INTERRELATIONS OF CADMIUM CONTENTS AND 
HISTOPATHOLOGICAL CHANGES IN KIDNEYS 
FOLLOWING SINGLE INTRAVENOUS INJECTION OF 
CADMIUM-SATURATED METALLOTHIONEIN II IN RATS

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Abstract — An interrelation of Cd-contents with hepato- and nephrotoxicities, and a 
mechanism for Cd-exclusion from kidneys were investigated in rats that received a single 
intravenous injection of either CdCl₂ or Cd-saturated metallothionein II (Cd-MT-II) 
with doses of 0.1 and 0.3 mg Cd/kg body weight (b.w.). Between the livers and 
kidneys, higher uptake of Cd was observed in the liver in terms of CdCl₂, and in the 
kidney in terms of Cd-MT-II. The CdCl₂ at the two doses hardly showed any 
histopathological alterations in the livers and kidneys. The 0.1 mg Cd/kg b.w. of 
Cd-MT-II showed a slight injury in the kidneys, while hardly in the livers. At Day 1 of 
the 0.3 mg Cd/kg b.w. of Cd-MT-II, the renal Cd-contents reached the maximal value of 
8.22±0.36 µg/g wet tissue, and degeneration including necrosis and defluxion of 
proximal tubular cells were most highly observed. At Day 5 of the 0.3 mg Cd/kg b.w., 
the renal Cd-contents were lowered to 2.40±0.24 µg/g wet tissue, and fibrosis 
and regeneration of the proximal tubular cells were remarkably found. These findings 
strongly suggested that, in the case of administration of Cd-MT-II, the Cd taken into the 
kidneys was eliminated from there mainly by cellular death of the proximal tubulus and 
by their resultant defluxion.

KEY WORDS: Cadmium, Metallothionein, Hepatotoxicity, Nephrotoxicity, Rat.

INTRODUCTION

Acute exposure to a high dose of cadmium (Cd) produces hepatic injury (Hoffman et al., 
1975; Dudley et al., 1982). In contrast, chronic exposure to Cd causes renal injury (Stowe et al., 
1972; Friberg, 1984; Dudley et al., 1985; Gatta et al., 1989). This difference is surmised to be 
ascribable to Cd-binding metallothionein (Cd-MT) that is synthesized by the liver, subsequently 
released into the plasma and taken up by the kidney (Dudley et al., 1985). In fact, a single 
administration of Cd-MT has been known to produce renal injury (Nordberg et al., 1975; 
Maitani et al., 1988; Suzuki and Cherian, 1989; Sudo et al., 1994), but no hepatotoxicity (Dudley 
et al., 1985). Nevertheless, it is not completely known as to whether the Cd-content taken into 
the kidney interrelates with the injured extent, and how the Cd is eliminated from the kidney.

Thus, in order to explore these problems, we first tried to purify MT-II from the livers of the 
rats that were treated with CdCl₂, and to prepare
Cd-saturated MT-II (Cd-MT-II). Because the authors in the above reports obtained the MTs by purification from livers of the animals that were treated with inorganic Cd, and did not replace Zn in MTs with Cd. In this case, the MTs obtained were considered to contain 5-g atoms of Cd and 2-g atoms of Zn per 1-g molecule of MTs, that is, Cd₅, Zn₂-MTs (Dunn et al., 1987). The Zn is variously reported not only to play a protective role in case of Cd-intoxication (Suzuki and Cherian, 1989), but also to cause renal dysfunction (Vander, 1963). Thus, in order to rule out a confusing factor of the Zn, and to simplify the experimental design, we prepared the Cd-saturated MT-II (Cd₇-MT-II). Then, administering either CdCl₂ or Cd-MT-II to rats, we studied alterations of Cd-contents in the kidneys. In addition to our previous report (Sudo et al., 1994), we histopathologically investigated as to the degenerated and regenerated extents in the renal cells. Furthermore, in order to ascertain a possible difference in injuries between the kidneys and liver, the livers were also comparably investigated.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats weighing 180±10 g were obtained from Charles River Japan (Tokyo), and allowed free access to water and standard diet pellets (CE-2; Clea Japan, Tokyo) during the periods of experiments.

Purification and Cd-Saturation of MT-II: MT-II was purified from the rat livers by gelfiltration- and anion-exchange ion chromatographies (Minkel et al., 1980; Templeton and Cherian, 1984). The MT-II was saturated by Cd according to the metal-exchange method (Abel et al., 1987). The MT-II saturated by Cd (Cd-MT-II) was freeze-dried, and kept at −80°C until use.

General Procedures: Rats received single intravenous injections of either CdCl₂ or Cd-MT-II dissolved by saline through the tail veins: doses being 0.1 and 0.3 mg Cd/kg body weight (b.w.) (Maitani et al., 1988; Suzuki and Cherian, 1989; Dorian et al., 1992). The rats that received saline were defined as the control.

Protocol 1: At Days 1 and 5 after the administrations, the body weights were estimated. Then, the abdominal cavity was opened under ether-anesthesia, and liver and left kidneys were removed and weighed. The organs were used for histopathological examinations.

Protocol 2: At Days 1 and 5 after the administrations, the abdominal cavity was opened under ether-anesthesia, and liver and left kidneys were perfused by saline in order to eliminate Cd from the blood components. Then, the organs were homogenized by a glass-teflon Potter homogenizer with 0.25 M sucrose, in order that 1 ml of homogenate contained 0.1 g wet weight of the tissue.

Histopathological Examinations: Slices of livers and kidneys were fixed in 10 % phosphate-buffered formalin (pH 7.0), dehydrated in ethanol and xylene, embedded in paraffin, sectioned at 4 to 5 μm in thickness, and stained with the hematoxylin-eosin procedure for microscopic examinations.

Determination of Cd-contents in Organs: Cd-contents of the homogenates of livers and kidneys were determined by the wet-digested mineralization and cation-exchange high-performance liquid chromatography with post-column reaction by 4-(2-pyridylazo)-resorcinol (Sudo et al., 1991).

Statistics: The degrees in renal injury were expressed in (−), (+), (++) and (+++), according to the five criteria described in Result section (Table 5) (Sudo et al., 1994). To define statistically significant differences among the groups, the data were subjected to the Chi-squared method for multiple samples.

Renal Cd contents in the groups were statistically analyzed by unpaired Student's t-test. In both statistical analyses, p values of less than 0.05 were considered to be significant.

RESULTS

Protocol 1: In the first series of experiments, changes of body weights, kidney weights, necropsy and histopathological changes of livers and kidneys, were investigated in the rats that received a single intravenous injection of either CdCl₂ or Cd-MT-II at the doses of 0.1 and 0.3 mg Cd/kg b.w.

As compared with the control groups, the 0.3 mg Cd/kg b.w. groups of Cd-MT-II showed a fall of the body weights at Day 1 (Table 1), a rise of
### Table 1. Changes of body weight following single intravenous injection of cadmium chloride and cadmium-saturated metallothionein II.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>206±3</td>
<td>245±4</td>
</tr>
<tr>
<td>CdCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg Cd/kg b.w.</td>
<td>213±3</td>
<td>250±3</td>
</tr>
<tr>
<td>0.3 mg Cd/kg b.w.</td>
<td>202±5</td>
<td>244±7</td>
</tr>
<tr>
<td>Cd-MT-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg Cd/kg b.w.</td>
<td>196±3</td>
<td>236±4</td>
</tr>
<tr>
<td>0.3 mg Cd/kg b.w.</td>
<td>188±1*</td>
<td>225±4</td>
</tr>
</tbody>
</table>

Abbreviations: body weight, b. w.; cadmium-saturated metallothionein II, Cd-MT-II. Values represent the mean ± S.E. Number of rats in each group was 4. Body weights of the Control rats at Day 0 were 193 ± 3 g. Statistical analysis was done by comparing CdCl₂ and Cd-MT-II groups with the Control one in identical time-points: * , P<0.05.

### Table 2. Changes of absolute and relative weight of left kidney following single intravenous injection of cadmium chloride and cadmium-saturated metallothionein II.

<table>
<thead>
<tr>
<th></th>
<th>Absolute Weight (g wet weight)</th>
<th>Relative Weight (g wet weight/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
</tr>
<tr>
<td>Control</td>
<td>0.86±0.02</td>
<td>1.02±0.04</td>
</tr>
<tr>
<td>CdCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg Cd/kg b.w.</td>
<td>0.85±0.03</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>0.3 mg Cd/kg b.w.</td>
<td>0.87±0.01</td>
<td>1.03±0.04</td>
</tr>
<tr>
<td>Cd-MT-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg Cd/kg b.w.</td>
<td>0.89±0.05</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td>0.3 mg Cd/kg b.w.</td>
<td>1.08±0.02**</td>
<td>1.14±0.16</td>
</tr>
</tbody>
</table>

Abbreviations: body weight, b. w.; cadmium-saturated metallothionein II, Cd-MT-II. Values represent the mean ± S.E. Number of rats in each group was 4. Statistical analysis was done by comparing CdCl₂ and Cd-MT-II groups with the Control one in identical time-points: ** , P<0.01.
absolute weights of the left kidneys at Day 1 (Table 2), and rises of relative weights of the left kidneys at Days 1 and 5 (Table 2). The other administered groups showed no significantly different changes of the above items in comparison to the control ones at Days 1 and 5.

In the necropsy examinations, any changes in the livers were not found in all the control and administered groups at Days 1 and 5. In contrast, in terms of the kidneys (Table 3), the 0.1 mg Cd/kg b.w. groups of CdCl₂ showed slight discolorless in 1 case at Day 1. The 0.3 mg Cd/kg b.w. groups of CdCl₂ showed slight discolorless in 1 case at Day 1 and in 1 case at Day 5. The 0.1 mg Cd/kg b.w. groups of Cd-MT-II showed slight discolorless in 1 case at Day 1 and in 1 case at Day 5. The 0.3 mg Cd/kg b.w. groups of Cd-MT-II showed slight discolorless in 2 cases at Day 1, moderate discolorless in 3 cases at Day 5, severe discolorless in 2 cases at Day 1 and in 1 case at Day 5, reddish-colorless in medulla in 1 case at Day 1 and in 2 cases at Day 5, hemorrhage in subcapsule in 1 case at Day 5, and, granulation in surface in 1 case at Day 5.

In the histopathological examinations of the livers, any changes including single-cell necrosis, focal necrosis, microgranulation and mitosis in parenchymal cells were not observed in the control groups, in the 0.1 and 0.3 mg Cd/kg b.w. groups of CdCl₂, and in the 0.1 mg Cd/kg b.w. groups of Cd-MT-II at Days 1 and 5. The 0.3 mg Cd/kg b.w. of Cd-MT-II showed slight microgranulation in parenchyma in 4 cases at Day 1, while did not show any such changes at Day 5.

In terms of the kidneys, the degree in renal injury was estimated by the five criteria described in Table 4. The control group showed no histopathological changes at Days 1 and 5 (Photo. 1). The CdCl₂ groups showed (±) in 1 case at 0.1 mg Cd/kg b.w. and in 2 cases in 0.3 mg Cd/kg b.w. at Day 1: the case of more than (+) was not observed at any doses of CdCl₂ at Days 1 and 5.

The Cd-MT-II groups showed (±) in 3 cases and (+) in 1 case at 0.1 mg Cd/kg b.w. at Day 1; (+++) in 1 case and (+++) in 3 cases at 0.3 mg Cd/kg b.w. at Day 1; (±) in 2 cases and (+) in 1 case at 0.1 mg Cd/kg b.w. at Day 5; (+++) in 2 cases and (+++) in 2 cases at 0.3 mg Cd/kg b.w. at Day 5. In general, the injured extent was severer in the Cd-MT-II groups in comparison to that in the CdCl₂ ones, and depended on the doses.

In detail, at Day 1, the 0.1 mg Cd/kg group of Cd-MT-II showed sporadically localized degenerating and necrotic changes in the proximal convoluting tubular cells in the inner cortex. The 0.3 mg Cd/kg group showed changes of degeneration, necrosis and defluxion of the tubular cells, tubular obliteraction resulted by hyaline casts and debris, and tubular dilation in the widespread areas of the cortex (Photo. 2): in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>CdCl₂</th>
<th>Cd-MT-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg Cd/kg b.w.)</td>
<td>1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>5</td>
<td>1 5</td>
</tr>
<tr>
<td>Discolored</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>0</td>
<td>0</td>
<td>1 0</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Reddish-Colored in Medulla</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Hemorrhage in Subcapsule</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Granulation in Surface</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Abbreviations: body weight, b.w.; cadmium-saturated metallothionein II, Cd-MT-II.
Number of rats in each group was 4.
Table 4. Histopathological findings in renal cortex following single intravenous injection of cadmium chloride and cadmium-saturated metallothionein II.

Criteria of Histopathological Changes.

(-) : No abnormal changes were observed.

(±) : Slight alterations were observed in tubular cells.

(+) : Focal necrosis, degeneration and/or basophilic changes in tubular cells, tubular dilation were observed. The lesion was small, and any hyaline casts were not observed in tubular lumens.

(+++) : Moderate necrosis and/or regeneration of tubular cells was observed.

(++++) : Severe necrosis of tubular cells was observed. The lesion was widely extended, and hyaline casts were often observed in tubular lumens.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>CdCl₂</th>
<th>Cd-MT-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 5</td>
<td>0.1  0.3</td>
<td>0.1  0.3</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td>1 5</td>
<td>1 5</td>
</tr>
<tr>
<td>(-)</td>
<td>4 4</td>
<td>3 4</td>
<td>4 2</td>
</tr>
<tr>
<td>(±)</td>
<td>1 2</td>
<td>3 2</td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>1 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+++)</td>
<td>1 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(++++)</td>
<td>3 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant Differences

- to 0.1 mg Cd/kg b.w. of CdCl₂ at Day 1
- to 0.1 mg Cd/kg b.w. of CdCl₂ at Day 5
- to 0.3 mg Cd/kg b.w. of CdCl₂ at Day 1
- to 0.3 mg Cd/kg b.w. of CdCl₂ at Day 5
- to 0.1 mg Cd/kg b.w. of Cd-MT-II at Day 1
- to 0.1 mg Cd/kg b.w. of Cd-MT-II at Day 5

Abbreviations: body weight, b.w.; cadmium-saturated metallothionein II, Cd-MT-II.

Number of rats in each group was 4.
Statistics: a), P<0.05 ; b), P<0.01.

particular, partial disappearance, discontinuity and depression of the basal membranes were observed in the tubular cells where the necrotic changes were severely brought.

At Day 5, the 0.1 mg Cd/kg b.w. group of Cd-MT-II sporadically showed focal regeneration and basophilic changes of cytoplasm in the proximal tubular cells in inner cortex. The 0.3 mg Cd/kg b.w. group of Cd-MT-II showed degeneration, necrosis and regeneration in the tubular cells, tubular obliteration resulted by hyaline casts and debris, and tubular dilation in the widespread areas of cortex (Photo. 3): the severely necrotized foci revealed partial disappearance and discontinuity of basal membranes in tubular cells, and fibrosis in the marginal areas neighboring there.

Any histopathological changes were hardly observed in the glomeruli of the two dose groups at Days 1 and 5.

Protocol 2: In the second series of experiments, Cd-contents in livers and kidneys were investigated separately using the other rats that received single intravenous injection of either CdCl₂ or Cd-MT-II in the same manner as in the Protocol 1.

In the livers (Fig. 1), the CdCl₂ and Cd-MT-II groups showed dose-dependent increments of Cd-contents at Days 1 and 5. The 0.1 and 0.3 mg Cd/kg b.w. groups of CdCl₂ showed higher Cd-contents than the identical doses ones of Cd-MT-II at identical time-points.

In the kidneys (Fig. 2), the CdCl₂ and Cd-MT-II groups showed dose-dependent incre-

Photo. 2. Renal cortex at Day 1 following a single intravenous injection of cadmium-saturated metallothionein II at dose of 0.3 mg Cd/kg body weight. Hematoxylin-eosin staining. Magnification, ×200.

Photo. 3. Renal cortex at Day 5 following a single intravenous injection of cadmium-saturated metallothionein II at dose of 0.3 mg Cd/kg body weight. Hematoxylin-eosin staining. Magnification, ×200.
Nephrotoxicity of Cd-Metallothionein

DISCUSSION

In the present study, a single intravenous administration of CdCl₂ at dose-levels of 0.1 and 0.3 mg Cd/kg b.w. revealed higher uptake of Cd in the livers than in the kidneys, and hardly showed any histopathological alterations in the both organs. Also, a single intravenous administration of Cd-MT-II at dose-levels of 0.1 and 0.3 mg Cd/kg b.w. revealed higher uptake of Cd in the kidneys than in the livers: this was contrary to the case of CdCl₂. These results were well in accordance with those of the precedent reports employed the Cd₃, Zn₂-MT-II (Maitani et al., 1988; Suzuki and Cherian, 1989; Dorian et al., 1992).

As to our histopathological examination with use of Cd-MT-II, its single intravenous administration showed slight and severe damages at dose-levels of 0.1 and 0.3 mg Cd/kg b.w., respectively. The below explanation has been done to the difference on nephrotoxic potentials between the CdCl₂ and Cd-MT-II: Inorganic Cd binds albumin in plasma (Friberg, 1984; Dunn et al., 1987). The Cd-bound albumin, because of largeness of its molecular weight, cannot pass through the glomerular basement membranes into the proximal tubular lumens, and is accessible to the basolateral membranes (Dorian et al., 1992). In contrast, the Cd-MT-II, because of smallness of its molecular weight, can easily pass through the glomerular basement membranes into the proximal tubular lumens (Abel et al., 1987; Dorian et al., 1992; Cherian et al., 1976), and can exclusively bind to the brushborder membranes of proximal tubules (Dorian et al., 1992; Selenke and Foulkes, 1981). Namely, the Cd-MT-II can be taken through both the basolateral and the brushborder membranes into the proximal tubular cells, while the Cd-bound albumin can be taken only through the basolateral membranes into the cells. This difference in the cellular uptake of Cd might bring, in part, the difference of the nephrotoxic potentials between the two (Fowler and Nordberg, 1978; Squibb et al., 1984).

Nevertheless, we do not adopt an idea that the Cd-contents taken up into the cells decide the extent in the cellular damage. It is reported...
that, when inorganic Cd is chronically administered to the rats, the renal critical content of Cd that produces kidney injury is 100–200 μg Cd/g wet tissue (Tohyama et al., 1987; Goyer et al., 1989). Our present study denotes that the renal critical content of Cd that causes the renal injury is several μg Cd/g wet tissue in the case of a single intravenous administration of Cd-MT-II. Accordingly, it is considered to be meaningless that the renal critical Cd-content which is required to produce renal damages with a similar extent is compared between the inorganic Cd and Cd-MTs. Because the intracellular partitioning of Cd and the binding of Cd to the cellular components are surmised to be quite different between when Cd enters through the basolateral membranes and when it enters through the brush-border membranes. As regards this point, further investigations remain to be done. In addition, our histopathological results gave us an impression that the extent of renal injury caused by Cd-MT-II (Cd₇-MT-II) appeared to be severer in comparison with that caused by Cd₅,Zn₂-MT-II described in the precedent reports (Maitani et al., 1988; Suzuki and Cherian, 1989; Dorian et al., 1992), although the dose-levels of Cd were equivalent. Therefore, a comparable study concerned with nephrotoxic potentials between Cd₇-MT-II and Cd₅,Zn₂-MT-II, also, should be done in future.

A major finding in terms of the kidney in the present study was of the case in the administration of Cd-MT-II at the dose level of 0.3 mg Cd/kg b.w.: At Day 1, the renal Cd-content revealed the maximal value of 8.22 ± 0.36 μg/g wet tissue, and degenerating alterations including necrosis and defluxion of proximal tubular cells were most highly observed. In contrast, at Day 5, the renal Cd-content was lowered to 2.40 ± 0.24 μg/g wet tissue, and, not only the similar histopathological changes to those at Day 1 but also fibrosis and regeneration of the proximal tubular cells were remarkably found in the renal cortex. These findings strongly suggested that the Cd-MT-II and/or Cd ions which were taken into the proximal tubular cells were eliminated from the kidney mainly by the cellular death and by the resultant defluxion.

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REFERENCES


