divalent metallic elements induced by Mn in main tissues and organs (i.e., liver, spleen, kidney, thighbone and iliac bone, cerebral cortex, hippocampus, testes and blood) [46]. Thereby, it is postulated that PAS-Na could improve the spleen coefficient by correcting the Mn-induced iron disorder, which requires further research. Moreover, following the interventional treatment with PAS-Na during the Mn exposure, the up-regulated serum levels of IL-1β and TNF-α induced by Mn were markedly decreased in our study. Our previous research found that PAS-Na inhibited Mn-induced neuroinflammation in rats by modulating the MAPK and COX-2 signaling pathways [47]. In addition, PAS-Na can also attenuate Mn-induced reductions in the activities of catalase and glutathione peroxidase (GSH-Px), the key of anti-oxindant enzymes to inhibit oxidative stress [75]. A useful approach to evaluate the DNA damage is the single-cell gel (SCGE) assay. Previous studies from this laboratory have demonstrated that PAS-Na can decrease both tail DNA and olive tail moments and maintain the DNA stability [45,76]. Therefore, there is a theoretical support that PAS-Na has anti-inflammatory effect through reducing the production of DAMPs.

In conclusion, excessive Mn exposure increases the blood Mn concentration, suppresses the growth of experimental rats evidenced by the slow weight gain and abnormal organ coefficients in vital organs such as heart, liver, spleen, and kidney. Excessive Mn also triggers the body to produce excessive inflammatory factors. However, PAS-Na could ameliorate most variations induced by Mn. The exact underlying molecular mechanisms remain unclear and further in-depth study is required.

Conflicts of interest

None declared.

Transparency document

The Transparency document associated with this article can be found in the online version.

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References

[1] T.W. Clarkson, Metal toxicity in the central nervous system, Environ. Health Perspect. 75 (1987) 59–64.
[2] E. Nam, J. Han, J.M. Suh, Y. Yi, M.H. Lim, Link of impaired metal ion homeostasis to mitochondrial dysfunction in neurons, Curr. Opin. Chem. Biol. 43 (2018) 8–14.
[3] M. Aschner, T.R. Guilarte, J.S. Schneider, W. Zheng, Manganese: recent advances in understanding its transport and neurotoxicity, Toxicol. Appl. Pharmacol. 221 (2) (2007) 131–147.
[4] R.O. Wright, A. Baccarelli, Metals and neurotoxicology, J. Nutr. 137 (12) (2007) 2809–2813.
[5] W.J. Firdaus, A. Wyttenbach, P. Giuliano, C. Kretz-Remy, R.W. Currie, A.P. Arrigo, Huntingtin inclusion bodies are iron-dependent centers of oxidative events, FEBS J. 273 (23) (2006) 5428–5441.
[6] D. Milatovic, S. Zaja-Milatovic, R.C. Gupta, Y. Yu, M. Aschner, Oxidative damage and neurodegeneration in manganese-induced neurotoxicity, Toxicol. Appl. Pharmacol. 240 (2) (2009) 219–225.
[7] J.L. Aschner, M. Aschner, Nutritional aspects of manganese homeostasis, Mol. Aspects Med. 26 (4–5) (2005) 353–362.
[8] T.R. Guilarte, Manganese and Parkinson's disease: a critical review and new findings, Environ. Health Perspect. 118 (8) (2010) 1071–1080.
[9] V.A. Fitsanakis, N. Zhang, J.G. Anderson, K.M. Erikson, M.J. Avison, J.C. Gore, M. Aschner, Measuring brain manganese and iron accumulation in rats following 14 weeks of low-dose manganese treatment using atomic absorption spectroscopy and magnetic resonance imaging, Toxicol. Sci. 103 (1) (2008) 116–124.
[10] S.L. O'Neal, J.W. Lee, W. Zheng, J.R. Cannon, Subacute manganese exposure in rats is a neurochemical model of early manganese toxicity, Neurotoxicology 44 (2014) 303–313.
[11] V.A. Fitsanakis, C. Au, K.M. Erikson, M. Aschner, The effects of manganese on glutamate, dopamine and gamma-aminobutyric acid regulation, Neurochem. Int. 48 (6-7) (2006) 426–433.
[12] P.K. Seth, S.V. Chandra, Neurotransmitters and neurotransmitter receptors in developing and adult rats during manganese poisoning, Neurotoxicology 5 (1) (1984) 67–76.
[13] S. Banerjee, C.M. Chaturvedi, Specific neural phase relation of serotonin and dopamine modulate the testicular activity in Japanese quail, J. Cell. Physiol. 234 (3) (2018) 2866–2879.
[14] G. Daniel, A. Musso, E. Tsika, A. Fiser, L. Glauser, O. Pletnikova, B.L. Schneider, D.J. Moore, Alpha-Synuclein-induced dopaminergic neurodegeneration in a rat model of Parkinson's disease occurs independent of ATP13A2 (PARK9), Neurobiol. Dis. 73 (2015) 229–243.
[15] T.B. Aydemir, M.H. Kim, J. Kim, L.M. Colon-Perez, G. Banan, T.H. Mareci, M. Febo, R.J. Cousins, Metal transporter Zip14 (Slc39a14) deletion in mice increases manganese deposition and produces neurotoxic signatures and diminished motor activity, J. Neurosci. 37 (25) (2017) 5996–6006.
[16] K.M. Streifel, J.A. Moreno, W.H. Hanneman, M.E. Legare, R.B. Tjalkens, Gene deletion of nos2 protects against manganese-induced neurological dysfunction in juvenile mice, Toxicol. Sci. 126 (1) (2012) 183–192.
[17] D.S. Kim, H. Jin, V. Anantharam, R. Gordon, A. Kanthasamy, A.G. Kanthasamy, p73 gene in dopaminergic neurons is highly susceptible to manganese neurotoxicity, Neurotoxicology 59 (2017) 231–239.
[18] X. Yang, H. Yang, F. Wu, Z. Qi, J. Li, B. Xu, W. Liu, Z. Xu, Y. Deng, Mn inhibits GSH synthesis via downregulation of neuronal EAAC1 and astrocytic xCT to cause oxidative damage in the striatum of mice, Oxid. Med. Cell. Longev. 2018 (2018) 4235695.
[19] Q. Song, Y. Deng, X. Yang, Y. Bai, B. Xu, W. Liu, W. Zheng, C. Wang, M. Zhang, Z. Xu, Manganese-disrupted interaction of dopamine D1 and NMDAR in the striatum to injury learning and memory ability of mice, Mol. Neurobiol. 53 (10) (2016) 6745–6758.
[20] V. Venkataramani, T.R. Doeppner, D. Willkommen, C.M. Cahill, Y. Xin, G. Ye, Y. Liu, A. Southon, A. Aron, H.Y. Au-Yeung, X. Huang, D.K. Lahiri, F. Wang, A.I. Bush, G.G. Wulf, P. Strobel, B. Michalke, J.T. Rogers, Manganese causes neurotoxic iron accumulation via translational repression of Amyloid Precursor Protein (APP) and H-Ferritin, J. Neurochem. 147 (6) (2018) 831–848.
[21] R.M. Gorojod, A. Alaimo, S. Porte Alcon, C. Pomilio, F. Saravia, M.L. Kotler, The autophagic- lysosomal pathway determines the fate of glial cells under manganese-induced oxidative stress conditions, Free Radic. Biol. Med. 87 (2015) 237–251.
[22] D. Wang, J. Zhang, W. Jiang, Z. Cao, F. Zhao, T. Cai, M. Aschner, W. Luo, The role of NLRP3-CASP1 in inflammasome-mediated neuroinflammation and autophagy dysfunction in manganese-induced, hippocampal-dependent impairment of learning and memory ability, Autophagy 13 (5) (2017) 914–927.
[23] C.S. Lu, C.C. Huang, N.S. Chu, D.B. Calne, Levodopa failure in chronic manganism, Neurology 44 (9) (1994) 1600–1602.
[24] J. Crossgrove, W. Zheng, Manganese toxicity upon overexposure, NMR Biomed. 17 (8) (2004) 544–553.
[25] Y.M. Jiang, X.A. Mo, F.Q. Du, X. Fu, X.Y. Zhu, H.Y. Gao, J.L. Xie, F.L. Liao, E. Pira, W. Zheng, Effective treatment of manganese-induced occupational Parkinsonism with p-aminosalicylic acid: a case of 17-year follow-up study, J. Occup. Environ. Med. 48 (6) (2006) 644–649.
[26] I. Mena, J. Court, S. Fuenzalida, P.S. Papavasiliou, G.C. Cotzias, Modification of chronic manganese poisoning. Treatment with L-dopa or 5-OH tryptophane, N. Engl. J. Med. 282 (1) (1970) 5–10.
[27] Z. Xu, K. Jia, B. Xu, A. He, J. Li, Y. Deng, F. Zhang, Effects of MK-801, taurine and dextromethorphan on neurotoxicity caused by manganese in rats, Toxicol. Ind. Health 26 (1) (2010) 55–60.
[28] N. Ahmadi, V. Ghanbarinejad, M.M. Ommati, A. Jamshidzadeh, R. Heidari, Taurine prevents mitochondrial membrane permeabilization and swelling upon interaction with manganese: implication in the treatment of cirrhosis-associated central nervous system complications, J. Biochem. Mol. Toxicol. (2018) e22217.
[29] C.L. Lu, S. Tang, Z.J. Meng, Y.Y. He, L.Y. Song, Y.P. Liu, N. Ma, X.Y. Li, S.C. Guo, Taurine improves the spatial learning and memory ability impaired by sub-chronic manganese exposure, J. Biomed. Sci. 21 (2014) 51.
[30] J. Johnson Jr, E. Pajarillo, P. Karki, J. Kim, D.S. Son, M. Aschner, E. Lee, Valproic acid attenuates manganese-induced reduction in expression of GLT-1 and GLAST with concomitant changes in murine dopaminergic neurotoxicity, Neurotoxicology 67 (2018) 112–120.
[31] R.M. Gorojod, A. Alaimo, S. Porte Alcon, J.H. Martinez, M.E. Cortina, E.S. Vazquez, M.L. Kotler, Heme oxygenase-1 protects astroglia against manganese-induced oxidative injury by regulating mitochondrial quality control, Toxicol. Lett. 295 (2018) 357–368.
[32] J. Chen, P. Su, W. Luo, J. Chen, Role of LRRK2 in manganese-induced neuroinflammation and microglial autophagy, Biochem. Biophys. Res. Commun. 498 (1) (2018) 171–177.
[33] Y. Deng, C. Jiao, C. Mi, B. Xu, Y. Li, F. Wang, W. Liu, Z. Xu, Melatonin inhibits manganese-induced motor dysfunction and neuronal loss in mice: involvement of oxidative stress and dopaminergic neurodegeneration, Mol. Neurobiol. 51 (1) (2015) 68–88.
[34] S. Gulab, H.R. Kayyali, Y. Al-Said, Atypical neurologic phenotype and novel SLC30A10 mutation in two brothers with hereditary hypermanganesemia, Neuropediatrics 49 (1) (2018) 72–75.
[35] M. Stamelou, K. Tuschl, W.K. Chong, A.K. Burroughs, P.B. Mills, K.P. Bhatia, P.T. Clayton, Dystonia with brain manganese accumulation resulting from SLC30A10 mutations: a new treatable disorder, Mov. Disord. 27 (10) (2012) 1317–1322.
[36] K. Tuschl, P.T. Clayton, S.M. Gospe Jr., S. Gulab, S. Ibrahim, P. Singhi, R. Aulakh, R.T. Ribeiro, O.G. Barsottini, M.S. Zaki, M.L. Del Rosario, S. Dyack, V. Price, A. Rideout, K. Gordon, R.A. Wevers, W.K. Chong, P.B. Mills, Syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia caused by mutations in SLC30A10, a manganese transporter in man, Am. J. Hum. Genet. 90 (3) (2012) 457–466.
[37] Y.K. Tai, K.C.M. Chew, B.W.Q. Tan, K.-L. Lim, T.W. Soong, Iron mitigates DMT1-mediated manganese cytotoxicity via the ASK1-JNK signaling axis: implications of iron supplementation for manganese toxicity, Sci. Rep. 6 (1) (2016).
[38] Z. Mate, E. Horvath, A. Papp, K. Kovacs, E. Tombacz, D. Nesztor, T. Szabo, A. Szabo, E. Paulik, Neurotoxic effects of subchronic intratracheal Mn nanoparticle exposure alone and in combination with other welding fume metals in rats, Inhal. Toxicol. 29 (5) (2017) 227–238.
[39] N.L. Schlageter, R.E. Carson, S.I. Rapoport, Examination of blood-brain barrier permeability in dementia of the Alzheimer type with [68Ga]EDTA and positron emission tomography, J. Cereb. Blood Flow Metab. 7 (1) (1987) 1–8.
[40] S.Q. Ky, H.S. Deng, P.Y. Xie, W. Hu, A report of two cases of chronic serious manganese poisoning treated with sodium para-aminosalicylic acid, Br. J. Ind. Med. 49 (1) (1992) 66–69.
[41] W. Zheng, Y.M. Jiang, Y. Zhang, W. Jiang, X. Wang, D.M. Cowan, Chelation therapy of manganese intoxication with para-aminosalicylic acid (PAS) in Sprague-Dawley rats, Neurotoxicology 30 (2) (2009) 240–248.
[42] J. Singh, S.V. Chandra, S.K. Tandon, Chelation in metal intoxication II: in vitro and in vivo effect of some compounds on brain, liver, and testis of rats treated with manganese sulphate, Bull. Environ. Contam. Toxicol. 14 (4) (1975) 497–503.
[43] L. Hong, C. Xu, S. O'Neal, H.C. Bi, M. Huang, W. Zheng, S. Zeng, Roles of P-glycoprotein and multidrug resistance protein in transporting para-aminosalicylic acid and its N-acetylated metabolite in mice brain, Acta Pharmacol. Sin. 35 (12) (2014) 1577–1585.
[44] A.P. Santos, R.L. Lucas, V. Andrade, M.L. Mateus, D. Milatovic, M. Aschner, M.C. Batoreu, Protective effects of ebselen (Ebs) and para-aminosalicylic acid (PAS) against manganese (Mn)-induced neurotoxicity, Toxicol. Appl. Pharmacol. 258 (3) (2012) 394–402.
[45] F. Wang, C. Wang, Y. Jiang, X. Deng, J. Lu, S. Ou, Protective role of sodium para-amino salicylic acid against manganese-induced hippocampal neurons damage, Environ. Toxicol. Pharmacol. 37 (3) (2014) 1071–1078.
[46] Z.X. Yuan, H.B. Chen, S.J. Li, X.W. Huang, Y.H. Mo, Y.N. Luo, S.N. He, X.F. Deng, G.D. Lu, Y.M. Jiang, The influence of manganese treatment on the distribution of metal elements in rats and the protection by sodium para-amino salicylic acid, J. Trace Elem. Med. Biol. 36 (2016) 84–89.
[47] S.J. Li, W.X. Qin, D.J. Peng, Z.X. Yuan, S.N. He, Y.N. Luo, M. Aschner, Y.M. Jiang, D.Y. Liang, B.Y. Xie, F. Xu, Sodium P-aminosalicylic acid inhibits sub-chronic manganese-induced neuroinflammation in rats by modulating MAPK and COX-2, Neurotoxicology 64 (2018) 219–229.
[48] E. Bonilla, Flameless atomic absorption spectrophotometric determination of manganese in rat brain and other tissues, Clin. Chem. 24 (3) (1978) 471–474.
[49] R. Lucchini, D. Placidi, G. Cagna, C. Fedrighi, M. Oppini, M. Peli, S. Zoni, Manganese and developmental neurotoxicity, Adv. Neurobiol. 18 (2017) 13–34.
[50] I.S. Afonina, Z. Zhong, M. Karin, R. Beyaert, Limiting inflammation—the negative regulation of NF-κB and the NLRP3 inflammasome, Nat. Immunol. 18 (8) (2017) 861–869.
[51] S. Bouabid, A. Tinakoua, N. Lakhdar-Ghazal, A. Benazzouz, Manganese neurotoxicity: behavioral disorders associated with dysfunctions in the basal ganglia and neurochemical transmission, J. Neurochem. 136 (4) (2016) 677–691.
[52] E.N. Haynes, H. Sucharew, P. Kuhnell, J. Alden, M. Barnas, R.O. Wright, P.J. Parsons, K.M. Aldous, M.L. Praamsma, C. Beidler, K.N. Dietrich, Manganese exposure and neurocognitive outcomes in rural school-age children: the

communities actively researching exposure study (Ohio, USA), Environ. Health Perspect. 123 (10) (2015) 1066–1071.
[53] L. Lu, L.L. Zhang, G.J. Li, W. Guo, W. Liang, W. Zheng, Alteration of serum concentrations of manganese, iron, ferritin, and transferrin receptor following exposure to welding fumes among career welders, Neurotoxicology 26 (2) (2005) 257–265.
[54] A. Kollias, M. Dafni, E. Poulidakis, A. Ntineri, G.S. Stergiou, Out-of-office blood pressure and target organ damage in children and adolescents: a systematic review and meta-analysis, J. Hypertens. 32 (12) (2014) 2315–2331 discussion 2331.
[55] H. Guan, M. Wang, X. Li, F. Piao, Q. Li, L. Xu, F. Kitamura, K. Yokoyama, Manganese concentrations in maternal and umbilical cord blood: related to birth size and environmental factors, Eur. J. Public Health 24 (1) (2014) 150–157.
[56] A.M. Mora, B. van Wendel de Joode, D. Mergler, L. Cordoba, C. Cano, R. Quesada, D.R. Smith, J.A. Menezes-Filho, B. Eskenazi, Maternal blood and hair manganese concentrations, fetal growth, and length of gestation in the ISA cohort in Costa Rica, Environ. Res. 136 (2015) 47–56.
[57] X.F. Liu, L.M. Zhang, H.N. Guan, Z.W. Zhang, S.W. Xu, Effects of oxidative stress on apoptosis in manganese-induced testicular toxicity in cocks, Food Chem. Toxicol. 60 (2013) 168–176.
[58] Y. Du, Y. Zhu, X. Teng, K. Zhang, X. Teng, S. Li, Toxicological effect of manganese on NF-kappaB/iNOS-COX-2 signaling pathway in chicken testes, Biol. Trace Elem. Res. 168 (1) (2015) 227–234.
[59] D.A. Vousden, E. Cox, R. Allemang-Grand, C. Laliberte, L.R. Qiu, Z. Lindenmaier, B.J. Nieman, J.P. Lerch, Continuous manganese delivery via osmotic pumps for manganese-enhanced mouse MRI does not impair spatial learning but leads to skin ulceration, Neuroimage 173 (2018) 411–420.
[60] I. Markiewicz-Gorka, L. Januszewska, A. Michalak, A. Prokopowicz, E. Januszewska, N. Pawlas, K. Pawlas, Effects of chronic exposure to lead, cadmium, and manganese mixtures on oxidative stress in rat liver and heart, Arhiv za higijenu rada i toksikologiju 66 (1) (2015) 51–62.
[61] D. Huang, K. Chen, Y. Lyu, B. Xia, F. Wang, C. Su, Y. Zou, X. Yang, Effects of chronic manganese sulfate toxicity test on myocardial ultrastructure and heart organ index of rats, Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chin. J. Ind. Hygiene Occup. Dis. 33 (5) (2015) 327–331.
[62] S. Nascimento, M. Baierle, G. Goethel, A. Barth, N. Brucker, M. Charao, E. Sauer, B. Gauer, M.D. Arbo, L. Altknecht, M. Jager, A.C. Dias, J.F. de Salles, T. Saint' Pierre, A. Gioda, R. Moresco, S.C. Garcia, Associations among environmental exposure to manganese, neuropsychological performance, oxidative damage and kidney biomarkers in children, Environ. Res. 147 (2016) 32–43.
[63] D. Hernandez-Bonilla, C. Escamilla-Nunez, D. Mergler, S. Rodriguez-Dozal, M. Cortez-Lugo, S. Montes, L.A. Tristan-Lopez, M. Catalan-Vazquez, A. Schilmann, H. Riojas-Rodriguez, Effects of manganese exposure on visuoperception and visual memory in schoolchildren, Neurotoxicology 57 (2016) 230–240.
[64] K.S. Kirkley, K.A. Popichak, M.F. Afzali, M.E. Legare, R.B. Tjalkens, Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity, J. Neuroinflammation 14 (1) (2017) 99.
[65] S. Sarkar, E. Malovic, D.S. Harischandra, H.A. Ngwa, A. Ghosh, C. Hogan, D. Rokad, G. Zenitsky, H. Jin, V. Anantharam, A.G. Kanthasamy, A. Kanthasamy, Manganese exposure induces neuroinflammation by impairing mitochondrial dynamics in astrocytes, Neurotoxicology 64 (2018) 204–218.
[66] T.R. Guilarte, N.C. Burton, T. Verina, V.V. Prabhu, K.G. Becker, T. Syversen, J.S. Schneider, Increased APLP1 expression and neurodegeneration in the frontal cortex of manganese-exposed non-human primates, J. Neurochem. 105 (5) (2008) 1948–1959.
[67] P. Zhang, K.M. Lokuta, D.E. Turner, B. Liu, Synergistic dopaminergic neurotoxicity of manganese and lipopolysaccharide: differential involvement of microglia and astroglia, J. Neurochem. 112 (2) (2010) 434–443.
[68] C.A. Dodd, N.M. Filipov, Manganese potentiates LPS-induced heme-oxygenase 1 in microglia but not dopaminergic cells: role in controlling microglial hydrogen peroxide and inflammatory cytokine output, Neurotoxicology 32 (6) (2011) 683–692.
[69] D. HaMai, A. Campbell, S.C. Bondy, Modulation of oxidative events by multivalent manganese complexes in brain tissue, Free Radic. Biol. Med. 31 (6) (2001) 763–768.
[70] J.P. de Rivero Vaccari, W.D. Dietrich, R.W. Keane, Activation and regulation of cellular inflammasomes: gaps in our knowledge for central nervous system injury, J. Cereb. Blood Flow Metab. 34 (3) (2014) 369–375.
[71] Z. Zhong, A. Umemura, S.-L. Elsa, S. Liang, S. Shalapour, J. Wong, F. He, D. Boassa, G. Perkins, S.R. Ali, M.D. McGeough, M.H. Ellisman, E. Seki, A.B. Gustafsson, H.M. Hoffman, T.D.-M. Maria, J. Moscat, M. Karin, NF-kappab restricts inflammasome activation via elimination of damaged mitochondria, Cell 164 (5) (2016) 896–910.
[72] C.J. Chen, S.L. Liao, Oxidative stress involves in astrocytic alterations induced by manganese, Exp. Neurol. 175 (1) (2002) 216–225.
[73] R.A. Beckman, A.S. Mildvan, L.A. Loeb, On the fidelity of DNA replication: manganese mutagenesis in vitro, Biochemistry 24 (21) (1985) 5810–5817.
[74] L.H. Rodan, M. Hauptman, A.M. D'Gama, A.E. Qualls, S. Cao, K. Tuschl, F. Al-Jasmi, J. Hertecant, S.J. Hayflick, M. Wessling-Resnick, E.T. Yang, G.T. Berry, A. Gropman, A.D. Woolf, P.B. Agrawal, Novel founder intronic variant in SLC39A14 in two families causing Manganism and potential treatment strategies, Mol. Genet. Metab. 124 (2) (2018) 161–167.
[75] S.J. Li, Y. Li, J.W. Chen, Z.X. Yuan, Y.H. Mo, G.D. Lu, Y.M. Jiang, C.Y. Ou, F. Wang, X.W. Huang, Y.N. Luo, S.Y. Ou, Y.N. Huang, Sodium para-aminosalicylic acid protected primary cultured basal ganglia neurons of rat from manganese-induced oxidative impairment and changes of amino acid neurotransmitters, Biol. Trace Elem. Res. 170 (2) (2016) 357–365.
[76] S.J. Li, Y.N. Luo, Y. Li, J.W. Chen, Y.H. Mo, Z.X. Yuan, S.Y. Ou, C.Y. Ou, Y.M. Jiang, X.F. Deng, Sodium para-aminosalicylate protected cultured basal ganglia astrocytes from manganese-induced DNA damages and alteration of amino acid neurotransmitter levels, J. Toxicol. Sci. 41 (5) (2016) 573–581.