Respiratory Toxicity of Copper

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Respiratory toxicity of copper was tested in Wistar rats by spraying copper sulfate (330 g/l spray) for daily periods of 1 hr in a self-contained chamber for up to 10 days. The respiratory toxicity was compared with that from intraperitoneal administration of 1 mg Cu/mg body weight and with adequate control rats. Analysis of tissue Cu and Zn was done in lung, liver, kidney, and plasma by using atomic absorption spectrophotometry. Similar organ and subcellular distribution of both elements were found between the two treated groups, and only statistically significant higher levels of Cu were found in plasma and liver. After exposure, Cu and Zn were basically associated with a low-molecular-weight component, which eluted as metallothionein in the postmicrosomal fractions. — Environ Health Perspect 102(Suppl 3):339–340 (1994).

Key words: copper, zinc, copper sulfate, urine, sprayers

Introduction

The use of copper-containing pesticides is traditional along the Mediterranean coastline. Copper, mostly in the form of sulfate (i.e., the traditional Bordeaux broth), has been known for centuries to inhibit the growth of parasites, especially fungi. Its use is still common and is most widely spread along the Mediterranean coast of Spain. However, only scanty information has been obtained on the effect of this practice upon people who have been traditionally spraying these pesticides. Presumably, the route of entry of copper into the body during spraying is by inhalation, and to the best of our knowledge no indications of respiratory toxicity of copper have been published to date.

It has to be considered that copper homeostasis is finely regulated, due to its essential nature as well as to its toxicity (1). This regulation is carried out mostly by changes in intestinal absorption and biliary excretion, when the entry into the body follows the normal route, i.e., gastrointestinal intake (2). However, during spraying, entry into the body is mostly through the lungs, which implies a bypass of the liver, which regulates metabolism (1,2), resulting in an unusual handling of copper and thus an unknown metabolic situation.

The aim of the present study was to set up a model of spraying similar to what is used in our area. The model used male Wistar rats. To analyze respiratory toxicity, a second group of rats was treated with copper by intraperitoneal administration to compare the results with a better-known model. Due to the well-known interaction between Cu and Zn, we analyzed these elements in parallel.

Materials and Methods

Male Wistar rats (180 ± 20 g) were randomly divided into four groups. Groups were sprayed (330 g CuSO_4/l, 0.5 l of spray in three dosages, chamber volume 142 l, 1 hr daily), intraperitoneally injected (CuCl_2, daily 1 mg Cu/kg weight), and two control groups (no treatment, and injected with phosphate-buffered saline). After spraying, animals were washed with distilled water to avoid licking of copper retained in the skin. Animals were sacrificed by chloroform overdose and samples of lung, liver, plasma, and kidneys were obtained. Separation of particulate and soluble fractions was carried out by centrifugation at 100,000g for 60 min. Soluble fractions were further characterized by column chromatography of Sephadex G-75 (Pharmacia, Uppsala, Sweden), using phosphate buffer as eluant (20 mM, pH 7.4). Characterization of the column was done by the appropriate molecular-weight standards plus purified metallothionein, which was kindly provided by Dr. M.A. Liberatore (DuPont Biosystems, Billerica, MA). Unless otherwise stated all the chemicals were from Sigma, Saint Louis, MO.

All samples were analyzed by atomic absorption spectrophotometry for Cu and Zn after wet digestion with ultrapure nitric acid (Merck, Darmstadt, Germany) for 24 hr at 120°C. Metal determinations were carried out on an IL551 atomic absorption spectrophotometer with air-acetylene flame and deuterium correction for zinc estimations. Protein in tissue homogenates and subcellular fractions was assayed by the Bradford method (3). Statistical analysis of the results was carried out by one way

![Figure 1. Sephadex G-75 elution profiles of Cu in (a) liver, (b) kidney, and (c) lung soluble fractions. Copper levels in control (●), intraperitoneally injected (●), and inhalation exposure (●) are shown. Arrow shows the metallothionein elution volume.](image-url)
ANOVA and the Scheffe’s S-test when appropriate. Statistical degree of significance was set at $p \leq 0.05$.

**Results and Discussion**

Control groups did not show any difference, and the values were pooled. As can be seen in Table 1, higher Cu and Zn levels could be detected in all the organs after Cu treatment, although only liver and plasma Cu were statistically significant. The increases of copper in these organs are similar to those reported in similar situations of copper administration (4). Liver Cu increased dramatically to levels of up to 280 ppm, whereas the levels of Zn only doubled. Although in kidneys the large individual variation did not allow statistically significant differences, it is obvious that an increase is produced with time in the animals exposed to copper through inhalation. Finally, in the lung only Cu levels were slightly higher after respiratory exposure. These results agree with the central role of liver in copper metabolism as the main organ in initial copper storage and detoxification (2,5). The similarity in the increases of copper and zinc highlights the common trends in their metabolism and the possibility of similar mechanisms of accumulation or immobilization of excess metal that could involve both elements. The increases observed in kidneys probably relate to the urinary excretion of these elements, which is high in populations of copper-sulfate sprayers (6). The subcellular partitioning of both elements between the particulate and the soluble fractions showed that most of the additional metal was accumulated in the soluble fraction, similar to the results reported by other groups for similar exposures (4).

The distribution of copper in the soluble fractions of liver and kidney (Figure 1) clearly shows the accumulation of copper in a low-molecular-weight protein, probably metallothionein. However, no zinc eluted in that fraction (results not shown) and the increases in its levels in both tissues were centered in the void volume fractions. These results agree both with the known higher affinity of metallothionein for copper than for zinc (2) and for the higher increase of copper levels in both organs. Copper toxicity has been clearly correlated to increased metallothionein levels in both organs (7). However, in the lungs the increase of copper was only evident in high-molecular-weight fractions, without any indication of metallothionein or metal associated with it in this organ. To date, no indication of metallothionein presence has been observed in the lung, and probably this organ does not easily accumulate metals.

Although exposure to copper both intraperitoneally and through the respiratory system shows similar results, the metal accumulates to higher concentrations in the latter. Handling of the element seems to proceed by the same mechanisms, and accumulation and excretion are mostly through liver and kidneys. Paradoxically, little or no accumulation occurs in the lungs, where the absence of copper associated with metallothionein is well known.

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**Table 1. Levels of copper and zinc in rats after intraperitoneal injection or respiratory exposure to Cu.**

| Treatment | Liver Cu | Liver Zn | Kidney Cu | Kidney Zn | Lung Cu | Lung Zn | Plasma Cu | Plasma Zn |
|-----------|----------|----------|-----------|-----------|---------|---------|-----------|-----------|
| Control   | 5.0 ± 1.5 | 23.4 ± 3.6 | 6.4 ± 4.3 | 12.6 ± 2.0 | 2.8 ± 0.4 | 14.0 ± 2.1 | 1.1 ± 0.1 | 1.6 ± 0.1 |
| Injected  | 44.6 ± 9.4 | 25.9 ± 2.2 | 7.2 ± 1.6 | 19.4 ± 5.8 | 2.4 ± 0.5 | 9.5 ± 1.4 | 2.3 ± 0.4 | 1.9 ± 0.6 |
| Inhalation| 6 hr     | 31.7 ± 9.8 | 32.2 ± 3.6 | 8.6 ± 3.4 | 23.1 ± 7.1 | 3.2 ± 0.5 | 12.3 ± 0.5 | ND        | ND        |
|           | 24 hr    | 37.9 ± 5.1 | 51.1 ± 6.1 | 6.8 ± 3.0 | 18.1 ± 5.8 | 3.1 ± 0.8 | 10.8 ± 3.0 | 2.4 ± 0.3 | 2.1 ± 0.4 |
|           | 5 days   | 84.2 ± 8.2 | 57.6 ± 14.2 | 18.7 ± 7.4 | 23.4 ± 4.7 | 5.1 ± 0.6 | 11.9 ± 3.1 | 2.0 ± 0.3 | 2.1 ± 0.4 |
|           | 10 days  | 284.8 ± 77.8 | 52.3 ± 15.2 | 16.2 ± 1.6 | 21.8 ± 3.7 | 4.0 ± 0.5 | 16.0 ± 1.1 | ND        | ND        |

ND=Not determined. *Values are expressed in μg/g or ml in plasma as average ± SD of three to four animals per group. **p<0.05.

**Table 2. Levels of copper and zinc in the particulate and soluble fractions of the different organs after copper exposure.**

| Organ   | Copper |                   | Zinc         |
|---------|--------|------------------|--------------|
|         | Control | Injected | Inhalation | Control | Injected | Inhalation |
| Liver   | 0.020  | 0.194  | 1.914      | 0.131   | 0.123   | 2.493      |
|         | 0.008  | 0.108  | 0.098      | 0.031   | 0.075   | 0.102      |
| Kidney  | 0.0030 | 0.040  | 0.201      | 0.127   | 0.114   | 0.457      |
|         | 0.009  | 0.018  | 0.089      | 0.032   | 0.046   | 0.166      |
| Lung    | 0.008  | 0.013  | 0.195      | 0.047   | 0.065   | 0.652      |
|         | 0.015  | 0.041  | 0.087      | 0.072   | 0.065   | 0.340      |

*Values are expressed as μg/mg protein and are the values of pooled samples from three to four animals.