Biochemical Pharmacology, Vol. 41, No. 5, pp. 775–780, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

SEX DIFFERENCES IN HEPATIC AND RENAL CADMIUM ACCUMULATION AND METALLOTHIONEIN INDUCTION

ROLE OF ESTRADIOL

MARK E. BLAZKA and ZAHIR A. SHAIKH*
Department of Pharmacology and Toxicology, University of Rhode Island, Kingston, RI 02881, U.S.A.

(*Received* 2 *July* 1990; *accepted* 11 *September* 1990)

Abstract—The role of estradiol in sex differences in hepatic and renal cadmium accumulation and metallothionein (MT) induction was investigated. Male and female rats and castrated males pretreated with estradiol were injected either i.v. or s.c. with 10 μmol $CdCl_2$/kg. Sex differences in cadmium accumulation and MT induction were apparent after s.c. but not i.v. administration. The female rats accumulated a significantly greater concentration of cadmium in their liver than did the males, as early as 1 hr after the s.c. injection. The elevated levels of cadmium in the females were maintained for at least 10 days. Pretreatment of castrated males with estradiol caused a similarly greater accumulation of cadmium in the liver. Hepatic MT levels peaked in the females at 24 hr and in males 48–72 hr after the cadmium injection and then declined to lower levels. This superinduction of MT occurred only after the s.c. administration of cadmium. MT levels in both sexes plateaued 5 days after the s.c. injection to the levels that were similar to those seen in male and female rats 24 hr after an i.v. injection. In animals injected s.c. with cadmium the renal cadmium levels continued to rise for 5–10 days; however, in animals injected i.v. the levels stabilized within 2 hr. The renal MT levels in the females were significantly higher than in the males. Estradiol pretreatment induced renal MT but did not affect renal cadmium accumulation. Thus, the sex differences in hepatic cadmium accumulation and MT induction are affected by the route and time after the administration of cadmium. Furthermore, estradiol causes a more rapid uptake of cadmium by the liver and also an enhanced induction of MT in both the liver and kidney.

Sex differences in cadmium accumulation are reported in environmentally exposed Japanese populations [1, 2] and in experimental animals exposed to cadmium [3–9]. In Japanese autopsy tissues the cadmium levels in liver and kidney were found to be significantly higher in women, as compared to men [1]. Similarly, in rats the whole body retention of orally administered cadmium is higher in females and castrated males than in normal males [5]. Other studies in rats reported greater cadmium absorption as well as hepatic and/or renal accumulation in females than in males [3, 4, 6–9]. Thus, it appears that sex hormones are involved in cadmium disposition.

In liver, kidney and other tissues the majority of cadmium is sequestered by metallothionein (MT). Greater cadmium accumulation in the females may thus be related to induction of MT by the female sex hormones. Nishiyama *et al.* [10] did observe renal MT induction by estradiol in castrated males. However, they failed to observe any effect of estradiol on hepatic MT or cadmium accumulation [4], probably because they used normal males rather than the castrated males. Lack of MT induction in response to estradiol was also reported by Stacey [11]. However, this study was carried out in cultured hepatocytes, whereas estradiol may have an indirect effect on MT in the intact animal. We reported previously that in both control and cadmium-injected female rats hepatic as well as renal MT levels are significantly higher than in the males [3]. In the present investigation we compared the time–course of cadmium accumulation as well as MT induction in normal males and females and evaluated the effect of estradiol on cadmium and MT levels in the castrated males.

* Author to whom correspondence and requests for reprints should be addressed.

MATERIALS AND METHODS

Materials. Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), 10- to 12-weeks-old, were provided with water and rat chow *ad lib.* The animal rooms were illuminated on a 12-hr light/dark cycle. The cadmium injection solution consisted of 20 μmol/mL $CdCl_2$ (Alfa Products, Danvers, MA) and 1.5 μCi/mL $^{109}CdCl_2$ (New England Nuclear, Boston, MA).

The hormone solutions contained 0.5 mg/mL estradiol or testosterone (Sigma Chemical Co., St. Louis, MO) in dimethyl sulfoxide (DMSO) (Fisher Scientific Co., Medford, MA). Borate, sodium chloride, Tween-20, bovine serum albumin (BSA) (Sigma Chemical Co.), and Pansorbin (Calbiochem, San Diego, CA) were used in the radioimmunoassay (RIA).

Animal dosage. For the time–course studies the animals were injected with 10 μmol $CdCl_2$/kg between 8:00 and 10:00 a.m. and were killed up to 24 hr after the i.v. injection or up to 10 days after the s.c. injection.

For the hormone study male rats were castrated

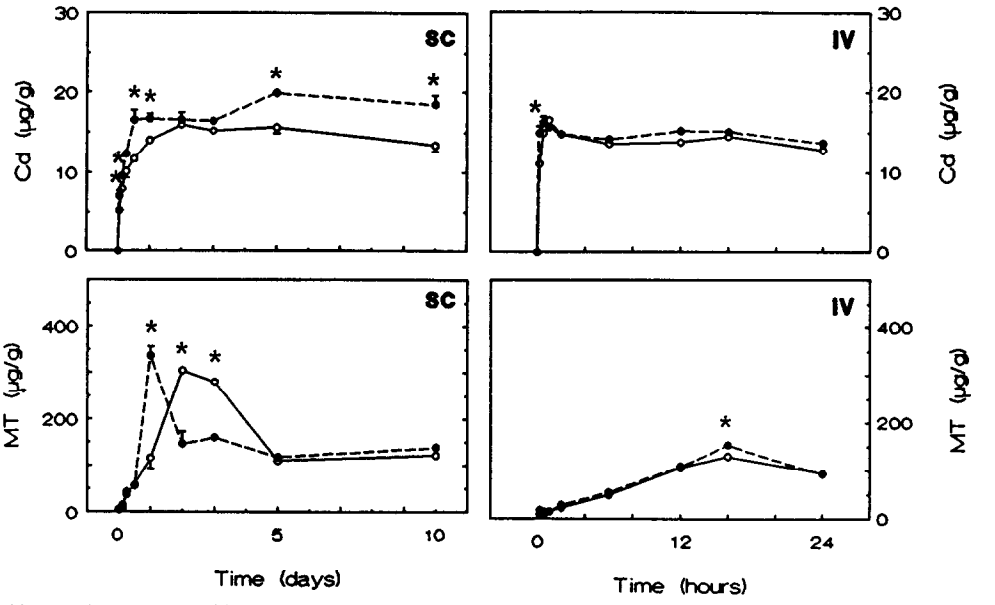

Fig. 1. Time–course of hepatic cadmium accumulation and changes in MT levels. Male (○) and female (●) rats were injected s.c. or i.v. with 10 μmol $CdCl_2$/kg. Values are means ± SEM, N = 3–7. Key: (*) significant sex difference at this time point ($P < 0.05$).

and 3 days later treated with estradiol, testosterone, or DMSO, twice a day for 6 consecutive days. The sham-operated rats received only DMSO. On day 6 of hormone treatment a single s.c. injection of either 10 μmol $CdCl_2$/kg or 0.5 mL saline/kg was administered to treated and control rats, respectively, and the animals were killed 24 hr later.

Methods. The animals were killed by exsanguination under ether anesthesia. The liver and kidneys were removed and weighed. ^{109}Cd levels in the tissues were determined with a gamma spectrometer (United Technologies-Packard, Downers Grove, IL), and the tissues were frozen at −20° until further analysis.

For MT determinations, the tissues were thawed and a portion was homogenized (5 or 10%, w/v) in 125 mM borate-NaOH buffer, pH 8.6, containing 0.1% Tween and 0.04% BSA. The analysis of MT by RIA was carried out as previously reported [12], with the exception that 200 μL of 1:20 diluted Pansorbin was used to separate the bound antigen [13].

Statistical analysis. In the time–course study, the male and female cadmium levels at each time point were compared by the Wilcoxon Rank Sum Test. The remaining data were analyzed by the general linear models procedure (SAS Institute, Cary, NC). When the analysis indicated a significant difference, the data were examined further by two- and one-way ANOVA, as appropriate.

RESULTS

Time–course of hepatic cadmium and MT levels. As shown in Fig. 1, hepatic cadmium accumulation was nearly completed within 24 hr after s.c. administration and 2 hr after i.v. administration. After s.c. injection the hepatic cadmium accumulation was significantly higher in females than in males as early as 1 hr after the injection and remained so even after 10 days. In comparison, the only sex difference after i.v. administration was at the 10-min time point; otherwise both sexes had the same concentration of cadmium.

MT was induced earlier in females than in males after the s.c. cadmium administration (Fig. 1). The MT levels in the females reached a peak at 24 hr, decreased by day 2 and then leveled off. The rise in hepatic MT concentration in males occurred at a slower rate than in the females and reached the maximum between days 2 and 3. As in the females, the MT levels in the males also declined. By day 5 the MT levels in both males and females were the same.

The increase in hepatic MT was much smaller after i.v. than after s.c. administration, and the protein reached peak levels in both sexes at 16 hr (Fig. 1). The females had slightly but significantly higher MT levels than the males at this time point. The MT levels in the i.v. group at 24 hr were similar to those in the s.c. group at 5–10 days.

Time–course of renal cadmium and MT levels. After s.c. administration, the renal cadmium concentration continued to rise for 5 days in males and 10 days in females (Fig. 2). The cadmium accumulation was similar in males and females except at 3 and 5 days at which time the males had higher cadmium levels than the females. Upon i.v. injection, cadmium accumulated rapidly and reached a plateau within 2 hr (Fig. 2). No significant sex differences in cadmium levels were seen at any of the time points in the i.v. group.

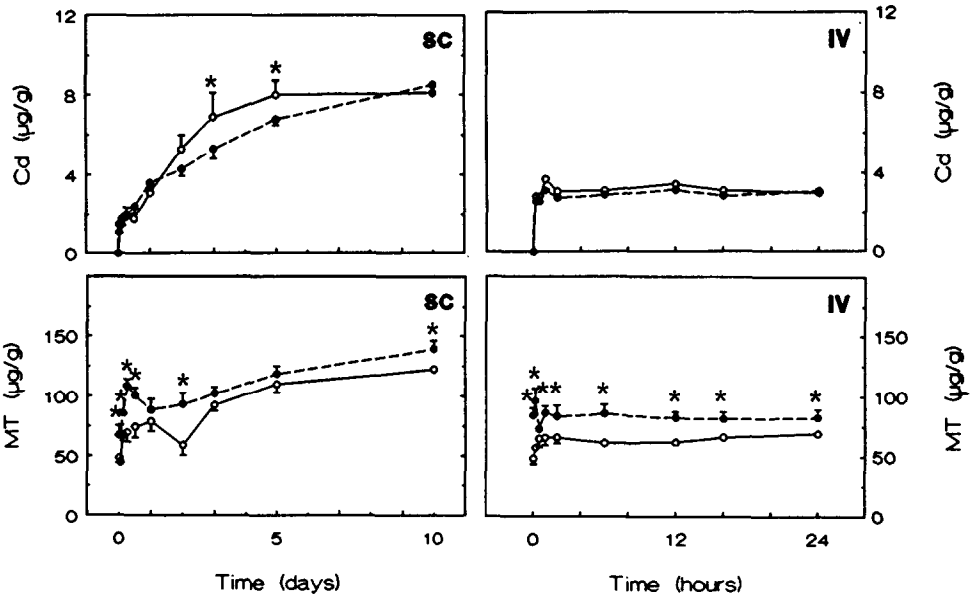

Fig. 2. Time–course of renal cadmium accumulation and changes in MT levels. Male (○) and female (●) rats were injected s.c. or i.v. with 10 μmol $CdCl_2$/kg. Values are means ± SEM, N = 3–7. Key: (*) significant sex difference at this time point ($P < 0.05$).

Following s.c. administration, MT levels in females were significantly higher than in males at most time points (Fig. 2). The renal MT continued to rise for the duration of the study in both males and females.

Following i.v. administration of cadmium, the renal MT levels were also higher in females than in males (Fig. 2). However, there was no further induction of MT in either sex during the 24 hr.

Effect of hormones on hepatic cadmium and MT levels. Estradiol treatment lowered the body weight of castrated males by as much as 14% without significant reduction in liver or kidney weight. Since the dose of cadmium was based on the body weight of the animals at the time of cadmium injection, the liver and kidney cadmium and MT concentrations were normalized to 100 g body weight.

As shown in Fig. 3, neither sham operation, nor testosterone or DMSO pretreatments of castrated males had any significant effect on hepatic cadmium accumulation in 24 hr. On the other hand, estradiol pretreatment resulted in significant enhancement of cadmium accumulation to levels similar to those found in the females.

The hepatic MT level in non-cadmium-treated control males was 6.4 ± 0.5 μg/g. Castration increased these levels to 17.8 ± 1.1 μg/g. Administration of testosterone or estradiol to the non-cadmium-treated castrated animals caused no further induction of MT (data not shown).

Estradiol pretreatment markedly increased the MT levels of cadmium-treated castrated males to those of the females (Fig. 3). In addition, sham operation, but not DMSO or testosterone pretreatment of castrated males caused an increase in MT levels upon cadmium injection.

Effect of hormones on renal cadmium and MT levels. Estradiol pretreatment of castrated control

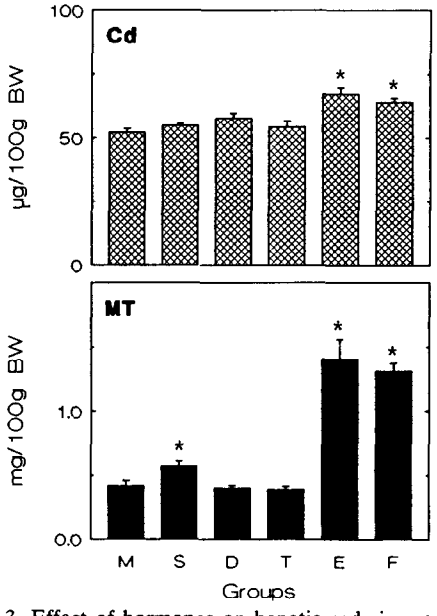

Fig. 3. Effect of hormones on hepatic cadmium and MT levels in normal and castrated males. The animals were injected s.c. with 10 μmol $CdCl_2$/kg, 24 hr before being killed. See Materials and Methods for additional details. Groups are: M, control males; S, sham-operated males pretreated with DMSO; D, castrated males pretreated with DMSO; T, castrated males pretreated with testosterone; E, castrated males pretreated with estradiol; and F, control females. Values are means ± SEM, N = 6. Key: (*) significantly different from control males ($P < 0.01$).

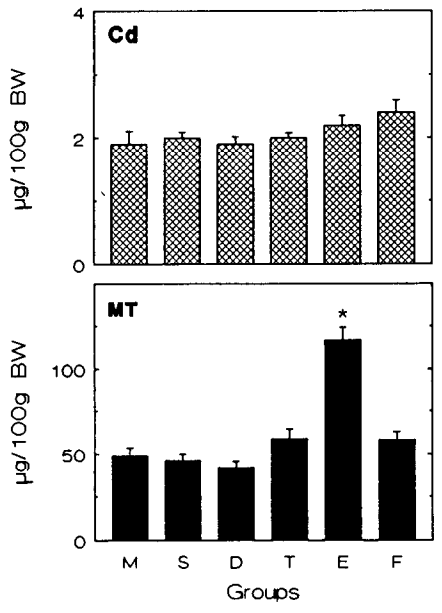

Fig. 4. Effect of hormones on renal cadmium and MT levels in normal and castrated males. The animals were injected s.c. with 10 μmol $CdCl_2$/kg, 24 hr before being killed. See Materials and Methods for additional details. Groups are: M, control males; S, sham-operated males pretreated with DMSO; D, castrated males pretreated with DMSO; T, castrated males pretreated with testosterone; E, castrated males pretreated with estradiol; and F, control females. Values are means ± SEM, N = 6. Key: (*) significantly different from the other groups ($P < 0.01$).

males alone caused a 2-fold increase in MT levels (31.8 ± 3.2 vs 65.2 ± 4.6 μg/100 g body wt). However, it had no effect on renal cadmium accumulation (Fig. 4). Estradiol and cadmium given together to castrated males raised the MT levels to greater than those in females or castrated males given estradiol or cadmium alone (Fig. 4).

DISCUSSION

In agreement with our previous study [3] and those of several others [4, 7, 8], the female rats accumulated more cadmium in their liver than the males. Furthermore, this study demonstrates that the castrated males treated with the female sex hormone, estradiol, accumulated as much cadmium in their liver after s.c. administration as did the females. This effect was not noted after i.v. injection of cadmium. Nishiyama *et al.* [4] also did not find a sex difference in cadmium disposition in the liver at 24 hr after i.v. administration although they did so at 4 and 10 hr. Since a previous study with freshly isolated hepatocytes showed that addition of estradiol to the medium did not affect cadmium uptake [11], it appears that the hormone accomplishes its *in vivo* effect on cadmium accumulation through some indirect mechanism.

We found no sex difference in renal cadmium accumulation following either an i.v. or s.c. dose. Also, estradiol-pretreatment did not enhance cadmium accumulation in the kidney. This is in agreement with *in vivo* studies of Nishiyama *et al.* [4] who also observed no sex difference 24 hr after an i.v. injection, but is contrary to their reported increase in renal cadmium in male rats who were treated with estradiol prior to receiving the metal.

Unlike other studies which reported that DMSO is capable of MT induction in the presence [14] or absence [15] of cadmium, our results are similar to those of Bracken and Klaassen [16], in that DMSO did not cause any significant change in MT levels. These apparent differences can be attributed to the experimental conditions. In the former case [14], the investigators examined the direct effect of DMSO in hepatocytes *in vitro*, under conditions in which the cells were in log phase of growth, while in the latter case [15], the rats were exposed to amounts of DMSO five times greater than those used in our study.

Estradiol alone had no effect on hepatic MT levels in the castrated males. This is in disagreement with Nishiyama *et al.* [10]. These investigators appeared to have used non-castrated males for estradiol dose–response and administered the estradiol in 30% ethanol. A synergistic effect between estradiol and ethanol on MT induction is a possibility. While estradiol alone is a poor inducer of hepatic MT *in vitro* and a concentration of 10^{-4} M is needed for the induction [16], ethanol is capable of inducing hepatic MT [17, 18].

In agreement with other *in vivo* studies [4, 10], we found that pretreatment with estradiol alone increased renal MT levels 2-fold. In addition, it was observed that a further induction of MT occurred when cadmium was administered along with the estradiol. Therefore, in the kidney, estradiol appears to act synergistically with cadmium in inducing MT. It is noteworthy, however, that the elevated levels of renal MT did not affect renal cadmium accumulation. These results along with the *in vitro* studies of Liu *et al.* [19] in hepatocytes suggest that the intracellular MT does not affect cadmium uptake.

Hepatic cadmium accumulation following the s.c. injection continued for up to 48 hr, while this process was completed within 2 hr of the i.v. injection. In agreement with other studies which have shown hepatic cadmium to have a half-life of 60–73 days [20, 21], no marked decrease in hepatic or renal cadmium levels in either sex following s.c. or i.v. injection was observed. This is contrary to Nishiyama *et al.* [4] who reported a marked decrease in hepatic cadmium levels in female rats between 10 and 24 hr of an i.v. injection.

It is well established that cadmium regulates hepatic MT synthesis at the level of transcription [22–26]. Upon entering a cell, non-MT bound cadmium binds to a metal-responsive factor which then binds to one of the metal-regulatory elements on the MT gene [27–29] causing gene amplification and enhancement of the transcription rate [30, 31]. This process is regulated by the amount of metal present in the cell [32]. We postulate that following s.c. cadmium injection, superinduction of hepatic MT mRNA occurred because of the continued uptake of cadmium. The process of superinduction resulted in the production of excess MT which was

degraded over 2 days in the females and 3 days in the males. However, when the cadmium was administered i.v., the superinduction of hepatic MT synthesis did not occur as the cadmium accumulation was completed within 2 hr—well before the induction of MT synthesis [22]. Also, since cadmium distribution was similar in both sexes of the intravenously injected animals, the MT levels were also very similar.

The pattern of renal MT induction was different from that observed in the liver of the subcutaneously injected animals. Unlike the liver, no superinduction of MT synthesis occurred due to the presence of a higher basal MT level. The initial influx of cadmium was sequestered by the existing MT without requiring any additional synthesis. Other investigators have also reported that the MT induction in the kidney is less responsive to metal exposure than in the liver [33]. As the kidneys continued to receive additional cadmium over 10 days, the MT levels did rise over this period possibly through translation of the existing mRNA [22].

Since the majority of the cadmium accumulated in the kidney within 1 hr after the i.v. injection, as opposed to the continued influx over 5–10 days observed after the s.c. injection and because the accumulated cadmium was about a third of what was found after the s.c. dose, the existing MT was able to sequester the metal. Even though there were no sex differences in cadmium accumulation in the kidney after the i.v. dose, the sex differences in MT levels that existed before cadmium uptake prevailed, further suggesting that cadmium did not induce MT synthesis in the kidney after i.v. administration.

In summary, this study shows that (a) after s.c. administration hepatic accumulation of cadmium and induction of MT occurred faster in females than males, (b) these differences in cadmium and MT levels were not seen if the cadmium was delivered to the tissues as an i.v. bolus, (c) estradiol enhanced hepatic but not renal cadmium accumulation, and (d) estradiol caused induction of hepatic as well as renal MT. We conclude that estradiol is an important hormone in regulating the disposition of cadmium and may explain the increased incidence of the toxic manifestations of cadmium in the exposed populations [34].

Acknowledgement—This work was supported by a U.S. Public Health Service Grant (ES03187).

REFERENCES

1. Sumino K, Hayakawa K, Shibata T and Kitamura S, Heavy metals in normal Japanese tissues. *Arch Environ Health* **30**: 487–494, 1975.
2. Kido T, Nogawa K, Honda R, Tsuritani I, Ishizaki M, Yamada Y and Nakagawa H, The association between renal dysfunction and osteopenia in environmental cadmium-exposed subjects. *Environ Res* **51**: 71–82, 1990.
3. Blazka ME, Nolan CV and Shaikh ZA, Developmental and sex differences in cadmium distribution and metallothionein induction and localization. *J Appl Toxicol* **8**: 217–222, 1988.
4. Nishiyama S, Onosaka S, Taguchi T, Konishi Y, Tanaka K and Kinebuchi H, Stimulation of cadmium uptake by estradiol in the kidney of male rats treated with cadmium. *Biochem Pharmacol* **37**: 3091–3096, 1988.
5. Kello D, Dekanic D and Kostial K, Influence of sex and dietary calcium on intestinal cadmium absorption in rats. *Arch Environ Health* **34**: 30–33, 1979.
6. Petering HG, Murthy L, Sorenson JRJ, Levin L and Stemmer KL, Effect of sex on oral cadmium dose responses in rats: Blood pressure and pharmacodynamics. *Environ Res* **20**: 289–299, 1979.
7. Murthy L, Rice DP and Petering HG, Sex differences with respect to the accumulation of oral cadmium in rats. In: *Trace Element Metabolism in Man and Animals—3* (Ed. Kirchgessner M), pp. 557–560. ATW, Weihenstephan, 1978.
8. Stonard MD and Webb M, Influence of dietary cadmium on the distribution of the essential metals copper, zinc and iron in tissues of the rat. *Chem Biol Interact* **15**: 349–363, 1976.
9. Cempel M and Webb M, The time–course of cadmium-thionein synthesis in the rat. *Biochem Pharmacol* **25**: 2067–2071, 1976.
10. Nishiyama S, Taguchi T and Onosaka S, Induction of zinc-thionein by estradiol and protective effects on inorganic mercury-induced renal toxicity. *Biochem Pharmacol* **36**: 3387–3391, 1987.
11. Stacey NH, Effects of ethinyl estradiol on substrate uptake and efflux by isolated rat hepatocytes. *Biochem Pharmacol* **35**: 2495–2500, 1986.
12. Nolan CV and Shaikh ZA, Determination of metallothionein in tissues by radioimmunoassay and by cadmium saturation method. *Anal Biochem* **154**: 213–223, 1986.
13. Shaikh ZA, Radioimmunoassay for metallothionein in biological fluids and tissues. In: *Metallobiochemistry—Vol. 2, Methods in Enzymology* (Eds. Vallee BL and Riordan JF), Academic Press, New York, in press.
14. Waalkes MP and Wilson MJ, Increased synthetic capacity for metallothionein in cultured liver cells following dimethyl sulfoxide pretreatment. *Exp Cell Res* **169**: 25–30, 1987.
15. Hidalgo J, Campmany L, Borras M, Garvey JS and Armario A, Metallothionein response to stress in rats: Role in free radical scavenging. *Am J Physiol* **255**: E518–E524, 1988.
16. Bracken WM and Klaassen CD, Induction of metallothionein by steroids in rat primary hepatocyte cultures. *Toxicol Appl Pharmacol* **87**: 381–388, 1987.
17. Bracken WM and Klaassen CD, Induction of metallothionein in rat primary hepatocyte cultures: Evidence for direct and indirect induction. *J Toxicol Environ Health* **22**: 163–174, 1984.
18. Waalkes MP, Hjelle JJ and Klaassen CD, Transient induction of hepatic metallothionein following oral ethanol administration. *Toxicol Appl Pharmacol* **74**: 230–236, 1984.
19. Liu J, Kershaw WC and Klaassen CD, Rat primary hepatocyte cultures are a good model for examining metallothionein-induced tolerance to cadmium toxicity. *In Vitro Cell Dev Biol* **26**: 75–79, 1990.
20. Matsubara-Khan J, Compartmental analysis for the evaluation of biological half-lives of cadmium and mercury in mouse organs. *Environ Res* **7**: 54–67, 1974.
21. Shaikh ZA, Metallothionein as a storage protein for cadmium: Its toxicological implications. In: *Biological Roles of Metallothionein* (Ed. Foulkes E), pp. 69–76. Elsevier North Holland, New York, 1982.
22. Shaikh ZA and Smith JC, The mechanisms of hepatic and renal metallothionein biosynthesis in cadmium-exposed rats. *Chem Biol Interact* **19**: 161–171, 1977.
23. Durnam DM and Palmiter RD, Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. *J Biol Chem* **256**: 5712–5716, 1981.

24. Dunn MA, Blalock TL and Cousins RJ, Metallothionein. *Proc Soc Exp Biol Med* **185**: 107–119, 1987.
25. Klaassen CD and Lehman-McKeeman LD, Induction of metallothionein. *J Am Coll Toxicol* **8**: 1315–1321, 1989.
26. Hamer DH, Metallothionein. *Annu Rev Biochem* **55**: 913–951, 1986.
27. Petering DH and Fowler BA, Discussion summary. Roles of metallothionein and related proteins in metal metabolism and toxicity: Problems and perspectives. *Environ Health Perspect* **65**: 217–224, 1986.
28. Chiu R, Imbra R, Imagawa M and Karin M, Metallothionein structure and function in regulating the trace element in human. In: *Essential and Toxic Trace Elements in Human Health and Disease* (Ed. Prasad AS), pp. 393–406. Alan R. Liss, New York, 1988.
29. Karin M, Haslinger A, Heguy A, Dietlin T and Cooke T, Metal-responsive elements act as positive modulators of human metallothionein-II_A enhancer activity. *Mol Cell Biol* **7**: 606–613, 1987.
30. Koropatnick J, Winning R, Wiese E, Heschl M, Gedamu L and Duerksen J, Acute treatment of mice with cadmium salts results in amplification of the metallothionein-I gene in liver. *Nucleic Acids Res* **13**: 5423–5439, 1985.
31. Koropatnick J, Amplification of metallothionein-I genes in mouse liver cells *in situ*: Extra copies are transcriptionally active. *Proc Soc Exp Biol Med* **188**: 287–300, 1988.
32. Kagi JHR and Schaffer A, Biochemistry of metallothionein. *Biochemistry* **27**: 8509–8515, 1988.
33. Sendelbach LE and Klaassen CD, Kidney synthesizes less metallothionein than liver in response to cadmium chloride and cadmium-metallothionein. *Toxicol Appl Pharmacol* **92**: 95–102, 1988.
34. Shaikh ZA, Kido T, Kito H, Honda R and Nogawa K, Prevalence of metallothioneinuria among the population living in the Kakehashi River Basin in Japan—An epidemiological study. *Toxicology*, **64**, 59–69, 1990.