The Role of Metallothionein in Hard Tissue

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Abstract:We studied the metallothionein (MT) induction by cadmium (Cd) in the dental pulp and bone in rats. In order to clarify the cell response to Cd in the hard tissue, the isoform-specific expression of MT mRNAs (MT-I and MT-II) was examined by reverse transcriptase-polymerase chain reaction (RT-PCR) method, and the localization of MT was confirmed by immunohistochemical studies. Both MT-I and MT-II mRNA levels were increased within 3 hours by Cd administration in both the dental pulp and bone. In the dental pulp, MT protein was localized in the specific cell type of odontoblasts (secretory odontoblasts and resting odontoblasts). In the bone, MT-positive cells were time-dependently increased, and the positive cells were mainly localized in osteocytes. The cell-specific induction of MT may be associated with Cd accumulation and Cd-induced the hard tissue injury in vivo.

Key words: Metallothionein, Cadmium, Odontblast, Osteocyte, Osteoclast

Introduction

Metallothionein (MT) is well known as a protein that is induced by cadmium (Cd) and binds Cd with high affinity. Due to its high affinity for Cd, MT is believed to play a role in protection against Cd toxicity. MT is induced in many tissues after Cd administration, such as liver, kidney, dental pulp and bone [1, 2]. It has already known that Cd is transferred to the dental pulp and bone within 1 hour after Cd administration in rats [3]. MT-like proteins or mRNAs for MT were detected in the dental pulp and bone *in vivo* and *in vitro* [4, 5]. However, there were no detailed reports concerning MT mRNA expression and the localization of MT in the dental pulp and bone in relation to Cd toxicity.

In this study, MT expression was examined in the dental pulp and bone cells after a single Cd injection. To investigate the mRNA expression of each isoform of MT (MT-I and MT-II), we used MT-I and MT-II mRNA-specific primers with the reverse transcriptase-polymerase chain reaction (RT-PCR) method. Furthermore, the localization of MT (MT-I/MT-II) in the dental pulp and bone (femur and tibia) was examined by immunohistochemical staining.

Materials and Methods

Male Wistar rats (6-week-old) were injected subcutaneously (*s.c.*) with cadmium chloride ($CdCl_2$, 4.5 mg Cd /kg) dissolved in saline. Control rats were injected *s.c.* with saline. All injections were in a volume of 2 ml/kg.

The dental pulp and bone were collected at the indicated time after Cd injection, and total RNA was isolated using AGPC method. Isolated RNA was treated with DNase I. The expression levels of MT-I and MT-II mRNA were measured by RT-PCR. The specific primers were constructed according to the known sequences [6]. The house-keeping gene, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as internal control. The [α-^{32}P] *d*CTP was added to each reaction mixture for quantification of amplified DNA fragments. PCR products were electrophoresed on 8% polyacrylamide gel. The gels were stained with 0.5 μg/ml ethidium bromide solution followed by viewing under UV illumination, then dried, exposed to an imaging plate, and analyzed using a BAS2000.

For immunochemical study, rats were anesthetized and perfused with 4% paraformaldehyde at indicated time after Cd injection. The incisors and bones (the proximal ends of tibiae and femora) were removed. After demineralization with 10% formic acid at 4ˆC for 2 or 3 weeks, these samples were dehydrated and embedded in paraffin. Sections 5 mm thick were cut from the blocks and reacted with anti-MT monoclonal antibody. The antibody was specifically reactive with a conserved epitope common to several mammalian species of MT-I and MT-II [7]. After washing, the sections were incubated with biotinylated rabbit anti-mouse immunoglobulin, ABComplex/HRP and followed by staining with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Sections were counterstained with hematoxylin. The specificity of the staining reaction was checked by omission of anti-MT monoclonal antibody from the procedure.

Results and Discussion

Both MT-I and MT-II mRNA were expressed in the dental pulp and bone. The expression of G3PDH mRNA was not changed by Cd treatment. The expression levels of MT-I and MT-II mRNA after Cd injection increased within 3 hours in both tissues, and remained at high levels until 6 hours in the bone, but decreased at 6 hours in the dental pulp.

The location of MT in rat hard tissues was determined by immunohistochemical staining. In the dental pulp, MT protein was detected in Cd-treated rat odontoblasts at late maturation stage. We classified odontoblasts into three types based on the morphology, *i.e.* preodontoblasts, secretory odontoblasts and resting odontoblasts. MT positive cells were secretory odontoblasts (Fig.1B) and resting odontoblasts. Secretory odontoblasts beginning mineral-

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ization, preodontoblasts and the pulp cells were not MT positive. In control rat tissue, each cell in the dental pulp was negative for MT immunostaining (Fig.1A).

In the bone, some osteoclasts were positive for MT immunostaining in the trabecular and cortical bone of both control and Cd-treated rats (Fig.2A). In the control rats, no staining was observed in osteocytes or osteoblasts. According to the time course study, the number of MT-positive osteocytes increased in a time-dependent manner until 48 hours after Cd injection. Furthermore, a few MT-positive osteoblasts were detected at 48 and 72 hours after injection (Fig.2B). Moreover, staining for MT was observed in both the cytoplasm and the nucleus, and nucleus staining was prominent in some odontoblasts and osteoblasts.

The cell-specific induction of MT may be associated with Cd accumulation and the protection against Cd-induced hard tissue injury *in vivo*.

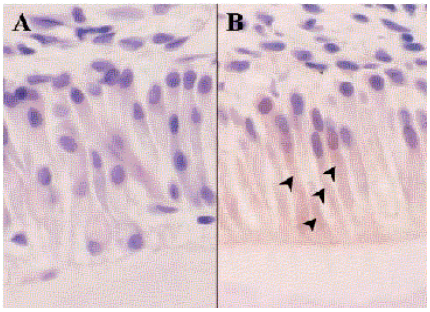

Fig.1. Immunohistochemical localization of MT in the dental pulp. Sections from a control rat (A) and a Cd-treated rat (4.5 mg Cd /kg, *s.c.*) (B : at 24 hrs after Cd injection).

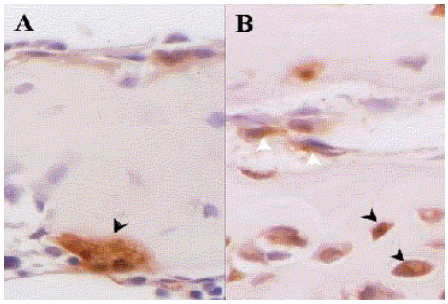

Fig.2. Immunohistochemical localization of MT in rat bones. Sections through the trabecular bone from a control rat (A) and a Cd-treated rat (4.5 mg Cd /kg, *s.c.*) (B: at 48 hrs after Cd injection). Note the MT-positive osteoclasts (A), and MT-positive osteocytes (black arrow head) and osteoblasts (white arrow head) in Cd-treated rats (B).

References

1. Hamer DH. Ann Rev Biochem 55:913-951, 1986
2. Klaassen CD, Liu J, Choudhuri S. Annu Rev Toxicol 39:267-294, 1999
3. Bryan SE, Hidalgo HA. Biochem Biophys Res Commun 68:858-866, 1976
4. Miyahara T, Yamada M, Ando R, Nemoto SHisham F, Gerburg K, Gerald W Hisham F, Gerburg K, Gerald W, Kaji T, Mori M, Kozuka H, Itoh N, Sudo H. Toxicol Lett 32:19-27, 1986
5. Sogawa CA, Sogawa N, Oda N, Furuta HJ. Okayama Dent Soc 19:255-260, 2000
6. Andersen RD, Burren BW, Taokutz SJ, Herschman HR. Mol Cell Biol 6:302-314, 1986
7. Jasani B, Elmes ME. Method Enzymol. 205:205-215, 1991