Clotrimazole blocks apoptosis on human erythrocytes when exposed to lead(II): an AFM imaging study

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Abstract

Atomic force microscopy (AFM) has been applied to the characterization of human red blood cells (RBC) at 23ºC. Erythrocytes were obtained by centrifugation and washing of blood samples extracted from healthy donors, attached to ethanol-washed glass coverslips and fixed with glutaraldehyde to obtain AFM images (Figure 1), so that cell diameters and thicknesses could be measured. When Pb^{2+} was applied at 10 μM at 37ºC for 10 minutes (all lead incubations were previous to fixation), erythrocytes underwent a morphological change due to the start of RBC apoptosis (eryptosis), losing their typical biconcave form to become essentially planar, including phosphatidylserine (PS) exposure to the outer monolayer of the cell membrane. Moreover, when Pb^{2+} incubation was increased to 1 hour, erythrocytes became totally spherical (spherocytes) (Figure 2). A progressive decrease in diameter was observed. Spiculated echinocytes appeared during this process when the incubation time was over 10 minutes.

Interestingly, the use of clotrimazole, a suggested anti-apoptotic agent, inhibited Pb^{2+} effect, completely preserving morphology even after 20 minutes of lead exposure. Furthermore, when clotrimazole-treated RBC were exposed to lead overnight, morphology was still preserved (no echinocytes or spherocytes were seen) but a slight decrease in diameter was detected and RBC started to slightly lose biconcavity. As opposed to previous *in vivo* studies in mice exposed to Pb^{2+} [1] no additional reagents other than clotrimazole were needed to achieve inhibition of lead effect in human RBC. In addition, clotrimazole appeared not to have any significant effect on untreated erythrocytes. Lead-induced eryptosis is considered to be ceramide-related, like other mechanisms of RBC death [2]. This data is relevant in the context of sphingolipid signaling and cell death.

References

[1] Mandal S, Mukherjee S, Chowdhury KD, Sarkar A, Basu K, Paul S, Karmakar D, Chatterjee M, Biswas T, Sadhukhan GC, Sen G. "S-allyl cysteine in combination with clotrimazole downregulates Fas induced apoptotic events in erythrocytes of mice exposed to lead." *Biochim Biophys Acta* **1820**: 9-23 (2012)

[2] Montes LR, López DJ, Sot J, Bagatolli LA, Stonehouse MJ, Vasil ML, Wu BX, Hannun YA, Goñi FM, Alonso A "Ceramide-enriched membrane domains in red blood cells and the mechanism of sphingomyelinase-induced hot-cold hemolysis" *Biochemistry* **47**:11222-30 (2008)

Figures

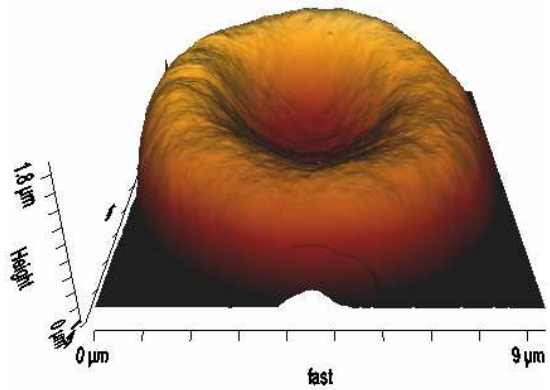

Figure 1: AFM 3D image of control red blood cell (RBC) with typical biconcave morphology

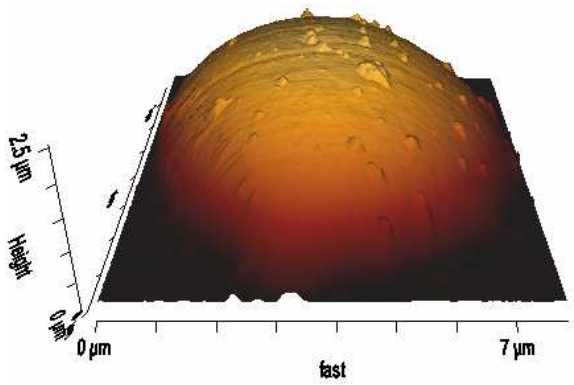

Figure 2: AFM 3D image of spherocyte obtained after 1 hour of lead(II) incubation of RBC