THE EFFECT OF MANGANESE ON MAMMALIAN MITOCHONDRIA

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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 replaced 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 ·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 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 cause 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.

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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 bc_1 complex	ubiquinol : ferricytochrome-*c* oxidoreductase (EC 1.10.2.2)
BSA	bovine serum albumin (fraction V powder)
C	cytosine
CCCP	carbonyl cyanide *m*-chlorophenylhydrazone
CO1	cytochrome *c* oxidase subunit 1 gene
CR	control ratio (= rate after DNP addition/rate before DNP addition)
cytochrome oxidase	ferrocytochrome *c* : 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_2O_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)
$O_2^{\cdot-}$	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
Taq polymerase	*Thermus aquaticus* DNA polymerase
Tris	2-amino-2-(hydroxymethyl)propane-1,3-diol
$\Delta\mu_H^+$	transmembrane proton electrochemical potential
tRNA	transfer RNA
U	unit
UQ	ubiquinone
UQH	ubiquinol
UV	ultraviolet
V	volts