ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research, 2010, Vol 4, No 1, 001-010
www.jpccr.eu

Influence of prolonged manganese intoxication on memory processes in hypoxic mice

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Abstract: Exposure to high levels of manganese in the workplace can result in the development of neurotoxic symptoms in humans. It is also known that exposure to heavy metals, including manganese, can lead to learning and memory deficits. Recently, it has been demonstrated that hypoxic mice in the model of bilateral clamping of the carotid arteries (BCCA) can be more vulnerable to the effects of lead or cadmium. The purpose of the present study was to examine the effect of prolonged manganese intoxication – up to 10 days – on memory processes in mice exposed to transient cerebral oligemic hypoxia induced by 30 min of BCCA. In the current study, long-term memory was evaluated using the step-through passive avoidance task. Spatial working memory was assessed by recording spontaneous alternation in the Y-maze test. In the passive avoidance task, manganese administered at a dose of 7.95 mg/kg i.p. did not impair retention in BCCA mice. Manganese at the same dose of 7.95 mg/kg i.p. did not alter alternation behaviour in the Y-maze either. These findings suggest that hypoxia induced by BCCA combined with prolonged manganese intoxication does not affect memory functions of mice.

Key words: manganese, cerebral oligemic hypoxia, passive avoidance, spontaneous alternation

INTRODUCTION

The neuronal damages, activation of neurotransmitter systems and behavioural disturbances are well known processes after severe cerebral ischemia [1, 2, 3]. In contrast to cerebral ischemia, cerebral oligemic hypoxia as induced by bilateral clamping of the carotid arteries (BCCA) generally does not produce neuronal necrosis in vulnerable brain structures such as the hippocampus [4]. The hippocampus, which is thought to be involved in spatial learning and memory processes [5], is a structure affected mostly by severe ischemia, as well as cerebral hypoxia in BCCA model [6]. It is noteworthy that, similarly to severe ischemia, cerebral oligemic hypoxia leads to changes in neurotransmitters turnover and/or content in certain brain areas in rodents [3]. Transiently reduced oxygen supply induced by BCCA causes disturbances in the cholinergic [4], GABAergic [7] and dopaminergic [8] systems in the brain. BCCA also leads to increased aspartate and glutamate, and the production of hydroxyl radicals in hippocampal and striatal tissues [9]. As severe ischemia, cerebral oligemic hypoxia affects cognitive functions. For example, spatial learning deficiencies in a water maze have been reported after BCCA [3]. It is important to note, that the BCCA procedure is suggested to be a model of transient ischemic attacks in humans [9].

Inorganic manganese (Mn) is an essential ingredient in steel and is used in the production of dry-cell batteries, glass, fireworks, manufacturing of chemicals, in the leather and textile industries, and as a fertilizer. Organic forms of manganese are used as fungicides, fuel-oil additives, smoke inhibitors, an anti-knock additive in petrol, and as a medical imaging agent [10]. Exposure to excessive manganese involves the central nervous system which has been considered to be a critical system [11]. Manganese toxicity from industrial exposure can cause a Parkinson-like condition related to impaired dopaminergic system by this metal [11]. Further, humans exposed to excess levels of manganese express psychiatric problems and deficits in attention, learning and memory [12]. The neurological effects associated with prolonged low-level manganese exposure generally have been subtle changes, also including deficits in tests of neuromotor or cognitive functions [10]. Animals exposed to high levels of manganese develop neurological abnormalities and neuropathological lesions in the brain [13]. The dopaminergic system in the basal ganglia is particularly vulnerable to the effects of manganese [13]. Similarly to humans, chronic exposure to manganese can impair learning processes in rodents [14]. It has been suggested that the inhibitory effect of manganese on the N-methyl-D-aspartate (NMDA) receptor can produce neurological dysfunction, including learning deficits [12]. Further, it has been previously demonstrated that neurotoxicity of heavy metals such as cadmium and lead can be affected by BCCA [15, 16]. Therefore, the aim of this study was to evaluate the effect of prolonged manganese exposure, another heavy metal, on memory processes in mice subjected to cerebral oligemic hypoxia. As chronic manganese intoxication [14] and BCCA [3] can disturb memory in rodents, it was hypothesized that the amnesic effect of manganese could be exacerbated by cerebral oligemic hypoxia. For this purpose we used the Y-maze test and the passive avoidance task [17, 18]. These 2 tests of learning and memory are commonly used in rodents.

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Received: 2 March 2010; accepted: 21 June 2010

MATERIAL AND METHODS

Animals and manganese exposure. Female Swiss mice, weighing 20-26 g, were used in this study. The animals were kept in colony cages with free access to food and tap water. They were exposed to a 12 h light/dark cycle in a room with a temperature of 21±1 °C, relative humidity 50-60%. The experimental groups consisted of 9 mice. The number of operated and used mice (BCCA and sham) in total was 36. The following drug was used: manganese chloride ($MnCl_2 \cdot 4H_2O$) (PPH Polskie Odczynniki Chemiczne, Gliwice, Poland) administered intraperitoneally (i.p.) once daily for 9 or 10 days (depending on the test). First, the mice were tested in the Y-maze, and subsequently, on the next day, in the passive avoidance task. Manganese chloride ($MnCl_2 \cdot 4H_2O$) was injected at a prolonged dose of 7.95 mg/kg, e.g. a mouse weighing 26 g received 0.05738 mg Mn daily. The dose of 7.95 mg/kg was 5 % of 159 mg/kg, a 2-week mean lethal dose (LD_{50}). The drug was administered in a volume of 10 ml/kg. All experimental procedures used in this study were approved by the University Ethics Committee for Animal Experiments.

Experimental model of cerebral oligemic hypoxia. Bilateral clamping of the carotid arteries (BCCA) by using thread was performed in mice. Throughout the surgery the animals were anesthetised by ketamine (Ketanest, Parke-Davis, Berlin, 50 mg/ml, i.p.) + xylazinum (Rometar, Spofa, Praha, 20 mg/ml, i.p.). The cessation of carotid blood flow was controlled visually. The occlusion period lasted for 30 min after which the threads were removed, circulation returned, and the surrounding skin sutured. The carotid arteries of the sham operated control mice were exposed for the same period of time, but were not clamped. During anesthesia and surgery, the mice were breathing spontaneously and the rectal temperature was kept at 37°C by a heating pad. Each task was performed on the following groups: sham, BCCA, sham + Mn and BCCA + Mn.

Passive avoidance task. The step-through passive avoidance task was used in the current study. The passive avoidance test is generally regarded as a measure of long-term memory [18]. The mice were trained on the passive avoidance task 9 days after BCCA or sham surgery and manganese was injected once daily at a dose of 7.95 mg/kg for 10 consecutive days. The first injection was given on the surgical day (60 min after recirculation), and the last one administered 60 min before training in the passive avoidance task on the 9th post-surgical day. During a single training trial the mice were individually placed in an illuminated box (15 × 12 × 15 cm) connected to a darkened box (15 × 12 × 15 cm) equipped with an electric grid floor. A 4 × 5 cm doorway was located at floor level in the centre of the common wall. Immediately after the mouse entered the darkened box, it was punished by an electric foot shock (0.6 mA for 2 s). On the next day (after 24 h) the retention test was conducted in which the same animals were placed in the illuminated box and the latency (time) to enter the darkened box was recorded. The trial ended when the mouse entered the darkened box or until 180 s had elapsed, whichever occurred first. Mice avoiding the dark compartment for 180 s were considered as remembering the task.

Y-maze test. Spontaneous alternation was assessed in the Y-maze test [17]. The mice were tested on the Y-maze 8 days after BCCA or sham surgery, and manganese was injected once daily at a dose of 7.95 mg/kg for 9 consecutive days. The first injection was given on the surgical day (60 min after recirculation), and the last one administered 60 min prior to the Y-maze test session on the 8th post-surgical day. The mice were individually placed in the Y-maze (3 compartments measuring 10 × 10 × 10 cm), which did not have a floor, on a clean sheet of paper. A clean sheet of paper was used after each animal to prevent odour cues. The Y-maze session lasted 8 min. Alternation (defined as consecutive entries into all 3 sections without repetitions) and the total number of section entries (locomotor activity) were scored. Locomotor activity was collected cumulatively over 8 min. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of section entries – 2) × 100. The ability to alternate requires that the mice know which sections have been visited. Therefore, alternation behaviour can be regarded as a measure involving spatial working memory [17].

Statistical analysis. A Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test was used to calculate data from the passive avoidance task. Results from the Y-maze were evaluated by one-way analysis of variance (ANOVA) and Duncan's *post hoc* test for multiple comparisons. $P < 0.05$ was considered as significant. Statistical evaluation of data was performed using software GraphPad (passive avoidance task) and STATISTICA (Y-maze).

RESULTS

Table 1 shows that the latency in the passive avoidance task was not affected in mice subjected only to BCCA. The combination of manganese (7.95 mg/kg) with BCCA did not impair learning of passive avoidance either. In the Y-maze, the performance of the task in BCCA mice did not differ significantly from sham-operated animals (Tables 2 and 3). Administration of manganese (7.95 mg/kg) in BCCA mice did not impair spontaneous alternation (Table 2) or locomotion (Table 3) in the Y-maze.

Table 1 Effect of prolonged manganese intoxication (7.95 mg/kg) on learning of the passive avoidance task in sham and BCCA mice.

	Sham	BCCA	Sham + Mn	BCCA + Mn
Latency (s)	180 (180, 180)	180 (180, 180)	180 (180, 180)	180 (180, 180)

Results are presented as the median values together with the 25th and 75th percentiles. Number of mice in each group n = 9. P > 0.05 vs. sham group (Kruskal-Wallis non-parametric ANOVA followed by Dunn's test).

Table 2 Effect of prolonged manganese intoxication (7.95 mg/kg) on spontaneous alternation in the Y-maze in sham and BCCA mice.

	Sham	BCCA	Sham + Mn	BCCA + Mn
Alternation (%)	62.8 ± 4.45	66.5 ± 2.63	66.7 ± 2.55	65.9 ± 4.27

Data are shown as the means ± S.E.M. Number of mice in each group n = 9. P > 0.05 vs. sham group (ANOVA followed by Duncan's test).

Table 3 Effect of prolonged manganese intoxication (7.95 mg/kg) on locomotor activity in the Y-maze in sham and BCCA mice.

	Sham	BCCA	Sham + Mn	BCCA + Mn
No. of section entries	41.5 ± 3.48	46.6 ± 3.07	37.8 ± 2.85	43.2 ± 3.09

Data are expressed as the means ± S.E.M. Number of mice in each group n = 9. P > 0.05 vs. sham group (ANOVA followed by Duncan's test).

DISCUSSION

The BCCA model is thought to be appropriate for studying the functional consequences of a rather moderate reduction in cerebral blood flow [7]. As already mentioned, this reduction in blood flow does not produce neuronal necrosis in brain vulnerable structures both in rats and mice [4, 19]. The absence of neuronal damage is probably due to the high efficiency of posterior communicating vessels in providing a collateral blood flow [20]. However, memory deficits can be observed following cerebral oligemic hypoxia. BCCA leads to spatial memory deficits in a water maze [3] and impairment of reference memory in the hole-board test in rats [4]. Although BCCA itself does not affect the performance of mice in the passive avoidance task and the Y-maze [19], in combination with CGP 37849, a competitive NMDA receptor antagonist, produced profound impairment of alternation behaviour [21]. Similarly, BCCA when combined with scopolamine, a cholinergic antagonist, impaired spontaneous alternation in this task [19]. Lack of memory deficits in mice subjected only to BCCA in the current study is consistent with earlier reports.

A number of studies indicate that exposure to manganese can cause morphological changes, alterations in levels of neurotransmitters and behavioural impairments both in animals and humans. It is well known that Mn-induced neurotoxicity is mediated by disruption of mitochondria initiating both apoptosis and necrotic cell death via formation of highly reactive oxygen species [22]. Further, reduced concentrations of dopamine in the brain, mainly in the caudate, globus pallidus and putamen, have been reported as a result of manganese exposure [23]. Recently, it has been hypothesized that the atypical form of parkinsonism induced by manganese, is not associated with the degeneration of nigrostriatal dopaminergic neurons, but rather with the ability of manganese to disrupt the presynaptic release of dopamine [23]. Additionally, it is known that GABAergic and glutamatergic systems in the brain are affected by increases in brain manganese levels. Manganese treatment damages the GABAergic neurons in the globus pallidus and causes a decrease in the glutamine transport, which potentially can result in the deregulation of glutamate homeostasis [24]. Resent findings suggest that the inhibitory effect of manganese on the NMDA receptor may produce a deficit in glutamatergic transmission in the brain, which can be responsible for memory impairment [12]. On the other hand, a non-competitive NMDA receptor antagonist, MK-801, is capable of blocking lesions produced by intrastriatal injections of manganese, which confirms the involvement of the glutamatergic system in Mn-induced neurotoxicity [25]. In general, subchronic manganese exposure can lead to hypoactivity, decreased memory performance and diminished sensorimotor reaction [26]. However, there is a lack of information about the effects of manganese intoxication on animals subjected to cerebral ischemia/hypoxia. Research data show that cerebral oligemic hypoxia induced by BCCA may exacerbate the amnesic effects of heavy metal salts. In rats, intrastriatal injections of ferric chloride, after a 60 min oligemic episode induced by BCCA, produced pronounced deficits of spatial memory in a water maze [27]. Co-exposure to 30 min of cerebral oligemia hypoxia and systemic cadmium chloride or lead acetate administration caused impairments in passive avoidance and alternation behaviour in mice [15, 16]. However, combined treatment of BCCA with manganese chloride at the dose of 7.95 mg/kg (5 % of LD_{50}) for up to 10 days, was ineffective in the current study. Although it was previously reported that prolonged cadmium chloride (dose 5 % of LD_{50}) impaired alternation behaviour [15]. Based on other reports concerning manganese exposure [14, 28], it can be suggested that the used prolonged dose of manganese chloride might have been too small to produce learning or locomotor disturbances in performed tests. Chronic manganese chloride poisoning at 20 mg/kg or 50 mg/kg p.o. for one month, led to significant impairment of learning processes in a multipath maze in rats [14]. Nam and Kim [28] reported that manganese chloride administration in the dose range of 20-40 mg/kg, significantly decreased motor activity in mice after 5 days of treatment. It should be noted that locomotor activity, which is closely related to dopaminergic neurotransmission is often disturbed in manganese-intoxicated rodents [29].

In conclusion, the current results show that cerebral oligemic hypoxia induced by BCCA, does not seem to exacerbate prolonged manganese intoxication in mice. However, it might be of interest to carry out further experiments on this subject, involving other behavioural tests and neurochemical studies.

REFERENCES

1. Smith MZ, Auer RN, Sjesjo BK: The density and distribution of ischemic brain injury in rats following 2-10 min of forebrain ischemia. *Acta Neuropathol* 1984, **64**, 319-332.
2. Ginsberg MD, Takagi K, Globus MY-T: Release of neurotransmitters in cerebral ischemia: relevance to neuronal injury. **In:** Krieglstein J, Oberpichler-Schwenk H (Eds.): *Pharmacology of cerebral ischemia*. Wiss Verl-Ges, Stuttgart, 1992, 177-190.
3. Sontag K-H, Heim C, Block F, Sieklucka M, Schmidt-Kastner R, Melzacka M, Osborne N, Läer S, Hüther G, Kunkel M, Ulrich F, Bortolotto Z, Weiner N, Wesemann W: Cerebral oligemic hypoxia and forebrain ischemia-common and different long-lasting consequences. **In:** Krieglstein J, Oberpichler-Schwenk H (Eds.): *Pharmacology of Cerebral Ischemia*. Wiss Verl-Ges, Stuttgart, 1992, 471-479.
4. Heim C, Sieklucka M, Block F, Schmidt-Kastner R, Jaspers R, Sontag K-H: Transient occlusion of carotid arteries leads to disturbed spatial learning and memory in the rat. **In:** Krieglstein J, Oberpichler H (Eds.): *Pharmacology of Cerebral Ischemia*. Wiss Verl-Ges, Stuttgart, 1990, 53-61.
5. Morris RGM, Garrund P, Rawlins JNP, O'Keefe J: Place navigation impaired in rats with hippocampal lesions. *Nature* 1982, **297**, 681-683.
6. Block F, Sieklucka M, Schmidt-Kastner R, Heim C, Sontag K-H: Electrophysiological, metabolic and biochemical approaches to the effects of bilateral clamping of carotid arteries in normotensive rats. **In:** Krieglstein J, Oberpichler H (Eds.): *Pharmacology of Cerebral Ischemia*. Wiss Verl-Ges, Stuttgart, 1990, 47-52.
7. Sieklucka M, Heim C, Block F, Sontag K-H: Transient reduction of cerebral blood flow leads to long-lasting increase in GABA content in vulnerable structures and decreased susceptibility to bicuculline induced seizures. *J Neural Transm* 1992, **88**, 87-94.
8. Läer S, Block F, Hüther G, Heim C, Sontag K-H: Effect of transient reduction of cerebral blood flow in normotensive rats on striatal dopamine-release. *J Neural Transm* (Gen Sect) 1993, **92**, 203-211.

9. Heim C, Zhang J, Lan J, Sieklucka M, Kurz T, Riederer P, Gerlach M, Sontag K-H: Cerebral oligaemia episode triggers free radical formation and late cognitive deficiencies. *Eur J Neurosci* 2000, **12**, 715-725.
10. ATSDR: Toxicological profile for manganese. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, Georgia, USA 2008.
11. Dobson AW, Erikson KM, Aschner M: Manganese neurotoxicity. *Ann N Y Acad Sci* 2004, **1012**, 115-128.
12. Guilarte TR, Chen MK: Manganese inhibits NMDA receptor channel function: implications for psychiatric and cognitive effects. *Neurotoxicology* 2007, **28**, 1147-1152.
13. Bird ED, Anton AH, Bullock B: The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. *Neurotoxicology* 1984, **5**, 59-65.
14. Shukakidze A, Lazriev I, Mitagvariya N: Behavioral impairments in acute and chronic manganese poisoning in white rats. *Neurosci Behav Physiol* 2003, **33**, 263-267.
15. Łukawski K, Nieradko B, Sieklucka-Dziuba M: Effects of cadmium on memory processes in mice exposed to transient cerebral oligemia. *Neurotoxicol Teratol* 2005, **27**, 575-584.
16. Łukawski K, Sieklucka-Dziuba M: Effect of lead exposure on memory processes in mice after cerebral oligemia. *Pharmacol Rep* 2007, **59**, 691-698.
17. Sarter M, Bodewitz G, Stephens DN: Attenuation of scopolamine-induced impairment of spontaneous alternation behavior by antagonist but not inverse agonist and agonist β-carbolines. *Psychopharmacology* 1988, **94**, 491-495.
18. Venault P, Chapouthier G, Prado de Carvalho L, Simiand J, Morre M, Dodd RH, Rossier J: Benzodiazepine impairs and β-carboline enhances performance in learning and memory tasks. *Nature* 1986, **321**, 864-866.
19. Jóźwiak L, Sieklucka-Dziuba M, Kleinrok Z: Behavioral studies of the effects of moderate oligemic hypoxia caused by bilateral clamping of carotid arteries in mice. Impairment of spatial working memory. *Pol J Pharmacol* 1998, **50**, 279-289.
20. Bortolotto ZA, Heim C, Sieklucka M, Block F, Sontag K-H, Cavalheiro EA: Effects of bilateral clamping of carotid arteries on hippocampal kindling in rats. *Physiol Behav* 1991, **49**, 667-671.
21. Jóźwiak L, Łukawski K, Czuczwar SJ, Sieklucka-Dziuba M: Competitive NMDA receptor antagonists and agonists: effects on spontaneous alternation in mice exposed to cerebral oligemia. *Pol J Pharmacol* 2004, **56**, 59-66.
22. Roth JA, Garrick MD: Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. *Biochem Pharmacol* 2003, **66**, 1-13.
23. Guilarte TR: Manganese and Parkinson's disease: a critical review and new findings. *Environ Health Perspect* 2010, **118**, 1071-1080.
24. Roth JA: Are there common biochemical and molecular mechanisms controlling manganism and parkinsonism? *Neuromolecular Med* 2009, **11**, 286-296.
25. Brouillet EP, Shinobu L, McGarvey U, Hochberg F, Beal MF: Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. *Exp Neurol* 1993, **120**, 89-94.
26. Vezér T, Kurunczi A, Náray M, Papp A, Nagymajtényi L: Behavioral effects of subchronic inorganic manganese exposure in rats. *Neurotoxicology* 2007, **28,** 1147-1152.
27. Heim C, Kolasiewicz W, Sontag K-H: The effects of the 21-aminosteroid U-74389 on spatial orientation in rats after a cerebral oligemic episode and iron-induced oxidative stress. *J Neural Transm* 2000, **107**, 95-104.
28. Nam J, Kim K: Abnormal motor function and the expression of striatal dopamine D2 receptors in manganese-treated mice. *Biol Pharm Bull* 2008, **31**, 1894-1897.
29. Fitsanakis VA, Au C, Erikson KM, Aschner M: The effects of manganese on glutamate, dopamine and gamma-aminobutyric acid regulation. *Neurochem Int* 2006, **48**, 426-433.