# METABOLISM OF INTRAVENOUSLY INJECTED CADMIUM-BINDING PROTEIN

M. George Cherian* and Zahir A. Shaikh

Department of Pharmacology and Toxicology,
University of Rochester School of Medicine and Dentistry,
Rochester, New York 14642

Received April 25,1975

Summary Metallothionein was induced in rats in response to cadmium and labeled in vivo with either $^{109}Cd$, $^{14}C$ or $^{35}S$. The labeled proteins were isolated from liver tissue and their metabolism was investigated after i.v. injection in rats. Tissue distribution and urinary excretion of cadmium from $^{109}Cd$-metallothionein and $^{109}CdCl_2$ were different. The deposition of cadmium in kidney was higher than in liver and a significant urinary excretion of the metal was observed when administered in the form of metallothionein. In kidney and in urine $^{109}Cd$ remained bound to metallothionein. The distribution and excretion of the protein moiety was very similar to that of the metal. However, gel filtration of kidney soluble fraction and urine indicated some degradation of the protein.

Introduction The distribution of cadmium in different organs and its excretion from the body have been studied in different laboratories after parenteral and oral administration of cadmium salts (1-3). These studies show that high concentration of the metal is accumulated in liver and kidneys. It is also known that repeated administration of small doses of cadmium salts can induce the synthesis of a low molecular weight cytoplasmic protein known as metallothionein (4,5). The specific role of this protein in the metabolism and toxicity of cadmium and other heavy metals is not yet understood. Earlier studies have suggested that this protein may have a protective effect against cadmium toxicity and may act as a transport mechanism for cadmium (1,6). In the present investigation an attempt is made to explore the role of this protein in the transport of cadmium.

Materials and Methods Female Sprague-Dawley rats weighing 150-200 g were purchased from Charles River Breeding Laboratories, Boston, Mass. Carrier-free $^{109}CdCl_2$ and uniformly labeled L-[$^{14}C$]cystine (250 mCi/mmole) were obtained from New England Nuclear Corporation (NEN), while

*Present address: Dept. of Pathology, Univ. of Western Ontario Medical Center, London, Ontario, Canada.

L-[$^{35}$S]cystine (256 mCi/mmole) was from Amersham Searle Corporation. All injection solutions were prepared in isotonic saline.

The synthesis of metallothionein was induced in rats by s.c. injection with 10 µmoles $CdCl_2$/Kg at 24 hour intervals for 5 days. To label the protein with $^{109}$Cd the injection solution was spiked with carrier-free $^{109}$Cd. For labeling the induced protein with $^{14}$C or $^{35}$S, the animals received, in addition to the $CdCl_2$ dose, a simultaneous daily i.p. injection of 20 µCi of radiolabeled cystine solution. The animals were sacrificed 24 hours after the last injection of Cd. Metallothionein was isolated from liver tissue as described elsewhere (7). Cadmium and protein contents of metallothionein were estimated by atomic absorption (8) and by colorimetric procedure (9), respectively.

Rats were anesthetized with sodium pentobarbital (30 mg/Kg). The jugular vein and common hepatic bile duct were cannulated with No. 10 polyethylene tubing. Isotonic saline was infused during the experiment and animals were kept on a heated operating table. Labeled metallothionein or $^{109}CdCl_2$ solutions was injected through the cannulated jugular vein at various dose levels as described in the results section. Bile and urine samples were collected while the animals were still under anesthesia.

Three hours after the injection, rats were sacrificed by exsanguination. Additional urine was collected by emptying the bladder. Liver, kidneys, pancreas, and spleen were removed and weighed. Twenty percent homogenates of each tissue was prepared in cold 0.25 M sucrose solution in TKM buffer, pH 7.6. Duplicate aliquotes of homogenates were taken for counting. The kidney homogenates were fractionated by centrifuging at 105,000 x g for 30 min. Unless stated otherwise in the text (see legend for Fig. 2), kidney supernatant and urine samples were chromatographed on a 0.9 x 60 cm calibrated Sephadex G-75 column equilibrated with 0.1 M phosphate buffer, pH 7.4. The elution was monitored at 254 nm and 1 ml fractions were collected.

$^{109}$Cd was counted in a well-type Packard gamma spectrometer equipped with a NaI crystal at an efficiency of 50%. The quantity of Cd in tissues was calculated from the specific activity of the injected dose. For measuring $^{14}$C and $^{35}$S radioactivity tissue samples were solubilized with Protosol (NEN). Bile and urine samples were decolorized with hydrogen peroxide. Aquasol (NEN) was used as the scintillation fluid and radioactivity was measured with an efficiency greater than 80% in a Packard liquid scintillation spectrometer.

Results Remarkable differences in the distribution and excretion of cadmium were observed when injected as cadmium chloride or as Cd-metallothionein complex (Table 1). More than half of the injected $^{109}CdCl_2$ was accumulated in liver within three hours. A comparable dose of cadmium injected as $^{109}$Cd-metallothionein resulted in much lower deposition of cadmium in the liver (16.2%). The biliary excretion of cadmium was also depressed when cadmium was administered as metallothionein.

As expected from previous observation in this laboratory (10) and as reported by Shaikh and Lucis (11), after $CdCl_2$ administration, only a small amount of $^{109}$Cd was accumulated in the kidney as compared to liver. However, cadmium bound to metallothionein behaved markedly different in its distribution and more $^{109}$Cd was accumulated in kidney than in liver at all dose levels

TABLE 1

Distribution and Excretion of Cd Injected as $^{109}CdCl_2$ or as $^{109}$Cd-metallothionein

| | Injected Dose | | Micrograms of Injected Cadmium | | | |
|---|---|---|---|---|---|---|
| | μgCd | mg Protein | Liver | Bile | Kidney | Urine |
| Cadmium chloride | 200 | ---- | 103.40 | 4.0 | 3.0 | 0.006 |
| Metallothionein | 10 | 0.12 | 0.4 | ND+ | 5.8 | 0.1 |
| | 30 | 0.37 | 1.4 | ND+ | 13.0 | 7.1 |
| | 200 | 2.50 | 32.4 | 0.6 | 71.4 | 63.8 |

+Not determined.

studied. In accordance with published reports (10,11) the urinary excretion of cadmium after administration of $CdCl_2$ was not significant (0.003%). However, in rats injected with cadmium as metallothionein, considerable amount of $^{109}$Cd was excreted in urine. The deposition of $^{109}$Cd was small in pancreas and also in spleen and was very similar regardless of the form in which cadmium was injected.

The fate of the thionein moiety of metallothionein was investigated by injecting $^{14}$C- and $^{35}$S-labeled preparations of metallothionein. Results summarized in Table 2 show that both $^{14}$C and $^{35}$S labels appeared in kidney, liver, pancreas and spleen in decreasing order of uptake. More than one-third of the injected radioactivity was recovered in the urine within 3 hours. Excretion in bile was negligible. At the end of 3 hours, no radioactivity could be detected in the blood.

Since kidneys took up a significant amount of both $^{109}$Cd and $^{35}$S radioactivity from injected metallothionein, the tissue was subfractionated to explore the cytoplasmic distribution of the isotopes. Further fractionation of the kidney supernatants by gel filtration showed that about 85% of the

TABLE 2

Distribution and Excretion of $^{14}C$- and $^{35}S$-Labeled Metallothionein

| | Injected Dose | | Percent of Injected Radioactivity | | | | |
|---|---|---|---|---|---|---|---|
| | μgCd | mg Protein | Liver | Kidney | Urine | Spleen | Pancreas |
| $^{14}C$-metallothionein | 40 | 0.5 | 5.4 | 15.6 | 35.4 | 0.7 | 1.2 |
| | 80 | 1.0 | 13.5 | 20.6 | ND+ | 0.6 | 3.4 |
| $^{35}S$-metallothionein | 320 | 4.0 | 13.4 | 14.1 | 41.4 | 0.8 | 3.0 |

+Not determined.

supernatant $^{109}Cd$ (45% of the tissue $^{109}Cd$) was bound to metallothionein (Fig. 1A) and the remaining radioactivity was associated with higher molecular weight proteins. Figure 1B shows the Sephadex elution pattern of the kidney supernatant from a rat injected with $^{35}S$-metallothionein. Contrary to the $^{109}Cd$ distribution, only 40% of the supernatant radioactivity (15.1% of the kidney $^{35}S$) was recovered in metallothionein elution region; the remaining $^{35}S$ radioactivity was found in fractions with molecular weight lower than metallothionein.

Fractionation of urine samples from rats injected with $^{109}Cd$, $^{14}C$ or $^{35}S$-labeled metallothionein on Sephadex columns showed that the major radioactivity peak was in the metallothionein elution region, though a minor peak was detected in $^{14}C$ and $^{35}S$ experiments in low molecular weight fraction (Fig. 2). In all the urine samples studied, a distinct UV absorption peak (254 nm), characteristic of metallothionein was observed corresponding to the major radioactivity peak.

Discussion The results presented in this report show that inorganic cadmium

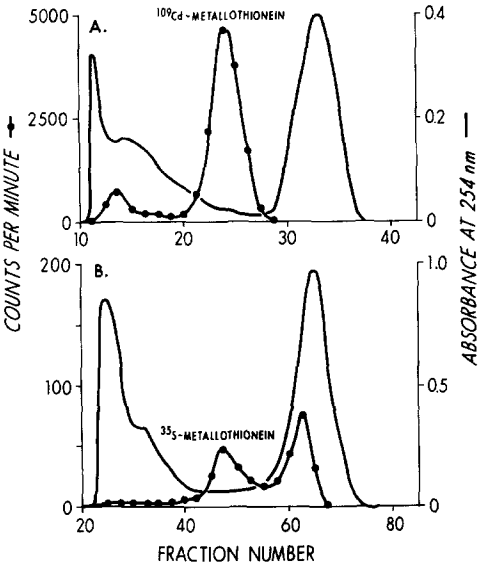


Fig. 1. Gel filtration of kidney supernatant from animals injected with (A) $^{109}$Cd-labeled metallothionein and (B) $^{35}$S-labeled metallothionein. The volumes of supernatants chromatographed were 0.25 ml and 5 ml, respectively. Sample B was chromatographed on a 1.5 x 90 cm column and 3.2 ml fractions were collected.

---

is metabolized differently than metallothionein-bound Cd. While the former is mainly taken up by liver, the latter is deposited in kidney. Considerable urinary excretion of cadmium also results when it is administered as metallothionein.

Earlier studies by Webb (14) suggest that metallothionein is not affected by proteinases in vitro. The present report clearly demonstrates that intravenously injected $^{14}$C- or $^{35}$S-labeled metallothionein is partly degraded in kidney within three hours. When $^{109}$Cd-metallothionein is administered cadmium is excreted in urine only in the form of metallothionein as shown by a single cadmium peak with absorption at 254 nm in the 10,000 molecular weight region. The cadmium released from the breakdown of metallothionein is sequestered by the higher molecular weight proteins in the kidney. In both $^{14}$C and $^{35}$S labeling experiments, there is a definite radioactivity peak in low molecular weight region, again showing that the metallothionein is catabolized in vivo.

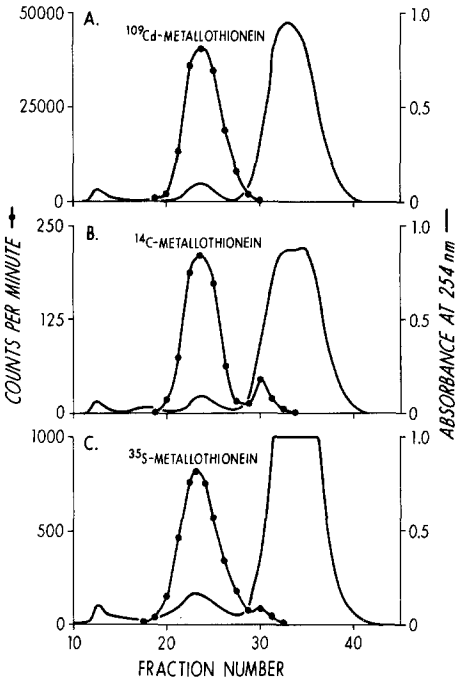


Fig. 2. Gel filtration of urine collected from animals administered metallothionein labeled with (A) $^{109}$Cd, (B) $^{14}$C and (C) $^{35}$S. Sample A and B were 0.25 ml, while 0.5 ml of sample C urine was chromatographed.

---

The present study supports the findings of Nordberg and Goyer (15) on selective deposition of cadmium in the kidney after administration of metallothionein. Nordberg et al (12) and others (13) have reported the presence of trace amounts of a low molecular weight Cd-binding protein in red blood cells and plasma and have suggested that the protein is involved in the transport of cadmium from liver to kidneys. Rapid clearance of metallothionein from the blood and significant excretion of the protein in urine, in our studies, do not suggest a transport role for metallothionein. This is also supported by recent observations by Shaikh and Smith (16) that metallothionein is not only synthesized in the liver but is also actively synthesized in the kidney. It appears that metallothionein which is produced in response to cadmium (5) is a nonsecretive, intracellular protein and may have a protective role against cadmium toxicity as reported by others (1,6).

Acknowledgements The excellent technical assistance of Judi Allen and Thomas Hanley is greatly appreciated. This study was supported by a USPHS grant GM 1590. One of us (Z.A.S.) received a NIH fellowship ES 01840.

References

1. Friberg, L., Piscator, M., Nordberg, G.F., and Kjellstrom, T. (1974) Cadmium in the environment, Second Edition, C.R.C. Press, Cleveland.
2. Shaikh, Z.A., and Lucis, O.J. (1972) Arch. Environ. Health, 24, 419-425.
3. Stowe, H.D., Wilson, M., and Goyer, R.A. (1972) Arch. Path., 94, 389-405.
4. Piscator, M. (1964) Nord. Hyg. Tidskr., 45, 76-82.
5. Shaikh, Z.A., and Lucis, O.J. (1972) Fed. Proc., 29, 298.
6. Nordberg, G.F. (1971) Environ. Physiol. Biochem., 1, 171-187.
7. Cherian, M.G. (1974) Biochem. Biophys. Res. Comm., 61, 920-926.
8. Pulido, P., Fuwa, K., and Vallee, B.L. (1966) Anal. Biochem., 14,393-404.
9. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randell, R.J. (1951) J. Biol. Chem., 193, 265-269.
10. Cherian, M.G., and Vostal, J.J. (1974) Toxicol. Appl. Pharmacol., 29, 141.
11. Shaikh, Z.A., and Lucis, O.J. (1972) Arch. Environ. Health, 24, 410-418.
12. Nordberg, G.F., Piscator, M., and Nordberg, M. (1971) Acta. Pharmacol. Toxicol., 30, 289-295.
13. Piscator, M., and Axelssen, B. (1970) Arch. Envir. Hlth., 21, 604-608.
14. Webb, M. (1972) Biochem. Pharmacol., 21, 2751-2765.
15. Nordberg, G.F., and Goyer, R.A. (1975) Arch. Pathol., 99, 192-197.
16. Shaikh, Z.A. and Smith, J.C. (1975) Fed. Proc., 34, 266.