Dichotomous Effects of Lead Acetate on the Expression of Metallothionein in the Liver and Kidney of Mice

Jiaming YU,* Hitomi FUJISHIRO,* Hideki MIYATAKE,* Tomohiro Max OYAMA,* Tatsuya HASEGAWA,* Yoshiyuki SEKO,* Nobuhiko MIURA,* and Seiichiro HIMENO*+.

* Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University; Yamashiro, Tokushima 770–8514, Japan; + Department of Environmental Biochemistry, Yamanashi Institute of Environmental Sciences; Kenmarubi, Kamiyoshida, Fujiyoshida, Yamanashi 403–0005, Japan; and $ Mechanism of Health Effects Research Group, National Institute of Occupational Safety and Health, Japan; Tama-ku, Kawasaki 214–8585, Japan. Received January 5, 2009; accepted March 21, 2009; published online March 25, 2009

Metallothionein (MT) is a low-molecular-weight cysteine-rich protein which has a high affinity for metals and plays important roles in the protection against metal toxicity. As little information is available concerning the mechanism of MT induction by lead (Pb) compounds, we investigated the induction of MT by Pb acetate both at mRNA and protein levels in mice. Administration of Pb increased the levels of MT-I mRNA in the liver and kidney in six strains of mice. However, MT protein was detected only in the liver, and little or no increases in MT protein were detected in the kidney of any strains of mice. Speciation of metals in the liver cytosol showed that the major metal bound to MT was zinc but not Pb. The increases in plasma concentrations of interleukin-6 suggested that the production of interleukin-6 by Pb administration is involved in the induction of MT in the liver. Treatment of renal cells with Pb in vitro also resulted in the increase in MT mRNA but little increase in MT protein. These data suggest that Pb exerts a dual effect on MT expression; enhancement of MT gene transcription both in the liver and kidney and suppression of MT mRNA translation in the kidney.

Keywords lead; metallothionein; induction; kidney

Lead (Pb) is a toxic metal that induces a broad range of physiological, biochemical and neurological dysfunctions in humans.1) Although atmospheric Pb pollution due to tetraethyllead from gasoline has been improved over the last two decades, humans are still exposed to Pb via contaminated foods and water and through industrial activities.2) Precise mechanisms of Pb toxicity as well as the protective measures against Pb toxicity still remain to be solved.

Metallothionein (MT) is a low-molecular-weight metal-binding protein and is known to play an important role in the protection against heavy metal toxicity.3) In addition to the detoxification of toxic metals such as cadmium (Cd) and mercury (Hg), MT is involved in the maintenance of homeostasis of essential trace elements such as zinc (Zn) and copper (Cu), and in the scavenging of free radicals. MT gene expression is induced by many factors, including metal ions such as Cd, Zn, Cu and Hg, alkylating agents, UV irradiation, and other chemical and physical stressors. The role of a specific transcription factor, metal response factor 1 (MTF1), in the activation of MT gene transcription by potent MT-inducing metals such as Zn and Cd has been extensively investigated.4,5) On the other hand, the mechanisms of MT induction by other weak MT-inducing metals such as manganese (Mn), nickel (Ni), cobalt (Co) or iron (Fe) have not been fully investigated recently. We have demonstrated that interleukin-6 (IL-6) produced by various metal compounds such as Mn, cerium (Ce) and vanadium (V) plays an important role in the induction of MT in the liver.6—8)

However, little information is available concerning MT induction by Pb compounds. An early study in 1980s showed that intraperitoneal administration of Pb increased the protein amount of Zn-thionein in the liver of rats.9) However, no information is available until now on the effects of Pb administration on MT gene expression. The purpose of the present study is to investigate tissue-specific expression of MT mRNA and protein in mice treated with Pb. Here we show that Pb administration caused an increase in MT mRNA both in the liver and kidney, but produced MT protein only in the liver, suggesting a dichotomous role of Pb in the transcription and translation of MT.

MATERIALS AND METHODS

Treatment of Animals Six-week-old male ICR, C57Bl/6, C3H/He, Balb/c, A/J, DBA/2 mice were obtained from Japan SLC Co. (Hamamatsu, Japan) and housed in plastic cages at 23—24 °C with a 12-h light and dark cycle. Mice were given free access to a commercial diet and tap water. All animal experiments were performed according to protocols approved by the Animal Care and Use Committee of Tokushima Bunri University. Male ICR mice were intraperitoneally injected with Pb acetate at doses of 100, 200, and 300 μmol/kg, and sacrificed 4, 8 and 24 h after Pb treatment. Control mice were given saline. In a time-course experiment, ICR mice were sacrificed at 1, 2, 3, 4, 6, 12, 24, and 48 h after Pb acetate (300 μmol/kg) administration.

Heparinized blood samples were collected under anesthesia and centrifuged at 3500 rpm for 10 min. The plasma samples were stored at −80 °C until subsequent measurements. The liver and kidney tissues of mice were removed, immediately immersed in liquid nitrogen, and stored at −80 °C.

RNA Isolation and RT-Polymerase Chain Reaction (PCR) Total RNA was isolated from the frozen liver and kidney tissues by the method of guanidium thiocyanate–phenol–chloroform extraction.10) Reverse transcription (RT) was performed in a mixture containing 50 mM Tris–HCl pH 8.3, 70 mM KCl, 3 mM MgCl2, 106 mM dithiothreitol, 1 mM each of deoxyxymecloside triphosphate (dNTP) 1 μM, 4 units of RNase inhibitor, 2 μg of total RNA, 0.5 μM Oligo(dT)15 primer 1 μl, and 5 units of reverse transcriptase in a total vol-
Cells with Lead Acetate

The proximal tubular cells in kidney were treated with CdCl2 (0.1, 0.3, 1.0 μM), or ZnSO4 (50, 100 μM), and Pb acetate (50, 100, 200 μM) for 8 and 24 h. Total RNA was extracted from cells at 8 h and used for MT-I mRNA determination. Cell lysates obtained at 24 h were used for MT protein determination. MT concentrations in cells were measured by Hg-binding assay basically in the same way as for the tissue samples with a minor modification as follows: the amounts of Hg and ovalbumin added to each assay tube for cellular MT determination were 1 nmol and 30 nmol, respectively (50 nmol and 100 nmol, respectively, for tissue MT determination).

Data Analyses All of data were expressed as mean ± S.D. Statistical significance for dose-dependency was determined by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test.

RESULTS

To investigate the effects of Pb administration on the levels of MT mRNA in the liver and kidney, semi-quantitative RT-PCR analysis of MT-I was performed. The expression of MT-I mRNA was detected not only in the liver but also in the kidney of male ICR mice 4 and 8 h after the administration of Pb acetate (200 μmol/kg) (Fig. 1A). The mRNA levels of MT-I increased dose-dependently both in the liver and kidney 8 h after Pb administration (Fig. 1B). Reflecting the mRNA levels of MT-I, the protein levels of MT in the liver increased...
in a dose-dependent manner (Fig. 1C). However, the protein levels of MT in the kidney did not show significant increases even at the highest dose (300 μmol/kg). Since MT-I mRNA levels in the kidney at 24 h were higher than those in the liver (Fig. 1B), the low levels of MT protein in the kidney may not be caused by a rapid decrease in MT mRNA. Concentrations of MT protein were also very low in the kidney at 8 h or 48 h after Pb administration (data not shown), suggesting that the low values of MT protein in the kidney was not caused by a rapid degradation or delayed synthesis of MT in the kidney.

To confirm whether this discrepancy in mRNA and protein levels of MT in the kidney occurs in other strains of mice, five strains of mice were administered Pb acetate (300 μmol/kg), and the levels of MT mRNA and MT protein in the liver and kidney were examined. As shown in Fig. 2A, all strains of mice showed increases in MT mRNA not only in the liver but also in the kidney. The mRNA levels of β-actin were not changed by Pb treatment in any strains of mice (data not shown). On the other hand, the protein levels of MT in the kidney showed only slight increases in any strains of mice after Pb treatment (Fig. 2B).

Figure 3 shows time-dependent changes in Pb and Zn concentrations in the liver and kidney of ICR mice after Pb administration. Concentrations of Pb increased substantially in the liver, while only slight increases were detected in the kidney. The increases in hepatic Zn concentration may reflect the increased amount of MT in the liver, while no increase in renal Zn may reflect the lack of MT protein synthesis in the kidney.

As there is a possibility that Hg-binding assay for MT protein determination may be unable to detect Pb-induced MT, we examined elution profiles of metals in the cytosol of liver and kidney tissues by using HPLC-ICP-MS (Fig. 4). The peaks of MT-I and MT-II were confirmed by the elution profile of the cytosol obtained from the liver of Cd-injected mice. Pb administration increased the amounts of Zn in the fractions of MT-I and MT-II in the liver, but not those of Pb in the same fraction, suggesting that the hepatic MT induced by Pb administration is Zn-thionein. In the kidney, no clear peaks of Zn, Cu or Pb were found in the fractions of MT-I or MT-II. Thus, both Hg-binding assay and metal speciation analysis by HPLC-ICP-MS confirmed the absence of MT protein in the kidney of mice treated with Pb.

As our previous study showed that MnCl₂ administration induced MT in the liver via the production of IL-6, we examined plasma levels of IL-6 and SAA, the latter is an acute-phase protein regulated by proinflammatory cytokines such as IL-6. Concentrations of plasma IL-6 increased after the administration of Pb with a peak at 3 h (Fig. 5). The increase in plasma ALT activity at 12 h suggests a moderate liver injury by Pb administration. Concentrations of SAA markedly increased with a peak at 24 h. These results indicate that the administration of Pb caused a transient inflammation in the liver, and that the induction of MT may be mediated, at least partly, by the production of IL-6 as in the cases of hepatic MT induction by Ce, V, and Mn.

To examine whether in vitro treatment of renal cells with Pb also induce MT expression, we established immortalized mouse proximal tubular cells (PT3 cells). As shown in Fig. 6,
the addition of Pb acetate to the medium of PT3 cells increased MT mRNA dose-dependently. The highest dose of Pb (200 μM) enhanced MT mRNA levels to the degree comparable to those treated with 0.3 μM Cd or 100 μM Zn, suggesting that Pb can directly enhance MT gene expression in renal cells. On the other hand, no significant increases in MT protein concentration were observed in the cells treated with Pb. Thus, the suppression of MT protein synthesis in spite of high expression of MT mRNA in renal cells was observed also in an in vitro system.

DISCUSSION

In the present study, we showed dichotomous effects of Pb on the expression of MT in the liver and kidney of mice. In the liver, both MT mRNA and MT protein were increased by Pb administration partly due to the production of IL-6 by Pb. On the other hand, high levels of MT mRNA were induced by Pb treatment, but MT protein was not synthesized in the kidney (Figs. 1, 2). The induction of MT mRNA without MT protein synthesis was also observed in cultured renal cells treated with Pb (Fig. 6).

To date, most studies on the mechanism of MT expression have focused on the activation of transcription factor MTF-1 by potent MT-inducing metals such as Zn²⁺, Cd²⁺, Cu²⁺, Hg²⁺, Ag⁺, and Bi³⁺. Other metals such as Fe³⁺, Cr³⁺, Mn²⁺, Ni²⁺, and Co²⁺ have also been shown to induce MT in vivo, but not all of them induced MT in vitro, suggesting the involvement of indirect mechanisms such as inflammatory responses in MT induction by these metals. Recently, we have shown that IL-6 is the most important inflammatory cytokine in the induction of MT by Ce³⁺, V²⁺, and Mn²⁺ in mice. Especially, the induction of MT by MnCl₂ injection...
was completely suppressed in IL-6 null mice. In the present study, administration of Pb resulted in the increase in plasma IL-6 with a peak at 3 h after the injection (Fig. 5). As IL-6 is a potent inducer of hepatic MT, the enhanced expression of MT in the liver may be partly mediated by the IL-6 production by Pb. The elevation of plasma levels of SAA, an acute-phase protein synthesized in the liver under the control of IL-6, also confirmed that IL-6 activated the expression of target genes in the liver. Furthermore, as IL-6 is also known to enhance the expression of hepatic ZIP14, a Zn transporter responsible for cellular Zn incorporation into the liver, the synthesis of Zn-thionein in the liver (Fig. 4) might have been accelerated by the increased Zn influx to the liver of Pb-treated mice.

However, the Pb-induced increase in MT mRNA in the kidney may not be explained by IL-6 since administration of IL-6 to mice is known to induce MT specifically in the liver probably due to the efficient deposition of circulating IL-6 in the liver. We also showed that administration of Ce3+, V5+, and Mn2+, the producers of IL-6, induced MT preferentially in the liver. As renal concentrations of Pb after the administration were very low (Fig. 3), the other factors than Pb accumulation may account for the induction of MT mRNA in the kidney. Further studies are required to examine the possible roles of other cytokines, reactive oxygen species, and protein kinase C activated by Pb treatment in the induction of MT mRNA in the kidney.

The most prominent finding of this study is the lack of MT protein synthesis in the kidney in spite of high levels of MT mRNA (Figs. 1, 2, 6). These data suggest a negative role of Pb in post-transcriptional control of MT synthesis specifically in the kidney. Although the mechanism of this phenomenon remains to be answered, several possibilities should be noted here.

A few studies have suggested the role of translational regulation of MT synthesis based on the discrepancy between mRNA and protein levels of MT in the tissues of metal-treated animals. Vasconcelos et al. reported that CdCl2 injection caused increases in MT mRNA levels both in the liver and kidney, while only a small increase in MT protein was observed in the kidney. Zalups et al. showed that administration of HgCl2 caused increases in MT mRNA expression both in the liver and kidney, but the correlation between MT mRNA and MT protein in the kidney was poor or absent, suggesting that post-transcriptional control plays more important role in the kidney than in the liver in the process of MT protein synthesis. These studies and our data suggest that renal MT induction by metals is readily modified at the step of translation and that the metals including Pb may exert complicit influences on the translational processes of MT in the kidney.

Another possible reason for the suppression of MT protein synthesis by Pb is that Pb deprived kidney cells of free Zn ions necessary for the synthesis of Zn-thionein. Pb is known to displace Zn in the active site of δ-aminolevulinic acid dehydratase, leading to the depression of the enzyme activity. DNA-binding of Sp1 or GATA-1 via Zn-finger motif was also suppressed by Pb treatment. However, this hypothesis may not explain the kidney-specific suppression of MT protein synthesis.

It has been known that treatment of cells with cycloheximide, a protein synthesis inhibitor, causes a superinduction of MT mRNA with no increase in MT protein. This phenomenon implies the existence of a cycloheximide-sensitive, negative regulator of MT gene transcription, though not yet identified. In vitro studies, Pb has been shown to inhibit protein synthesis through the phosphorylation of eIF2α, an initiation factor for protein synthesis. However, Pb administration did not increase phosphorylation of eIF2α either in the liver or kidney (data not shown), suggesting that the superinduction of MT mRNA in vivo by Pb treatment may not have occurred.

Recently, Heo et al. reported that Pb treatment resulted in an increase in interferon-γ (IFNγ) mRNA but a decrease in IFNγ protein via the inhibition of IFNγ protein synthesis, suggesting a dichotomous role of Pb in the transcription and translation of IFNγ. In the present study, we showed a suppression of MT protein synthesis in the presence of high levels of MT mRNA in the kidney of Pb-treated mice and in Pb-treated renal cells. These experimental models may provide a good tool for studying the post-transcriptional control of MT induction especially in the kidney.

Acknowledgements This study was supported by a Grant-in-Aid for Scientific Research (B-19390169), and by a Grant-in-Aid for JSPS Fellows (P05485) from Japan Society for the Promotion of Science (JSPS). J.Y. was supported by a JSPS postdoctoral fellowship.

REFERENCES


