Effect of the Binding Capacity of Metallothionein for Various Metals on Induction of Heme Oxygenase Activity

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It is well known that metallothionein (MT), which is involved in protection from metal toxicity and homeostasis of essential metals, is induced by various metals. These metals can be divided into two groups, those that bind to MT and those that do not *in vivo*. Our previous study indicated that a single treatment with cadmium (Cd), a typical MT-binding metal, increased the activity of heme oxygenase (HO), the rate-limiting enzyme of heme degradation, 11-fold as compared with the control, while daily administration of Cd for 3 d caused an increase of only 4-fold. Consequently, we considered that there may be a relation between MT content and HO activity. Therefore, it was of interest to investigate whether the binding capacity of MT for various metals regulates the induction of HO. Cd, zinc (Zn), copper (Cu) and silver (Ag) were used as MT binding metals, whereas cobalt (Co) and nickel (Ni) were used as nonbinding metals.

Adult male rats of the Wistar strain, weighing 250 g, were used for these experiments. Metals and doses used were as follows: $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ (3 mg/kg), $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (33 mg/kg), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (15 mg/kg), AgNO_3 (10 mg/kg), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (20 mg/kg) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (40 mg/kg).

MT content and HO activity were significantly increased, namely MT was 55.6-, 33.9-, 35.3- and 34.4-fold and HO was 11.0-, 2.6-, 4.4- and 5.2-fold increased, at 24 h after a single injection of Cd, Zn,

Cu and Ag, respectively, as compared with the control. On the other hand, cytochrome P-450 (P-450) content was decreased to 60, 80, 50 and 60% of the control by the above treatments, respectively. At 72 h after the injection, both MT content and HO activity were slightly restored toward the control level. However, no marked change was observed in the P-450 content when compared to that 24 h after the injection. After repeated treatment with metals daily for 3 d, MT content was markedly increased by 95.4-, 53.8-, 31.0- and 61.3-fold at 24 h after the last injection of Cd, Zn, Cu and Ag, respectively, as compared to the control. However, HO activity was 4.1-, 1.5-, 2.3- and 3.9-fold increased over the control. No significant change in P-450 content was noted as compared to that after the single injection.

In the case of a single injection of Co and Ni, MT content was increased 4.9- and 9.1-fold at 24 h after treatment, while HO activity was also enhanced 2.4- and 5.7-fold, respectively. However, at 72 h after the injection, MT content and HO activity were restored nearly to the control level. The repeated injection of Co and Ni daily for 3 d caused marked increases in the MT content, 12.3- and 24.7-fold at 24 h after the last injection, respectively. In addition, HO activity was enhanced 4.8- and 10.0-fold as compared to the control.

On Cd treatment of Zn-, Cu- or Ag-pretreated rats, the MT content was markedly increased as compared with that after Cd injection alone, but the HO activity was very much lower. At 24 h after the injection of Ni into Zn-pretreated rats, both MT content and HO activity were increased as compared with those after Ni treatment alone. These results clearly indicate that the MT-binding capacity for various metals *in vivo* is related to the induction of HO.