Short Communication

Lead-induced Changes of Cation-osmotic Hemolysis in Rats

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Abstract. Erythrocyte microrheology changes were measured using cation-osmotic hemolysis (COH) in healthy rats and rats after 6 and 12 months of lead ingestion Using COH, properties of two membrane constituents, spectrine membrane skeleton and membrane bilayer were studied COH in rats after 12 months of lead ingestion was significantly lower only in the area with lower ionic strength (15 4 61 6 mmol l^{-1} of NaCl) ($p < 0\ 01$ resp $p < 0\ 05$) No changes in COH were found after 6 months of continuous lead ingestion The relation between cation-osmotic hemolysis and erythrocyte deformability is being discussed

Key words: Lead — Cation-osmotic hemolysis — Spectrine skeleton — Rats

Lead is a potent toxic heavy metal that is an occupational hazard and environmental pollutant It is know to induce a broad range of physiological, biochemical and behavioral dysfunctions in animals and humans (Bornschein et al 1980a, Bornschein et al 1980b, Sollinway et al 1996, Gurer et al 1999) One of the targets for lead toxicity is the hematological system It was found that lead induces changes in the red blood membrane lipids and proteins (Jehan and Motlag 1995, Belloni-Olivi et al 1996), changes in activity of some membrane-associated enzymes (Jehan and Motlag 1995) or ions transport mechanisms (Calderon-Salinas et al 1999) Moreover, lead may inhibit hemoglobin synthesis and induce anaemia (Piomelli et al 1982, Gurer et al 1998) Furthermore, it was documented that chronic exposure to lead can result in an erythrocyte deformability decrease (Horiguchi et al 1991 Terayama 1993) This last effect may also be involved in the lead-induced anemia because of more intensive sequestration and destruction of less deformable erythrocytes in spleen network (Terayama 1993)

Erythrocyte deformability (ED) is an important physiological factor which plays an essential role in delivery of oxygen to the tissues ED depends upon the

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following three factors 1 viscoelastic properties of the membrane, 2 geometric properties and 3 internal viscosity (Mokken et al 1992) Under normal conditions, ED allows individual red cells to traverse nutritive capillaries and supply the tissues with oxygen Any decrease in deformability may result in impaired perfusion of peripheral tissues (Ernst et al 1991, Gdovinová et al 1994)

The aim of this study was to determine lead dependent changes of erythrocyte microrheology in rats using cation-osmotic hemolysis

One month old juvenile Wistar albino rats of both sexes (average body weight 128 ± 9 g/male and 119 ± 8 g/female, $n = 6/6$) were housed in a temperature-controlled environment (22 ± 2°C) and exposed to 12 h light/12 h dark cycle They had free access to a commercial, balanced stock diet (Larsen diet) and water The drinking water containing $PbCl_2$ (100 0 μmol l^{-1}) was given to the experimental animals for 182 and 364 days As a control group, animals housed under the same conditions were used but water was free of lead

Erythrocyte membrane biophysical properties were estimated using the method of cation-osmotic hemolysis (COH) described by Nicak and Mojžiš (1992)

The analysis of data for statistical significance of the differences found was carried out using the Student's t-test The $p < 0\ 05$ was selected as the point of minimal statistical significance

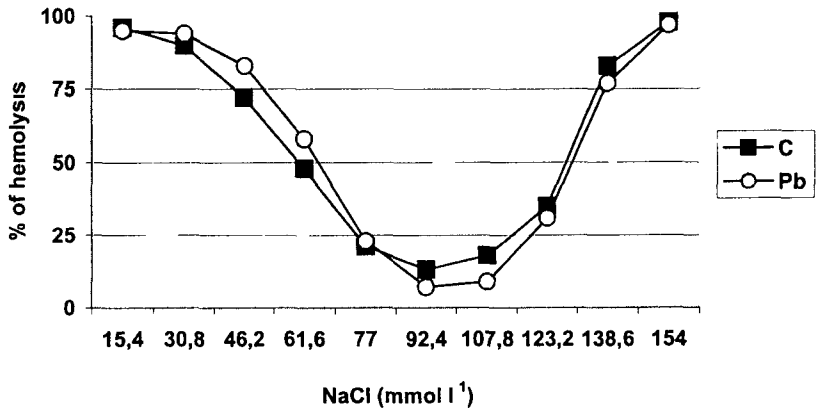

Figure 1. Cation-osmotic hemolysis in lead-treated rats (Pb) after 6 months of lead ingestion in comparison with control group (C)

There were no significant differences in COH between control and experimental group after 6 months of lead ingestion (Fig 1) Comparison of COH between the group of lead-treated animals and the control group after 12 months of lead ingestion is shown in Fig 2 There is a significant decrease in COH in the media with low ionic strength (15 4–61 6 mmol l^{-1} of NaCl) ($p < 0\ 01$ resp $p < 0\ 05$)

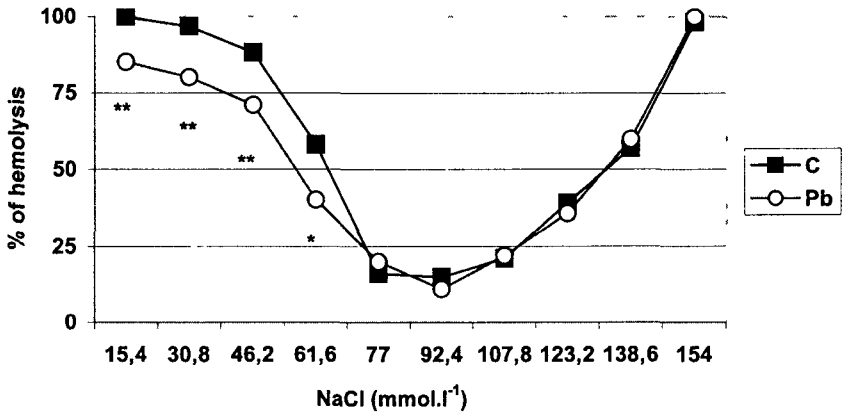

Figure 2. Cation-osmotic hemolysis in lead-treated rats (Pb) after 12 months of lead ingestion in comparison with control group (C), $*p < 0.05$, $** p < 0\ 01$.

However, in the media with high ionic strength (92.4–154.0 mmol·l^{-1} of NaCl), no significant effect of lead on COH was observed.

The interest in erythrocyte deformability (ED) has rapidly increased in the last years, and because the clinical importance of ED became apparent, many methods of its measuring have been developed. Erythrocyte filtration is currently the most widely used technique (Mokken et al. 1992). A few years ago we developed the method of cation-osmotic hemolysis (Nicák and Mojžiš 1992). Later, we showed that membrane deformability and COH were closely related (Mirossay et al. 1997), and we believe that COH reflects basic information on the erythrocyte deformability. Moreover, this method was used in many experimental or clinical studies (Dolobáč et al. 1998; Mojžiš et al. 1998; Mojžiš et al. 1999; Mojžiš and Ništiar 2000). We also showed that the method of COH, in relation to the NaCl concentration in the incubating medium, enables determination of the biophysical state of the spectrin skeleton and the lipid bilayer biophysical state according to the first and the second maximum of hemolysis at the low and high NaCl concentration, respectively (Nicák and Mojžiš 1993). Decrease of ED was also documented in some human and animal studies (Horiguchi et al. 1991; Terayama 1993; Osterode 1996). In our experiments there were no changes in COH after 6 months of continuous lead application. On the other hand, 12-month pretreatment of animals with lead significantly decreased COH (i.e. deformability) in the media with low ionic strength (15.4–61.6 mmol·l^{-1}). Decrease of COH in media with low ionic strength indicates that mainly spectrin skeleton was affected. Precise mechanism of lead-induced erythrocyte rigidity is not known and there is not any published evidence for direct lead-spectrin interaction. Explanation of the effect of lead on erythrocyte deformability is rather speculative. Lead induces production of free radicals and decreases activity of antioxidant enzymes (Jendryczko 1994; Hunaiti

and Soud 2000) and it is well known that free radicals produce spectrin cross-linking and thus cytoskeleton-associated alterations of red blood cell deformability (Snyder et al. 1985, Nash and Gratzer 1993; Baskurt et al. 1998). Moreover, various mechanisms including decrease in ATP concentration or inhibition of Ca^{2+}-ATPase (Grabowska and Gumińska 1996) with subsequent disturbances of intracellular calcium homeostasis may contribute to this effect of lead.

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