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THE EFFECT OF MANGANESE ON MAMMALIAN MITOCHONDRIA

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Biochemistry
at Massey University, New Zealand

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1999

ABSTRACT

Manganese (Mn) is an essential trace element, but excessive inhalation can cause serious disorders of the central nervous system, lungs and liver, and results in the condition known as manganism. The general population is exposed to Mn through its use in the fungicide Maneb and MMT, which is used as an anti-knock agent to replace lead in petrol. Also there have been a number of reports of Mn contaminated drinking water. Victims of Mn poisoning suffer from serious neurological disorders, such as an intermittent tremor of small amplitude, speech impairments and disruption of postural reflexes, which are caused by damage to certain regions of the brain. After prolonged exposure severe symptoms develop that generally resemble those associated with Parkinson's disease.

The action of Mn on the brain is not well understood, although three possible mechanisms have been proposed:

1. Inhibition of the mitochondrial electron transfer chain following Mn accumulation by mitochondria.
2. Neuronal degradation by free radicals such as $O_2^{\cdot -}$ and $\cdot OH$ causing lipid peroxidation and damage to DNA and protein.
3. Induction of mutation of the mitochondrial genome, as has previously been shown in both eukaryotes and prokaryotes.

It has been shown in this study that Mn inhibits the mitochondrial electron transfer chain. An overall ionic strength inhibition of the entire electron transfer chain was observed, probably mediated by an interference of the electrostatic interactions between cytochrome *c* and the cytochrome *bc*₁ complex or cytochrome oxidase. Also a direct inhibition of succinate dehydrogenase, NADH dehydrogenase and cytochrome oxidase was observed. This inhibition would be associated with a decrease in the production of ATP and could be sufficient to cause the degradation of brain tissue seen in victims of Mn poisoning.

It seems likely that if Mn can inhibit the mitochondrial electron transfer chain, this inhibition would lead to an increase in the generation of free radical species by the mitochondria. However, this was not shown in this work, due to difficulties with

detector molecules. It was observed that sheep liver mitochondria can oxidise and reduce acetylated cytochrome *c*, which may not have been previously reported.

The effect of Mn on isolated mtDNA showed a decrease in the intensity of PCR products after exposure to Mn, which may have been caused by an interference of the activity of *Taq* polymerase. It has previously been shown that Mn interferes with the activity of both *Taq* polymerase and chicken liver mitochondrial polymerase- γ and, if it could interfere with the activity of mitochondrial DNA polymerase, this would also decrease further both the number of functional mitochondria and the production of ATP.

A decrease in the production of ATP by mitochondria, or a decrease in the production of functional mitochondria, would lead to cellular death of affected cells and could provide an explanation of the symptoms observed in victims of Mn poisoning.

ACKNOWLEDGEMENTS

I wish to thank my supervisor Dr Simon Brown for his advice, encouragement, guidance and support during the last two years.

I would also like to thank Dr Mark Patchett for his advice throughout this work, especially with regard to the molecular biological aspects of this project.

I must thank those people around the Institute of Molecular Biosciences and the old Department of Biochemistry, especially those in the “Twilight Zone”, the Biochemistry Prep Room and those in the Bioenergetics Lab who have helped me throughout this project.

To my friends, thank you for the support, especially those who have proof read some of my essays and listened to my speeches, I know they didn’t make any sense to you but it helped me a lot.

Finally I must thank my parents for helping to make this possible, and the rest of my family for their support and interest.

This work was supported in part by the Massey University Graduate Research Fund. I would also like to thank the Massey University Alumni Association, for their award that contributed towards my personal expenses.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF FIGURES	viii
LIST OF TABLES	ix
ABBREVIATIONS	x
CHAPTER 1 : INTRODUCTION	1
1.1 Manganese	3
1.1.1 Manganese poisoning	4
1.1.2 Relationship between manganism and Parkinson's disease	7
1.2 Mechanism of Manganese Poisoning	8
1.2.1 Active accumulation of manganese by mitochondria	8
1.2.2 Inhibition of mitochondrial electron transfer	10
1.2.3 Induction of mitochondrial mutations	12
1.2.4 Influence on levels of free radicals	14
1.2.5 Association of the three proposed mechanisms of manganese toxicity	17
1.3 Aims of this Project	17
CHAPTER 2 : MATERIALS AND METHODS	20
2.1 Materials	20
2.1.1 Chemicals and solvents	20
2.1.2 Enzymes	20
2.1.3 Primers	20
2.1.4 Miscellaneous products	21
2.1.5 Sources of liver	21
2.2 General methods	21
2.2.1 Preparation of reagents	21
2.2.2 Calibration of the Aminco DW2a spectrophotometer	21
2.2.3 Preparation of reduced cytochrome <i>c</i>	22
2.2.4 Determination of reduced cytochrome <i>c</i> concentration	22
2.3 Preparation and storage of mitochondria	21
2.3.1 Preparation of mitochondria	22

2.3.2	Preparation of sub-mitochondrial particles	23
2.3.3	Storage of mitochondria	23
2.4	Determination of mitochondrial quality	23
2.4.1	Protein determination of extracted mitochondria	24
2.4.2	Measuring oxygen consumption of mitochondria	24
2.4.3	Determining the quality of mitochondria produced	25
2.5	Measurements of mitochondrial electron transfer	24
2.5.1	Assays of electron transfer chain activities	25
2.5.2	Measurement of electron transfer from succinate to cytochrome <i>c</i>	25
2.5.3	Measurement of electron transfer from malate/pyruvate to cytochrome <i>c</i>	26
2.5.4	Measurement of succinate dehydrogenase activity	26
2.5.5	Measurement of cytochrome <i>c</i> oxidase activity	26
2.6	Free radical preparation and measurement	25
2.6.1	Production of superoxide radicals	26
2.6.2	Measurement of superoxide radicals using NBT	27
2.6.3	Measurement of superoxide radicals using acetylated cytochrome <i>c</i>	27
2.6.4	Production of hydroxyl radicals	27
2.7	Molecular biological techniques	27
2.7.1	Isolation of mitochondrial DNA	28
2.7.2	Agarose gel electrophoresis	28
2.7.3	DNA digestion with restriction endonucleases	29
2.7.4	DNA amplification	29
2.7.5	Treatment of mitochondrial DNA with manganese chloride, magnesium chloride and reactive oxygen species	30
2.7.6	Preparation of PCR products for automated DNA sequencing	30
CHAPTER 3 : THE EFFECTS OF MANGANESE ON MITOCHONDRIAL ELECTRON TRANSFER CHAIN ENZYMES		32
3.1	The quality of isolated sheep liver mitochondria	32
3.2	The effect of manganese on whole chain electron transfer	33
3.2.1	Manganese inhibition of mitochondrial electron transfer in coupled and uncoupled mitochondria	34
3.2.2	The effect of varying the concentration of manganese and magnesium salts on succinate-dependant electron transfer	36
3.2.3	The effect of manganese chloride and magnesium chloride on succinate-dependant electron transfer in a phosphate-free buffer	38
3.2.4	The effect of manganese chloride and magnesium chloride on malate/pyruvate-dependant electron transfer	38
3.3	The effect of manganese on specific partial reactions	38
3.3.1	The effect of manganese on electron transfer from succinate and malate/pyruvate to cytochrome <i>c</i>	41
3.3.2	The effect of manganese on succinate dehydrogenase activity	44
3.3.3	The effect of manganese on cytochrome oxidase activity	46

3.4	Discussion	47
CHAPTER 4 : THE ROLE OF FREE RADICAL SPECIES IN MANGANISM		54
4.1	Generation and measurement of free radical species	53
4.1.1	Production of superoxide radicals	54
4.1.2	Production of hydroxyl radicals	58
4.2	Discussion	59
CHAPTER 5 : THE EFFECT OF MANGANESE ON MITOCHONDRIAL DNA		62
5.1	Isolation and treatment of mitochondrial DNA with manganese and free radical species	61
5.1.1	Isolation of mitochondrial DNA	62
5.1.2	Restriction endonuclease digest of mitochondrial DNA	64
5.1.3	Design of PCR primers	64
5.1.4	PCR of COI using COX1for and COX1rev	64
5.1.5	Treatment of isolated mitochondrial DNA with manganese chloride and reactive oxygen species	67
5.2	Discussion	70
CHAPTER 6 : DISCUSSION		74
6.1	Inhibition of the mitochondrial electron transfer chain	76
6.2	Generation of free radicals species	79
6.3	Mutation of the mitochondrial genome	80
6.4	Summary	82
REFERENCES		84
APPENDICES		92
Appendix I	Oligonucleotide primers	93
Appendix II	Tissue press	94
Appendix III	mtDNA sequence of the sheep cytochrome c oxidase subunit 1 gene	95
Appendix IV	Results of automated DNA sequencing	96

LIST OF FIGURES

		Page
Figure 1.1	The enzymes of the inner mitochondrial membrane involved in energy transduction	2
Figure 1.2	The relationship between the three models of manganese neurotoxicity	18
Figure 3.1	Representative oxygen electrode traces showing ADP stimulation of oxygen consumption	33
Figure 3.2	Representative oxygen electrode traces showing DNP stimulation of oxygen consumption	34
Figure 3.3	Representative oxygen electrode traces showing the effect of manganese on whole chain electron transfer	35
Figure 3.4	The effect of various manganese and magnesium salts on the relative rate of whole chain succinate-stimulated electron transfer	37
Figure 3.5	The effect of MnCl ₂ and MgCl ₂ on whole chain succinate-dependant electron transfer	39
Figure 3.6	The effect of MnCl ₂ and MgCl ₂ on whole chain malate/pyruvate-stimulated electron transfer	40
Figure 3.7	The effect of MnCl ₂ and MgCl ₂ on electron transfer from succinate dehydrogenase to cytochrome <i>c</i>	42
Figure 3.8	Graph showing the effect of MnCl ₂ and MgCl ₂ on electron transfer from malate/pyruvate to cytochrome <i>c</i>	43
Figure 3.9	Representative traces showing the reduction of DCPIP in the presence of MnCl ₂ and MgCl ₂	44
Figure 3.10	The effect of MnCl ₂ and MgCl ₂ on succinate dehydrogenase activity	45
Figure 3.11	Representative traces showing the oxidation of reduced cytochrome <i>c</i> in the presence of MnCl ₂ and MgCl	47
Figure 3.12	The effect of MnCl ₂ and MgCl ₂ on cytochrome oxidase activity	48
Figure 4.1	Representative traces showing the rapid degradation of O ₂ ^{•-}	55
Figure 4.2	Representative traces showing the reduction of detector molecules by O ₂ ^{•-} in the absence of mitochondria	56
Figure 4.3	Representative traces showing the production of O ₂ ^{•-} by a mitochondrial suspension	57
Figure 4.4	Representative spectrophotometric traces showing the production of ·OH	59
Figure 5.1	Sample of isolated sheep liver mitochondrial DNA	63
Figure 5.2	The 1593 bp PCR product amplified from sheep liver mitochondrial DNA with COX1for and COX1rev primers	65
Figure 5.3	Diagnostic digest of 1593 bp PCR product (A) and a schematic representation of expected digest products (B)	66
Figure 5.4	The 1593 bp PCR products produced after various treatments of mitochondrial DNA	68
Figure 5.5	Digest of pre-treated 1593 bp PCR product (A) and a schematic representation of expected digest products (B)	69
Figure 6.1	The relationship between the three models of Mn neurotoxicity	81

LIST OF TABLES

	Page	
Table 1.1	The neurological effects of Mn poisoning in 15 patients	6
Table 3.1	Inhibition of substrate oxidation by Mn compounds reported in the literature	49
Table 3.2	Apparent inhibition constants ($K_{1/2}$) of various partial reactions of the mitochondrial electron transfer chain	52
Table 5.1	Treatments of mtDNA before PCR of COI	67

ABBREVIATIONS

6-OHDA	6-hydroxydopamine
A	adenine
A _{xxx}	absorbance (XXX-wavelength of measurement)
ADP	adenosine 5'-diphosphate
AMProp	2-amino-2-methyl-1 propanol
ATP	adenine triphosphate
bp	base pair
cytochrome <i>b_c1</i> complex	ubiquinol : ferricytochrome- <i>c</i> oxidoreductase (EC 1.10.2.2)
BSA	bovine serum albumin (fraction V powder)
C	cytosine
CCCP	carbonyl cyanide <i>m</i> -chlorophenylhydrazone
CO1	cytochrome <i>c</i> oxidase subunit 1 gene
CR	control ratio (= rate after DNP addition/rate before DNP addition)
cytochrome oxidase	ferrocytochrome <i>c</i> : oxygen oxidoreductase (EC 1.9.3.1)
DCPIP	2,6-dichlorophenol-indo-phenol
DMSO	dimethyl sulfoxide
DMPO	5,5-dimethyl-1-pyrroline N-oxide
DNA	deoxyribonucleic acid
DNP	2,4-dinitrophenol
EDTA	ethylene diamine tetra-acetic acid
EGTA	ethylene glycol-bis(β-aminoethylether)-N,N,N',N'-tetra-acetic acid
EPR	electron paramagnetic resonance
FECN	potassium ferricyanide
G	guanine
GSH	glutathione
H ₂ O ₂	hydrogen peroxide
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
·OH	hydroxyl radical
kb	kilobase

KO ₂	potassium superoxide
L-DOPA	L-3,4-dihydroxyphenylalanine
Maneb	[ethylenebis(dithiocarbomato)]manganese
$\Delta\psi$	membrane potential
Mg	magnesium
MMT	methylcyclopentadienyl manganese tricarbonyl
Mn	manganese
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	mitochondrial DNA
NAD ⁺	nicotinamide adenine dinucleotide (oxidised form)
NADH	nicotinamide adenine dinucleotide (reduced form)
NADH dehydrogenase	NADH : ubiquinone oxidoreductase (EC 1.5.5.3)
NBT	2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-[3,3'-dimethoxy-4-4'-diphenylene]-ditetrazolium chloride
RAPD	random amplified polymorphic DNA
RCR	respiratory control ratio
RFLP	restriction fragment length polymorphism
SOD	superoxide dismutase (EC 1.15.1.1)
O ₂ ⁻	superoxide radical
PCR	polymerase chain reaction
PD	Parkinson's disease
succinate dehydrogenase	succinate : ubiquinone oxidoreductase (EC 1.3.5.1)
T	thymine
TE buffer	Tris-HCl (10 mM) EDTA (1 mM) pH 8.0
TAE buffer	Tris (40 mM) acetate (20 mM) EDTA (1 mM) pH 8.0
<i>Taq</i> polymerase	<i>Thermus aquaticus</i> DNA polymerase
Tris	2-amino-2-(hydroxymethyl)propane-1,3-diol
$\Delta\mu_{\text{H}}^+$	transmembrane proton electrochemical potential
tRNA	transfer RNA
U	unit
UQ	ubiquinone
UQH	ubiquinol
UV	ultraviolet
V	volts