DOCTORAL THESIS

Protective action of metallothionein against chemically induced toxicity

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Protective Action of Metallothionein Against Chemically Induced Toxicity

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Abstract

The protective action of MT against chemically induced cell injury was studied using both *in vitro* and *in vivo* systems.

At the molecular level, the protective action of MT against the $[(OP)_2Cu^+]$ complex induced DNA cleavage was demonstrated by an inhibition of the conversion of scDNA to ocDNA. The IC_{50} for inhibiting the DNA damage was 0.1 μM for CdZn-MT, 0.2 μM for Zn-MT. Cysteine and sodium azide also inhibited the DNA cleavage. However, the IC_{50} for cysteine was about 3 mM. Sodium azide can only inhibit the cleavage by 40% at the concentration of 25 mM. Spectrophotometric and Sephadex chromatography analyses showed that MT may disturb either the formation of the $DNA{\cdot}[(OP)_2Cu^+]$ complex or dissociate the $DNA{\cdot}[(OP)_2Cu^+]$ complex formed through binding Cu ions.

At the cellular level, MT can reduce the amount of free radicals produced in RH-35 cells stimulated with PMA. However, the inhibition of free radicals requires the presence of the protein. Zn or Cd ions, on the other hand, can only reduce PMA induced free radicals through MT induction.

In rat liver, $CdCl_2$ (2 mg/kg, i.p) administered for 3 days resulted in the significant increases in both MT and GSH level, and the decreases in SOD and catalase activities. The results implied that the increases in MT and GSH were closely related to the decreases in SOD and catalase activities. Administration of $CuCl_2$ also significantly increased the MT and GSH, but the levels of MT and GSH were significantly lower than those after the administration of $CdCl_2$. There were no changes in SOD and catalase activities observed. These results seemed to suggest that MT may function as a metal chelator to store the increased metal while antioxidant molecules are maintained at normal level. To further demonstrate a perturbation of tissue antioxidative function, catalase activity was inhibited by 3-AT (1 g/kg, i.p) for 24 hr after a 2-day treatment with $CdCl_2$. A significant decrease in SOD was observed. In the case, MT and GSH level were decreased, but MDA was increased when compared with that in the treatment with $CdCl_2$ alone. The decrease of MT induction may be evidence for MT as an antioxidant to give protection against the excess production of MDA, while the lack of antioxidants was caused by 3-AT. The administration of cysteine and methionine can reverse the decreases of MT and GSH caused by 3-AT. The simultaneous changes of MT and GSH suggested that the role of MT is closely related to GSH.

Finally, the ability of Zn-MT to remove intracellular Cd from RH-35 cells pre-treated with $CdCl_2$ for 2 days was studied. Zn-MT added to incubation medium significantly improved Cd extrusion from the treated cells. When compared to DTC, Zn-MT was significantly more efficient in removing intracellular Cd.

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