Copper overload modulates energetic state and induces OXPHOS remodeling in the erythropoietic cell line K562

Ruiz, L.M.1; Rossel, Y.1; Elorza, A.1

1Dep de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile

Copper is an important trace metal in cell physiology as an enzyme cofactor and generator of ROS. In mitochondria, copper ion is mainly used by cytochrome oxidase (Complex IV) and superoxide dismutase (SOD1). Mitochondria synthesize ATP, heme and ROS playing a key role in erythropoiesis. Copper imbalance is related with several diseases, like Wilson disease, sideroblastic anemia and β-Thalassemia. It has been shown copper overload increase ROS generation, cellular proliferation and induced anemia. Despite copper is essential for mitochondrial physiology and hereby erythropoiesis nothing is known about how copper imbalance might alters the mitochondrial function. Our goal is to study the effect of copper overload on bioenergetics parameters in the erythropoietic human cell line K562.

K562 cells were treated with CuCl₂ (50 µM – 200 µM), differentiation was induced with Butyrate. Cell proliferation, differentiation and death were assessed by trypan blue, benzidine staining and CD71 marker. Cell respiration and glycolysis were measured with the XF24 technology. Mitochondrial membrane potential and superoxide production were measured with TMRE and Mitosox probes by confocal microscopy and protein oxidation by Oxyblot. NADH autofluorescence was measured by flow cytometry. The remodeling of respiratory complexes was assessed by BN-PAGE, Western blot and in-gel activity.

Copper overload induces cellular proliferation and decreases differentiation in K562 cells. Moreover, copper overload increases basal respiration and leaking along with an increase in the glycolytic rate and decrease in mitochondrial membrane potential and NADH content. These results suggest an increase in ATP demands along with mitochondrial uncoupling. In addition, it was observed a higher generation of superoxide anion. These bioenergetics changes were correlated with an increase in protein expression of the respiratory complexes (CI-CIV) and ATP synthase (CV) along with changes in their supramolecular organization from single complexes to supercomplexes.

Word keys: Mitochondria, erythropoiesis, copper.
Supported by: COCHILCO-FONDECYT 1100995; DI 10-10R. Postdoctorado Fondecyt 2011 3110171