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Dose-dependent Induction of Metallothionein in Kidneys of Mice injected with Indium and Nickel Ions

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The induction of renal metallothionein (MT) by indium and nickel loadings was studied by the use of a high performance liquid chromatograph coupled with an atomic absorption spectrophotometer. The amount of MT detected in the kidneys at 24 h post-injection showed a linear dose-dependence up to 100 and 260 μmol/kg body weight of indium and nickel, respectively, given by intraperitoneal (*i.p.*) injection. The results suggested that the renal MT was synthesized in the kidneys *per se*. The quantities of MT induced in the liver and kidneys by indium loading were correlated well with hepatic and renal indium concentrations, respectively, after *i.p.* or intravenous (*i.v.*) injection. Although the kidneys accumulated a high concentration of indium after repeated administrations, indium was not detected in the MT fraction on gel filtration chromatography. Only tiny MT peaks were observed at 7 d compared to those at 1 d after a single *i.v.* injection of indium.

Keywords——metallothionein; zinc-thionein; indium; nickel; HPLC; mouse; kidney

Metallothionein (MT) can be induced by the injection of various metals.[1] The metals may be classified into two groups. One (abbreviated hereafter as group A) consists of ,at present, the seven metals (Zn, Cd, Hg, Cu, Ag, Au and Bi) which are detected as loaded-metal-containing MT (namely, MT which contains the loaded metal and zinc (and copper[2]).[3–5] The other (abbreviated as group B) is the set of almost all heavy metals[6] other than those in group A.[7–9] These metals induce MT probably as a result of some kind of stress[10] without being incorporated into the induced MT (namely, MT which contains only zinc (zinc-thionein, Zn–Th)).

The induction of MT by group A metals has been widely investigated in various tissues. Liver and kidneys were most frequently studied and sometimes the spleen,[11,12] pancreas,[13] small intestine[14–16] and other tissues.[17] On the other hand, only a few studies have been reported on the MT-induction by group B metals in tissues other than the liver.[9] We have examined the comparative ability of several heavy metals in group B to induce Zn–Th in the liver.[6] Indium ion was the most potent inducer among the group B metals tested, when they were injected intraperitoneally (*i.p.*) at an equimolar dose. Moreover, indium ion has the same electron configuration ($4d^{10}$) as cadmium ion, which is a typical group A metal. Therefore, we were very interested in the MT-inducing character of indium. The present study was undertaken to investigate the induction of Zn–Th in the kidneys *per se* by loading with indium, in the hope of finding a dose-response relationship between Zn–Th induction and loading of indium, which has been classified as a group B metal.[7] In addition to indium ion, nickel ion, which induced a relatively high quantity of Zn–Th in the liver[6] and was reported to induce renal MT,[11] was also studied. The detection of induced MT was performed by the use of a high performance liquid chromatograph (equipped with a gel permeation column) coupled with an atomic absorption spectrophotometer as a detector (HPLC–AAS)[18] for cadmium-replaced kidney supernatants.[19]

Materials and Methods

Single Injection into Mice——Male ICR mice (JCL, Clea Japan, Tokyo) were injected *i.p.* (unless otherwise noted) with indium sulfate, zinc acetate and nickel acetate dissolved in physiological saline solution. Control mice were given saline solution. The animals were killed 24 h or 7 d after the injection under ether anesthesia, and the kidneys and liver were excised.

One of the kidneys from each mouse was combined in the appropriate group and the pooled kidneys were homogenized in 3 volumes of 0.1 M Tris-HCl buffer solution (pH 7.4, 0.25 M glucose) with a Polytron homogenizer under ice-water cooling in an atmosphere of nitrogen. The homogenates were centrifuged at $170000 \times g$ for 60 min at 2°C. In the case of the liver, a 0.4 g portion of the tissue was combined in each group and the pooled material was homogenized as mentioned above.

Repeated Injections into Rats——Female rats of the Wistar strain (JCL, Clea Japan, Tokyo) were injected with indium sulfate solution at a dose of 75 μmol/kg body weight 5 times on every other day and killed 24 h after the last injection. A 0.3 g portion of the kidneys from each rat was cut off for metal determination and the remainder was combined and homogenized in 3 volumes of 0.1 M Tris-HCl buffer solution. The homogenate was centrifuged.

Gel Filtration Chromatography——The kidney supernatant obtained from rats injected repeatedly with indium was applied to a Sephadex G-75 column (2.6 × 90 cm), and eluted with 10 mM Tris-HCl buffer solution (pH 8.6 at 25°C) at 6°C. Fractions of 5 ml each were collected. Absorbances at 254 and 280 nm were determined on a Hitachi 220A spectrophotometer with a sample sipper. Metal concentrations in the eluate were determined by AAS (Hitachi 170-50A).

Metal Determinations of Tissues——The liver (about 0.3 g portion) and one kidney (or a 0.3 g portion in the case of rats) from each animal were digested with 1 and 0.5 ml (or 1 ml in the case of rats) of mixed acid (HNO_3: $HClO_4$, 5: 1, v/v) and then the solutions were diluted to 10 and 5 ml (or 5 ml in the case of rats), respectively, with doubly distilled water. All the metal concentrations except indium in the digested solutions were determined with an inductively coupled plasma-atomic emission spectrophotometer (Jarrell-Ash Model 975 Plasma Atomcomp). Indium concentration was determined by AAS (Hitachi 170-50A).

HPLC-AAS——Cadmium acetate solution (1000 ppm Cd, 10 μl) was added to 300 μl of the original supernatant in each case to convert Zn–Th to Cd–Th, and the excess cadmium bound to high-molecular-weight proteins was removed by heat treatment (70°C, 5 min) and centrifugation ($10000 \times g$, 1 min).[20] A 100 μl portion of the Cd-replaced supernatant was subjected to HPLC (Toyo Soda HLC 803A machine equipped with a gel permeation column (TSK GEL SW 3000 column, Toyo Soda, 7.5 × 600 mm with a precolumn, 7.5 × 75 mm)), and the column was eluted with 50 mM Tris-HCl buffer solution (pH 8.0 at 25°C) at a flow rate of 1 ml/min. The cadmium level of the eluate was continuously monitored by AAS (Hitachi 170-50A)[12,18].

Results and Discussion

MT was detected in the kidneys after the *i.p.* injection of indium ion into mice as shown in Fig. 1A. The amount of the detected MT was dose-dependent up to the dose of 100 μ mol/kg body weight. The dose-dependency strongly suggests that the detected MT was induced in the kidneys *per se*. At a higher dose (150 μ mol/kg body weight), MT deviated from the dose-dependent relationship, though the injected indium was still recovered dose-dependently even at this dose as shown in Fig. 2. The ratio of the amount of MT-II to MT-I in the kidneys was considerably smaller than that in the liver (Fig. 1C). The difference also suggests that the MT detected in the kidneys was not transferred from the liver but was synthesized in the kidneys *per se*, though a difference in relative transfer of MT-I and -II from the liver to the kidneys could also account for the above result.

The amount of MT induced in the kidneys by indium loading was almost the same as that on zinc loading (Fig. 1B), though this is not a direct reflection of the ability to induce MT, since the amounts of the two metals transferred to the kidneys are not equal. The high ability to induce MT seems to be related to the high toxicity of the metal[21] (one mouse was dead within 24 h after injection at the dose of 150 μ mol/kg).

Hepatic MT-induction on indium loading was already reported.[6,7] However, no dose-dependent induction was observed at the doses used in the present study (the induction of hepatic MT was already at a plateau level). Namely, the doses of 25 and 100 μ mol/kg body weight showed almost the same degree of MT-induction in the liver, as also reported in the

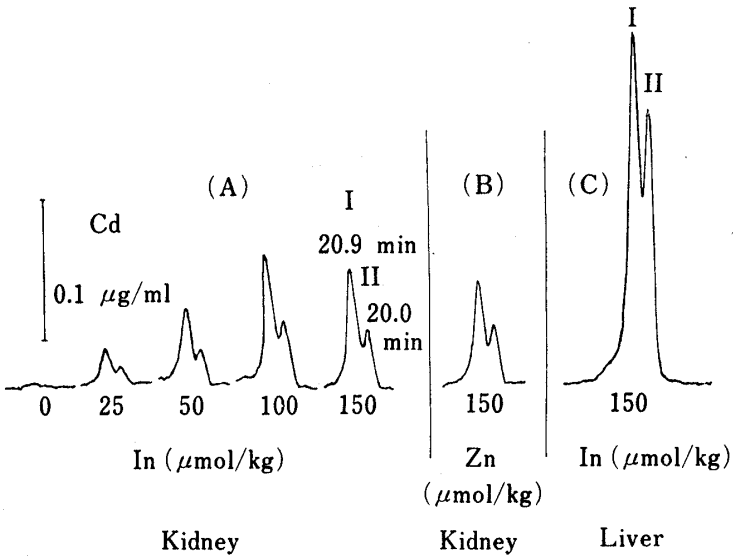

Fig. 1. Gel Permeation –Cd Atomic Absorption Chromatograms of Cd-replaced Kidney (A and B) and Liver (C) Supernatants after Injection of Indium (A and C) or Zinc (B) Ion Solution

Mice were injected with the metals at the indicated doses and were killed 24 h after the injection. Tissues were homogenized in 3 volumes of 0.1 M Tris–HCl buffer solution and the homogenates were centrifuged at 170000×*g* for 60 min. Cd solution was added to the original supernatants and the excess Cd was removed by heat-treatment and centrifugation. A 100 μl of the Cd-replaced supernatants was subjected to HPLC–AAS. The vertical bar indicates the absorption peak detected with a standard solution (0.1 μg Cd/ml) on the AAS detector. I and II indicate MT-I and -II, respectively, and figures immediately below the Roman numbers and below the peaks give the retention times of the peaks and the dose, respectively.

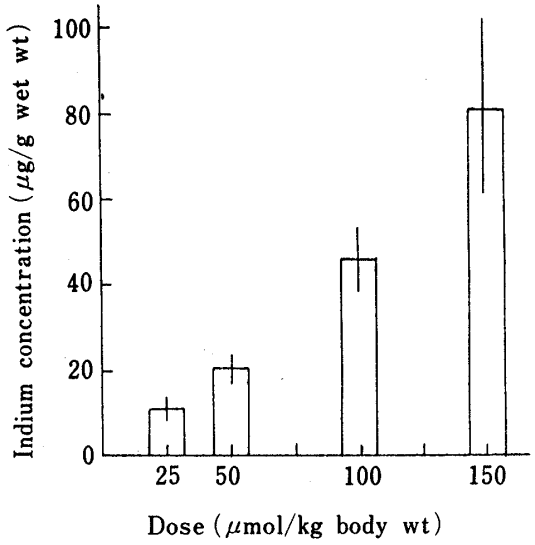

Fig. 2. Indium Concentration in the Kidneys after Indium Loading at Various Doses

Mice were killed 24 h after the *i.p.* injection and the tissues were digested with mixed acid (HNO_3: $HClO_4$, 5: 1, v/v). Vertical bars indicate S.D. of six mice.

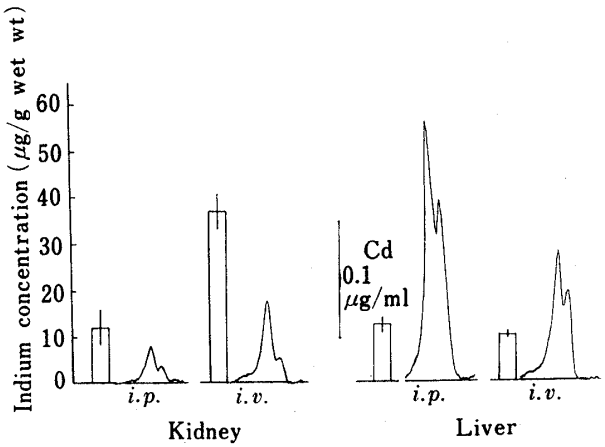

Fig. 3. The Indium Concentration and the Amount of Induced Metallothionein in the Kidneys after *i.p.* and *i.v.* Injections of Indium

Mice were injected with indium at a dose of 30 μmol/kg body weight and killed 24 h after the injection. The detector sensitivity of AAS was set as indicated by the vertical bar. See the legends to Figs. 1 and 2.

preceding paper,[6] though the detected hepatic indium concentration at the higher dose was twice that at the lower dose. In general, various stressful conditions are reported to produce little change in renal MT content.[10] Therefore, the doses at which dose-dependent MT-induction are observed in the kidneys may be far higher than those in the liver even on indium loading.

The dose (amount of indium in the tissue)-dependent induction of MT by indium loading was observed irrespective of the injection route, as shown in Fig. 3. The injection route that resulted in a higher indium concentration gave a larger MT peak in a given tissue. Namely, renal MT peak area after intravenous (*i.v.*) injection was larger than that after the *i.p.* injection, in accord with the much higher renal indium level. On the other hand, the *i.v.* injection produced less MT in the liver than the *i.p.* injection, in agreement with the lower hepatic indium level. The results also suggest that the observed renal MT was synthesized in the kidneys, not transferred from the liver. Liver also showed a higher ability to induce MT than kidneys after the *i.v.* injection. Although the quantity of induced MT was correlated to the indium concentration in every tissue, the *i.p.* injection gave a larger MT peak per unit concentration of detected indium in both tissues.

The electron configuration of the indium (III) ion is $4d^{10}$ which is the same as that of the cadium (II) ion. Moreover, indium ion tends to form polynuclear complexes[22] with sulfur-containing ligands.[23] Therefore, indium (III) ion might be a group A metal and so induce a large quantity of MT. Consequently an experiment was undertaken to detect indium ion in the MT fraction obtained from kidney supernatant. Fig. 4 shows the Sephadex G-75 elution profile of kidney supernatant from rats subcutaneously injected five times with indium. Although the kidneys showed a marked accumulation of indium (79±13 μg/g wet weight), indium was detected only in the high and low molecular weight fractions and no indium peaks were observed in the MT fraction[7] (the sensitivity of atomic absorption measurement for indium is one order poorer than that for cadium or zinc, and the mean noise level corresponded to about 20 ppb indium). Moreover, the Zn–Th level observed was much lower than that expected. This result suggests that Zn–Th (formed in response to stress) cannot be induced in the kidneys after subcutaneous injection of indium or that even if it can be induced, it cannot be accumulated.

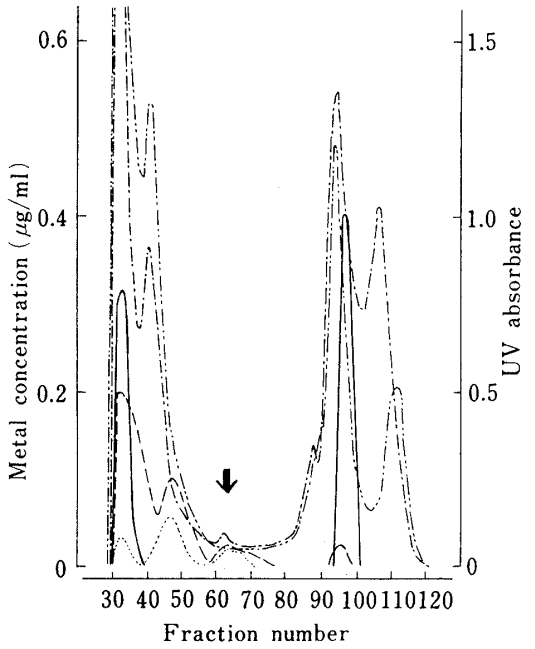

Fig. 4. Sephadex G-75 Elution Profile of Indium-containing Rat Kidney Supernatant

Rats were injected subcutaneously with indium sulfate at a dose of 75 μmol/kg body weight 5 times on every other day and killed 24 h after the last injection. The kidneys were homogenized in 3 volumes of 0.1 M Tris–HCl buffer solution (pH 7.4, 0.25 M glucose) and the homogenate was centrifuged at 170000 ×*g* for 60 min. The supernatant was applied to a Sephadex G-75 column, eluted with 10 mM Tris–HCl buffer solution (pH 8.6), and collected (5 ml/fraction). The arrow indicates the metallothionein fraction. ——, In; —— —— ——, Zn; -----, Cu; —— - ——, 254 nm; —— - - ——, 280 nm.

In general, when Zn–Th is induced by the injection of various substances, the MT level in liver and kidneys reaches its maximum about 1 d after the injection and then decreases to the control level within a week.[24] The liver and kidney supernatants obtained from mice injected with indium (30 μmol/kg body weight, *i.v.* injection) and killed 7 d after the injection both gave only tiny MT peaks on HPLC–AAS. Thus, the time-courses of MT level observed after indium loading were those

of Zn–Th.

A dose-dependent induction of MT in the kidneys was also detected on nickel loading at doses of 0—260 μmol/kg body weight (Fig. 5). However, a higher dose (340 μmol/kg) deviated from the linear relationship despite the linear increase of renal nickel concentration up to this dose (Fig. 6). On the nickel loading, a dose-dependent induction of hepatic MT was observed only at the lower doses (0—170 μmol/kg body weight). The injection of higher doses showed almost the same magnitude of MT peaks as that at 170 μmol/kg body weight, though the hepatic nickel level increased steeply with increase of the dose (hepatic nickel concentrations were 1.6, 8 and 22 μg/g wet weight at doses of 170, 260 and 340 μmol/kg body weight). The hepatic MT peaks observed at doses of less than 260 μmol/kg body weight (at these doses, MT was detected dose-dependently in both tissues) were about six times higher than those in the kidneys at each dose, despite the 3—5 times lower nickel concentration. Namely, the liver exhibited a much greater ability to induce MT than kidney per unit concentration of nickel. Although Ni–Th can be prepared from nickel ion and apothionein *in vitro*, the nickel ion in MT is readily displaced by added zinc ion.[25] Therefore, even if Ni–Th is formed *in vivo*, Zn–Th seems to be found as MT even after nickel loading. Hence, nickel ion is classified as a group B metal.

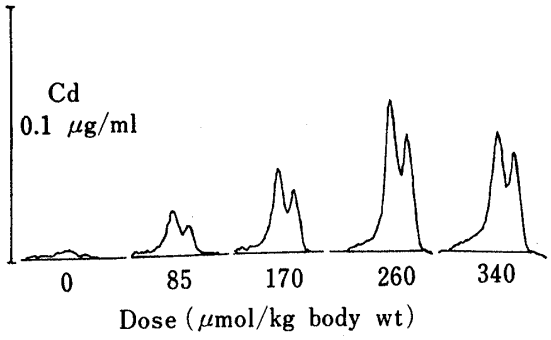

Fig. 5. Gel Permeation-Cd Atomic Absorption Chromatograms of Cd-replaced Kidney Supernatant after Nickel Loading at Various Doses

Mice were injected with nickel at the indicated doses, and were killed 24 h after the injection. The detector level of AAS was set as indicated by the vertical bar. Figures below the peaks give the dose. See the legend to Fig. 1.

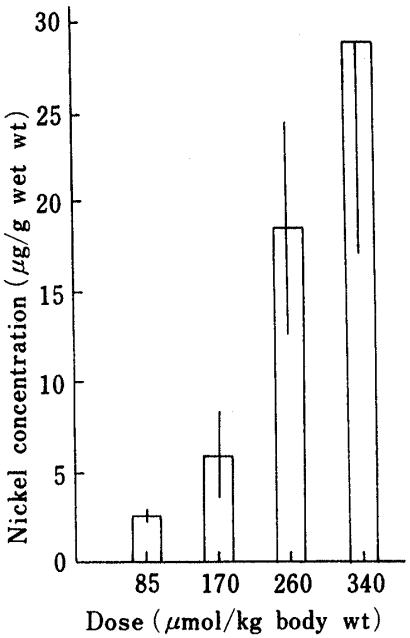

Fig. 6. Nickel Concentration in the Kidneys after Nickel Loading at Various Doses

Mice were killed 24 h after the injection and the tissues were digested with mixed acid. Vertical bars indicate S.D. of six mice.

Thus, the dose-dependent induction of MT was observed in the kidneys after the injection of the two group B metals (there is no evidence that the metals can be classified into group A), which seems to suggest the induction of Zn–Th in the kidneys *per se*. However, a significant ($p<0.05$) increase of zinc concentration in the kidneys was not observed in any case. This may be because only a small amount of MT[6] was induced in the kidneys as compared to the liver, or because the distribution pattern of zinc ion was merely altered, while the total amount remained constant.[26]

It seems noteworthy that the above-mentioned classification (groups A and B) holds

good only for the metals in a simple and soluble state. The metals in group A exhibit their ability to induce MT which contains the loaded metal when they are administered in the above state. However, they induce Zn–Th in the liver, like group B metals, when injected as methylmercury[27] or cadmium suspension.[28]

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