BioMetals 1997, **10**, 331–335

Lead effects on structural and functional cellular parameters in human red cells from a prenatal hematopoiesis stage

Raquel E. Serrani, Inés A. Gioia & Juan L. Corchs

Cátedra de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Santa Fe, Rosario, Argentina

Received 12 January 1997; accepted for publication 15 April 1997

An attenuation (or reversion) of the prolytic effect of lead on neonatal red cells is observed in iso- or hypotonic low ionic strength media. This effect correlates neither with concomitant activation of K^+ (Ca^{2+} or Pb^{2+}) channels nor with volume reduction. Neonatal erythrocytes were used in this study owing to their greater cellular density, as compared with adult red cells, for the above mentioned channels. The attenuation–reversion effect would be mediated through lead interactions with the cytoskeleton, a structure that is the limiting factor for red cell lysis in low ionic strength media.

Keywords: erythrocytes, ionic strength, K^+ transport, lead

Introduction

The margin of safety for lead toxicity is extremely narrow; risk blood levels have been reexamined in recent years and values above 30 μg dl^{-1} (1.5 μM) are now regarded as high (Simons 1993). Moreover, childhood lead poisoning has been redefined as a blood level of 0.48 μmol l^{-1} (10 μg dl^{-1}) (Rosen 1992).

Silbergeld (1992) described a direct link between low-level lead exposure during early development and deficits in neuro behavioral–cognitive performance evidenced later in childhood and adolescence. The finding of a significant and positive correlation between lead concentration in maternal and cord blood, as well as the possibility of transfer of heavy metals from mothers to young infants (Ong *et al*. 1993), are indicative of a potential health hazard.

Lead blood levels are determined by environmental and/or occupational exposures, and 99% of the cation present in human blood is located in the erythrocyte. This distribution between serum and erythrocytes may reflect both the binding of the element to cellular components and its transport across the erythrocyte membrane. Lead can readily pass through the erythrocyte membrane via the anion exchanger but it is also actively extruded from the cells by the Ca^{2+} pump (Simons 1983a,b, 1984, 1985, 1993).

In the present paper, the effect of lead on the lysis phenomena as well as its effect on the passive transport of potassium across the plasma membrane of neonatal erythrocytes were studied. Red cells from umbilical cord blood were used because the proportion of transport units affected by lead is greater in these cells than in adult erythrocytes (Serrani *et al*. 1995). The upper limit of the concentration range within which lead induces activation of [K^+(Ca^{2+})] channels was selected to study the effect of the medium ionic strength on the structural (susceptibility to lysis) parameters. Evidence is presented suggesting that the effect of lead on cellular integrity is independent of the changes in cellular volume induced by activation of the K^+–Ca^{2+} channels. Furthermore, at the lead concentration assayed, the ionic strength of the incubation media diminished (or reverted) its effect on lysis.

Address for correspondence: R.E. Serrani, Cátedra de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Santa Fe 3100, Rosario, Argentina. Tel: (+54) 41 371 741; Fax: (+54) 41 253 753.

© *1997 Rapid Science Publishers Ltd London*

Materials and methods

Blood samples from human umbilical cord belonging to normal newborn babies were used. They were processed within 12 h after extraction. Each sample was centrifuged to remove the plasma, and the cellular pellet was washed three times with an isotonic buffered solution to ensure that the erythrocytes were free of plasma and other cellular elements.

Incubation conditions. Efflux of potassium

The cells were resuspended at a hematocrit value of 3–5%, in a medium the composition of which varied to obtain a specific ionic strength. Cell suspensions were incubated at 37°C in a thermostatized water bath shaker, in the appropriate medium with or without 20 μM Pb^{2+}, and with the circumstantial addition of quinidine. The incubation media utilized had either a normal ionic strength (μ) (1.46×10^{-1} M, components expressed in mM: $NaNO_3$, 120; KNO_3, 1; $Ca(NO_3)_2$, 1; Tris–MOPS pH 7.4 at 37°C, 10; glucose, 10); or a near null ionic strength (6.3×10^{-3} M: KNO_3, 1; $Ca(NO_3)_2$, 1; Tris–MOPS, 10; sucrose, 290).

The efflux of potassium was determined in aliquots of the cell suspensions. The aliquots were centrifuged in a Beckman microfuge and the supernates were used to measure K^+ by atomic absorption spectrophotometry in a Perkin Elmer spectrophotometer model 2380.

Cellular lysis in hypotonic media. Incubation conditions and data adjustment

After incubating aliquots of the cell suspensions in isotonic media NO_3^-/Pb^{2+} or NO_3^-/Pb^{2+}/quinidine) as stated above, they were resuspended and incubated at 27°C during 5 min in media with decreasing tonicity (the concentration of $NaNO_3$ was reduced from 0.3 to 0.0 mM; the range was covered with at least 14 different concentrations). At the end of this treatment the samples were centrifuged and hemoglobin was measured in the supernatant by spectrophotometry at 414 nm. Hemoglobin concentrations were expressed as a percentage of the maximum hemolysis obtained with the minimum value of tonicity assayed (bidistilled H_2O = 100% lysis). Data were adjusted with a logistic function (Corchs *et al.* 1993). Break points were calculated as described elsewhere (Shanubhogue *et al.* 1992).

Estimation of the cellular volume (relative scale)

This variable was determined in aliquots of cell suspensions with a high hematocrit value (> 40%) to increase the accuracy of the measurement. The cellular volume, before and after the incubation, was estimated with the relationship Hb/Hc (hemoglobin concentration/hematocrit) as described by Dunham & Ellory (1981). Hemoglobin was measured as cyanmethemoglobin (Drabkin reaction) at 540 nm in a Beckman spectrophotometer, model DU2.

Results and discussion

Changes in cellular volume and potassium content induced by lead. Sensitivity to quinidine

At the concentration tested, lead induced significant reductions in cellular volume and in potassium loss in an isoosmotic, normal ionic strength medium. Both effects were reversed by quinidine (Figure 1A and 1B).

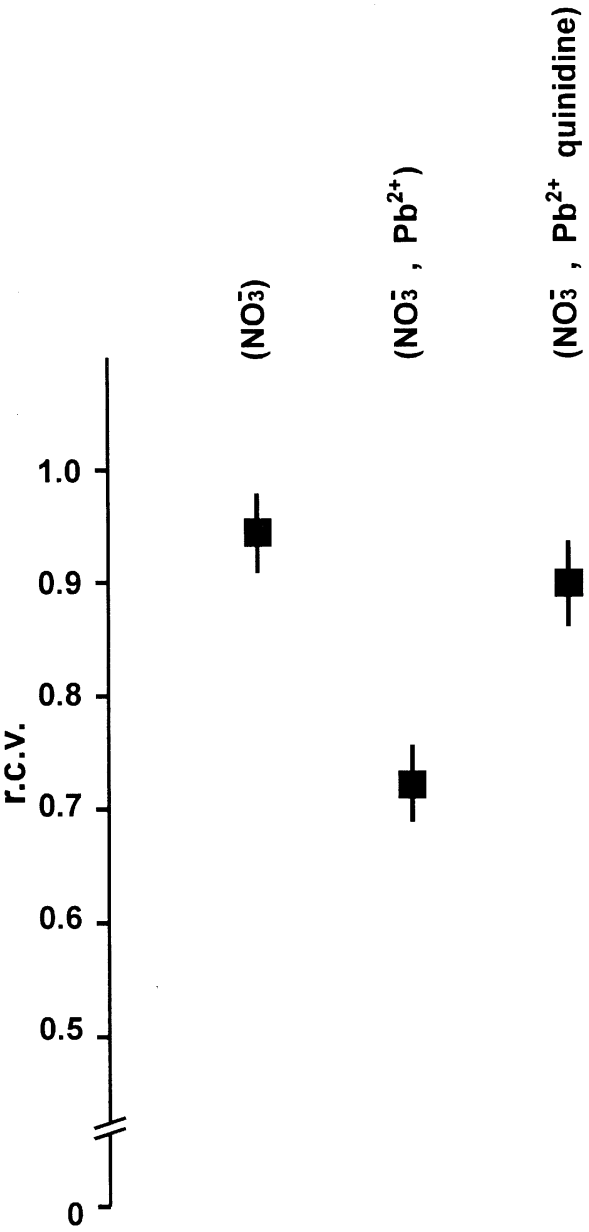

Figure 1A. Relative cellular volume of cells incubated as described in Materials and methods in isotonic–normal ionic strength media, containing NO_3^- as the main anion, with or without lead (20 μM) and lead/quinidine (20 μM). Data are shown as mean ± standard error, using seven individual samples in each treatment group.

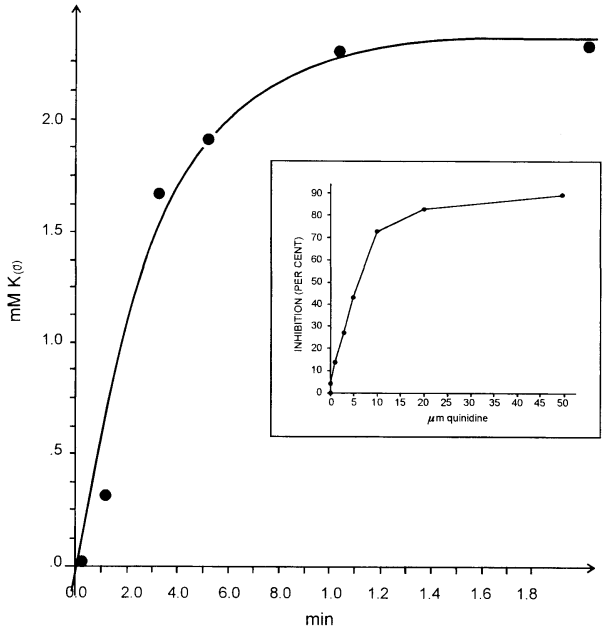

Figure 1B. Extracellular potassium concentration of cell suspensions incubated for varying periods in isotonic–normal strength media containing NO_3^-, with 20 μM Pb^{2+}. *Insert*: Cells incubated as described in the presence of increasing concentrations of quinidine. The extracellular steady-state potassium concentration was expressed as a fraction of the value obtained in the medium lacking quinidine. A representative result for each experimental condition is presented.

Influence of lead on the prolytic action of hypotonic, low ionic strength media. Independence of quinidine action

Figure 2 shows that incubation of erythrocytes in lead-containing media diminished their sensitivity to lysis when tonicity and ionic strength decrease. The addition of quinidine does not revert the effect; rather, it seems to enhance the resistance to lysis.

Effect of lead on the prolytic activity of an isotonic, near null ionic strength medium. Independence of modifications in cellular volume

As observed in Figure 3, isotonic–normal ionic strength media showed an increased prolytic action (Figure 3A) associated with a significant decrease in cellular volume (Figure 3B). On the contrary, the prolytic action of isotonic–null ionic strength media lessened (Figure 3A), while no significant changes in cellular volume were demonstrated (Figure 3B).

Dependence of cellular potassium loss on the medium ionic strength

The loss of cellular potassium induced by lead was estimated by measuring the extracellular concentration of potassium at the end of the incubation period. This variable increased as a function of time, reaching 3.0 ± 0.4 mM (normal ionic strength) and 0.5 ± 0.2 mM (null ionic strength) after 60 min incubation (Figure 4). The latter did not differ from the value at time 0 (control value). Media with half-normal or normal ionic strength affected potassium loss similarly.

Lead, acting as a calcium-simil ion, activates [K^+ (Ca^{2+})] channels and induces cellular potassium loss and volume reduction (Fehlau *et al.* 1989, Schwarz *et al.* 1989). These actions are reverted at concentrations greater than 20 μM (Schwarz *et al.* 1989). Since this lead level has been reported to be found in cord blood (Ong *et al.* 1993), this concentration was selected to study the effect of the cation on cellular structural stability, potassium loss and volume modifications, using red blood cells from the prenatal hemopoietic stages (Karai *et al.* 1982, Göran Eriksson & Beving 1993, Song *et al.* 1993).

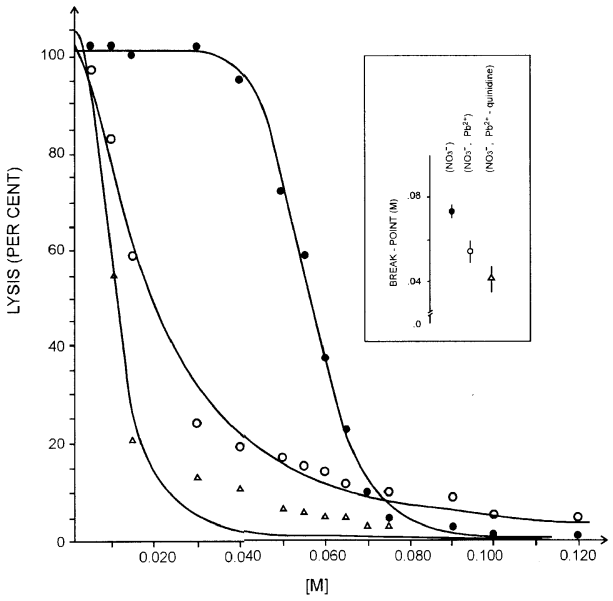

Figure 2. Cellular lysis curves in media of decreasing tonicity and ionic strength: (●) NO_3^-; (○) NO_3^- with 20 μM Pb^{2+}; (△) NO_3^- with 20 μM Pb^{2+} and 20 μM quinidine. Data from a representative experiment are depicted. *Insert*: break points (see Materials and methods) of cellular lysis curves in the media described. Data are expressed as mean ± SE, with a sample size of five individuals per treatment group.

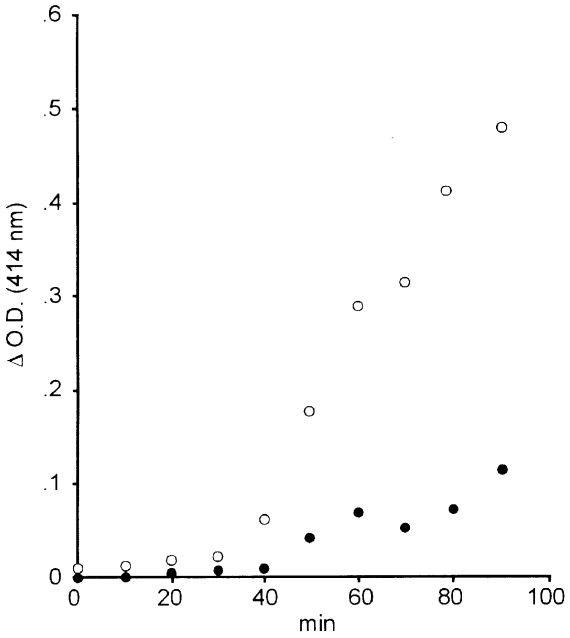

Figure 3A. Cellular suspensions incubated isotonic NO_3^- media (○). The difference in absorbance values (OD 414 nm) of extracellular media (suspension incubated in the presence (20 μM) and without Pb) is plotted as a function of incubation time. (●): *idem* but in sucrose media.

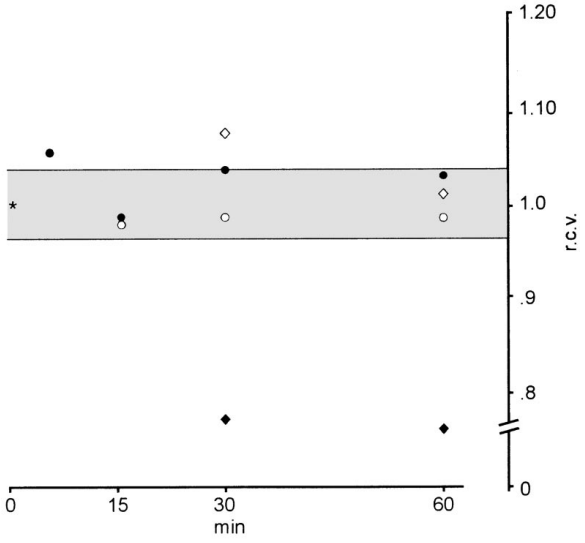

Figure 3B. Relative cell volume as a function of the incubation time in the following experimental conditions: (◇) isotonic NO_3^- medium without Pb^{2+}; (◆) isotonic NO_3^- medium with 20 μM Pb^{2+}; (○) sucrose medium ($\mu \cong 0$) without Pb^{2+}; (●) sucrose medium ($\mu \cong 0$) with 20 μM Pb^{2+}; (*) reference value (rcv = 1). The shaded area corresponds to the error calculated as the variance of a function of random variables. The symbols represent means of experiments done with five different individuals.

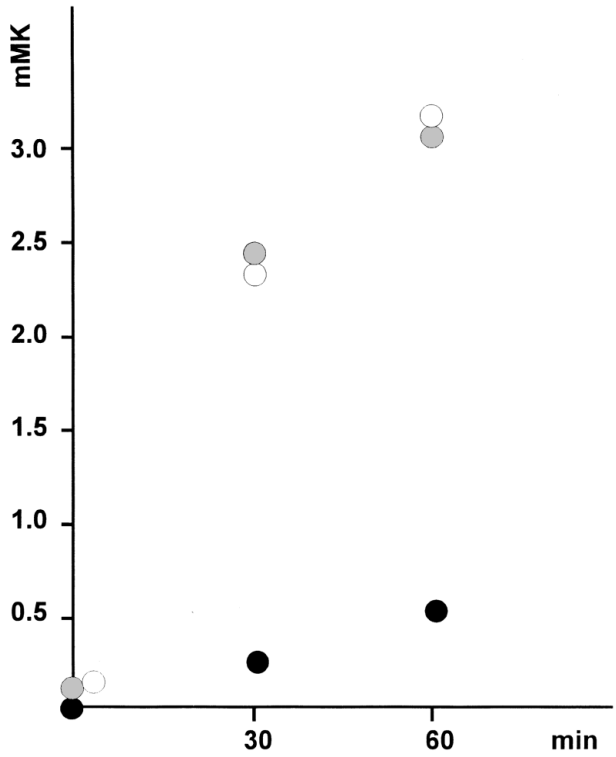

Figure 4. Cell suspensions incubated as described in Materials and methods. Extracellular concentration of potassium as a function of incubation time, in the following conditions: (●) isotonic sucrose medium ($\mu \cong 0$); (○) isotonic NO_3^- medium (normal μ, 0.146 M); (●) isotonic NO_3^- medium (normal μ/2: 0.073 M). All media contained 20 μM Pb^{2+}. Symbols represent mean values and standard errors (included in the symbol) of a set of five to seven individual determinations per group.

In isotonic–normal ionic strength media lead induced K^+ loss and cellular volume reduction (Figures 4 and 3B). Neither K^+ loss nor cellular volume reduction were observed in isotonic–near null ionic strength (Donlon & Rothstein 1969); however, cellular stability increased (lysis decreased) in this last condition (Figure 3A).

Lead lowered hypotonic lysis sensitivity in media of decreasing tonicity and ionic strength. This effect was not reverted by the addition of quinidine, an inhibitor of K^+–Ca^{2+} channels (Figure 2) (Gardos 1968). These findings suggest that lead alters the sensitivity of neonatal red cells to lysis independently of its effect on the K^+–Ca^{2+} channels.

Cytoskeleton alterations induce an increased susceptibility to lysis (Jay & Rowlands 1975, Bennett 1985, Pooler 1985, Mays *et al*. 1994). In low ionic strength media, electrostatic phenomena, such as charge unscreening, induce cytoskeleton disruption.

In these conditions, cytoskeleton structure constitutes the limiting step for lysis (Araki & Rifkind 1981, Degrado *et al*. 1982, Sambasivarao *et al*. 1986). The effect of lead on lysis in this paper could be interpreted as due to cytoskeleton strengthening.

Acknowledgements

This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina.

References

Araki K, Rifkind JM. 1981 The rate of osmotic hemolysis. A relationship with membrane bilayer fluidity. *Biochim Biophys Acta* **645**, 81–90.

Bennett V. 1985 The membrane skeleton of human erythrocytes and its implications for more complex cells. *Ann Rev Biochem* **54**, 273–304.

Corchs JL, Corchs MJ, Serrani RE. 1993 Neonatal red blood cell lysis induced by hypertonic low ionic strength media. *Arch Int Physiol Bioch Biophys* **101**, 249–252.

Degrado WF, Musso GF, Lieber M, Kaiser ET, Kézdy FJ. 1982 Kinetic and mechanism of hemolysis induced by melittin and by a synthetic melittin. *Biophys J* **37**, 329–338.

Donlon J, Rothstein A. 1969 The cation permeability of erythrocytes in low ionic strength media of various tonicities. *J Membrane Biol* **1**, 3752.

Dunham PB, Ellory JC. 1981 Passive transport in low potassium sheep red cells: dependence upon cell volume and chloride. *J Physiol Lond* **318**, 511–530.

Fehlau R, Grygorczyk R, Fuhrman GF, *et al*. 1989 Modulation of Ca^{2+} or Pb^{2+} activated K^+ selective channels in human red cells. II Parallelisms to modulation of activity of a membrane-bound oxidoreductase. *Biochim Biophys Acta* **978**, 37–42.

Gardos G. 1968 The function of calcium in the potassium permeability of human erythrocytes. *Biochim Biophys Acta* **30**, 653–654.

Göran Eriksson LE, Beving H. 1993 Calcium- and lead-activated morphological changes in human erythrocytes: a spin label study of the cytoplasm. *Arch Biochim Biophys* **303**, 296–301.

Jay AWL, Rowlands S. 1975 The stages of osmotic haemolysis. *J Physiol* **252**, 817–832.

Karai I, Fukumoto K, Kageyamak K, *et al*. 1982 Effect of lead *in vitro* on water metabolism and osmotic fragility of human erythrocytes. *Brit J Ind Med* **39**, 295–299.

Mays R, Beck K, Nelson J. 1994 Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. *Current Opinion in Cell Biology* **6**, 16–24.

Ong CN, Chia SE, Foo SC, *et al*. 1993 Concentrations of heavy metals in maternal and umbilical cord blood. *Biol Met* **6**, 61–66.

Pooler JP. 1985 The kinetics of colloid osmotic hemolysis. I. Nystatin induced lysis. *Biochim Biophys Acta* **812**, 193–198.

Rosen JF. 1992 Health effects of lead at low exposure levels. *HJDC* **146**, 1278–1280.

Sambasivarao D, Rao NM, Sitaraman V. 1986 Anomalous permeability and stability characteristics of erythrocytes in non-electrolyte media. *Biochim Biophys Acta* **857**, 48–60.

Schwarz W, Keim H, Fehlau R, *et al*. 1989 Modulation of the Ca^{2+} or Pb^{2+} activated K^+ selective channels in human red cells. I Effect of propranolol. *Biochim Biophys Acta* **978**, 32–36.

Serrani RE, Gioia IA, Corchs JL. 1995 The homogeneous effect of calcium ionophore A23187 on potassium loss in human fetal red cell populations. *Physiol Res* **44**, 275–280.

Shanubhogue A, Rajarshi, MJ, Core AP, Sitamaran V. 1992 Statistical testing of equality of two break-points in experimental data. *J Biochem Biophys Meth* **25**, 95–112.

Silbergeld EK. 1992 Mechanisms of lead neurotoxicity, or looking beyond the lamppost. *FASEB J* **6**, 3201–3206.

Simons TJB. 1983a The transport of lead ions across human red cell membranes. *J Physiol* **345**, 108.

Simons TJB. 1983b The influence of lead ions on red cell cation permeability. *J Physiol* **343**, 91–92.

Simons TJB. 1984 Active transport of lead by human red blood cells. *FEBS* **172,** 250–254.

Simons TJB. 1985 Influence of lead ions on cation permeability in human red cell ghosts. *Membr Biol* **84**, 61–71.

Simons TJB. 1993 Lead transport and binding by human erythrocytes *in vitro*. *Phl Arch* **423**, 307–313.

Song LY, Ahkong QF, Baldwin JM, *et al*. 1993 Divalent cations, phospholipid asymmetry and osmotic swelling in electrically induced lysis, cell fusion and giant cell formation with human erythrocytes. *Biochim Biophys Acta* **1148**, 30–38.