TRANSPORTATION OF COPPER IN RABBIT BLOOD

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Abstract: Copper sulfate was given to male rabbits intraperitoneally and the copper concentrations in the plasma, red cells and EtOH-CHCl₃ treated fraction of the cells were determined.

The concentration of copper in the plasma increased markedly and in the red cells increased slightly after administration of 1 mg copper/kg of body weight, while the copper level in the EtOH-CHCl₃ treated fraction remained constant. In this experiment, the ceruloplasmin activity in the plasma increased, while the superoxide dismutase activity in the red cells remained unchanged.

The form of the copper present in the plasma was also examined by gel filtration on a Sephadex G-150 column. Most of the copper absorbed in the plasma appeared at first in the albumin fractions and was then incorporated into ceruloplasmin fractions.

Key words: Copper—Blood—Albumin—Ceruloplasmin—Superoxide dismutase—Transportation—Rabbit

INTRODUCTION

Moderate intake of copper in the diet has little effect on the blood concentration of copper in most species, while hypercupremia occurs in some species as a result of extremely high copper intake by the massive injection of copper salts. It is also known that high levels of copper in the blood occur on taking it with certain substances such as molybdenum and sulfate¹. Recently, the role of copper has been discussed in relation to the function of metallothionein in cadmium poisoning².

In this paper, we examine the behavior and the form of the copper present in plasma and red cells in order to clarify the detoxication mechanism in rabbits following intraperitoneal administration of large amounts of copper.

MATERIALS AND METHODS

Male rabbits (2.5—3.5 kg) were used for the experiments. Blood samples were collected from the earvein, heparin was added to the whole blood (2 units/ml), and each sample was separated into plasma and cells by centrifugation at 3000 r.p.m. for 5 min.
The cells were then washed three times with 0.9% saline to remove the majority of the white cells, and red cells were obtained. An aliquot of the red cells was treated with ethanol and chloroform, and the fraction which contained superoxide dismutase (SOD) was then obtained according to the method previously reported by us3).

Determination of the copper in the plasma, red cells and EtOH–CHCl₃ treated fraction was performed by the technique of flameless atomic absorption spectrophotometry developed in this laboratory3).

SOD activity was measured by the xanthine oxidase method as improved by us4) and ceruloplasmin activity was measured by the p-phenylenediamine method5).

The gel chromatographic conditions were as follows: gel bed, Sephadex G-150 2.2 cm i.d. × 85 cm; eluent, 0.01 M Tris·HCl-0.15 M NaCl (pH 7.8); flow rate, 9 ml/hr; fraction volume, 3 ml/tube.

RESULTS

Time-course of Copper Levels and Copper-enzyme Activities in the Blood after Intraperitoneal Administration

In order to examine the transportation and the form of the copper present in the blood, various amounts of copper (1-10 mg of Cu per kg body weight in the form of CuSO₄) were injected to rabbits intraperitoneally. Blood samples were withdrawn at various intervals following the administration, and the copper concentrations in the plasma, red cells and EtOH–CHCl₃ treated fraction were measured.

The absorption rate and concentration of copper in the blood after the intraperitoneal injection were found to be very variable and were not always proportional to the amounts of copper administered. One of the typical time-courses of copper concentration in the rabbit blood is presented in Fig. 1, following intraperitoneal administration of 1 mg Cu/kg body weight. The plasma copper level reached a maximum at 45 min after the injection and decreased rapidly within a period of 4 hr. Thereafter, about a 1.5-fold increase in the copper level was maintained for 4 hr to 4 days. In the case of the red cells, the copper concentration reached a maximum at 1 to 1.5 hr and then returned slowly to the baseline value during the first 8 hr after the injection. On the other hand, the copper concentration in the EtOH–CHCl₃ treated fraction showed no appreciable change during the period of the experiment (Fig. 1).

The activities of ceruloplasmin and SOD were also determined. The activity of ceruloplasmin increased to about twice the normal level at 24 hr after the administration and then decreased gradually, while the activity of SOD remained constant for 10 days (Fig. 2).

Gel Chromatographic Distribution of Plasma Proteins and Copper

Gel chromatographic separation of plasma samples obtained at adequate intervals after the administration of copper, was performed. Elution profiles of the proteins and copper are shown in Fig. 3. In each chromatogram, two main peaks of copper can be
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Fig. 1. Blood copper levels in the rabbit following intraperitoneal administration of copper in the form of copper sulfate.

Fig. 2. Ceruloplasmin and SOD activities in rabbit plasma following intraperitoneal administration of copper.
The copper absorbed in the plasma at first appeared in the albumin fractions and then decreased at 4 to 24 hr after the administration. On the other hand, the copper content in the ceruloplasmin fractions gradually increased. The ceruloplasmin activity was correlated to the amounts of copper and the correlation coefficient was 0.975.

Fig. 3. Elution profiles of the copper and protein in plasma on a Sephadex G-150 column.

--- Absorbance at 280 nm, --- Copper

Peak 1: Ceruloplasmin fraction, Peak 2: Albumin fraction
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DISCUSSION

The results in Fig. 3 suggest that most of the absorbed copper is bound to albumin, transported to the liver and finally incorporated into ceruloplasmin. This agrees with the metabolic pathway of copper reported previously by many workers. When the copper in the red cells increased, that in the EtOH-CHCl₃ treated fraction remained constant. These findings support the hypothesis of Cartwright shown in Fig. 4. The stable copper in the red cells shown in the figure may correspond to the EtOH-CHCl₃ treated fraction copper, while the difference in the total and EtOH-CHCl₃ treated fraction copper may represent the copper in labile pool.

Although details are not given in the results, administration of large amounts of copper (10 mg Cu/kg body weight) caused death in rabbits after 4 hr and they suffered from hemolysis. In this case, the amounts of copper may have exceeded the binding capacity for copper of albumin.

One of the important roles of the albumin in plasma appears to be the transportation and detoxication of copper.

REFERENCES