

COPPER - INDUCED CYTOTOXICITY AND TRANSCRIPTIONAL ACTIVATION OF STRESS GENES IN HUMAN LIVER CARCINOMA CELLS

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Abstract: Copper is a naturally occurring element found as a component of many minerals. It is an essential nutrient that is normally present in a wide variety of tissues. In humans, ingestion of large quantities of copper salts may cause gastrointestinal, hepatic, and renal effects with symptoms such as severe abdominal pain, vomiting, diarrhea, hemolysis, hepatic necrosis, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma, and death. The chronic toxicity of copper has been characterized in patients with Wilson's disease, a genetic disorder causing copper accumulation in tissues. Although the clinical manifestations of Wilson's disease (cirrhosis of the liver, hemolytic anemia, neurologic abnormalities, and corneal opacities) are known, the cellular and molecular events associated with copper toxicity are poorly understood. In the present study, we used human liver carcinoma (HepG₂) cells as a model to study the cytotoxicity, and the potential mechanisms of copper-induced toxicity and carcinogenesis. We hypothesized that copper-induction of stress genes may play a role in the cellular and molecular events leading to toxicity and tumor formation in liver cells. To test this hypothesis, we performed the MTT-assay for cell viability, the CAT-Tox(L) assay for gene induction, to assess the transcriptional activation of stress genes. Data obtained from the MTT assay indicated a strong dose-response relationship with respect to copper toxicity. Upon 48 hrs of exposure, the chemical dose required to cause 50% reduction in cell viability (LD₅₀) was computed to be about 200 ug/mL copper sulfate. The CAT-Tox (L) assay showed statistically significant inductions ($p < 0.05$) of a significant number of stress genes including GSTY α , *c-fos*, HMTIIA, NF-kB, HSP70, RARE, GADD153, and GRP78. These data support previous research indicating that copper overload is cytotoxic to liver cells. The CAT-Tox data on the other hand indicate that copper overload induces proteotoxic effects (HMTIIA, HSP70, GRP78), inflammatory reactions/oxidative stress (*c-fos*, NF-kB), and growth arrest and DNA damage (GADD153). The induction of GSTY α indicates the potential involvement of copper sulfate in phase II biotransformation pathway in the liver, while the activation of RARE points to the potential developmental effects associated with copper over-exposure.

Key words: Copper, Wilson disease, cytotoxicity, gene expression, HepG₂ cells

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