

## THE EFFECTS OF CHRONIC MANGANESE ADMINISTRATION ON BLOOD PRESSURE IN RATS

IONELA LACRAMIOARA SERBAN<sup>1</sup>, MIHAI HOGAS<sup>1</sup> and ALIN CIOBICA<sup>2,3</sup>

<sup>1</sup> Department of Physiology, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi 700115, Romania

<sup>2</sup> "Alexandru Ioan Cuza" University, Iasi, 700506, Romania

<sup>3</sup> Center of Biomedical Research of the Romanian Academy, Iasi Branch, Iasi, 700506, Romania

**Abstract** - Recently it was observed that several trace elements, including manganese (Mn), can affect the cardiovascular system and have been implicated in certain cardiovascular disease mechanisms. However, to date the effects of Mn on the vascular system, such as in the control of blood flow and blood pressure, are not completely understood. 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 show a significant time effect ( $p<0.001$ , ANOVA, repeated measures) on blood pressure during the 45 days of chronic Mn treatment with two doses of Mn (3 mg/kg/day; 10 mg/kg/day). Additionally, we observed significant differences in blood pressure, 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.

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

### INTRODUCTION

Manganese (Mn) is a multifunctional trace element and well-known neurotoxic agent that participates 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 of 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; they have been 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). Aside from the putative mechanisms of manganese action on vascular tone and blood pressure, this multifunctional trace element could mimic the superoxide dismutase (SOD) enzyme systems (Kasten et al., 1994, Gray and Carmichael, 1992) and thereby potentiate the activity of nitric oxide (NO) on vascular tone (Bild et al., 2013, Kasten et al., 1994). 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 largely unknown (reviewed by: Klimis-Tavantzis et al. (Taylor et al., 1997, Klimis-Tavantzis et al., 1993, Yang et al., 1998 a,b), with many gaps in our knowledge on the mechanistic processes and signaling pathways (Kalea et al., 2005).

For this reason, 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 in male Wistar rats.

## MATERIALS AND METHODS

### *Animals*

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 ( $23 \pm 2^\circ\text{C}$ ; a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania; 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 of rats in 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 measurements were performed with an electronic system (HUGO SACHS D 7806) especially designed to non-invasively measure the blood pressure in rats by the tail-cuff method.

Measurements (all sampling was done three times on average) were performed two times before starting the Mn administration (day 1 and 2) and

eight times (on days 4, 9, 16, 20, 24, 28, 32 and 43) during the 45 days of manganese administration.

### *Data Analysis*

The total values of blood pressure measurements were statistically analyzed by 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 were statistically analyzed by Student's t-test (two-tailed, unpaired). All results are expressed as mean $\pm$ SEM.  $P < 0.05$  was considered as statistically significant. The analyses were performed using the SPSS program (version 17.0).

## RESULTS

When we first analyzed the total values of blood pressure, we observed no statistical differences (ANOVA, repeated measures; treatment effect,  $p = 0.8$ ; days effect,  $p < 0.001$ ) between the values of the blood pressure measurements during the entire period of treatment (both before and after starting the Mn treatment) between the two different doses we chose to use in our experiment (3 mg/kg/day and 10 mg/kg/day) (Fig.e 1). However, as can be seen from the  $p$  value for the time effect (number of days), a significant effect exists between the doses used in our experiment.

Additionally, when we used the Student's t-test, two-tailed and 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

The effects of manganese exposure on blood pressure and vascular contraction have been studied in a very few instances. Kasten et al. (1994) showed that the administration of Mn decreased blood pressure. However, these effects were diminished by N-nitro-



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

L-arginine, which leads to the idea that Mn increases the duration of NO half-life, probably through some mechanisms that involving the stimulation of SOD that in turn increases the effects of NO on the vascular domain. These aspects are also explained by the observation that different species of SOD contain manganese, copper or zinc in its active sites (Ciobica et al., 2012). In addition, Jamieson et al. (1983) demonstrated that manganese reduced blood pressure, as studied on guinea-pig isolated trachea. These effects were explained as the result of interaction between Mn and ion channels, as well as neurotransmitters and specific receptors (Kalea et al., 2005).

Additionally, in a study involving approximately 700 children 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 way below the necessary reference needed (Schutte

et al., 2003). It was also reported that dietary manganese influences the vascular contraction mechanisms in the thoracic aorta of rats. Kalea et al. (2005) demonstrated in Sprague-Dawley rats that were either fed with a deficit of Mn or supplemented with Mn, that dietary Mn influences the receptor signaling pathways and contractile characteristics of vascular smooth muscle cells by a mechanism mainly involving the  $\alpha_1$  adrenergic receptor. Interestingly enough, 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; the vessel reactivity reached its highest values in the case of the Mn-deficient group. This confirms that despite the existence of some important studies in this area of research, the current knowledge regarding the blood pressure and vascular contraction mechanisms are quite limited and waiting to be further established.

Our results describe a significant time effect on blood pressure during 45 days of chronic Mn treatment (3 mg/kg/day; 10 mg/kg/day). Significant dif-

ferences in blood pressure were observed on days 2, 9, 24, 28 and 43.

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