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# Improvement of Acute Cadmium Toxicity by Pretreatment with Copper Salt

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The toxicity of Cd compounds has been thoroughly reviewed. Furthermore, modification of the toxicity by other metals is well known (Yoshikawa 1968). For example, pretreatment with Zn significantly decreases the lethality of Cd (Yoshikawa 1968). Testicular injuries induced by Cd are improved by simultaneous injection of Zn or Se (Parizek 1957). Thus, such preventive action might be expected as a result of prior or simultaneous injection of Cu salts.

Hill et al (1963) reported that supplementation of the basal diet (1 ppm Cu) with 40 ppm copper sulphate markedly reduced Cd-induced lethality. Gunn and Gould (1970) reported that Cu affords protection against testicular injuries caused by Cd. Recently, Kaji et al (1992) found that Cu could prevent Cd cytotoxicity in cultured vascular endothelial cells.

On the other hand, Irons and Smith (1976) reported previously that injection of Cu along with Cd decreases the binding of Cd to hepatic metallothionein (MT) and increases the toxicity of the Cd. An interactive increase in toxicity caused by a similar mechanism was observed in embryonic chick bone treated with both Cd and Cu in a culture system (Miyahara et al 1983). Accordingly, we should accumulate further data to understand the preventive effect of Cu against Cd toxicity. The aim of this study was to determine the effect of Cu pretreatment on the acute toxicity of Cd in mice. We focused on two organs, the liver and testis.

## MATERIALS AND METHODS

Specific-pathogen-free (SPF) male ICR mice were maintained on a 12 hr light-dark cycle. Food and water were provided ad libitum. Seven weeks after birth, the animals were randomly allocated into one of four groups,

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each consisting of 5 mice. In the Cu and Cd groups, the mice were injected subcutaneously (sc) with $CuCl_2$ (2.0 mg/kg as Cu) for 3 days (one time per day, at 24-hr intervals) and injected sc with $CdCl_2$ (1.5 mg/kg as Cd) one time only, respectively. Mice in the Cu+Cd group were given Cu at 24-hr intervals for 3 days, and then given Cd 24 hr after the final Cu injection. The control group was injected with deionized water. All mice were sacrificed 24 hr after the final injection to excise the blood, liver and testis.

To assess hepatic function, the activities of aspartate aminotransferase [E.C. 2.6.1.1] (ASAT) in the serum and thiobarbituric acid reactive substance (TBARS) in the liver and testis were determined by using commercial kits (Wako, Osaka, Japan) and the method of Uchiyama and Mihara (1978), respectively. The liver (0.4 to 0.5 g) and the testis (one side) were digested with an acid-mixture (nitric acid:perchloric acid=5:1) to measure metal (Cu, Cd and Zn) concentrations. These metal concentrations were determined with a flame (type 208, Hitachi, Tokyo, Japan) or flameless (type 180-80, Hitachi) atomic absorption spectrophotometer.

A part of the liver (about 0.5 g) was homogenized with 0.25 M sucrose solution in a Teflon/glass homogenizer. The homogenate was centrifuged at 100,000 g for 60 min. Part of the cytosol fraction was used to determine metal concentrations in the MT fraction, which was isolated by the method of Bartsch et al (1990). Protein concentration was estimated by the method of Lowry et al (1951). Results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test (Dunnett 1964). When results were obtained only from two groups, comparisons between them were performed using Student's t-test. The significance levels were ascertained at $p<0.05$.

**RESULTS AND DISCUSSION**

Serum aspartate aminotransferase (S-ASAT) and thiobarbituric acid reactive substances (TBARS) are parameters that indicate cell damage following Cd treatment (Sugawara et al 1989). S-ASAT and hepatic TBARS levels in the Cd group were significantly higher than in the other three groups (Table 1). The enhancement induced by Cd injection was restored to the control level by Cu pretreatment (Table 1). The testicular TBARS level increased by Cd injection was slightly improved by Cu injection (Table 1). On the other hand, an increase of the testicular TBARS concentration was found in the Cu group (Table 1). These opposite effects could not be explained with certainty here. Direct and indirect interactions between Cd and other metals have to be taken into consideration for the detoxication of Cd.

Table 1. ASAT in the serum, and TBARS in the liver and testis.

| Groups | n | ASAT | TBARS | |
|---|---|---|---|---|
| | | serum | liver | testis |
| Control | (5) | 20.5± 3.2[a] | 44.5± 9.7[a] | 43.1± 0.5[a] |
| Cu+Cd | (5) | 43.8±19.9[a] | 46.9± 7.5[a] | 108.4±36.2[b] |
| Cd | (5) | 112.6±46.8[b] | 72.9±15.0[b] | 171.2±26.0[c] |
| Cu | (5) | 33.2± 6.5[a] | 40.6± 6.4[a] | 68.8± 4.4[d] |

Data: mean±SD. (n): number of mice. ASAT: aspartate aminc transferase (Karmen Units). TBARS: thiobarbituric acid reactive substance (nmole/g tissue). Significantly different between means with a different superscript letter at $p<0.05$.

Table 2. Concentrations of Cd, Cu and Zn in the liver.

| Groups | n | Cd | Cu | Zn |
|---|---|---|---|---|
| Control | (5) | ND | 5.93± 0.8[a] | 19.71±1.6[a] |
| Cu+Cd | (5) | 23.16±5.5[a] | 41.08±13.7[b] | 29.71±3.5[b] |
| Cd | (5) | 17.71±3.0[a] | 6.49± 1.2[a] | 26.11±2.4[b] |
| Cu | (5) | ND | 30.62± 8.4[b] | 24.79±3.0[b] |

Data: mean±SD. (n): number of mice. Concentration: µg/g liver. ND: not detected by flame AAS. Significantly different between means with a different superscript letter at $p<0.05$.

Irons and Smith (1976) reported Cu failure to sequester Cd in metallothionein (MT), resulting in an increase of Cd toxicity. Their hypothesis (Irons and Smith 1976) may be supported by the different binding affinities of Cd and Cu to MT (Waalkes et al 1984). However, our results shown in Table 1 revealed that pretreatment with Cu was effective for the challenge dose of Cd.

Recently, Kaji et al (1992) hypothesized that the reduction of Cd cytotoxicity by Cu salts is due to the decreased uptake of Cd into endothelial cells. However, our results indicated that the hepatic Cd concentration

Table 3. Concentrations of Cd, Cu and Zn in the hepatic metallothionein fraction.

| Groups | n | Cd | Cu | Zn |
|---|---|---|---|---|
| Control | (4) | ND | 4.01± 1.2[a] | ND |
| Cu+Cd | (4) | 196.37±15.0[a] | 314.75±60.1[b] | 94.65± 8.9[a] |
| Cd | (4) | 133.90±19.3[b] | 52.58±11.0[c] | 87.45±13.5[a] |
| Cu | (4) | ND | 261.73±58.7[b] | 101.53± 5.6[a] |

Data: mean±SD. (n): number of mice. metal: μg/g protein. ND: not detected by flame AAS. Significantly different between means with a different superscript letter at $p<0.05$.

was slightly higher in the Cd+Cu group (23.1 μg/g) than in the Cd group (17.7 μg/g), although the difference between them was not significant (Table 2). The increased Cd may be due to the enhanced incorporation of Cd into the MT fraction stimulated by the preinjection of Cu (Table 3). Accordingly, the recovery of hepatic dysfunction due to the pretreatment (Table 1) may be related to the hepatic MT-bound Cd.

The reduction of Cd toxicity by pretreatment with metals is related to the induction of metallothionein (MT) (Cherian and Goyer 1978). Pretreatment with a small dose of Cd or Zn stimulates the induction of MT by the following challenge dose of Cd and, furthermore, the sequestration of Cd in the MT fraction (Suzuki and Yoshikawa 1974). Accordingly, the reduction of Cd toxicity is not due to the decreased uptake of Cd but to the fact that Cd becomes inert by binding to MT. Our results suggest that two metals, Cu and Cd, have a synergistic effect on the uptake of the metals into the liver (Table 2) and MT fraction (Table 3).

The hepatic Zn concentration was moderately increased by the injection of Cd and/or Cu (Table 2). The increase (Table 2) may be related to the appearance of Zn in the MT fraction (Table 3) but not linked to the improvement of hepatic function, although an injection of exogenous Zn is effective for treatment of Cd toxicity (Webb 1972). The increased hepatic Zn is an index for MT induction but not for injury (Tables 1 and 3).

Acute injection of Cd into experimental animals causes severe testicular injury accompanied by decreased concentrations of Zn, K and Mg (Sugawara and Sugawara 1986). Accordingly, a decrease of the testicular Zn concentra-

Table 4. Concentrations of metals in the testis.

| Groups | n | Cd | Cu | Zn |
|---|---|---|---|---|
| Control | (4) | ND | 1.68±0.1[a] | 28.15±0.9[a] |
| Cu+Cd | (5) | 0.293±0.03[a] | 1.60±0.2[a] | 24.16±0.7[b] |
| Cd | (5) | 0.285±0.04[a] | 1.58±0.1[a] | 21.74±1.7[b] |
| Cu | (5) | ND | 1.69±0.3[a] | 22.04±2.2[b] |

Data: mean±SD. n: number of mice. ND: not found by flame AAS. metal: μg/g testis. Significantly different between means with a different superscript letter at $p<0.05$.

tion is a clue to the understanding of the testicular injury caused by Cd. Pre- or simultaneous treatment with Se, Mn and Co prevents testicular injury by Cd (Gunn and Gould 1970). Parizek (1957) reported that a large dose of Cu (200 times that of Cd) does not prevent Cd injury to the testis. Our results (Table 1) indicated that although the Cu dose was not so large, the metal slightly prevented the lipid peroxidation induced by Cd, though the Zn level did not return to the control level (Table 4). It is of interest to note that although concentrations of Cu and Cd were not influenced by the pretreatment with Cu (in the Cu+Cd group) (Table 4), a preventive effect by Cu was found (Table 1).

In conclusion, injection of Cu prior to Cd injection prevented hepatic and testicular lipid peroxidation induced by Cd. The preventive effect is not due to the reduction of Cd uptake into the liver and testis. In the liver, the increased binding of Cd to the MT fraction may be related to the reduction of Cd toxcity. Further investigations concerning the combined effect of Cu and Cd are being performed in our laboratory.

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