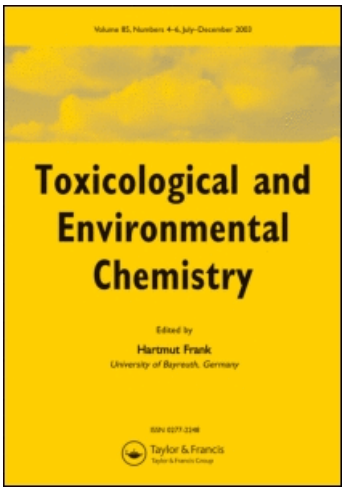

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Changes in serum, liver, and brain high and low molecular weight alkaline phosphatase following manganese toxicity in rats

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The relationship between manganese (Mn) treatment and changes in the activities of serum, liver, and brain high and low molecular weight alkaline phosphatase (ALP) activity was investigated. Results obtained showed that every other day intrapritoneally injection of 175 μmol kg^{-1} Mn to male rats for two consecutive weeks produced decreased levels of liver and brain ALP activity, while a serum enzyme activity was significantly elevated. Chronic exposure to 68.7 μmol kg^{-1} Mn produced a significant fall in liver and brain levels of ALP activity and a rise in serum activity. Using gel filtration chromatography technique with sephacryl S_{300} showed that, in comparison to control, serum and liver homogenate from Mn-treated groups displayed a significant level of high molecular weight ALP, which might be considered as a potential biomarker for Mn toxicity.

Keywords: manganese; alkaline phosphatase; high molecular weight alkaline phosphatase; liver; brain

Introduction

The serum of patients with excess manganese (Mn) may contain high molecular weight alkaline phosphatase (ALP). The incidence of this form of ALP may be associated with hepatic malignancy (Bhudhisawasdi, Musik, and Areejitransuorn 2004), and thus may be considered as a biomarker in such patients. As a model for hepatic damage, the existence of this isoenzyme was investigated in sera of Mn-treated animals, which is the major aim of the current study.

Manganese is the second most common naturally occurring element in the environment and essential for maintaining the function of many enzymes, including pyruvate carboxylase, arginase, superoxide dismutase and glycosyltransferase (Crossgrove and Allen 2003; Erikson et al. 2007; Gerber, Leonard, and Hantson 2002).The main route of Mn absorption is the gastrointestinal tract. The liver may be an important depot for Mn that serves as a source for the brain. Mn readily concentrates in the brain, especially in basal ganglia and produces an irreversible neurological syndrome similar to Parkinson's disease (Aschner 2000; Takeda 2003). Increased brain Mn levels were also reported to

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induce oxidative stress, as well as alterations in neurotransmitter metabolism with concurrent neurobehavioral and motor deficits (Erikson et al. 2004; Veźera et al. 2005). Moreover, Mn may play a role in the pathogenesis of chronic hepatic encephalopathy and intra-hepatic cholestasis (Krieger et al. 1995).

High molecular weight ALP was found in patients with extra-or intra-hepatic cholestasis, malignancy of liver, primary or metastatic carcinoma, Hodgkin's and non-Hodgkin's lymphoma, and/or leukemia (Bhudhiswaseli, Musik, and Areejitrnsuorn 2004; Wolf 1990), and was suggested as a tumor marker for liver (Moshtaghie, Ani, and Soltani 1996), and colorectal cancer (Wei, Chaung, and Wei 1993). The existence of hepatobiliary dysfunction in those patients with Mn overload (Crossgrove and Zheng 2004; Krieger et al. 1995) lead us to explore and compare the probable occurrence of high molecular weight ALP in the sera, liver, and brain of rats treated with Mn.

Materials and methods

All chemicals used in this study were purchased from Sigma Chemical Company. Twenty-eight male Wistar rats weighing ~200–220 g were purchased from Pasteur Institute (Tehran, Iran), kept in the university animal house at standard conditions 22–24°C, 40–60% relative humidity and 12 h light–dark cycle, and fed with standard rat food and water *ad libitum* through the whole experimental period.

Rats were divided randomly into two groups, labeled short-term and chronic exposure to Mn, respectively. Each group had a specific control group. In the short-term study, the control group received intrapritoneally (ip) injection of sterile normal saline (0.1 mL) every other day for two consecutive weeks. Simultaneously, the treated group was administered ip 175 μmol kg^{-1} of Mn ($MnCl_2 \cdot 4H_2O$). The chronic study was carried out using 68.7 μmol kg^{-1} of this Mn for a period of seven weeks, as described in the method for the short-term groups. Rats were then killed by decapitation at the end of their treatment periods. Blood samples were collected and sera were separated from cells by centrifugation, and used for enzyme and protein assays.

Brain and liver tissues were removed, washed with cold (+4°C) saline solution, and homogenized (10% w/v) in a buffer solution containing 10 mM Tris, and 0.25 M sucrose, pH 7.4 at 4°C. The homogenates were then centrifuged at 13,000 *g* for 20 min at 4°C, the resultant supernatants removed and used for the enzyme and protein determination (Yazar and Tras 2001).

Alkaline phosphatase activity was measured at 410 nm and 37°C by the formation of para-nitrophenol (pNP) from para-nitrophenol phosphate (pNPP) as substrate, and 2-amino-2-methyl-1-propanol (AMP) as buffer (Bomers and McComb 1975). Protein concentration was determined as described by Bradford (1976), with bovine serum albumin as standard.

Gel filtration chromatography

In order to separate high and low molecular weight isoenzymes of ALP, gel filtration chromatography on sephacryl S_{300} was used. Each sample (0.2 mL) was diluted with equal volume of Tris buffer (50 mM, pH 7.4), applied to a column (50 × 0.9 cm) loaded with sephacryl S_{300} and then eluted at 10 mL h^{-1} with Tris-HCl buffer (50 mM, pH 7.4). Fractions of 1 mL were then collected (Moshtaghie, Ani, and Soltani 1995). Alkaline phosphatase activity and protein concentrations in each fraction were

determined according to the methods mentioned earlier (Bomers and McComb 1975; Bradford 1976).

Statistical analysis

Analysis of data was accomplished using SPSS (version 11.5) statistical software package. Between-groups comparisons were performed with Student's *t*-test. All results were presented as mean ± SD and were considered statistically significant at $p < 0.05$.

Results

Studies showed that administration of Mn significantly elevated serum total ALP activity (Panel A of Table 1). Significant reduction in the liver and brain total ALP activities was seen when rats were treated with the same amount of Mn every other day for two weeks (Panel A of Table 1).

Chronic exposure to Mn produced significant elevation 16.6 in total serum ALP (Panel B of Table 1) and reduction in liver and brain ALP activities. Comparing data obtained from short-and long-term effects of Mn on the activity of the enzyme in serum, liver, and brain showed that changes in the enzyme activity were time- dependent with greater effects noted chronically.

Gel filtration chromatography technique

Experiments were conducted to separate high and low molecular weight ALP from sera, liver, and/or brain homogenate of both treated and untreated animals. Fractionation of the serum from Mn-treated and Mn-untreated control animals showed elevation in serum total ALP activity was mostly related to the high molecular weight ALP (Figure 1). When liver homogenate from both control and Mn-treated rat was chromatographed, a significant reduction in activity of low molecular weight ALP, and a significant elevation in high molecular weight ALP was found (Figure 2). A decrease in total liver homogenate ALP activity and concomitant elevation of high molecular ALP was also seen.

Table 1. Effects of Mn on the activity of serum, liver, and brain ALP specific activity.

	ALP (IU/mg tissue protein)		
	Serum	Liver	Brain
Panel A			
Control	1.94 ± 0.10	3.42 ± 0.26	2.02 ± 0.12
Treated	2.23 ± 0.23*	3.02 ± 0.18*	1.87 ± 0.08*
Panel B			
Control	2.10 ± 0.10	3.16 ± 0.26	2.27 ± 0.13
Treated	2.45 ± 0.14*	2.75 ± 0.13*	2.04 ± 0.1*

Notes: Rats were injected with Mn as ($MnCl_2 \cdot 4H_2O$) every other day for two weeks (Panel A) and for seven weeks (Panel B).
*Indicates statistically significant differences of ALP specific activity between Mn-treated animals and their controls ($p < 0.05$).
Data are presented as Mean ± SD.

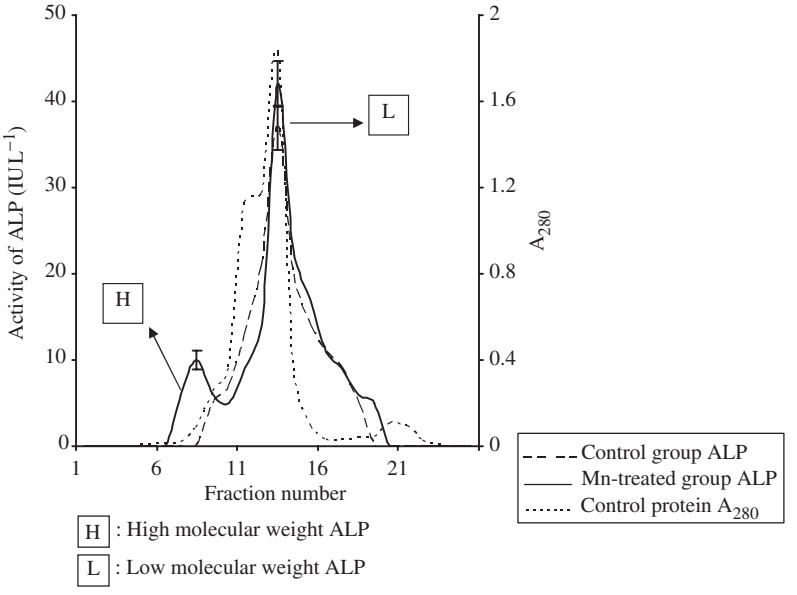

Figure 1. The elution profile of serum of control and Mn-treated groups. Serum was diluted with buffer and loaded on the top of the column containing sephacryl S_{300} and eluted with buffer *Tris*-HCl, 50 mM, pH 7.4) at 10 mL h^{-1} rate. ALP activity and A_{280} of all fractions were measured.

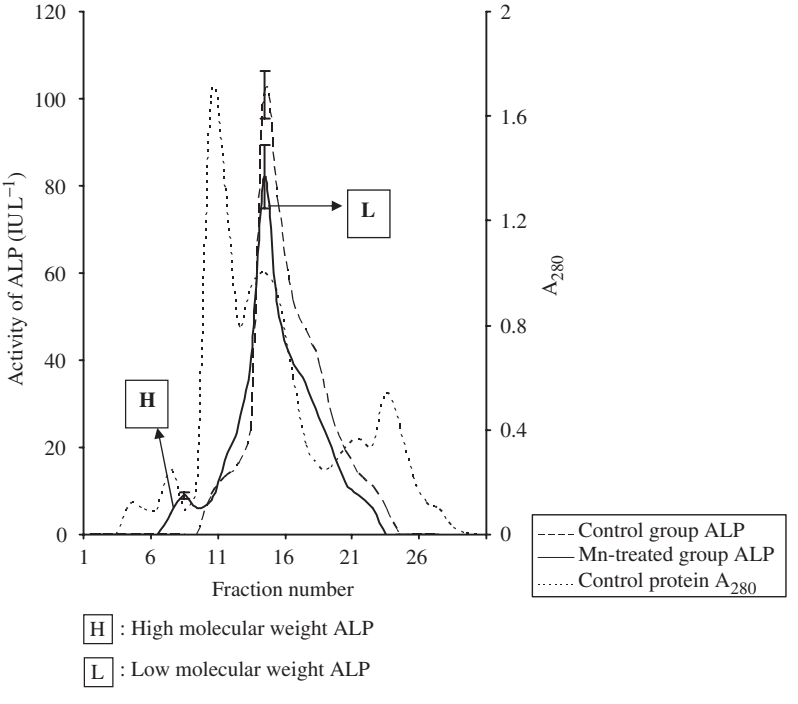

Figure 2. The elution profile of liver of control and Mn-treated groups. Liver from Mn-treated and Mn-untreated animals were homogenate as mentioned earlier and loaded on the top of column and eluted as already mentioned.

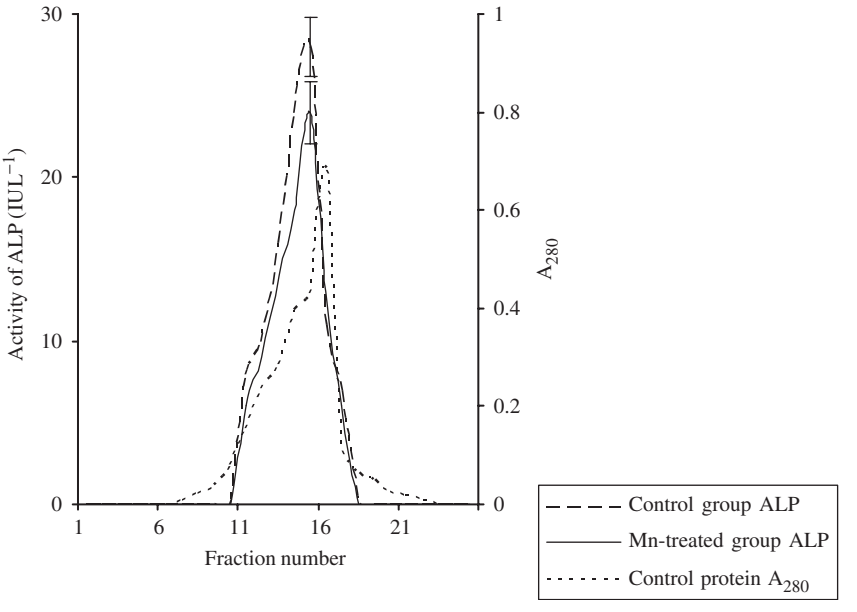

Figure 3. The elution profile of brain of control and Mn-treated groups. Brain from Mn-treated and Mn-untreated animals were homogenate as mentioned earlier and loaded on the top of column and eluted as mentioned in method section.

Figure 3 shows a significant reduction in low molecular weight brain ALP following Mn treatment, but no changes observed in the level of high molecular weight ALP when compared with control values.

Discussion

Measurement of the activities of ALP isoenzymes was used for the identification and monitoring of diseases associated with these isoenzymes. Biliary ALP or high molecular weight ALP was found in the serum of patients with biliary obstruction and metastatic liver cancer (Bhudhisawasdi, Musik, and Areejitransuorn 2004). Previous studies from our lab showed that high molecular weight ALP might be considered as a tumor marker for liver cancer (Moshtaghie, Ani, and Soltani 1996). This isoenzyme is regulated by steroid hormones (Moshtaghie, Ani, and Soltani 1995), and also affected by other elements such as aluminum and lead (Moshtaghie, Ani, and Mirhashemi 2006a, 2006b). However, no apparent data was presented concerning Mn toxicity and the induction of high molecular weight ALP in the serum of patients with Mn overload. Results presented in this study, revealed the relationship between Mn administration and changes in the sera, liver, and/or brain high molecular weight ALP.

Data presented showed that short- and long-term Mn administration to rat increased total serum ALP activity significantly, whereas, liver and brain total ALP activities decreased (Table 1). Similar results were reported by Salehi et al. (2003). It was demonstrated that Mn in body is primarily cleared by the liver and may play a role in the pathogenesis of chronic hepatic encephalopathy (Takeda, Sawashita, and Okada 1998). Rahman, Siddiqui, and Jamil (2000) suggested that the decrease in the activities of ALP in

different tissues might be due to increased permeability of plasma membrane or cellular necrosis.

When elevated serum total ALP activity from Mn-treated animals was fractionated, it showed that increase of serum total ALP activity was mostly due to the high molecular weight ALP (Figure 1). Elevated high molecular weight ALP was also found in liver homogenate in the Mn-exposed group (Figure 2), but there was no high molecular weight ALP in brain homogenate (Figure 3). The elevated high molecular weight ALP in serum may originate either from the liver and/or other tissues producing this enzyme. This may also be due to either damage of bile duct and/or synthesis of new molecules of high molecular weight ALP. Alternatively, association of low molecular weight ALP with other enzymes including 5′-nucleotidase, γ-glutamyltranspeptidase, and nucleotide-pyrophosphatase, might result from formation of high molecular weight ALP (Wulkan and Leijense 1986; Remaley and Wilding 1989). Comparing data obtained from liver and brain (Figure 2 versus Figure 3) showed that, although low molecular weight ALP was significantly decreased in brain of Mn-treated animals, no indication was seen in the production of high molecular weight ALP.

Data indicate that Mn may be involved in pathological damage to liver tissue, particularly, bile ducts leading to production and secretion of high molecular ALP. Thus, the appearance of this isoenzyme in sera may be considered as a suitable tool diagnosis of Mn toxicity.

References

Aschner, M. 2000. Manganese: Brain transport and emerging research needs. *Environmental Health Perspectives* 108 (Suppl. 3): 429–32.

Bhudhisawasdi, V., K. Musik, and P. Areejitransuorn. 2004. Clinical value of biliary alkaline phosphatase in non-jaundiced cholengiocarcinoma. *Cancer Research and Clinical Oncology* 130: 87–92.

Bomers, G.N., and R.B. McComb. 1975. Measurement of total alkaline phosphatase activity. *Clinical Chemistry* 21: 1988–95.

Bradford, M.M. 1976. A rapid and sensitive method or quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–54.

Crossgrove, J., and W. Zheng. 2004. Manganese toxicity upon overexposure. *NMR in Biomedicine* 17: 544–53.

Crossgrove, J.S., and D.D. Allen. 2003. Manganese distribution across the blood-brain barrier. *Neurotoxicology* 24: 3–13.

Erikson, K.M., K. Thompson, J. Aschner, and M. Aschner. 2007. Manganese neurotoxicity. A focus on the neonate. *Pharmacology & Therapeutics* 113: 369–77.

Erikson, K.M., A.W. Dobson, D.C. Dorman, and M. Aschner. 2004. Manganese exposure and induced oxidative stress in the rat brain. *The Science of the Total Environment* 334: 409–16.

Gerber, G.B., A. Leonard, and P. Hantson. 2002. Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Critical Reviews Oncology Hematology* 42: 25–34.

Krieger, D., S. Krieger, O. Jansen, and H. Lichtnecker. 1995. Manganese and chronic hepatic encephalopathy. *Lancet* 346: 270–4.

Moshtaghie, A.A., M. Ani, and S.M. Mirhashemi. 2006a. Comparative effects of lead on serum, liver and brain high molecular weight alkaline phosphatase in rats. *Pakistan Journal of Biological Science* 9: 2278–82.

Moshtaghie, A.A., M. Ani, and S.M. Mirhashemi. 2006b. Comparative effects of aluminium on serum, liver and brain high and low molecular weight alkaline phosphatase in rats. *Journal of Medical Science* 6(5): 848–52.

Moshtaghie, A.A., M. Ani, and M. Soltani. 1996. High molecular alkaline phosphatase as a tumor marker for liver cancer; A comparative study with bone and intestinal cancers. *Clin. Chem. Enzyme. Commun* 7: 9–13.

Moshtaghie, A.A., M. Ani, and M. Soltani. 1995, Study of the effect of steroid hormone on high and low molecular weight alkaline phosphatase, *Indian Journal of Pharmacology* 27: 152–8.

Rahman, M.F., M.K. Siddiqui, and K. Jamil. 2000. Acid and alkaline phosphatase activities in a novel phosphothionate (RPR-11) treated male and female rats. Evidence of dose and time dependent response. *Drug and Chemical Toxicology* 23: 497–509.

Remaley, A.T., and P. Wilding. 1989. Macroenymes: Biochemical characterization, clinical significance, and laboratory detection. *Clinical Chemistry* 35: 2261–70.

Salehi, F., D. Krewski, D. Mergler, and L. Normandin. 2003. Bioaccumulation and locomotor effect of manganese phosphate/sulfate mixture in Spargue-Dawly rats following subchronic (90 days) inhalation exposure. *Toxicology and Applied Pharmacology* 191: 264–71.

Takeda, A. 2003. Manganese action in brain function. *Brain Research Reviews* 41: 79–87.

Takeda, A., J. Sawashita, and S. Okada. 1998. Manganese concentration in rat brain: Manganese transport from the peripheral tissues. *Neuroscience Letters* 242: 45–8.

Veźera, T., A. Pappa, Z. Hoykb, C. Vargab, M. Ńarayc, and L. Nagymajťenyia. 2005. Behavioral and neurotoxicological effects of subchronic manganese exposure in rats. *Environmental Toxicology and Pharmacology* 19: 797–810.

Wei, J.S., N.C. Chaung, and L.L. Wei. 1993. High molecular alkaline phosphatase as a tumor marker for colorectal cancer: Comparison of two test methods. *Clinical Chemistry* 39: 540–3.

Wolf, P. 1990. High molecular weight alkaline phosphatase and alkaline phosphatase lipoprotein X complex in cholestasis and hepatic malignancy. *Archives of Pathology & Laboratory Medicine* 114: 557–9.

Wulkan, R.W., and B. Leijense. 1986. Alkaline phosphatase and cholestasis. *Annals of Clinical Biochemistry* 23: 405–12.

Yazar, E., and B. Tras. 2001. Effects of fluoroquinolone antibiotics on hepatic superoxide dismutase and glutathione peroxidase activities in healthy and experimentally induced peritonitis mice. *Revista de Medicina Veterinaria* 152: 235–8.