Pb(II) Induces apoptosis via scramblase activation and ceramide-domain generation in red blood cells

Pb(II) Induce la apoptosis a través la activación de la escramblasa y la generación de dominios de ceramida en células de eritrocitos.

Hasna Ahyayauch[1,2,3], Aritz B. García-Arribas[1,2], Jesús Sot[1], Jon V. Busto[1,2], F. Xabier Contreras[1,2], Adela Rendón-Ramírez[1,2], Cesar Martin[1,2], Alicia Alonso[1,2] & Félix M. Goñi[1,2]

1 Instituto Biofsika (CSIC, UPV/EHU), 48080, Bilbao, Spain.
2 Departamento de Bioquímica, University of the Basque Country (UPV/EHU), 48080, Bilbao, Spain.
3 Institut Supérieur des Professions Infermières et des Techniques de Santé, Rabat, Morocco

INTRODUCTION

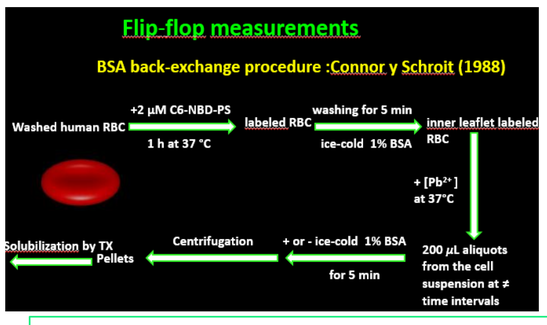
Lead (Pb2+) is one of the most abundant heavy metals on earth. It has been widely used throughout human history, posing a serious health problem to susceptible populations. Tis metal causes a broad range of biochemical, physiological and behavioural dysfunctions. In mammal erythrocytes lead has been associated with the induction of apoptosis (He et al. 2000). This apoptosis presumably contributes to a decrease in the life-span of erythrocytes and the development of anaemia in cases of lead poisoning (Kempe 2005 ; Lang et al. 2010 ; Aguilar-Dorado et al. 2014). It has been reported that micromolar or even submicromolar Pb2+ concentrations cause lipid scrambling and exposure of PS to the outer membrane leaflet in erythrocytes via a scramblase action, as well as calcium entry in proteoliposomes (Shettihalli, A. K. & Gummadi, 2013). However, the cellular mechanism of the process is not yet fully understood. In this work we provide insights about the lead-induced eryptotic mechanism.

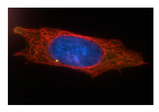
OBJECTIVE

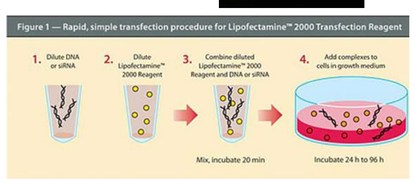
The objective of this study is to describe the mechanism of apoptosis in the red blood cell (RBC) and therefore lead-induced anemia

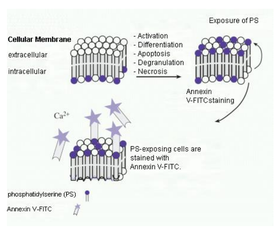
MATERIALS & METHODS

Translocation of cell membrane lipids from the inner to the outer leaflet









Measurement of cell [Ca2+]i . Cells were loaded with 4µM Fura2-AM at 37°C for 30min.

Confocal and AFM microscopies are used for cells visualization.

RESULTS

Lipid scrambling in living cells

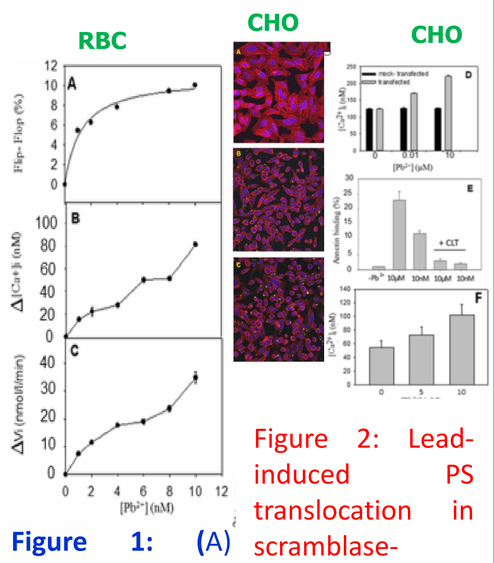


Figure 1: (A) Outward movement of C6-NBD-PS in human RBC (A). (B,C) Pb2+ - induced increase in intracellular Ca2+

Figure 2: Lead-induced PS translocation in scramblase-transfected CHO

✓ Exposure to Pb2+, increased significantly the translocation of PS and intracellular Ca2+

✓ The involvement of a scramblase activity in PS translocation

Morphological changes

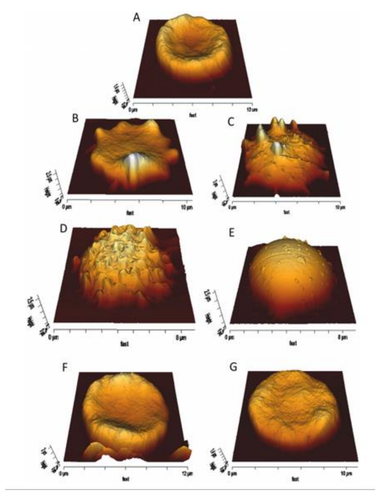


Figure 3. AFM monitorization of 10 µM Pb2+ effect on RBC. Exposure times: 0min (A), 6min (B), 30min (C), 45min (D), 60min (E). Lead (II) effect on CLT-treated RBC, 20min (F) and 15h (G).

✓ The biconcave shape is lost in the first minutes, and the cells become echinocytes) (Fig. 3C,D). Then after ~1 h the cells become spherical

✓ CLT-treated cells retain their biconcave form after treatment with 10µM Pb2+

Ceramide formation

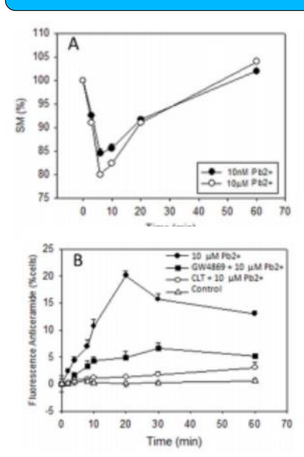
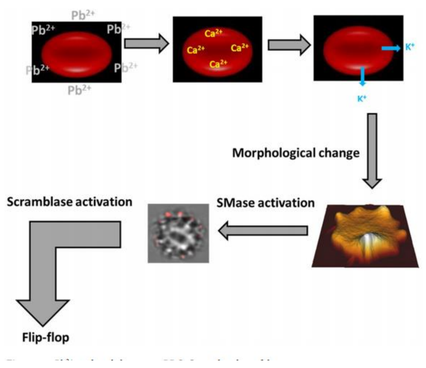


Figure 4. Quantitation of sphingomyelin (A) and ceramide (B) over time during Pb(II) incubation.

The morphological changes could perhaps induce a sphingomyelinase (SMase) activity in the cell membrane.

CONCLUSION



✓ Pb2+ causes Ca2+ entry which in turn causes K+ depletion from the cell.

✓ After K+ depletion, the red blood cells undergo a morphological change leads to the generation of echinocytes, and finally to spherocytes.

✓ Ceramide generation is linked to the morphological change

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Organizan

Financian