

CONTENTS

	Page
ABSTRACT.....	iii
ACKNOWLEDGEMENT.....	vii
LIST OF FIGURES.....	xviii
LIST OF ABBREVIATIONS.....	xxi
 CHAPTER 1: INTRODUCTION	
Introduction.....	1
Oxidative stress and neuronal dysfunction.....	2
Generation of ROS/RNS in brain tissues.....	2
The physiological role of ROS/RNS in brain tissue.....	3
Mitochondrial dysfunction and neurodegeneration.....	3
Adriamycin, a potent anticancer drug.....	4
Adriamycin and somnolence syndrome.....	4
Oxidative stress and Adriamycin.....	5

CONTENTS (continued)

Alzheimer's disease and somnolence syndrome.....	6
Alzheimer's disease and genetic defect.....	6
Oxidative stress and Alzheimer's disease.....	7
Tumor necrotic factor alpha (TNF), Adriamycin and neuronal dysfunction.....	9
TNF-alpha and neurodegeneration.....	10
Antioxidant prevention and neuroprotective System.....	10
Somnolence syndrome (Cognitive dysfunction).....	12
Summary and Experimental approaches.....	13

CHAPTER 2: ADRIAMYCIN-INDUCED TNF-MEDIATED CNS TOXICITY:

INSIGHTS INTO THE MECHANISM OF CHEMOBRAIN

Abstract.....	14
Introduction.....	15

CONTENTS (continued)

Materials and Methods.....	17
 Localization of adriamycin in brain tissues	17
 Enzyme-linked immunosorbent assay (ELISA).....	18
 Immunohistochemistry study.....	18
 Mitochondrial isolation and purification.....	18
 Mitochondrial respiration.....	19
 Preparation of brain homogenate.....	20
 Western Blot analysis.....	20
 Immunoprecipitation assays.....	21
 Caspase 3 activity assay.....	21
 TUNEL assay.....	22
 Statistical analysis.....	22

CONTENTS (continued)

Results.....	23
 Adriamycin accumulation in brain tissues.....	23
 Adriamycin-induced circulating TNF levels.....	23
 Increased TNF level in brain tissues.....	23
 Adriamycin-induced mitochondrial dysfunction.....	24
 Pro-survival and pro-apoptotic protein levels in mitochondria.....	24
 p53 forms specific complex with the protective Bcl-xL protein.....	25
 Cytochrome c release from the mitochondria to cytosol.....	25
 Increased caspase 3 activity and apoptosis.....	26
Discussion.....	27

CHAPTER 3: ADRIAMYCIN-INDUCED NO PRODUCTION AND

NITRATION OF MnSOD IN CNS

CONTENTS (continued)

Abstract.....	42
Introduction.....	43
Materials and Methods.....	46
Enzyme-linked Immunosorbent assay (ELISA).....	46
Mitochondrial Isolation and Purification.....	46
Mitochondrial Respiration.....	47
Preparation of brain homogenate.....	47
MnSOD activity assay.....	48
Western Blot Analysis.....	48
Immunoprecipitation Assays.....	48
Slot Blot Analysis.....	49
RT-PCR Analysis.....	50
Statistical Analysis.....	50

CONTENTS (continued)

Results.....	51
Adriamycin-induced circulating TNF levels.....	51
Adriamycin-induced TNF-mediated iNOS mRNA in brain tissues.....	51
Adriamycin-induced mitochondrial dysfunction.....	51
ADR-induced brain protein nitration.....	52
Adriamycin-induced MnSOD nitration/inactivation in brain tissues.....	52
MnSOD and CuZnSOD level in brain tissues.....	53
Discussion.....	54

CHAPTER 4: β -AMYLOID MEDIATED NITRATION OF MnSOD:

IMPLICATION FOR OXIDATIVE STRESS IN

A APP^{NLh}/APP^{NLh} x PS-1^{P264L} / PS-1^{P264L}

Abstract.....	66
Introduction.....	67

CONTENTS (continued)

Materials and Methods.....	69
Mutant mouse lines.....	69
Genotyping of mice.....	69
Immunocytochemistry for Aβ.....	70
Tissue preparation for Aβ ELISA.....	70
Sandwich ELISA to determine the levels of insoluble brain	
Aβ 1-40 and Aβ 1-42	70
Mitochondrial isolation	71
Measurement of mitochondrial respiration.....	71
Immunoprecipitation.....	72
Western blot analysis.....	72
MnSOD activity assay.....	72

CONTENTS (continued)

Statistical analysis.....	73
Results.....	74
A β deposition in APP/PS-1 mice.....	74
Increased levels of A β 1-40 and 1-42 fractions in APP/PS-1 mice.....	74
Unchanged MnSOD protein levels in WT and APP/PS-1 mice.....	74
Increased nitration of MnSOD in APP/PS-1 mice.....	75
Decreased SOD activity in APP/PS-1 mice.....	75
Decreased mitochondrial respiration in APP/PS-1 mice.....	75
Discussion.....	77

CHAPTER 5: SUMMARY AND FUTURE STUDIES

Summary and Future Studies.....	90
---------------------------------	----

CONTENTS (continued)

REFERENCES.....95

VITAE..... 123

LIST OF FIGURES

Figure 2.1: Localization of Adriamycin in mouse brain.....	30
Figure 2.2: Adriamycin increased circulating TNF.....	31
Figure 2.3: Adriamycin-induced TNF is increased in brain tissues.....	32
Figure 2.4: Immunofluorescence analysis of TNF localization in cortex following ADR treatment.....	33
Figure 2.5: Immunofluorescence analysis of TNF localization in hippocampus following ADR treatment.....	34
Figure 2.6: Adriamycin-mediated TNF elevation leads to mitochondrial dysfunction.....	35
Figure 2.7: Representative immunoblots showing the levels of p53, Bax, Bcl-xL, and succinate dehydrogenase in mitochondria.....	36
Figure 2.8: Representative immunoblots showing the prevention of p53 and Bax translocation to mitochondria by anti-TNF antibody.....	37
Figure 2.9: Representative co-immunoprecipitation of the	

LIST OF FIGURES (continued)

anti-apoptotic protein, Bcl-xL, in mitochondria.....	38
Figure 2.10: ADR induced cytochrome c released from mitochondria to cytosol.....	39
Figure 2.11: ADR-induced caspase 3 activity in brain tissues.....	40
Figure 2.12: ADR-induced TUNEL positive apoptotic cell death in cortical and hippocampal regions of the brain.....	41
Figure 3.1: Adriamycin increased circulating TNF.....	57
Figure 3.2: Adriamycin-induced iNOS mRNA expression and NO production in wild-type mice.....	58
Figure 3.3: Adriamycin-mediated TNF elevation leads to mitochondrial dysfunction.....	59
Figure 3.4: Adriamycin-mediated TNF elevation leads to mitochondrial dysfunction in wild-type mice.....	60
Figure 3.5: DPTA NONOate causes mitochondrial dysfunction in iNOSKO mice.....	61
Figure 3.6: ADR-induced protein nitration.....	62

LIST OF FIGURES (continued)

Figure 3.7: ADR-induced MnSOD Nitration.....63

Figure 3.8: ADR induced MnSOD inactivation.....64

Figure 3.9: No change in MnSOD and CuZnSOD protein levels in ADR-treated mice.....65

**Figure 4.1: Sections of frontal cortex from APP/PS-1
mice immunostained with 10D-5 antibody for A β84**

Figure 4.2: Levels of A β 1-40 and 1-42 in APP/PS-1 mice.....85

**Figure 4.3: Representative immunoblot (A) and
densitometry analysis (B) showing the levels of MnSOD.....86**

Figure 4.4: Immunoprecipitation of nitrotyrosine with MnSOD.....87

Figure 4.5: Decline in mitochondrial respiration via complex I in APP/PS-1 mice.....89

**Figure 5.1: A hypothetical model of Nitric oxide-mediated Manganese
superoxide dismutase inactivation: An insight into the mechanisms
of chemotherapeutic-induced neuronal dysfunction.....94**

LIST OF ABBREVIATIONS

ADR	Adriamycin
CNS	Central Nervouse System
ROS	Reactive oxygen species
RNS	Reactive nitrogen spcies
RONS	Reactive oxygen and nitrogen species
TNF-α	Tumor necrosis factor alpha
MnSOD	Manganese superoxide dismutase
NOS	Nitric oxide synthase
NOSs	Nitric oxide synthases
nNOS	Neuronal nitric oxide synthase
iNOS	Inducible nitric oxide synthase
eNOS	Endotelial nitric oxide synthase
iNOSKO	Mouse lacking inducible nitric oxide synthase gene
O₂	Oxygen
O₂⁻	Superoxide free radical
NO	Nitric oxide
NO[·]	Nitric oxide radical
ONOO-	Peroxynitrite
ONOOH	Peroxynitrous acid
OH[·]	Hydroxy radical
H₂O₂	Hydrogen peroxide
HOCl	Hypochlorus acid
Ca₂⁺	Calcium ion
AD	Alzheimer's disease
DNA	Deoxynucleic acid
RNA	Ribonucleic acid

LIST OF ABBREVIATIONS (continued)

Fe-S	Iron sulphur
MRP1	Multi-drug resistant protein 1
BBB	Blood brain barrier
NADH	Reduced nicotinamide adenine dinucleotide
GSH-Px	Glutathione peroxidase
GSH	Reduced glutathione
CAT	Catalase
Aβ	Amyloid β-peptide
SPs	Senile plaques
APP	Amyloid precursor protein
CSF	Cerebrospinal fluid
PHFτ	Hyperphosphorylated tau
NFTs	Neurofibrillary tangles
NTs	Neurophil threads
PS-1,2	Presinillin-1,2
COX2	Cyclooxygenase-2
NF-kB	Nuclear factor kappa B
AP-1	Activator protein-1
HIV	Humeral immune deficiency virus
FAEE	Ethyl-4-hydroxy-3-methoxy-cinnamic acid
MCI	Mild cognitive dysfunction
i.p	Intra-peritoneally injection
PBS	Phosphate buffer saline
ADP	Adenine dinucleotide phosphate
RCR	Respiration control ratio

LIST OF ABBREVIATIONS (continued)

TBST	Tween 20 and tris-buffer saline
TBS	Tris-buffer saline without Tween 20
SDHB	Succinate dehydrogenase
rTdT	Recombinant termination deoxynucleotidyl transferase
IgG	Immunoglobulin G
PTX	Pentoxiphylline
LPS	Lipopolysaccharide
NBT	Nitroblue tetrazolium
BCS	Bathocuproin sulfonate
Cu/ZnSOD	Copper Zinc superoxide dismutase
RT-PCR	Reversed transcriptase polymerase chain reaction
mRNA	Messenger ribonucleic acid
NMDA	N-methyl-D-aspartate
MLS	Multisclerosis
FAD	Familial Alzheimer's disease
P264L	Poline 264 Leucine, amino acid substitution
3-NT	3-Nitrotyrosine
MPO	Myeloperoxidase