ACUTE TOXICITY OF CADMIUM IN RATS
WITH OR WITHOUT CADMIUM PRETREATMENT

Hideaki HIRATSUKA*, **, Osamu KATSUTA*, **, Hiroshi IWATA*,
Junko MATSUMOTO* and Takashi UMEMURA**

* Mitsubishi-Kasei Institute of Toxicological and Environmental Sciences,

14 Sunayama, Hasaki, Kashima, Ibaraki 314-02, Japan

** Department of Veterinary Pathology, Faculty of Agriculture, Tottori University,

4-101 Koyama, Tottori 680, Japan

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ABSTRACT — The acute toxicity of cadmium (Cd) in male rats was examined with or
without Cd pretreatment. Firstly, the metallothionein (MT) contents in the liver and
kidney after Cd exposure (2.0 mg/kg, i.v.) were determined. The MT contents in the
liver increased immediately to a peak (36.0±5.5 n mol/g wet tissue) 2 days after Cd
exposure and were 55-fold higher than that at 0 day (0.64±0.25 n mol/g wet tissue).
On the other hand, the MT contents in the kidney increased slightly but steadily for 14
days after Cd exposure. In the study for comparison of Cd-induced toxicity, the LD50
value of the Cd-pretreatment group (Group II) was approximately 2-fold higher than
that of the non-pretreatment group (Group I). In microscopic findings, differences
between rats in Group I and Group II were recognized in the kidney. Cytoplasmic
vacuolation of the proximal tubular epithelium in the kidney was observed in Group I,
while degeneration or coagulative necrosis in the proximal tubular epithelium was
observed in some rats in Group II in addition to the cytoplasmic vacuolation. Because
the toxic changes other than in the kidney in Group II were almost equal to or less than
that in Group I, in spite of the doubled dosage of Cd, the toxic effects of Cd, except on
the kidneys, were considered to be reduced by the pretreatment with Cd.

KEY WORDS: Cadmium, Metallothionein, Acute toxicity, Nephrotoxicity,
Hepatotoxicity, Pretreatment with cadmium.

INTRODUCTION

Animals pretreated with a sublethal dose of cadmium (Cd) develop tolerance to a toxic
dose of Cd (Goering and Klaassen, 1983; Goering and Klaassen, 1984; Piscator, 1964; Probst et al.,
1977; Suzuki and Yoshikawa, 1974; Yoshikawa, 1973). The defensive mechanisms against Cd
toxicity are thought to be due to cellular adaptive responses to exposure to Cd. One of these
mechanisms can be considered as a cytoplasmic change involving a specific metal binding protein,
metallothionein (MT) (Cherian and Shaikh, 1975). MT is a low-molecular-weight protein
with a high affinity for Cd and is mainly synthesized in the liver and kidneys (Kagi and Vallee,
1961). There are many reports on the relationship between MT and Cd-induced toxicity
(Piscator, 1964; Suzuki and Yoshikawa, 1974; Yoshikawa, 1973). These reports have proposed
that MT acts as a scavenger to sequester Cd in the liver and decrease its toxic effects. In fact,
the 50% lethal dose (LD50) values for acute Cd
exposure following Cd pretreatment were elevated (Probst et al., 1977), but the differences in the quality and degree of Cd-induced toxic findings between rats with and without Cd-pretreatment is hardly known.

Therefore, we examined the differences in Cd-induced acute toxicity in male rats with and without Cd pretreatment.

MATERIALS AND METHODS

Animals: Five-week-old male Sprague-Dawley rats weighing 180-200 g were obtained from Charles River, Japan Inc., and housed in an environmentally controlled room (temperature ranging from 20 to 25°C, relative humidity ranging from 40 to 70%) with ventilation at the rate of 12 changes per hour, and illumination for 12 hr a day. Food (MF: Oriental Yeast Co., Ltd., Tokyo, Japan) and water were provided ad libitum.

Cadmium: Cd solutions were prepared by dissolving CdCl₂ (95% pure, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) in 0.9% NaCl.

Determination of MT contents in liver and kidney after a single dose of Cd: Rats were divided into seven groups of three animals each and were injected with a single dose of CdCl₂ (2 mg/kg) intravenously (iv). They were sacrificed at the designated time points (0, 6-hr, 1-, 2-, 4-, 7- and 14-day). The liver and kidney were removed to determine the MT concentration by the Cd-hem method (Onosaka et al., 1978). Fig. 1 shows the schema of this method.

MT contents in liver and kidney: The MT contents in the liver increased immediately to a peak (36.0±5.5 n mol/g wet tissue) at 2 days after Cd exposure, and thereafter the MT contents gradually decreased until 14 days after Cd exposure (Fig. 2). The MT contents in the liver at 2 days after Cd exposure were 55-fold higher than that at 0 day (0.64±0.25 n mol/g wet tissue). On the other hand, the MT contents in the kidney increased slightly but steadily for 14 days after Cd exposure (Fig. 2).

Cd-induced acute toxicity: The dose-mortality relation curve (Fig. 3) in Group II (pretreated with Cd) moved to a higher dose in parallel with that in Group I (pretreated with saline). The LD50 values for Groups I and II were 5.17 and 9.67 mg/kg, respectively. Pretreatment with Cd obviously elevated the lethal dose of Cd.

Since the LD50 value in Group II was approximately 2-fold higher than that in Group I, the degrees of Cd-induced toxic findings were fairly different between Group I and II at the same challenge dose. Therefore, the Cd-
induced toxic findings were compared between Groups I and II, which showed about the same mortality.

In the clinical observation after challenge exposure, the rats became excited and flushed their limbs and ears immediately. Then their spontaneous movement gradually decreased, and the rats crouched and fell into a lethargic state. Rats showing convulsions and gasping within 7 to 8 hr after Cd exposure finally died.

In the surviving rats, suppression of body weight gain or decrease in body weight was noted on the 3rd day after Cd exposure, but thereafter increased normally.

At necropsy, hemorrhage and congestion of the liver, and accumulation of pleural fluid were observed in the dead rats, and in surviving rats atrophy of the testis was mainly observed.

There were no marked differences between Groups I and II in clinical signs, body weight or gross findings.

Differences in histological findings between Groups I and II were recognized in the kidney and testis (Table 1). In the kidney, cytoplasmic vacuolation of the proximal tubular epithelium (Photo. 1a) was observed in Group I (dead rats of 5.0 and 7.1 mg/kg group), while degeneration or coagulative necrosis and/or regeneration of the proximal tubular epithelium (Photo. 1b) was observed in Group II (one surviving rat in the 10.0 mg/kg group and 3 rats in the 14.1 mg/kg group) in addition to the cytoplasmic vacuolation.
Table 1. Microscopic findings of surviving and dead rats.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group</th>
<th>Surviving Rats</th>
<th>Dead Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I (a)</td>
<td>II (b)</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td></td>
<td>1.8 2.5 3.5 5.0 5.0 7.1 10.0</td>
<td>5.0 7.1 10.0 10.0 14.1 20.0</td>
</tr>
<tr>
<td>Grade (c)</td>
<td></td>
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<td>+ + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>No. of Animals</td>
<td>5 5 5 3 5 2</td>
<td>2 5 5 3 5 5</td>
<td>200 (d) 500 000 110 210 030</td>
</tr>
</tbody>
</table>

Kidneys
- Cytoplasmic vacuolation of proximal tubular epithelium
- Tubular necrosis in cortex
- Regeneration of proximal tubular epithelium

Testes
- Degeneration / necrosis of seminiferous tubule
- Proliferation of interstitial cell

Liver
- Degeneration / necrosis of hepatocyte
- Regeneration of hepatocyte

a) Rats in Group I were pretreated with saline.  b) Rats in Group II were pretreated with CdCl₂.
c) +, slight; ++, moderate; ++++, severe.  d) No. of rats which showed findings.

In the testis, necrosis and calcification of the seminiferous tubules (Photo. 2a) were observed diffusely in Group I (3.5 mg/kg, surviving rat). However, not only necrotic tubules but also surviving tubules (Photo. 2b) were observed in Group II (7.1 mg/kg, surviving rat). In the liver, congestion and hemorrhage, degeneration of peri-portal hepatocytes and coagulative necrosis of mid-zonal hepatocytes were observed in dead rats in both Groups I and II.

DISCUSSION

We investigated the differences in Cd-induced acute toxicity in male rats with and without Cd pretreatment. Our findings confirm the previous reports (Goering and Klaassen, 1983; Goering and Klaassen, 1984; Piscator, 1964; Probst et al., 1977; Suzuki and Yoshikawa, 1974; Yoshikawa, 1973) that pretreatment with Cd elevated the lethal dose of Cd. The dose-mortality relation curve moved to a higher dose in parallel and the LD50 value was approximately 2-fold higher after Cd pretreatment. Pretreatment with Cd also reduced the toxic effects to the testis in microscopic findings. These detoxic phenomena are attributed to the MT synthesized in the liver by Cd pretreatment. Probst et al. (1977) reported that the Cd LD50 value in the mouse pretreated with Cd (2.0 mg of Cd/kg, i.p.) was elevated by about two times, and elevated LD50 values have been correlated with dose-related increases in hepatic MT. MT has a high affinity for Cd and one mol of MT can bind 6 g-atoms of Cd (Onosaka and Cherian, 1982). Several reports suggest that MT induced by Cd acts as an integral part of the detoxification system by sequestering the subsequent Cd dose in the liver and decreasing its distribution to other target organs (Piscator, 1964; Suzuki and Yoshikawa, 1974; Yoshikawa, 1973).

Toxic effects of Cd have been reported to develop in the kidney and liver (Piscator, 1964; Suzuki and Yoshikawa, 1974; Yoshikawa, 1973). Tubular nephropathy is one of the major complications of Itai-Itai disease attributed to chronic Cd poisoning. In the present experiment, the animals in Group I died without showing severe
degenerative changes of the tubular epithelium of the kidney due to intravascular injection of a massive dose of Cd. However, some rats in Group II exhibited severe renal damage: coagulative necrosis of the tubular epithelium. Webb and Etienne (1977) reported that the renal toxicity induced by the Cd-MT complex exceeds that produced by Cd itself. Most Cd administered parenterally as an inorganic salt is distributed to the liver, but Cd administered as a Cd-MT complex is distributed preferentially to the kidney (Cherian, 1983; Cherian and Nordberg, 1983; Suzuki, 1982; Suzuki and Yamamura, 1979; Tanaka et al., 1975). Therefore, the enhancement of Cd-nephrotoxicity in Group II in our experiment might be caused by the release of an elevated amount of MT in the form of a Cd-MT complex from the damaged hepatocytes in Group II. Thus, the Cd-MT complex might be located in the kidneys and produce renal injury.

REFERENCES


