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Toxicology

A study of dose response and organ susceptibility of copper toxicity in a rat model

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ABSTRACT

Copper (Cu) in higher concentration is toxic and results in various organ dysfunction. We report Cu concentration in liver, brain and kidney in the rat model following chronic exposure of oral copper sulphate at different subtoxic doses and correlate the tissue Cu concentrations with respective organ dysfunction. Fifty-four male wistar rats divided in 3 groups, the control group received saline water and the experimental group (Group-IIA and IIB) received oral copper sulphate in dose of 100 and 200 mg/kg Body Weight. At the end of 30 days, 60 days and 90 days of exposure, six rats were sacrificed from each group. The maximum peak force in grip strength, latency to fall in rotarod and percentage attention score in Y-maze were significantly reduced in the copper sulphate exposed rats compared to the controls at all time points and these were more marked in Group-IIB compared to Group-IIA. Cu concentration was significantly higher in liver, kidney and brain in the Group-II compared to the Group-I. The Cu concentration was highest in the liver (29 folds) followed by kidney (3 folds) and brain (1.5 folds). Serum ALT, AST and bilirubin correlated with liver Cu, BUN with kidney Cu, and grip strength, rotarod and Y-maze findings correlated with brain Cu level. In rats, chronic oral copper sulphate exposure at subtoxic level results in neurobehavioral abnormality and liver and kidney dysfunctions due to increased Cu concentration in the respective organs. Liver is the most vulnerable organ and copper toxicity increases with increasing dose and duration of exposure.

1. Introduction

Copper (Cu) is an essential trace metal that plays an important role in many biological functions and serves as a cofactor for several enzymes. Cu uptake, distribution within the cells, detoxification and removal are maintained by an elegant system. Mutations in genes involved in Cu homeostasis are responsible for disorders of Cu metabolism in humans. The best described human Cu toxicosis disorder is Wilson disease (WD) which is an autosomal recessive disease due to ATP7B gene mutation which leads to defective Cu transportation and excretion into the bile [1]. The main pathogenesis of cellular injury in WD is due to the presence of excess free Cu which results in cirrhosis of the liver, Kayser Fleischer ring, renal tubular dysfunction and brain damage [2]. Clinically, the WD patients have liver dysfunction in the first decade and neurological dysfunction in the second decade [3]. Many WD patients with neurological manifestation may not have a history of hepatic dysfunction. Central nervous system (CNS) is privileged as it is protected from most of the toxic agents and infections because of blood brain and blood cerebrospinal barriers. However, free Cu is capable of crossing blood brain barrier resulting in oxidative stress mediated cell damage [4,5]. On cranial MRI, certain areas of the brain such as corpus striatum, thalamus and substantia nigra are more frequently involved in WD suggesting vulnerability of these areas to Cu toxicity [6–8].

Recently Long Evans Cinnamon (LEC) rat and toxic milk mice have been developed to replicate the WD and to evaluate the various aspect of Cu induced toxicity [9–11]. The well-established model for WD, LEC rat has a deletion in the ATP7B gene leading to a non-functioning protein. The animals steadily accumulate Cu in the liver and develop hepatic manifestation at the age of about 3 months [11]. LEC rat has symptoms similar to hepatic manifestation but does not exhibit symptoms similar to neurological

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CNS, central nervous system; Cu, Copper; kgBWt, kg Body Weight; LEC, Long Evans Cinnamon; MRI, magnetic resonance imaging; WD, Wilson disease.

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manifestation of human disease. An animal model of Cu toxicity for longer duration may simulate the pathological changes similar to WD patients [12,13]. Intraperitonial injection of copper lactate leads to impaired spatial memory and neuromuscular coordination, swelling of astrocytes, decreased serum AChE activity, copper deposition in the choroid plexus, neuronal degeneration, and increased levels of Cu in the hippocampus of rats [14]. Cu induced cellular injury in both humans and animals may be similar and may be mediated by free radical and oxidative stress [15,16]. Previous experimental studies had reported Cu induced toxicity in the liver, kidney and brain in isolation [14,17–20]. Only few studies evaluated liver, brain and kidney dysfunction comprehensively in Cu induced toxicity [14,21–23]. It is important to learn whether there are simultaneous CNS and hepatic injury in chronic Cu toxicity or there is a sequential involvement of different organs. None of the experimental study comprehensively evaluated the vulnerability of different organs as well as different part of the CNS to chronic Cu toxicity. The experimental study of chronic Cu toxicity may help in understanding the vulnerability and sequential changes of different organ dysfunction. The present study therefore aimed to evaluate the Cu concentration in liver, kidney and brain at different time points following different doses of copper sulphate exposure in the rat. Cu level in different organs would also be correlated with respective biochemical and neurobehavioral changes.

2. Materials and methods

Male Wistar rats were acclimatized for 7 days to oral gavage feeding in the laboratory. The animals maintained in an air-conditioned room ($25 \pm 2\,^{\circ}C$) with 12 h light and dark cycle. All animals had free access to water and food. The study was approved by the Animal Ethics Committee, Central Drug Research Institute, India.

2.1. Study design

Fifty Four male Wistar rats (body weight = 205 ± 10 g, 2.5 months old) were included in this study. Animals were divided into three groups, 18 animals in each group. Group-I ($n = 18$, control group), rats were fed saline water daily through oral gavage. In experimental group, Group-IIA ($n = 18$) and Group-IIB ($n = 18$) were fed copper sulphate ($CuSO4{\cdot}5H2O$, Sigma–Aldrich, St. Louis, MO) in a dose of 100 mg/kg and 200 mg/kg Body Weight respectively, daily upto 90 days through oral gavage. The animals were examined daily and their body weight and behavioral changes were recorded at baseline, 30 days, 60 days and 90 days. Blood was collected at baseline, 30 days, 60 days and 90 days in heparinized and plain vial from the orbital vein.

Six rats from each group were sacrificed by decapitation at the end of 30 days, 60 days and 90 days of exposure. Just before the sacrifice, blood was also collected in heparinized and plain vial by cardiac puncture under anesthesia after an overnight fast. Thereafter, the rats were perfused with saline from the left ventricle through thoracotomy to remove whole blood from the organs. The liver, kidney, and brain were removed and were frozen in liquid nitrogen and then stored at $-80\,^{\circ}C$ until analysis.

2.2. Hematological and biochemical studies

Heparinized blood was used for measurement of hemoglobin and white cell count. Measurement of blood urea nitrogen (BUN) and serum bilirubin, transaminases, and creatinine, was done using clinical chemistry analyzer (Randox Imbola, Ireland, United Kingdom).

2.3. Behavioral studies

All the behavioral tests were performed during the light phase. The following behavioral studies were done at different time points.

2.4. Grip-strength test

The forelimb-grip strength of the rat was measured using a computerized grip strength meter (TSE-Systems, Bad Homburg, Germany). Each rat was gently placed on the mesh and pulled by holding the tail in the opposite direction until it released its grip from the mesh. The peak force developed before the release of grip was recorded in Newton (N). We calculated the mean of five measurements, allowing 30 s of recovery time in between the test.

2.5. Y-maze test

Y-maze was used for assessing learning and memory. Y-maze consists of three arms with an angle of 120° between each arm. The maze was placed in a separate room with minimal light, and the

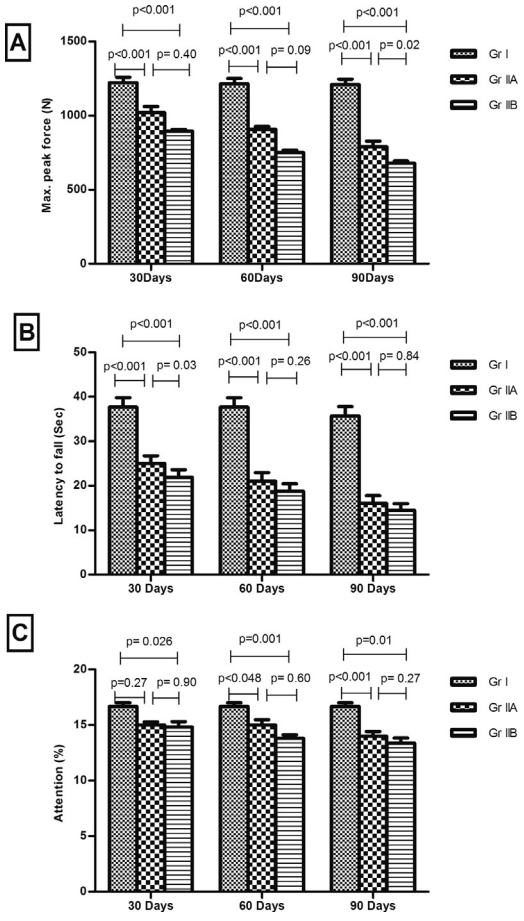

Fig. 1. Error bar diagram shows behavioral changes in the control and experimental group. (A) Grip strength: Maximum peak force declines in the Group-II compared to the Group-I. The grip strength was not significantly different in the Group-IIA and B at 30 and 60 days but at 90 days; grip strength was significantly reduced in Group-IIB compared to Group-IIA. (B) Rotarod: Latency to fall time was significantly decreased in Group-II compared to the Group I. The latency to fall time was significantly reduced in Group-IIB at 30 days compared to Group-IIB. (C) Y-maze: The percentage of attention was reduced in Group-II at all time points compared to Group-I; however this reduction was not significantly different between Group-IIA and B. The differences in the variables between the groups were analyzed by two-way ANOVA with Bonferroni post-hoc multiple comparison tests. Values are in Mean ± SEM.