Induction of Hepatic Metallothionein by Trivalent Cerium: Role of Interleukin 6

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Metallothionein (MT) is a small sulphydryl-rich protein that binds to and is inducible by heavy metals such as mercury, cadmium, zinc, and copper. However, little is known about the induction of MT by trivalent metals except for bismuth. In this study, we examined the induction of MT synthesis by cerium, a trivalent lanthanoid metal. Administration of cerium chloride (CeCl3) to mice resulted in accumulation of cerium and induction of MT in the liver in a dose-dependent manner. Distribution profiles of metals in the soluble fraction of the liver of CeCl3-treated mice analyzed by high performance liquid chromatography/inductively coupled argon plasma-mass spectrometry (HPLC/ICP-MS) demonstrated that the metal bound to MT-I and MT-II was zinc, but not cerium. Administration of CeCl3 caused increases in the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the levels of serum amyloid A (SAA), an acute phase protein. Among inflammatory cytokines examined, interleukin 6 (IL-6) exhibited a marked increase in the serum at 3 h after the CeCl3 administration. In order to evaluate the involvement of IL-6 in the induction of MT by cerium, we examined MT induction by CeCl3 in IL-6 null mice. Both the induction of hepatic MT and the increases in SAA levels were markedly suppressed in IL-6 null mice. These results suggest that IL-6 plays an important role in the induction of hepatic MT by cerium.

Key words cerium; metallothionein; interleukin 6; induction; liver

Metallothionein (MT) is a low molecular weight metal-binding protein characterized by a high content of cysteine. The synthesis of MT is induced by heavy metals, hormones, cytokines and a variety of stress.1 It has been suggested that the function of MT includes detoxification of heavy metals, metabolism of trace metals such as zinc and copper, and scavenging of free radicals.2,3 Monovalent and divalent metals, such as silver, cadmium, zinc, copper and mercury, are known to induce MT synthesis and bind to this protein.4,5 Among trivalent metals, bismuth is the only known metal to induce MT synthesis.5 However, little is known about whether other trivalent metals induce MT synthesis. In a preliminary study, we examined the MT induction by several trivalent metals in mice, and found that cerium chloride (CeCl3) induced hepatic MT to the same extent as zinc chloride (ZnCl2) did.

Cerium belongs to the light lanthanoids and is about 100 times more abundant than cadmium in the earth’s crust. The Clarke number of cerium is almost the same as those of cobalt, tin, vanadium and zinc.6 Cerium is used in a variety of industrial fields as catalysts for purification of exhaust gas of cars, lighters, glass additives, ceramics, magnets and abrasives.7,8 Also, cerium is used as an antiseptic drug for extensive burns.9,10 Studies on the toxicity of cerium have shown that cerium causes liver damage in rodents, characterized by fatty liver, jaundice, and elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.6,11 Arvela reported that induction of MT by cadmium decreased cytotoxicity of cerium.12 However, no study has thus far examined the induction of MT synthesis by cerium.

The induction of MT by heavy metals such as cadmium and zinc is primarily controlled at the step of transcription, which is regulated via enhancer sequences in MT genes designated metal responsive elements (MREs), and a heavy metal dependent transcription factor, MTF-1.13,14 The metals known to induce MT synthesis can bind to MT protein, but it is not clear whether trivalent metals such as cerium can bind to MT. In the present study, we demonstrated that cerium induced MT partly via the production of IL-6 and was not bound to the MT induced.

MATERIALS AND METHODS

Animals Male ICR mice were purchased from Charles River Japan, Inc. (Atsugi, Japan), and used at the age of eight weeks. IL-6 null mice and B6J129Sv mice as wild-type controls were purchased from Jackson Laboratories (Bar Harbor, ME, U.S.A.), and bred in the National Institute for Environmental Studies (NIES; Tsukuba, Japan). Eight-week-old male mice of both strains were used for experiments. All experiments were conducted according to the NIES guidelines for animal welfare and treatment.

Treatments Male ICR mice were administered saline or CeCl3 (100, 250, 500 and 750 μmol/kg, i.p.) dissolved in saline. The animals were sacrificed 24 h after treatment under anesthesia for collection of blood, liver and kidney samples. In a time-course experiment, male ICR mice were sacrificed 0, 1.5, 3, 6, 12 and 24 h after treatment with CeCl3.
(500 μmol/kg, i.p.). Blood samples of animals were collected and used for analyses of IL-6, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and serum amyloid A (SAA). To determine dose-dependent changes of serum IL-6 concentrations by CeCl3 administration, male ICR mice were injected i.p. with CeCl3 at doses of 0, 100, 250, 500 and 750 μmol/kg. Animals were sacrificed 3 h after treatment, and blood was collected under anesthesia. IL-6 null mice and B6J129Sv mice were treated i.p. with CeCl3 at doses of 0, 250 and 500 μmol/kg, sacrificed 24 h after treatment, and the serum concentration of SAA and the hepatic MT concentration were determined. All the samples were stored frozen at −80°C until subsequent analyses.

**Determination of Serum Activities of Hepatic Enzymes**

Serum ALT and AST activities were determined using an automatic analyzer (Model 7150, Hitachi Co., Tokyo, Japan).

**Determination of Serum Cytokines and SAA**

IL-6 and IFN-γ were measured using OptEIA ELISA kits from BD PharMingen (San Diego, CA, U.S.A.), and IL-1β and TNF-α were measured using ELISA kits from Genzyme-Techne (Minneapolis, MN, U.S.A.). SAA concentration was determined using Cytoscreen ELISA kit from BioSource International (Camarillo, CA, U.S.A.).

**Determination of Cerium and MT**

Concentrations of cerium in the liver and kidney were determined by an inductively coupled argon plasma-mass spectrometer (ICP-MS) apparatus (HP4500, Yokogawa Analytical Systems, Musashino, Japan) after acid digestion of tissue samples with nitric acid. Mass number of 140 was used for determination of cerium. MT concentration was determined by Hg-binding assay as modified from the original 203Hg-binding assay.

Mercury bound to MT was measured by atomic absorption spectrophotometry using mercury analyzer (RA-2A, Nippon Instruments, Tokyo, Japan) after digestion with nitric acid. Mass number of 140 was used for determination of MT bound to cerium. MT concentration was determined by Hg-binding assay as modified from the original 203Hg-binding assay.

**HPLC/ICP-MS Analysis for Hepatic MT**

The distribution profiles of cerium, cadmium, zinc and copper in the liver supernatant were analyzed by HPLC/ICP-MS as described by Suzuki with a modification. Portions of liver samples from three mice obtained 24 h after treatment with CeCl3 (750 μmol/kg, i.p.) were pooled and homogenized in four volumes of saline. Supernatant samples were prepared by ultracentrifuging the homogenate at 105000 g for 1 h at 4°C. The liver of mice obtained 24 h after treatment with CdCl2 (10 μmol/kg, s.c.) was prepared in the same manner. An aliquot (40 μl) of the liver supernatant was applied to a TSK gel G3000SW column (7.5×600 mm with a 7.5×75 mm guard column, Tosoh, Tokyo, Japan) and was eluted with 50 mM Tris–HCl buffer (pH 8.6 containing 0.1% sodium azide) at a flow rate of 0.8 ml/min on an HPLC Instrument (HP1100, Yokogawa Analytical Systems, Musashino, Japan). The eluate was introduced directly into the nebulizer capillary of an ICP-MS and the distributions of cerium, cadmium, zinc and copper were continuously determined at mass numbers of 140, 111, 63 and 65, respectively.

**Statistical Analysis**

Data were expressed as mean ± S.D. Statistical significance was determined by using either one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. Differences between groups were considered significant at p<0.05.

**RESULTS**

Figure 1 shows concentrations of cerium and MT in the liver and kidney of ICR mice treated with CeCl3. The administration of CeCl3 resulted in a dose-dependent increase in MT level in the liver. The level of hepatic MT at the highest dose (750 μmol/kg) of CeCl3 was 35 times that of control mice. On the other hand, only a small increase in MT level was detected in the kidney. Hepatic cerium concentrations increased markedly and dose-dependently (Fig. 1), while renal cerium concentrations hardly changed from the control level, suggesting that specific induction of MT in the liver by CeCl3 may reflect the preferential accumulation of cerium in the liver.

The distribution profiles of cerium, cadmium, zinc and copper in the liver supernatant of mice treatment with CdCl2 and CeCl3 were analyzed by HPLC/ICP-MS. As shown in Fig. 2, two isosfomts of MT were observed as two peaks of cadmium at retention times of 22.9 (MT-II) and 24.2 min (MT-I) in the liver supernatant of CdCl2-treated mice used as a positive control. On the other hand, in the liver supernatant of CeCl3-treated mice, cerium was detected only in the high-molecular weight fraction. Instead, two clear peaks of zinc (retention time 23.0 min and 24.3 min) were detected. These distributions of metals indicate that cerium was not bound to the MT induced by the administration of CeCl3, and that zinc was the major metal bound to MT in the CeCl3-treated mice.

The hepatic injury by administration of CeCl3 was examined using biochemical indicators obtained from blood. As shown in Table 1, serum ALT and AST activities increased dose-dependently in mice treated with CeCl3. To examine whether the hepatic injury was caused by inflammation, we measured concentrations of SAA, an acute phase protein. The concentrations of SAA increased markedly and dose-dependently in mice 24 h after treatment with CeCl3 (Fig. 3B). The SAA level began to increase 6 h after CeCl3 administration, and reached a plateau level at 12 h (Fig. 3A). These results suggest that administration of CeCl3 caused inflammation in the liver, leading to increases in the levels of SAA, ALT and AST.
As shown in Fig. 4A, serum concentrations of IL-6 increased after the administration of CeCl₃ with a peak at 3 h. The concentrations of IL-6 at 3 h increased dose-dependently (Fig. 4B). On the other hand, the concentrations of IL-1β, TNF-α or IFN-γ in the serum of CeCl₃-treated mice were close to the limits of detection at any time points.

To further examine the involvement of IL-6 in CeCl₃-induced MT synthesis, IL-6 null mouse were utilized. Administration of CeCl₃ to IL-6 null and control B6J129Sv mice resulted in increases in MT levels, but the MT levels in IL-6 null mice were about one third of those in control mice (Fig. 5A). On the other hand, the production of SAA by treatment with CeCl₃ was markedly reduced in IL-6 null mice (Fig. 5B), suggesting an important role of IL-6 in the production of SAA by treatment with CeCl₃.

The results obtained by using IL-6 null mice suggest that IL-6 production plays the most important role in MT induction in the liver of mice treated with CeCl₃. However, the contribution of other factors cannot be excluded.

DISCUSSION

In the present study, we demonstrated that the cerium, a trivalent lanthanoid metal, was a potent inducer of MT in the liver of mice. The maximum level of MT induced by CeCl₃ was almost the same as that induced by zinc. Accumulation of cerium was observed in the liver, but not in the kidney (Fig. 1). However, HPLC/ICP-MS analysis of the supernatant of the liver revealed that cerium was not bound to MT pro-
teins induced by cerium (Fig. 2). The major metal bound to MT protein was zinc. This means that CeCl₃ can induce MT effectively, but cannot bind to MT, and suggests a different mechanism of MT induction compared with that of other MT-inducing metals such as cadmium and zinc. The measurement of serum cytokine levels and the utilization of IL-6 null mice revealed that IL-6 plays an important role in the induction of MT by cerium (Figs. 4, 5).

To date, various agents such as organic solvent, endotoxin, hormones, and cytokines have been shown to induce MT in animals and cells. Inflammation or physical stress involves the induction of cytokines such as IL-1β, IL-6, TNF-α and IFN-γ. These cytokines are also inducers of MT in the liver of animals and hepatic cells. Especially, IL-6 plays a key role in the induction of hepatic MT by n-hexane, ultraviolet, partial hepatectomy, turpentine, ferric nitroltriacetate, and restraint stress. Cadmium also induces IL-6 as well as IL-1β and TNF-α, suggesting that the induction of MT by cadmium is mediated, at least in part, by inflammatory responses in hepatocytes, although the primary pathway for MT induction by cadmium is the activation of the transcription factor, MTF-1. Thus, many studies have suggested the involvement of inflammation in the induction of MT by various compounds, but the actual contribution of inflammation or cytokine production in MT induction by metal compounds has not yet been evaluated quantitatively.

In the case of cerium, hepatic MT concentration was suppressed by about 70% in IL-6 null mice (Fig. 5), suggesting the involvement of inflammation in the induction of MT compared with that of other compounds, but the actual contribution of cytokines and acute phase proteins by metals in the production of MT has not yet been elucidated.

In conclusion, trivalent cerium is a potent inducer of MT, but does not bind to MT probably due to its electronic configuration. The induction of MT synthesis by cerium is mainly mediated by the production of IL-6, which accompanied the production of acute phase proteins such as SAA. Further studies are warranted for the elucidation of the involvement of cytokine production in the mechanism of MT induction by heavy metals. Also, the roles of the production of cytokines and acute phase proteins by metals in the protection against metal toxicity should be clarified.

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