Apparent Quiescence of the Metallothionein Gene in the Rat Ventral Prostate: Association with Cadmium-induced Prostate Tumors in Rats

Timothy P. Coogan[1], Noriyuki Shiraishi[1], and Michael P. Waalkes[2]

Inorganic Carcinogenesis Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland.

Several chronic studies in rats indicating that cadmium exposure can induce tumors of the ventral prostate have recently been completed in our laboratory. In one such study, a single dose of cadmium, s.c., increased prostatic tumor incidence only at doses below 5.0 μmol/kg, the approximate threshold for cadmium-induced testicular damage. In a further study, prostatic tumors were elevated with higher doses of cadmium (30 μmol/kg, s.c.) if testicular damage was prevented by zinc pretreatment. Most recently, we found that dietary cadmium (25 to 200 μg/g) also can increase prostatic neoplastic lesions, but these were reduced by zinc-deficient diets. Thus it appears that cadmium produces prostatic tumors only if testicular function is maintained. Furthermore, we find that metallothionein (MT), a protein associated with cadmium tolerance, may be deficient in the rat prostate, and the prostatic MT gene, at least in the ventral lobe, is unresponsive to metal stimuli. In liver, MT gene expression, as assessed by MT-I mRNA, was quite apparent in control tissue and was induced in a dose-dependent manner 24 hr following cadmium exposure (1 to 10 μmol/kg, s.c.). However, in the ventral prostate very low constitutive levels of MT-I mRNA were detected and increases did not occur with cadmium exposure. Cadmium concentrations in the ventral prostate were in excess of those that cause significant induction in the liver. In sharp contrast to the gene in the ventral prostate, in the dorsal prostate the MT gene was quite active. The dorsal prostate is not susceptible to cadmium carcinogenesis. The association between tissue-specific quiescence of the MT gene and susceptibility to cadmium carcinogenesis deserves further study. — Environ Health Perspect 102(Suppl 3):137–139 (1994).

Key words: cadmium, prostate, metallothionein, carcinogenesis, tumors, rat, RNA

Introduction

Cadmium is a highly toxic metal that is cause for elevated environmental and occupational concern due to increasing exposure of humans. In animal models, cadmium is a potent carcinogen that is capable of inducing a variety of tumors (for review see *1*); both the lung (*2*) and more recently the prostate (*3–5*) have been identified as target sites for cadmium-induced tumors. In humans, cadmium is classified by the IARC as a suspected human carcinogen (Group 2A) with the lung and possibly the prostate as target organs (*6,7*).

The exact mechanism of cadmium carcinogenesis is unknown. However, the level of metallothionein (MT) gene expression may play a role in determining a tissues's susceptibility to cadmium-induced tumors. MT is a low molecular weight (6000 to 7000 daltons) metal binding protein, the amino acid content of which is approximately 30% cysteine residues (*8*). This high content of cysteinyl thiol groups accounts for the high metal affinity of MT (*8*). Although MT is found in a variety of eukaryotic cells, its physiologic functions are still unknown. However, MT appears to function, in part, in cellular defenses against metal toxicity (*9*). In particular, MT appears to play an important role in providing protection from cadmium toxicity. It is possible that reduced levels of MT gene expression may lead to an increase in a tissue's susceptibility to cadmium carcinogenesis.

A number of studies from our laboratory have demonstrated that the rat ventral prostate is a target site for cadmium-induced tumors. (*3–5*). The following will summarize these studies, as well as provide new data with regard to MT gene expression in this target for cadmium carcinogenicity.

In the first study, male Wistar [Crl:(WI)BR) rats were given a single s.c. dose of cadmium (0, 1, 2.5, 5, 10, 20, or 40 μmole/kg) and studied over a 2 year period (*3*). A strong positive correlation was established between the dose of cadmium and the incidence and multiplicity of prostatic tumors for doses up to 2.5 μmole/kg. A significant increase in prostatic tumor incidence was demonstrated at the 2.5 μmole/kg dose of cadmium with 8 adenomas/26 rats at risk (31%). Prostatic tumors occurred exclusively in the ventral lobe. At higher doses of cadmium (>2.5 μmole/kg), an increased incidence in prostatic tumors did not occur. However, these doses were associated with testicular

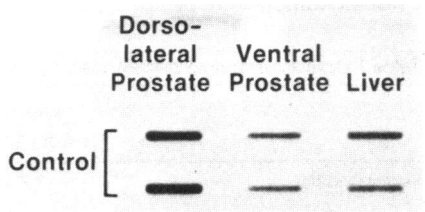

Figure 1. Basal metallothionein gene expression in various tissues of the Wistar rat. RNA was isolated, denatured, and slot-blotted onto Nytran. Blots were probed with a ^{32}P labeled p2A10 plasmid specific for the MT-I gene. Equal loading of RNA was confirmed by β-actin gene expression.

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Address correspondence to Dr. P. Waalkes, NCI-FCRDC, Building 538, Room 205E, Frederick, MD 21702-1201. Current address of Dr. Coogan is R.W. Johnson Pharmaceutical Research Institute, Spring House, PA.

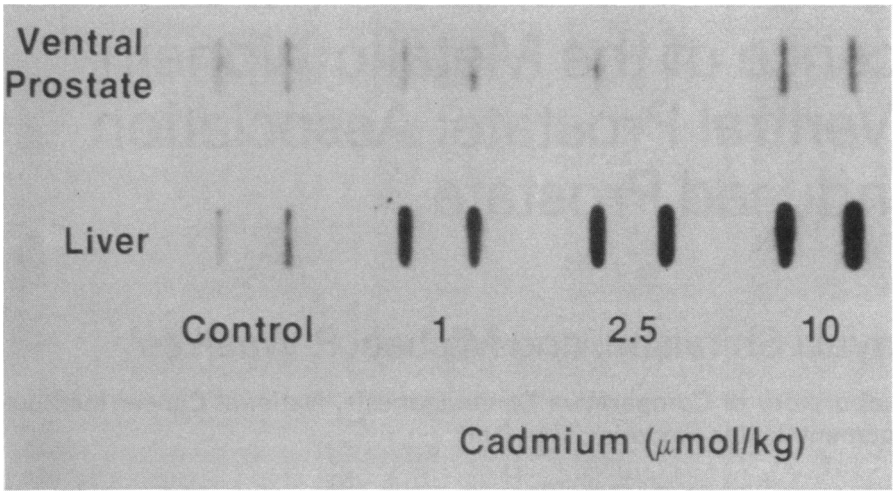

Figure 2. Effect of cadmium on metallothionein gene expression in liver and ventral prostate of the Wistar rat. Animals were treated with cadmium, sc, and metallothionein gene expression determined at 24 hr. RNA was isolated, denatured, and slot-blotted onto Nytran. Blots were probed with a ^{32}P labeled p2A10 plasmid specific for the MT-I gene. Equal loading of RNA was confirmed by β-actin gene expression.

degeneration and atrophy. A strong positive correlation between cadmium and multiplicity of hyperplastic foci was demonstrated at doses up to and including 20 μmole/kg. These data indicate that cadmium will induce preneoplastic lesions that develop into tumors only when doses of cadmium that are given are well below those which induce testicular degeneration and loss of function.

In a second study, the ability of zinc to modify the carcinogenic effect of cadmium was investigated in male Wistar rats (*4*). Groups of rats received a single s.c. or i.m. injection of cadmium (30 μmole/kg) and zinc was given in three separate s.c. doses of 100, 300, or 1000 μmole/kg. In groups in which cadmium-induced testicular tumors and chronic testicular degeneration were prevented by zinc (1000 μmole/kg), a marked elevation in prostatic tumors occurred (control, 9.6%; cadmium + high zinc, 29.6%). In addition, cadmium when administered i.m. did not result in testicular degeneration but induced an elevated incidence of prostatic tumors (42.3%). Prostatic tumors were exclusively adenomas and occurred only in the ventral prostate. When all cadmium treated groups were combined, regardless of other treatments, there was a significant increase in prostatic tumor incidence (51 tumor bearing rats/199 rats; 26%) as compared with controls (8/83; 10%).

In a third study, male Wistar (WF/NCr) rats received dietary cadmium (25 to 200 μg/g) mixed in diets either adequate (60 μg/g) or deficient (7 μg/g) in zinc (*5*). A significant elevation in the overall incidence of prostatic proliferative lesions (focal atypical hyperplasia and adenomas) occurred in animals given cadmium and zinc-adequate diets (12 rats with lesions/97 rats at risk; 12%) as compared to animals given cadmium and zinc-deficient diets (5/103; 5%) or controls (1/54; 2%). Lesions occurred exclusively in the ventral prostate. The lower incidence of prostatic proliferative lesions in the zinc-deficient rats was associated with an increase in prostatic atrophy, with adequate and deficient animals exposed to cadmium resulting in 24% and 39% atrophy, respectively.

Table 1. Basal zinc and metallothionein levels in various tissues of the male Wistar rat.

	Metallothionein[1,2] (μg/g wet weight)	Zinc[1,2] (μg/g wet weight)
Ventral Prostate	1.21 ± 0.35 (4)[A]	13.67 ± 7.3 (11)[a]
DorsolateralProstate	28.46 ± 8.63 (4)[B]	139.3 ± 32.1 (11)[b]
Liver	3.96 ± 1.03 (4)[C]	21.3 ± 2.8 (11)[a]

[1]Values represent the mean ± S.D. (n). [2]Values not sharing a common letter are significantly different. $p < 0.05$. Metallothionein, capital letters. Zinc, lower case.

These chronic studies in rats establish that the rat ventral prostate is a target for cadmium-induced tumors. Hyperplastic lesions were evident at high doses of cadmium in the absence of an increase in prostatic tumors (*3*), and the lack of increased prostatic tumor formation at higher doses parallels a loss of testicular function. These studies demonstrate the importance of testicular function in the development, but not initiation, of prostatic tumors.

Since MT is thought to provide protection against the toxic effects of cadmium, we hypothesized that the increased susceptibility to cadmium carcinogenicity demonstrated for the ventral prostate may be due to alterations in MT gene expression. One possible pathway for cadmium carcinogenicity may involve the interaction of cadmium with DNA, either directly or indirectly. Previous studies in our laboratory have shown that an increase in MT gene expression can reduce the level of DNA damage associated with cadmium exposure (*10*). Hence, a deficiency of MT may contribute to a tissue's susceptiblity to cadmium carcinogenicity. Therefore, we investigated MT production and gene expression in the ventral prostate as well as in the dorsolateral prostate and liver, two tissues that are not targets for cadmium carcinogenicity in the rat.

Metallothionein levels were determined using the Onosaka assay (*11*) as modified by Eaton and Toal (*12*). Basal MT levels were determined in liver, ventral prostate and dorsolateral prostate and are presented in Table 1. Zinc levels were determined by atomic absorption and basal tissue levels are presented also in Table 1. MT levels in

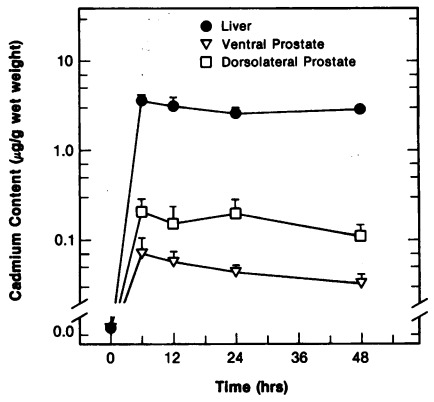

Figure 3. Cadmium accumulation in various tissue following administration of a dose of cadmium carcinogenic to the prostate. Animals were administered cadmium (2.5 μmole/kg, s.c.) using a dose known to induce prostatic tumors. Tissue cadmium content was determined as a function of time by atomic absorption spectrometry. Data represent the mean ± S.D. (n=4).

Table 2. Effect of increasing doses of cadmium on metallothionein levels in various tissues of the rat.

Cadmium (μmole/kg)	Liver[1,2]	Ventral prostate[1,2]
0	3.96 ± 1.03[A]	1.21 ± 0.35[a]
0.5	24.20 ± 10.6[B]	0.95 ± 0.09[a]
1.0	33.48 ± 6.99[C]	0.85 ± 0.48[a]
2.5	79.07 ± 5.45[D]	0.99 ± 0.27[a]
5.0	127.35 ± 5.06[E]	1.74 ± 0.19[a]
10.0	170.75 ± 41.2[F]	1.22 ± 0.31[a]

[1]Values represent the mean ± S.D. (n = 4 to 8). [2]Values not sharing a common letter are significantly different. $p < 0.05$. Liver, capital letters. Ventral prostate, lower case.

the dorsolateral prostate were significantly higher than those of either the liver or ventral prostate. In addition, liver MT levels were greater than that of the ventral prostate. In general, MT levels correlated with zinc tissue levels for the tissues examined. The dorsolateral prostate had the greatest zinc content and in turn, the highest MT levels. Although the liver and ventral prostate were not significantly different in zinc content, the liver did contain significantly greater basal MT levels. Ventral prostate contained very low basal levels of MT, almost at the limit of detection with this assay (*12*).

Analysis of MT mRNA from each tissue correlated with the data obtained from the MT protein assay (Figure 1). Total RNA was isolated using RNAzol, denatured with glyoxal and slot-blotted onto Nytran, as previously described (*10*). RNA integrity was determined by electrophoresis and message size confirmed by northern analysis. Northern transfers and slot-blots were probed for MT-I gene expression using a ^{32}P-labeled p2A10 plasmid for the MT-I gene generously supplied by Dr. H. Herschman (*13*). RNA loading was standardized to β-actin gene expression. MT-I gene expression was greatest in the dorsolateral prostate and was very low in the ventral prostate. Similar data were obtained with probes specific for MT-II RNA.

The ability of cadmium to induce MT was investigated in the liver and ventral prostate. MT levels are presented in Table 2. Animals were exposed to cadmium s.c. and MT levels were assessed at 24 hr. Increasing doses of cadmium resulted in increased MT and this induction was linear throughout the dose range examined. In contrast, MT was not induced in the ventral prostate following doses up to and including 10 μmole/kg. Analysis of MT-I gene expression resulted in findings similar to that for MT protein levels (Figure 2). Increased amounts of MT-I mRNA were detected in samples obtained from the livers of rats treated with various doses of cadmium. Increased levels of MT gene expression were not demonstrated in the ventral prostate 24 hr following administration of cadmium.

Since a dose of 2.5 μmole/kg cadmium resulted in an increased incidence of tumors in the ventral prostate but not the dorsolateral prostate or liver, the tissue distribution of cadmium following this dose was examined. These data are presented in Figure 3. Cadmium levels were maximal at the first time point examined, 6 hr, and were persistent through 48 hr. Liver cadmium levels were the highest of the tissues examined with values approximately 10-fold higher than that of the dorsolateral prostate and approximately 60-fold higher than that of the ventral prostate. Similar levels of cadmium as obtained in the ventral prostate were sufficient to stimulate the MT gene in the liver (not shown). Interestingly, accumulation of cadmium within the ventral prostate is the lowest of the three tissues examined although the ventral prostate is a target for cadmium carcinogenesis. Clearly, factors other than distribution of cadmium play an important role in the development of ventral prostatic tumors.

In summary, we have found that the rat ventral prostate is a target for cadmium carcinogenesis. Metallothionein gene activity is quiescent in the ventral prostate. This tissue-specific quiescence of the metallothionein gene may determine a tissue's susceptibility to cadmium carcinogenesis.

REFERENCES

1. Waalkes MP, Oberdörster G. Cadmium carcinogenesis. In: Biological Effects of Heavy Metals, Mechanisms of Metal Carcinogenesis, Vol II (Foulkes EC, ed). FL:CRC Press,1990;129–158.
2. Takenaka S, Oldiges H, Konig H, Hochrainer D, Oberdörster G. Carcinogenicity of cadmium chloride aerosols in W rats. J Natl Cancer Inst 70:367–371 (1983).
3. Waalkes MP, Rehm S, Riggs CW, Bare RM, Devor DE, Poirier LA, Wenk ML, Henneman JR, Balaschak MS. Cadmium carcinogenesis in male Wistar [Crl:(WI)BR] rats: dose-response analysis of tumor induction in the prostate and testes, and at the injection site. Cancer Res 48:4656–4663 (1988).
4. Waalkes MP, Rehm S, Riggs CW, Bare RM, Devor DE, Poirier LA, Wenk ML, Henneman JR. Cadmium carcinogenesis in the male Wistar [Crl:(WI)BR] rat: Dose-response analysis of effects of zinc on tumor induction in the prostate and in the testes and at the injection site. Cancer Res 49:4282–4288 (1989).
5. Waalkes MP, Rehm S. Carcinogenicity of oral cadmium in the male Wistar (WF/NCr) rat: effect of chronic dietary zinc deficiency. Fund Appl Toxicol 19:512–520 (1992).
6. IARC. Cadmium and cadmium compounds. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl 7. Lyon:International Agency for Research on Cancer 1987;139–142.
7. Elghany NA, Schumacher MC, Slattery ML, West DW, Lee JS. Occupation, cadmium exposure, and prostate cancer. Epidemiology 1:107–115 (1990).
8. Kägi JHR, Schäffer A. Biochemistry of metallothionein. Biochemistry 27:8509–8515 (1988).
9. Waalkes MP, Goering PL. Metallothionein and other cadmium-binding proteins: recent developments. Chem Res Toxicol 3:281–288 (1990).
10. Coogan TP, Bare RM, Waalkes MP. Cadmium induced DNA damage in cultured liver cells: Reduction in cadmium genotoxicity following zinc pretreatment. Toxicol Appl Pharmacol 113:227–233(1992).
11. Onosaka S, Tanaka K, Doi M, Okahara K. Simplified procedure for the determination of metallothionein in animal tissues. Eisei Kagaku 24:128–131 (1978).
12. Eaton DL, Toal BF. Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallo-thionein in biological tissues. Toxicol Appl Pharmacol 66:134–142 (1982).
13. Andersen RD, Birren BW, Ganz T, Piletz JE, Herschman HR Molecular cloning of the rat metallothionein 1 (MT-1) mRNA sequence. DNA 2:15–21 (1983).