COPPER-INDUCED APOPTOSIS IN HUMAN LIVER CARCINOMA (HEPG2) CELLS USING ANNEXIN V AND CASPASE-3

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Abstract: Copper, an essential element for all known living organisms, is a reddish metal that occurs naturally in rock, soil, water, sediment, and, at low levels, air. Exposure to excessive levels of copper can result in liver and kidney damage, anemia, immunotoxicity, and gastrointestinal developmental toxicity. In humans, ingestion of large quantities of copper salts may cause severe abdominal pain, nausea, vomiting, diarrhea, hemolysis, hepatic necrosis, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma, pulmonary fibrosis, increased vascularity of the nasal mucosa and death. The chronic toxicity of copper has been characterized in patients with Wilson's disease, an autosomal recessive genetic disorder associated with impaired copper metabolism. Although the clinical manifestations of Wilson's disease (hemolytic anemia, cirrhosis of the liver, neurologic abnormalities, and corneal opacities) are known, the cellular and molecular events associated with copper toxicity are poorly understood. In the present study, we hypothesize that copper may play a role in the production of programmed cell death in human liver carcinoma cells (HepG2). To test this hypothesis, human liver carcinoma (HepG2) cells were used to assess the responses following exposure to copper sulfate, CuSO4. The Annexin V assay for early apoptosis and the Caspase-3 assay for apoptosis were performed. Apoptosis is a cell death characterized by morphological and biochemical features such as loss of membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA that occur at different stages of a cells life. Once triggered, apoptosis may proceed with different kinetics depending on the cell types and culminates with the death of the cell. Data obtained from the Annexin V assay indicated a biphasic response relationship with respect to copper. Upon 8 hrs of exposure, the percentage apoptosis expressed was 0.59 ± 0.01, 0.86 ± 0.04, 1.07 ± 0.05, 0.14 ± 0.01, 0.03 ± 0.06, and 0.25 ± 0.03 at 0, 50, 100, 150, 200, and 250 μg/mL, respectively. A significant difference (p < 0.05) was expressed at 100 μg/mL. Caspases are a family of cysteine proteases that have been identified as regulators and effectors of apoptotic responses. If caspases are activated via an apoptotic signal it is either through cell surface specific receptors or intracellular pathways. This causes protein cleavage followed by cell dysfunction and cell death. During apoptosis, caspases select a set of proteins to target and cleavage occurs at specific peptide sequences. Protein destruction is genetically programmed and systematically carried out to ensure the proper death of the cell and debris disposal. Data obtained from the Caspase-3 assay also expressed a biphasic response with respect to copper. Upon 48 hrs of exposure, the percentage apoptosis expressed was 0.35 ± 0.02, 0.52 ± 0.02, 1.31 ± 0.05, 0.16 ± 0.04, 0.36 ± 0.06, and 0.39 ± 0.01 at 0, 50, 100, 150, 200, and 250 μg/mL, respectively. A significant difference (p < 0.05) was expressed at 100 μg/mL. These data support previous research indicating that copper overload is toxic to liver cells. These data further support a renewal capacity of copper sulfate on HepG2 cells.

Keywords: copper, annexin, caspase, apoptosis, HepG2 cells.