

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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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

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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 $[(OP)_2Cu^+]$ complex or dissociate the DNA $[(OP)_2Cu^+]$ 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.

Table of Contents

Declaration	i
Abstract	ii
Acknowledgment	iii
Table of Contents	iv
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xvi
Chapter 1 Research Background -----	1
1.1 Overview of Metallothionein -----	1
1.1.1 Classification of Metallothionein -----	1
1.1.2 Structural Characteristics of Metallothionein -----	6
1.1.3 Spectroscopic Characteristics -----	9
1.2 Reactivity of Metallothionein -----	10
1.2.1 Formation of Metal-thiolate Clusters -----	11
1.2.2 Metal-Exchange Reactions of Metallothionein -----	12
1.2.3 Ligand Substitution Reactions of Metallothionein -----	15
1.2.4 Sulfhydryl Reactivity of Metallothionein -----	17
1.3 Possible Function of Metallothionein -----	18
1.4 The Role of Metal Ions in Biological Systems -----	19
1.4.1 Essentiality and Toxicity -----	19
1.4.2 The Role of Metal Ions -----	20
1.5 Roles of Free Radicals in the Toxicities Induced by Toxic Chemicals ---	24
1.5.1 Mechanisms of Injuries Related to Free Radicals -----	26

1.5.1.1 Superoxide Anion (O_2^-)	-----	26
1.5.1.2 Hydrogen Peroxide (H_2O_2)	-----	27
1.5.1.3 Other Free Radicals	-----	28
1.5.2 Protection Against Free Radicals Induced Toxicity	-----	28
1.5.2.1 Protection by Enzymes	-----	28
1.5.2.2 Protection by Small Molecules	-----	29
1.5.2.3 Protection by Sequestration of Metal Ions	-----	29
1.6 Perspective of Metallothionein Against Chemically Induced Toxicity	---	29
1.7 The Objective of this Study	-----	35
Chapter 2 Materials and Methods	-----	36
2.1 Chemicals and Reagents	-----	36
2.2 Incubation Media	-----	37
2.2.1 Preparation of MEM Solution	-----	37
2.2.2 Preparation of PBS	-----	38
2.3 Cell Culture	-----	38
2.3.1 Cell Line Maintenance and Subculture	-----	39
2.3.2 Preparation of Cells for Metallothionein, Total Protein Cell Viability and Chromatography	-----	39
2.3.3 Preparation of Cells for Metal Determination	-----	40
2.4 Animal Preparation	-----	40
2.4.1 Animal Care	-----	40
2.4.2 Preparation of Animal Tissues for Protein and Metallothionein Determination	-----	41
2.4.3 Preparation of Animal Tissues for Metal Analysis	-----	42
2.5 Rabbit Liver Zn-Metallothionein Preparation	-----	42

2.6 DPP Assay for Metallothionein Determination	43
2.7 Protein Quantitation	47
2.8 Trypan Blue Exclusion Test	47
2.9 Sephadex G-75 Chromatography	47
2.10 Statistical Analysis	48
Chapter 3 Determination and Induction of Metallothionein	49
3.1 Introduction	49
3.2 Methods Specific to this Chapter	50
3.2.1 Cd-saturation Assay	50
3.2.2 Preparation of Cells for the Identification of DPP Responsive Protein Fractions	51
3.2.3 Induction of Metallothionein in RH-35 Cells	51
3.3 Results and Discussions	52
3.3.1 Comparisons of Metallothionein Determinations by Cd-saturation Assay and DPP	52
3.3.1.1 Comparison of Standard Metallothionein Determinations	52
3.3.1.2 Comparison of Metallothionein Determinations in RH-35 cells	52
3.3.2 Potential Interference of DPP	55
3.3.2.1 Effects of Cysteine and Glutathione on the Metallothionein Determination	59
3.3.2.2 Effects of Other Chemicals on the Metallothionein Measurement	59
3.3.3 MT Stability Studied by DPP	64
3.3.4 Quantification of Metallothionein Induction in RH-35 Cells by DPP	68

3.3.5 Applicability of DPP for Quantification of Metallothionein ----	72
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Chapter 4 Protective Action of Metallothionein against Copper-

1,10-Phenanthroline Complex Induced DNA Cleavage ----	73
4.1 Introduction -----	73
4.2 Methods Specific to this Chapter -----	75
4.2.1 Preparation of the Plasmid DNA -----	75
4.2.2 DNA Quantitation -----	76
4.2.3 DNA Cleavage Assay -----	76
4.2.3.1 Preparation of 1.0 % Agarose Gel -----	76
4.2.3.2 Protocol for DNA Cleavage Assay -----	76
4.2.4 Treatment Protocols for DNA Cleavage Activity -----	77
4.2.4.1 Effect of Metallothionein on DNA Integrity -----	77
4.2.4.2 Selecting a Proper Mixture of OP, CuCl ₂ and Ascorbic Acid for Subsequent Studies -----	78
4.2.5 Dose-dependent Action of Metallothionein in Protection against [(OP) ₂ Cu ⁺] Induced DNA Cleavage -----	78
4.2.6 Spectrophotometric Detection of [(OP) ₂ Cu ⁺] and DNA Interaction -----	79
4.2.6.1 Time Course Study on the Formation of DNA. [(OP) ₂ Cu ⁺] -----	80
4.2.6.2 Effect of Metallothionein on the Formation of DNA. [(OP) ₂ Cu ⁺] Complex -----	80
4.2.7 Identification of the Copper Containing Fraction after the Interaction of Metallothionein, DNA and [(OP) ₂ Cu ⁺] -----	81
4.3 Results -----	81
4.3.1 DNA Cleavage Activity of CdZn-Metallothionein -----	81
4.3.2 DNA Cleavage Activity of Zn-Metallothionein -----	84

4.3.3 [(OP) ₂ Cu ⁺] Induced DNA Cleavage Activity -----	84
4.3.4 Protective Effect of CdZn-Metallothionein on the DNA Cleavage by the [(OP) ₂ Cu ⁺] Complex -----	84
4.3.5 Protective Effect of Zn-Metallothionein on the DNA Cleavage by the [(OP) ₂ Cu ⁺] Complex -----	89
4.3.6 Protective Effects of Cysteine and Other Chemicals on the DNA Cleavage by the [(OP) ₂ Cu ⁺] Complex -----	89
4.3.7 Effect of MT on the Absorption Spectrum of the DNA [(OP) ₂ Cu ⁺] Complex -----	94
4.3.8 Effect of MT on the Cu Ions in the [(OP) ₂ Cu ⁺] Complex or DNA [(OP) ₂ Cu ⁺] Complex -----	95
4.4 Discussions -----	100
4.4.1 Comparison between the Protective Effects of CdZn- Metallothionein and Zn-Metallothionein -----	100
4.4.2 The Mechanism of Metallothionein against the [(OP) ₂ Cu ⁺] Induced DNA Cleavage -----	100
 Chapter 5 Protective Action of Metallothionein against Phorbol Myristate Acetate Induced Free Radical Generation in RH-35 Cells -----	 103
5.1 Introduction -----	103
5.2 Methods Specific to this Chapter -----	104
5.2.1 Assay for O ₂ ⁻ -----	104
5.2.2 Assay for H ₂ O ₂ -----	105
5.2.3 Treatment Protocols -----	106
5.2.3.1 Confirmation of Cell Viability under Different Study Conditions -----	106
5.2.3.2 Quantification of Metallothionein, Zn and Total Cellular Protein -----	106

5.2.3.3 Time Course Study on the Production of O_2^- Following Addition of PMA -----	107
5.2.3.4 Time Course Study on the Effect of Metallothionein on the Production of O_2^- Following Addition of PMA --	107
5.2.3.5 Dose-dependent Study on the Short Term Action of Metallothionein in Reducing PMA Induced O_2^- Production -----	108
5.2.3.6 Dose-dependent Study on the Short Term Action of Metallothionein in Reducing PMA Induced H_2O_2 Production -----	108
5.2.3.7 Cell Viability, Total Cellular Protein, Metallothionein and Zn Measurements after Pre-treatment (15 hr) with Metallothionein, Cd or Zn Ions -----	109
5.2.3.8 Reduction of PMA Induced O_2^- Generation after Pre-treatment (15 hr) with Metallothionein, Cd or Zn Ions -----	109
5.3 Results and Discussions -----	110
5.3.1 Protection of Metallothionein against PMA Induced Free Radical Generation -----	110
5.3.2 The Immediate Effect of Metallothionein on PMA Induced Free Radical Generation -----	116
5.3.3 Comparison of the Inhibitory Effects of a 15-hour Pretreatment with Metallothionein and $ZnCl_2$ on PMA Induced Free Radical Generation -----	122
 Chapter 6 Evidence for Metallothionein as an Antioxidant	
in SD Female Rats -----	126
6.1 Introduction -----	126
6.2 Methods Specific to this Chapter -----	127
6.2.1 Assay for GSH -----	127
6.2.2 Assay for Malonaldehyde -----	128
6.2.3 Assays for Catalase and Superoxide Dismutase -----	128

6.2.4 Treatment Protocols -----	129
6.2.4.1 Comparison of Metallothionein Inductions by CdCl ₂ in Male and Female Rats -----	129
6.2.4.2 Treatment of Rats with CuCl ₂ -----	130
6.2.4.3 Treatment of Rats with 3-AT -----	130
6.2.4.4 Effect of 3-AT on CdCl ₂ Induced Change in Antioxidant Parameters -----	130
6.2.4.5 Effect of Cysteine and Methionine on CdCl ₂ , and/or 3-AT Treatment -----	130
6.3 Results and Discussions -----	131
6.3.1 Comparison of Metallothionein Inductions between Male and Female Rats Treated with CdCl ₂ -----	131
6.3.2 Comparative Effects of Cd and Cu on Metallothionein and Other Antioxidant Molecules -----	131
6.3.3 Change in Liver Tissue Metallothionein Content after Inhibition of Catalase by 3-AT -----	139
6.3.4 Effects of Cysteine+Methionine on the Metallothionein Induction and GSH -----	139
 Chapter 7 Potential Function of Zn-Metallothionein as Chelator	
for Removing Intracellular Cadmium -----	145
7.1 Introduction -----	145
7.2 Methods Specific to this Chapter -----	146
7.2.1 Assessment of the Toxicities of Metallothionein, Metals and DTC -----	146
7.2.2 Cadmium Loading and Removal -----	146
7.3 Results -----	147
7.4 Discussion -----	155

Chapter 8 General Conclusion	-----	157
References	-----	159
Appendix	-----	175
Curriculum Vitae	-----	176