カドミウム摂取による精巣中メタロチオネイン量とmRNA発現量の変動

中村康宏[1)] 大川陽平[1)] 前島幸[1)] 中川妙子[1)] 太田久吉[1)2)]

1) 北里大学大学院医療系研究科環境毒医科学
2) 北里大学医療衛生学部産業保建学

Effects to amount of metallothionein and mRNA in testis by cadmium injection and oral administration to male rats

Yasuhiro Nakamura[1)], Youhei Ohkawa[1)], Yuki Maejima[1)], Taeko Nakagawa[1)], and Hisayoshi Ohta[1) 2)]

1)Department of Occupational Health and Toxicology, Graduate School of Medical Sciences, and 2)School of Allied Health Sciences, Kitasato University

Male Wistar rats were given cadmium by intraperitoneal (ip) injection (1mgCd/kg). Other male Wistar rats were given Cd by oral administration at a dose of 0, 1, 2, and 5 mgCd/kg/day. All rats were slaughtered at 24 hours after Cd injection, and the testis was extracted immediately. Animals of the control group were given distilled water. Metallothionein (MT) concentration in testis was measured by the Cd-Hem method and capillary electrophoresis. Total RNA in testis was extracted, and gene expression of iso-MT (MT I, II, and III) was checked by RT-PCR.

The increased MT concentration in the oral Cd administration group was found depending on the amount of Cd intake whereas the decreased MT level was found in the Cd ip injection group. Increased gene expression of MT I and III was found depending on the increase of Cd exposure dose in both groups of the Cd injection and the oral administration of Cd. However, the decreasing tendency of MT-II was found in the Cd injection (ip) group, whereas the tendency of increase or decrease depended on Cd ingestion were not found in the oral Cd administration group. In the testis, it was considered that Cd toxicity was mitigated by MT induced by oral administration of Cd. On the other hand, as the decreased protein amount of MT was found, it was thought that the proteolysis or the translation function of MT gene in the testis was disordered by the acute Cd toxicity. Moreover, the possibility of the MT-III induction according to the increased Cd accumulation in testis by oral Cd administration was suggested from the result of mRNA expression by RT-PCR.

Keywords: Cadmium intake, Metallothionein, Cadmium toxicity, Testicular damage, Gene expression
カドミウム摂取、メタロチオネイン、カドミウム毒性、精巣障害、遺伝子発現

<緒言>

精巣はカドミウム（Cd）毒性に対し感受性が高く、ラットへの$CdCl_2$の腹腔内注射により、出血性の炎症や壊死が起こる事、Cdの毒性軽減と体内蓄積に関してメタロチオネイン（MT）が関与している事が知られている[1,2]。本研究では、カドミウム（Cd）の精巣組織に及ぼす影響をイソ・メタロチオネイン（I,II,III）の蛋白の誘導合成と遺伝子発現の変動から検討した。

連絡先：中村康宏
神奈川県相模原市北里１－１５－１
北里大学医療系研究科環境毒医科学
TEL&FAX：042-778-8070
E-mail address：mm03037q@st.kitasato-u.ac.jp

論文受理日：平成16年9月13日

<実験方法>

Wistar系雄ラット３匹に、カドミウム（$CdCl_2$）1mg/kgを腹腔内注射し、注射から24時間後に屠殺し、精巣を摘出した。また、Cd経口投与群として1mg/kg、2mg/kg、5mg/kg

を６週間経口投与し、同様にして精巣を摘出した。なお対照群には蒸留水を摂取させた。精巣をホモジナナイズし、遠心分離（13000rpm 15min）後、上清を測定試料とし、Cd-Hem法とキャピラリー電気泳動によりMT濃度を測定した［3］。また、精巣内全ＲＮＡをRNAgents®Total RNA Isolation System (Promega)を用いて抽出し、iso-MT（MTⅠ、ⅡおよびⅢ）のmRNA発現量をRT-PCRにより確認した。

＜結果と考察＞

Cd注射群においてMT濃度の減少が見られたのに対し、Ｃｄ経口投与群では、Ｃｄの摂取量に依存したMT濃度の増加が認められた（Fig.1., Fig.3.）。ＭＴⅠ及びMTⅢのｍＲＮＡ発現量は、注射群、経口投与群共にＣｄ摂取量に依存して増加傾向にあった。しかし、ＭＴⅡについては注射群で減少傾向にあり、経口投与群では摂取量に応じた増減は認められなかった(Fig.2., Fig.4.)。精巣において、Ｃｄの経口投与によってMTが誘導合成され、Ｃｄ毒性が軽減されるものと考えられた。一方、Ｃｄの腹腔内注射では、ｍＲＮＡ量は増加するが、タンパク質量は減少しており、精巣障害により、タンパク質の分解や翻訳機能の低下が起きているものと考えられた。またＣｄの経口投与による精巣中Ｃｄ蓄積量の増加に応じた、ＭＴⅢの誘導の可能性が示唆された。

文献

1) Hisayoshi Ohta et al: Induction of metallothionein-Like cadmium-binding protein in the testis by oral cadmium administration in rats. Ind Health 35: 96-103, 1997.

2) Hisayoshi Ohta et al: Induction of metallothionein-like cadmium-binding protein in testis and its protective role against cadmium toxicity. Metallothionein Ⅳ : 301-307 , 1999.

3) Taeko Nakagawa et al : Basic research in an identification of metallothionein isoforms by capillary zone electrophoresis. Biomed Res Trace Elements: 13 (4) 270-271, 2002.

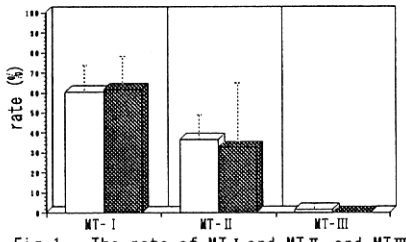

Fig. 1. The rate of MTⅠand MTⅡ and MTⅢ in total MT in an ip injection group.
□control ■1mg ip Cd injection

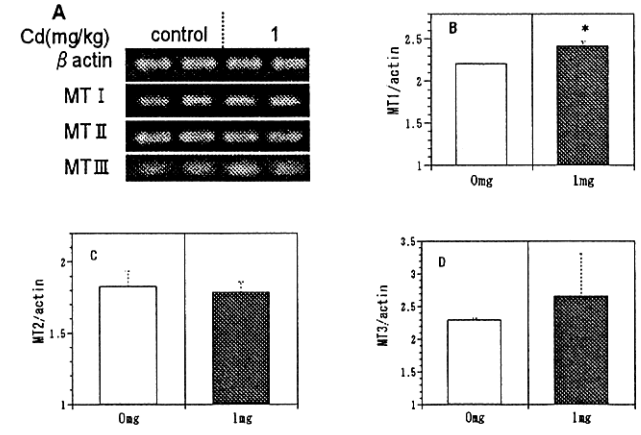

Fig.2. Gene expression of iso-metallothionein (iso-MT) in testis of intraperitoneal injection groupA: cDNA of iso-MT and β actin were staind by ethidium bromide and checked by electrophoresis. B,C and D: The rate of iso-MT to β actin was shown as bar graph. B: MT Ⅰ to β actin, C: MTⅡ to β actin, D: MTⅢ to β actin

□ control ▩ 1mg ＊: p<0.05 to control

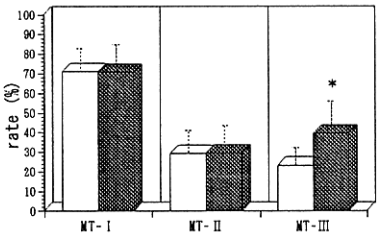

Fig. 3. The rate of MTⅠand MTⅡ and MTⅢ in total MT in an oral administering group.
□control ■1mg oral Cd administration
＊:p<0.05 to control

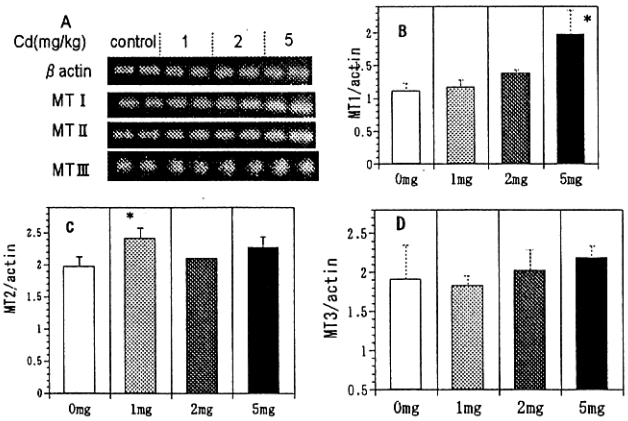

Fig.4. Gene expression of iso-metallothionein of an oral administering group. A: cDNA of iso-MT and β actin were staind by ethidium bromide and checked by electrophoresis.B,C and D: The rate of iso-MT to β actin was shown as bar graph. B: MTⅠto β actin, C: MTⅡ to β actin, D: MTⅢ to β actin

□ control ▩ 1mg ▩ 2mg ■ 5mg
＊: p<0.05 to control