Effect of Cadmium on Selective Removal of Copper by Tetrathiomolybdate in the Liver of LEC Rats

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Introduction

Mechanisms underlying the selective removal of Cu bound to metallothionein (MT) by tetrathiomolybdate (TTM) in the liver of LEC rats were studied using an animal model of Wilson disease. Two steps out of the three steps in the proposed mechanism, *i.e.*, i) the removal of Cu/TTM complex from the MT/TTM complex that is formed between Cu-containing MT and TTM with differing ratios, and ii) the binding of the Cu/TTM complex to albumin, were focused on. Effects of the presence of Cd bound to MT in the former step were examined *in vitro* by the HPLC/ICP-MS method and equilibrium dialysis and *in vivo* by injecting Cd before TTM treatment.

Methods

***In vitro* Experiments** — A 20% liver supernatant (Cu 25 μM , Zn 5 μM) prepared from LEC rats was incubated at 37 °C for 5 min with TTM (0–200 μM) in the presence of 25 μM of Cd or Zn. Distributions of Cu and Mo were determined together with Cd and Zn by HPLC/ICP-MS.

***In vivo* Experiments** — Male LEC rats 11 weeks old received a single subcutaneous injection of Cd at a dose of 3 mg/kg b.w. 3 d before an intraperitoneal injection of TTM at a dose of 10 mg/kg b.w., which was injected for 8 consecutive d. The rats were killed 1 d after the last injection, and the concentrations and distributions of the 4 metals in the liver and serum were determined by ICP-MS and HPLC/ICP-MS, respectively. Biochemical parameters for liver and kidney func-

tions, ALT, LDH and BUN were determined.

Equilibrium Dialysis —

Equilibrium dialysis between the serum of Wistar rats or Nagase analbuminemia rats (NAR) and the liver supernatant (Cu 20 μM) of LEC rats was conducted in the presence of TTM (0–2 mM) in the former cell and 20 μM of Cd or Zn in the latter cell at 4 °C for 6 h. Concentrations of Cu and Mo in both cells were determined.

Results and Discussion

The Cu/TTM complex was shown to be liberated completely from the MT/TTM complex in the presence of Cd by addition of more than TTM/Cu=1, while it remained bound to the MT/TTM complex in the presence of Zn but not Cd by the addition of TTM up to TTM/Cu=8; this suggested that Cd bound to MT accelerated liberation of the Cu/TTM complex from the MT/TTM complex *in vitro*. Cd injected into LEC rats before TTM treatment, however, did not accelerate the removal of Cu from the liver and significant effects were not observed on the biochemical parameters for the liver damage, indicating that Cu bound to MT in the liver of LEC rats can be removed by TTM without significant interference by either the presence or absence of Cd in MT.

Equilibrium dialysis between the sera of Wistar and NAR rats and the liver supernatant of LEC rats indicated that the presence of albumin accelerates liberation of the Cu/TTM complex from the MT/TTM complex by selectively binding the Cu/TTM complex, the ratio of Cu/TTM in the Cu/TTM/albumin complex being unity.