STUDIES OF CELL DEATH IN PARKINSON'S DISEASE USING ORGANOTYPIC CELL CULTURES

TUYET TB TRAN

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due references has been made in the text.

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PUBLICATIONS AND PRESENTATIONS

The following articles have been published or accepted for publication or presentation during the period of my PhD candidature, and sections of these articles are included in the present thesis.

Publications under review:

Tuyet T.B Tran, Peter C. Blumbergs and James A. Temlett (2008)

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ABBREVIATIONS

AA	-ascorbic acid
AA(A/D)C	-aromatic l-amino acid decarboxylase
Ab	-antibody
ACh	-acetylcholine
AChE	-acetylcholinesterase
AD	-Alzheimer's disease
AIF	-apoptosis-inducing factor
α-syn	-alpha synuclein
ALDH	-aldehyde dehydrogenase
AMPA	-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPT	-alpha-methyl-p-tyrosine
ANOVA	-analysis of variance
AP	-antero posterior
APTP	-1-amino-4-phenyl-1,2,3,6-tetrahydropyridine
APP+	-1-amino-4-phenyl-pyridinium
ARs	-aldose reductase
ART	-artemin
ATP	-adenosine triphosphate
Bax	- Bcl-2-associated protein X
BDNF	-brain-derived neurotrophic factor
bFGF	-basic fibroblast growth factor

BG	-basal ganglia
BJAB	-B lymphoma cell line
BMPs	-bone morphogenic factors
BSA	-bovine serum albumin
BSS	-balanced salt solution
Ca^{2+}	-calcium
cAMP	-3'-5'-cyclic adenosine monophosphate
CGC	-cerebellar granule cells
CoQ(10)	-coenzyme Q(10)
COMT	-catechol-O-methyl transferase
CNS	-central nervous system
Cu-SOD	-copper superoxide dismutase
cGMP	-Guanosine 3,5-cyclic monophosphate
DA	-dopamine
DAergic	-dopaminergic neurons
D1	-dopamine receptor, type 1
D2	-dopamine receptor, type 2
DAB	-3,3'-diaminobenzidine-Tetrahydrochloride
DAPI	-4,6-diamino-2-phenolindol dihydrochloride
DAT	-dopamine transport
DBS	-deep brain stimulation
DCF	-2',7'-dichlorofluorescin
DIV	-days in vitro

DMEM -modified basal medium, Eagle

DMSO -dimethyl sulfoxide

DLBD -dementia with LB disease

DOPAC -3,4-dihydroxyphenylacetic acid

DOPAL -dihydroxyphenylacetaldehyde

DOPET -3, 4-dihydroxyphenylethanol

DPX -DePex mounting medium

DTC -dithiocarbamate

DV -dorso ventral

E -embryonic

EDTA -ethylenediaminetetraacetic acid

ELISA -enzyme-linked immunosorbent assay

En-1 -engrailed

Enk -enkephalin

Epo -erythropoietin

ERKs -extracellular signal-regulated kinases

ETC -electron transport chain

FADD -Fas-associated death domain

FGF8 -fibroblast growth factor-8

FD -fluorodopa

FJC -fluoro jade C

Foxa2 -Forkhead box protein A2

FP -floor plate

FPD	- familial Parkinsons Disease
GABA	-gamma-aminobutyric acid
GADPH	- glyceraldehyde-3-phosphate dehydrogenase
GBSS	-Geys balanced salt solution
Gbx2	-gastrulation brain homeobox 2
GDNF	-glial-derived neurotrophic factor
GDF5	-growth/differentiation factor 5
GFAP	-glial fibrillary acidic protein
GLU	-glutamate
GM-CSF	-granulocyte macrophage colony-stimulating factor
GP	-globus pallidus
GPe	-external segment of the globus pallidus
GPi	-internal segment of the globus pallidus
GPx	-glutathione peroxidase
GSSG	-oxidised glutathione
GSH	-glutathione
h/hr	-hour
H&E	-haemoatoxylin and eosin
HBSS	-Hank's balanced salt solution
HB-EGF	-heparin-binding epidermal growth factor
Hesc	-human embryonic stem cells
HL-60	-human promyelocytic leukemia cell line
HP100	- H_2O_2-resistant cell line

HRP	-horseradish peroxidase
HT1080	-human fibrosarcoma cell line
H_2O_2	-hydrogen peroxide
H_2SO_4	- sulphuric acid
5-HT	-serotonin
6-OHDA	-6-Hydroxydopamine
Fe^{2+}	-iron
IBMX	-isobutylmethylxanthine
IMVS	-Institute of Medical and Veterinary Science
IFN-γ	-interferon gamma
IL-1β	-interleukin-1β
IL-6	interleukin-6
iNOs	-inducible nitric oxide synthase
IPD	-idiopathic Parkinsons's disease
JNK	-Jun N-terminal kinase
LB	-Lewy body
L-dopa	-levodopa
LDH	-lactate dehydrogenase
Lmx1	-Lim-domain transcription factors
LPS	-lipopolysaccharide
MAPK	-Mitogen-activated protein (MAP) kinases
mDNs	-midbrain/mesencephalic dopaminergic neurons
M	-molar

MAO-B	-monoamine oxidase B
MHB	-mid-hindbrain organizer
MHJ	-midbrain–hindbrain junction
μm	-micromolar
μl	-microlitres
mg	-milligrams
mins	-minutes
MN9D	-midbrain-derived dopaminergic neuronal cell line
Mn-SOD	-magnesium superoxide dismutase
Mn–EBDC	-manganese ethylene-bis-dithiocarbamate
MPDP	-1-methyl-4-phenyl-2,3-dihydropyridinium
MPPC	-1 -methyl-4-phenylpyridine
MPPP	-1-methyl-phenyl-propion-oxypiperidine
MPP^+	-N-methyl-1-4-phenylpyridinium ion
MPTP	-1-methyl-4-phenl-1,2,3,6-tetrahydropyridine
Mw	-molecular weight
NA	-noradrenaline
NAC	-N-acetylcysteine
NDI1	-single-subunit nicotinamide adenine dinucleotide (reduced) dehydrogenase of Saccharomyces cerevisiae
NMDA	-N-methyl-D-aspartic acid
NADPH	-Nicotinamide adenine dinucleotide phosphate
NADH	-Nicotinamide adenine dinucleotide

NADH-DH	-NADH-dehydrogenase
3-NT	-3-Nitrotyrosine
n	-number
nM	-nanomolar
NGF	-nerve growth factor
NM	-neuromelanin
$NO^{\bullet}$	-nitric oxide
NTF	-neurotrophic factor
NRT	-neurturin
NSAIDs	-nonsteroidal anti-inflammatory drugs
NT-3	-neurotrophin-3
NT-4/5	-neurotrophin-4/5
NMDA	-N-methyl-D-aspartate
NF- κB	-nuclear factor κB
Nurr1	-Nuclear receptor related 1
O_2	-oxygen
$OH^{\bullet}$	-hydroxyl radical
$\bullet O2^{-}$	-superoxide anion radical (superoxide)
ONOO–	-peroxynitrite
OS	-oxidative stress
Otx2	-homeobox protein
PARP	-poly (ADP-ribose) polymerase
Pax-	-paired-like homeodomain proteins

PBS	-phosphate buffered saline
PC12 cells	-rat pheochromocytoma cell line
PD	-Parkinson's disease
P7	-postnatal day 7 (seven day old)
P75	-low affinity neurotrophin receptor
PBS	-phosphate buffered saline
PET	-positron emission tomography
Pitx3	-paired-like homeodomain transcription factor 3
PKC	-Protein Kinase C
PPX	-pramipexole
PSP	-persephin
Ref	-reference
ROI	-reactive oxygen intermediates
ROP	-ropinirole
ROS	-reactive oxygen species
RRF	-retrorubral field
SAPK	-stress-activated protein kinase
SD	-standard deviation
SEM	-standard error of the mean
sFas	- soluble Fas
Shh	- Sonic hedgehog
SH-SY5Y	-human dopaminergic cells
SK-N-SH	-human neuroblastoma cell line

SN	-substantia nigra (A9)
SNpc	-substantia nigra pars compacta
SNpr	-substantia nigra pars reticulate
SOD	-superoxide dismutase
SP	-substance P
SPC	-Pierce peroxidase conjugated streptavidin tertiary
STN	-subthalamic nucleus
ST	-striatum
RPM	-revolutions per minute
TBARS	-thiobabituric acid reactive substances
TBS	-Tris buffered saline
TH	-tyrosine hydroxylase
TH-ir	-tyrosine hydroxylase immunoreactive
TGF-β	-transforming growth factor beta
TMB	-3,3′,5,5′-Tetramethylbenzidine
TNF	-tumor necrosis factor
TNFα-R1	- tumor necrosis factor alpha receptor 1
TRADD	-TNFRSF1A-associated via death domain
TUNEL	-terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
Trk	- tyrosine receptor kinase
UV	-ultraviolet
VM	-ventral mesencephalon
VMAT	-vesicular monoamine transporter

VMB	-ventral midbrain
VTA	-ventral tegmental area (A10)
Wnt1	-Wingless-type MMTV integration site family
WT	-wild type
Zn-SOD	-zinc superoxide dismutase
Z-DEVD-fmk	-Caspase-3 Inhibitor
^{o}**C**	-degrees Celsius

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ABSTRACT

In this study we aimed to investigate the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone neurotoxins on dopaminergic (DAergic) neuronal survival using ventral mesencephalic (VM) organotypic cell culture derived from postnatal rat pups (P4-5) and immunocytochemistry for tyrosine hydroxylase (TH) as a marker of DAergic cells. In addition, we examined the neuroprotective effects of glial cell line-derived neurotrophic factor (GDNF) on TH-ir cells exposed to MPTP and rotenone as a possible treatment for PD.

The TH-ir cells in co-cultures with striatum (ST) as a target grew better then when VM was cultured alone and that TH-ir cells in co-cultures could be maintained without using conditioned and trophic media. We treated 7 day and 14 day co-cultures at different times with varying MPTP and rotenone concentrations and found 14 day old cultures were more vulnerable than 7 day old co-cultures to the effects of either neurotoxin with TH-ir cell numbers significantly lower in 14 day cultures compared to 7 day cultures. Both neurotoxins induced a dose-dependent TH-ir cell reduction in the co-cultures. In addition we compared the toxicity of MPTP and its active metabolite 1-methyl-4-phenylpyridinium (MPP^+) as the neurotoxic effects of MPTP on DAergic cells depends on its conversion to MPP^+ by astrocytes. We found no significant difference in TH-ir cell reduction in co-cultures treated with MPTP and MPP^+. Rotenone was more toxic than MPTP with less TH-ir cell survival in the weeks post treatment. GDNF exposure produced increased cell size and significant increases in TH-ir cell branching in co-cultures in a dose-dependent manner. Post treatment of GDNF against MPTP and

rotenone provided significant neuroprotection as TH-ir cell survival was at the lower neurotoxin doses and not at the higher doses.