

MASTER'S THESIS

Cellular metabolism in in vitro toxicity and toxicology studies

Yu, Lok Chiu

Date of Award:
2005

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

Cellular Metabolism in *In Vitro* Toxicity and Toxicology Studies

YU Lok Chiu

A thesis submitted in partial fulfillment of the requirements

for the degree of

Master of Philosophy

Principal Supervisor: Dr. Mildred M.S. YANG

Hong Kong Baptist University

September 2005

Abstract

The aim of the present study is to clarify the role cellular metabolism plays in the course of cell death. Cellular metabolism is a study of inter-conversion of nutritional substrates to generate useful chemical molecules to maintain proper cellular function. The level of comprehensive analysis of these molecules would provide a complete picture of cellular metabolism. Because of the different variety of metabolites, the current study will concentrate on those related to energy and redox metabolism.

Using cell cultures as models, the present study aims to investigate metal induced cell death. Four objectives were set: (1) What are the energy and redox changes when cells were exposed to toxic metals? (2) Do all toxic metals affect cellular metabolism similarly? (3) Do all cells response similarly to a single toxicant? (4) What is the correlation between cellular metabolic changes and cell death?

We have studied the effect of Cd, Zn, Se and an organic hydroperoxide on the HepG2 hepatoma and the C6 glioma cells. The results demonstrated that during this initial phase of Cd and Zn administration, there was a significant increase in cellular energy state as demonstrated by an increase in ATP/TAN, and a decrease in AMP/TAN. The change was correlated to a decrease in GSH/GSSG. Se, on the other hand, caused an opposite change in cellular energy state suggesting that different toxicants act differently on cells. Upon exposure to Cd, the C6 glioma cells were more sensitive than the HepG2 hepatoma cells as demonstrated by a more sensitive decrease in GSH/GSSG. This correlated by a relatively low glutathione reductase activity in the C6 glioma cells. Finally, using Cd as a model, the dynamic change in cellular energy and redox metabolism was described over a 6-hr exposure to a 3-hr LC50 of Cd. The results correlated with the cell death through apoptosis.

Table of Contents

Declaration	i
Abstract	ii
Acknowledgement	iii
Table of Contents	iv
List of Tables	viii
List of Figures	ix
List of Abbreviation	xii
Chapter 1: Research Background	
1.1 Overview of Cellular Metabolism	1
1.2 Modes of Cell Death	3
1.2.1 Apoptosis	3
1.2.2 Necrosis	6
1.2.3 Alternative Modes of Cell Death	8
1.3 The Role of Cellular Metabolism and Cell Death	9
1.3.1 The Significance of Energy Metabolites in Studying Cell Death	11
1.3.2 The Significance of Redox Metabolites in Studying Cell Death	12
1.4 Metal-induced Cell Death	14
1.4.1 The Role of Cadmium	14
1.4.2 The Role of Zinc	18
1.4.3 The Role of Selenium	20
1.5 Organic-chemical-induced Cell Death	22
1.5.1 The Role of <i>tert</i> -butylhydroperoxide	22
1.6 Objectives	24

Chapter 2: Methods in Studying Cellular Metabolism *In Vitro*

2.1 Introduction	26
2.2 Cell Culture as Experimental Model	26
2.2.1 The HepG2 Hepatoma Cells	26
2.2.2 The C6 Glioma Cells	28
2.3 Correlation between Energy and Oxidative Subsets..... of Metabolites	29
2.3.1 Determination of Energy Metabolites	29
2.3.2 Analysis of Cellular Energy State	30
2.3.3 Determination of Cellular Oxidative State	31
2.3.4 Analysis of Cellular Oxidative State	32
2.4 Methods of Extracting Metabolites in Cultured Cells	33
2.5 Methods for Integrating Metabolomic Data in Cultured	37
Cells	

Chapter 3: Cadmium- and *tert*-butylhydroperoxide-Induced Changes in Energy and Redox States in HepG2 Cells: Correlation with Mode of Cell Death

3.1 Introduction	39
3.2 Material and Methods	40
3.2.1 Chemicals	40
3.2.2 Analysis of Cell Viability	40
3.2.3 Determination of Lipid Peroxidation	41
3.2.4 Extraction of Energy Metabolites	41
3.2.5 Analysis of Data	42
3.2.6 Detection of Apoptosis	42
3.3 Results.....	42

3.3.1 Effect of Cd and <i>t</i> -BHP on Cell Viability	42
3.3.2 Effect of Cd and <i>t</i> -BHP on Cellular Metabolism	43
3.3.3 Effect of Cd and <i>t</i> -BHP on Nucleus Staining	44
3.4 Discussion	50

Chapter 4: Energy and Redox States in C6 Glioma Cells following Acute Exposure to Zn, Se⁺⁴ and Se⁺⁶ and The Correlation with Apoptosis

4.1 Introduction	54
4.2 Materials and Methods	57
4.2.1 Chemicals	57
4.2.2 Cell Culture Model	57
4.2.3 Determination of Cell Viability	58
4.2.4 Extraction of Cellular Metabolites	58
4.2.5 Analysis Cellular Metabolites and Metabolic States	59
4.2.6 Nuclear Staining of Apoptotic Bodies	59
4.2.7 Statistical Analysis	60
4.3 Results	60
4.3.1 Effect of Zn on Cellular Metabolism	60
4.3.2 Effect of Se ⁺⁴ on Cellular Metabolism	61
4.3.3 Effect of Se ⁺⁶ on Cellular Metabolism	62
4.3.4 Apoptosis following Exposure to Different Metals	63
4.4 Discussion	71

Chapter 5: Cellular Metabolism and Enzyme Activity

5.1 Introduction	76
5.2 Materials and Methods	77
5.2.1 Chemicals	77
5.2.2 Quantitative Analysis of Cellular Metabolites	78
5.2.3 Quantitative Analysis of Glutathione Reductase Activity	78
5.3 Results	79
5.3.1 Effect of Cd on Metabolism in Different Cells	79
5.3.2 Analysis of Glutathione Reductase Activity	79
5.4 Discussion	84

Chapter 6: Redox Metabolism in Cadmium-induced Cell Death

6.1 Introduction	88
6.2 Materials and Methods	90
6.3 Results	90
6.3.1 Effect of Cd on Cellular Metabolism	90
6.3.2 Effect of Cd on Mitochondrial Transmembrane Potential (Ψ_m)	91
6.3.3 Nuclear Staining of Apoptotic Bodies	92
6.4 Discussion	97

Chapter 7: Summary	100
---------------------------------	-----

List of References	103
---------------------------------	-----

Curriculum Vitae	131
-------------------------------	-----

List of Publications	132
-----------------------------------	-----