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THE EFFECTS OF CHRONIC MANGANESE ADMINISTRATION ON BLOOD PRESSURE IN RATS

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Abstract - Several trace elements, including manganese (Mn), affect the cardiovascular system and are implicated in some cardiovascular disease mechanisms. The effects of Mn on the vascular system, such as in the control of blood flow and blood pressure, are not completely understood. Thus, the main objective of the present study was to determine the effects of a 45-day exposure to two different doses of Mn on blood pressure values of male Wistar rats. Our results showed a significant time effect ($p<0.001$, ANOVA, repeated measures) on the values of blood pressure measurements during 45 days of Mn treatment of rats with two doses (3 mg/kg/day and 10 mg/kg/day). Additionally, we observed significant differences in the values of blood pressure measurements, especially on days 2 ($p<0.001$), 9 ($p<0.05$), 24 ($p<0.05$), 28 ($p<0.01$) and 43 ($p<0.05$). Further studies are necessary in order to establish the mechanism and relevance of Mn in this area of research.

Key words: Manganese, chronic, blood pressure, rat.

INTRODUCTION

Manganese (Mn) is a multifunctional trace element and a well-known neurotoxic agent, participating in many fundamental processes in the cell (Mergler et al., 1997, Klos et al., 2006, Vukojević et al., 2009, Vezer et al., 2007, Shukakidze et al., 2003, Ponzoni et al., 2000). In our previous studies published in this journal, we reported that chronic manganese exposure in Wistar rats led to behavioral alterations consisting of working memory deficiencies in the Y-maze task and anxiety-like behavior in the elevated plus maze, but no motor disturbances as tested by the number of arm entries in the Y-maze (Hogas et al., 2011).

Recently it was demonstrated that several trace elements, including manganese, affect the cardiovascular system and are implicated in some cardiovascular disease mechanisms (Yan et al., 1998, 2001), by participating in cell signal transduction pathways, which further affect the biomechanical properties of the vessels (Kalea et al., 2005). It also seems that besides the putative mechanisms of manganese action on vascular tone and blood pressure, this multifunctional trace element could mimic the superoxide dismutase enzymes systems (Kasten et al., 1994, Gray and Carmichael, 1992), thereby potentiating the activity of nitric oxide (NO) on vascular tone (Bild et al., 2013, Kasten et al., 1994). However, it is generally believed, as reviewed by Klimis-Tavantzis et al. (Tay-

lor et al., 1997, Klimis-Tavantzis et al., 1993, Yang et al., 1998a,b), that the effects of Mn on the vascular system, such as the control of blood flow and blood pressure, coagulation, platelet aggregation, vessel permeability, wound healing and angiogenesis, are still mainly unknown, with many gaps on the mechanistic processes and signaling pathways (Kalea et al., 2005). Therefore, the main objective of the present study was to determine the effects of a 45-day exposure to two different doses of Mn on blood pressure values of male Wistar rats.

MATERIALS AND METHODS

Animals

Experimentally naive, male Wistar rats (n=12), weighing approximately 180-250 g at the beginning of the experiment were used. The animals were housed in a temperature- and light-controlled room (23 ± 2°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act of Romania, and all procedures complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethic Committee and efforts were made to minimize animal suffering and to reduce the number of animals used.

Drug treatment

Manganese (Sigma, USA) was injected intraperitoneally (i.p.) to two separate groups at doses of 3 mg/kg/day and 10 mg/kg/day for 45 consecutive days. A sample size of n=6 for each experimental group was used. Blood pressure measuring was performed with an electronic system (HUGO SACHS D 7806) especially designed to non-invasively measure the blood pressure in rats (the tail-cuff method).

Measurements (all samplings were measured three times and averaged) were performed two times before starting the Mn administration (days 1 and 2) and eight times (days 4, 9, 16, 20, 24, 28, 32 and 43) during the 45 days of manganese administration.

Data Analysis

The total values of the blood pressure measurements were statistically analyzed using two-way ANOVA repeated measures for dose effect (3 mg/kg/day vs. 10 mg/kg/day) and time effect (days of measurements). In addition, the differences for each day separately were statistically analyzed by using the Student's t-test (two tailed, unpaired). All results are expressed as mean ± SEM. $P < 0.05$ was regarded as statistically significant. The analyses were performed using the SPSS program (version 17.0).

RESULTS

When we first analyzed the total values of the blood pressure measurements using two-way ANOVA repeated measures, we observed no statistical differences (ANOVA, repeated measures; treatment effect, $p = 0.8$; days effect, $p < 0.001$) in the values during the entire period of treatment (both before and after starting Mn treatment) between the two different doses we chose to use in our experiment (3 mg/kg/day and 10 mg/kg/day) (Fig. 1). However, as can be seen from the p value for the time effect (number of days), it seems that when the day variable is considered, a significant effect exist between the doses used in our experiment. Additionally, when we used the Student's t-test, two tailed, unpaired (considering that we had only two groups to compare), we also observed significant differences in the values of the blood pressure measurements, especially on days 2 ($p < 0.001$), 9 ($p < 0.05$), 24 ($p < 0.05$), 28 ($p < 0.01$) and 43 ($p < 0.05$) (Fig. 1).

DISCUSSION

Kasten et al. (1994) showed that the administration of Mn generated decreased blood pressure values. However, these effects were diminished by N-nitro-L-arginine, which indicates that Mn is increasing the duration of NO half-life, probably through some mechanisms that involve the stimulation of superox-

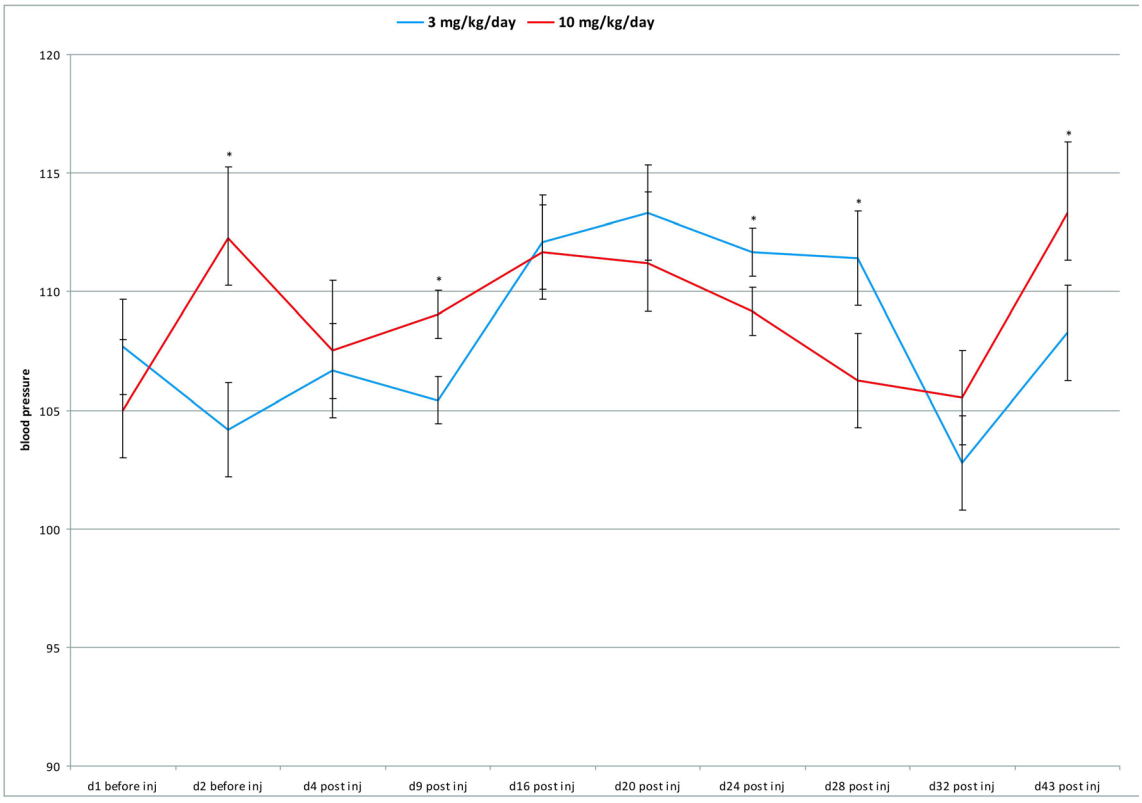

Fig. 1. Blood-pressure values during the 45 days of Mn administration in rat.

ide dismutase (SOD) that would in turn increase the NO effects on vascular domain. These aspects are also somehow explained by the fact that SOD could contain manganese, cooper or zinc in their active sites (Ciobica et al., 2012). Jamieson et al. (1983) demonstrated that manganese reduced the blood pressure, as studied in the guinea-pig isolated trachea. These effects could be mainly explained by the interaction between Mn and the ion channels, as well as with some neurotransmitters and specific receptors (Kalea et al., 2005).

In a study involving approximately 700 child patients from South Africa, it was demonstrated by regression analysis, that the cardiovascular parameters of hypertensive subjects significantly correlated with manganese (along with vitamin E, B12, A or iron) levels. In addition, the study showed that the dietary intake of these nutrients was below the necessary reference needed (Schutte et al., 2003).

It was also reported that dietary manganese influences vascular contraction mechanisms in the thoracic aorta of rats. Kalea et al. (2005) demonstrated in Sprague-Dawley rats that were fed either with a deficit of Mn or with supplemented Mn that dietary Mn influences the receptor signaling pathways and contractile characteristics of the vascular smooth muscle cells by a mechanism mainly involving the α1 adrenergic receptor. Interestingly, the maximum contractile force was obtained in the case of the Mn-adequate group of rats, while the lowest was reported for the Mn-supplemented group. Additionally, the vessel reactivity reached its highest values in the case of the Mn-deficient group.

These papers show that despite the existence of some important studies in this area of research, current knowledge regarding the blood pressure and vascular contraction mechanisms is quite limited and awaits further research.

We describe a significant time effect ($p<0.001$, ANOVA, repeated measures) in the values of the blood pressure measurements during chronic 45-day Mn-treatment, using two different doses of Mn (3 mg/kg/day and 10 mg/kg/day). We observed significant differences in the values of the blood pressure measurements, especially on days 2, 9, 24, 28 and 43. Further studies are necessary in order to establish the mechanism and relevance of Mn in this area of research.

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