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Different Inducibility of Metallothionein In Various Mammalian Cells In Vitro

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MT is heavy metal binding protein of low molecular weight. The apoprotein has high cystein content and no aromatic amino acids. The synthesis of MT may be induced not only in the liver and kidney of animals exposed to Cd or zinc (Zn), but also in primary cultured cells from rat liver, pig kidney and liver by exposure to Cd. It has been demonstrated that the heavy metal ion can mediate the induction of MT in established cell lines such as human skin (HE), rat hepatocyte (RLC) and human cervical carcinoma (HeLa). However, it is not known which cells have low inducibility of MT-synthesis by exposure known which cells have low inducibility of MT-synthesis by exposure to the heavy metal ion. In this paper differential inducibility of MT-synthesis among some mammalian cells exposed to Cd in vitro is described.

Adult human hepatocyte cell (Chang liver), human cervical carcinoma cell line (HeLa) and rabbit kidney cell (LLC–RK_1) were purchased from the Culture Center. Cell line (A-31) from a cloned continuous mouse embryonal line (BALB/3T3) transformed by the Kirsten strain of murine sarcoma virus in vitro, and cell line (FRD) from a BALB/c mouse with Rauscher virus-induced myelomonocytic leukemia were supplied by Dr. Ikawa. Human lymphocytes were prepared from fresh normal blood by using Ficoll paque.

Cell in 20 ml of Eagle's MEM or McCoy's 5a medium supplemented with 10% fetal calf serum were incubated at 37°C in a humid atmosphere of 5% CO_2 (v/v) in air. After 24 h preincubation, cells ($1 \cdot 10^6$/ml) were exposed to Cd (as $CdCl_2$) at 0, 1, 2.5, 5 or 10 μg/ml for 24 h. The number of surviving cells was measured by counting Trypan blue negative cells. ^{3}H-Thymidine was added to the medium to give a concentration of 0.005 μCi/ml together with Cd^{2+}. The radioactivity of the precipitate obtained from 24 h-exposed cells using trichloroacetic acid (10%) was measured with a liquid scintillation spectrometer. Amounts of DNA were determined by the method of Schmidt and Thanhauser.

Cells were incubated in the medium containing Cd (2.5 μg/ml and 0.1 μCi/ml) for 24 h. The 24 h-exposed cells collected from 5 dishes were washed 4 times in fresh medium by centrifugation. The pellets obtained were homogenized in 0.01 M Tris-HCl buffer, pH 8.6, containing 0.05 M NaCl and 0.2% Triton X-100, for 20 min at 37°C. The homogenate was

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centrifuged at 100000×*g* for 1 h. The supernatant obtained from the homogenate was applied to a column of Sephadex G-75 (2×95 cm), equilibrated with 0.01 M Tris-HCl buffer, pH 8.6, containing 0.05 M NaCl. The sample was eluted at a flow rate of 12 ml/h with the buffer. Each fraction was monitored by measuring absorption at 250 nm and counting radioactivity of ^{109}Cd with an auto-gamma spectrometer. Cells were incubated in the medium containing Cd (5 μg/ml) and ^{35}S-cystine (0.005 μCi/ml) for 24 h. The pellets obtained from the exposed cells were treated as described above, except for counting radioactivity of ^{35}S with the liquid scintillation spectrometer.

The preliminary gel filtration of rabbit liver MT established the location in the elution pattern of Sephadex G-75 column chromatogram (Ve=170 ml). The cytosol of Cd-exposed cells was eluted by the same procedure. Both patterns had two radioactive peaks of ^{109}Cd. One of them [P-I] at the void volume was associated with high molecular weight proteins and the other [P-II] at the Ve=170 ml corresponded to MT fraction. The elution patterns of ^{35}S from the cytosol of Cd-exposed cells in the medium containing [^{35}S]cystine overlapped the ^{109}Cd-elution patterns from the ^{109}Cd-exposed cells. Two of three ^{35}S-peaks were eluted at the same position of [P-I] and [P-II], respectively.

Table I shows the incorporation of ^{109}Cd into cells and distribution of ^{109}Cd to [P-I] and [P-II] found in the cytosol of Cd-exposed cells. 2 to 6.3% of given ^{109}Cd were recovered in 5 mammalian cells except mouse FRD cells after 24 h-exposure. Mouse FRD cells incorporated much more ^{109}Cd than the other cells. 70 to 90% of ^{109}Cd found in the cytosol of human liver, HeLa, rabbit kidney and mouse FRD cells were distributed in the [P-II] fraction in Sephadex G-75 gel filtration, which was recognized as MT fraction. However, 30% of ^{109}Cd found in both cytosols of human lymphocytes and mouse A-31 cells were in this fraction.

Hnman cells seem to be more resistant to Cd^{2+} than the other cells used in this experiment.

Utakoji has reported that lymphoblastic transformation is induced with heavy metal ions such as Hg^{2+}, Cd^{2+} and Zn^{2+} in human peripheral blood lymphocytes in vitro and that the ions stimulate the incorporation of thymidine into cells about 5 to 10 times as much as the control. Our results indicate that the level of [^{3}H] thymidine-uptake into the Cd-exposed lymphocytes increases with the dose of Cd and reaches a maximum at 5 μg of Cd/ml. Such elevation of [^{3}H]thymidine-uptake was not observed in the mammalian cells.

Rudd and Herschman have shown that Cd-binding in MT-fraction of RCC cells in culture is dependent on the time of exposure and concentration of Cd and increases in a linear fashion for 72 h at least when the cells are incubated in about 0.5 μg Cd/ml. In the present experiment the induction of MT-synthesis is compared among 6 mammalian cells exposed to 2.5 μg Cd/ml for 24 h. Bryan and Hidalgo have stated that cytoplasmic Cd obtained from the liver of Cd-exposed rat is bound to high molecular weight protein fraction (corresponding to [P-I]) early after the administration of Cd and appears progressively in the

low molecular weight fraction (corresponding to [P-II]) with time. They have also demonstrated that more than 90% of Cd in cytosol are associated with the low molecular weight protein fraction 24 h after the administration of Cd. As the ^{109}Cd found in [P-I] is not bound to MT in cytoplasm, the relative inducibility of MT-synthesis may be presented by the ratio of [P-I]^{109}Cd to [P-II]^{109}Cd. The values from human liver cells, lymphocytes, HeLa cells, rabbit kidney cells, mouse A-31 cells and FRD cells are calculated to be 4, 0.4, 4, 9, 0.4 and 2.3, respectively. The synthesis of MT is effectively induced with Cd in human liver cells, HeLa cells, rabbit kidney cells and mouse FRD cells. Though human lymphocytes and mouse A-31 cells can take up Cd from the cultured medium at a similar level to the other cells, they are not able to induce effectively the synthesis of MT with the metal under the condition of exposure used in this experiment. Lymphocytes and embryonic cells (mouse A-31) present lower or slower inducibility of MT with Cd in vitro than the other cells. These results suggest that the inducibility of MT-synthesis with Cd is different among various mammalian cells.

Table I Distribution of ^{109}Cd in cytosol of Cd-exposed Mammalian cells
The ^{109}Cd incorporation into the Cd-exposed cells is expressed as % of the radioactivity of isotope added to the medium. The distribution of ^{109}Cd in the cytosol of Cd-exposed cells is expressed as % of ^{109}Cd of [P-I] and [P-II] from gel filtration, respectively.

Cells	^{109}Cd incorporated into Cd-exposed cells (%)	Distribution of ^{109}Cd in cytosol of Cd-exposed cells (%)	
		P-I	P-II
Human liver	4.7	20	80
Human lymphocytes	3.6	70	30
Human HeLa	6.3	20	80
Rabbit kidney	2.0	10	90
Mouse A-31	3.0	70	30
Mouse FRD	21.8	30	70