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Effects of copper supplementation on the differentiation of HL-60 cells into granulocytic lineage

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Introduction Copper is an essential trace element for adequate cell functions. Neutropenia is one of the signs of severe copper deficiency results from impaired granulocyte maturation. This indicates copper may be a critical requirement for granulopoiesis although exact mechanism is not yet understood. When the differentiation of neutrophils was studied using human promyelocyte leukemic HL-60 cell, one of the copper dependent enzymes Cu/Zn-superoxide dismutase (Cu/Zn-SOD) activity was decreased. We asked what is copper's role in the development of neutrophils?

Materials and Methods Differentiation of cells into the granulocytic lineage was induced by incubating HL-60 cells with 1μM retinoic acid (RA) for 96 hours. At the same time, copper was supplemented to both noninduced and RA induced cells as either as 12 μM copper in 0.02% nitric acid or 2 μM ceruloplasmin. Intracellular copper levels, Cu/Zn-SOD activity and protein, respiratory burst activity were measured. Nitroblue tetrazolium reduction and differential cell counting were assessed under the oil immersion microscopy.

Results The respiratory burst activity, as a measure of cellular function and degree of differentiation, was increased more than 4 fold after 96 hr exposure to RA. Copper supplementation to RA induced cells resulted in significant increases in respiratory burst activity compared to RA induced cells not supplemented with copper. Morphological determination of cellular differentiation revealed that copper supplementation was associated with a greater percentage of cells that had differentiated. These findings indicate that cellular differentiation was enhanced by copper supplementation. However, nitroblue tetrazolium reduction determination, another functional indicator of cellular differentiation, failed to show the differentiation enhancing effect of copper. Upon differentiation, there was an increase in intracellular copper level with a concomitant decrease in Cu/Zn-SOD activity. These data demonstrate that copper was not utilized for Cu/Zn-SOD activity as a cofactor, but perhaps used for some aspect of differentiation. Copper supplementation resulted in a marked increase in specific activity of Cu/Zn-SOD in both noninduced and induced cells. These findings indicate that copper facilitates the differentiation process in *in vitro* condition by either the increased cellular copper available for differentiation or the enhanced cellular protection from oxygen radicals through increased Cu/Zn-SOD activity.

References

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