BCL-2 FAMILY OF PROTEINS AND CELL CYCLE REGULATORY GENES PLAY A ROLE IN THE REGULATION OF APOPTOSIS INDUCED BY LITHIUM AND CALYCULIN-A IN HL-60 CELLS

By

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree Master of Science has not previously been submitted by me for a degree at this or any other University, that this is my own work in design and in execution, and that all materials contained therein have been duly acknowledged.

Signed: _____

Date: _____

DEDICATION

This thesis is dedicated to my mother Matlhamu Sellina Ngobeni and my late father Tsakani Donald Tshabalala *etlela hi kurhula nkovana.*

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"*Ndzi nkhensile*"

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LIST OF ABBREVIATIONS

- Akt - Protein kinase B (PKB)
- Apaf-1 - Apoptotic protease activating factor-1
- ATCC - American type culture collection
- Bad - Bcl-2-X_L/Bcl-2 associated death promoter
- Bax - Bcl-2 associated protein-X
- BCA - Bicinchoninic acid
- Bcl-2 - B-cell leukaemia-2
- Bcl-X_L - Anti-apoptotic Bcl-2 family member
- Bcl-X_S - Anti-apoptotic Bcl-2 family member
- BH1-3 - Bcl-2 homologue regions 1, 2 and 3
- Bid - BH3-interacting domain death agonist
- Bik - Bcl-2 interacting killer
- CAPS - 3-[cyclohexylamino] -1- propanesulphonic acid
- Cdc2 - Cell division cycle-2 protein
- CDKs - Cyclin-dependent kinases
- cDNA - Complementary DNA
- CL-A - Calyculin-A
- CNS - Central nervous system
- CO_2 - Carbon dioxide
- -COOH - Carboxyl group
- ^oC - Degrees centigrade (Celsius)
- DAG - Diacylglycerol
- DISC - Death-inducing signaling complex
- DNA - Deoxyribose nucleic acid
- DNase - Deoxyribonuclease
- EDTA - Ethylenediaminetetraacetic acid
- dATP - Deoxyadenosine triphosphate
- dNTPs - Deoxyribonucleotide triphosphates

- FADD - Fas-associated death domain
- FasL - Fas ligand
- FBS - Foetal bovine serum
- FLICE - FADD-like interleukin-1-converting enzyme
- G0-phase - Gap-0 (zero) phase of the eukaryotic cell division cycle
- G1-phase - Gap-1 phase of the eukaryotic cell division cycle
- G2-phase - Gap-2 phase of the eukaryotic cell division cycle
- GSK-3 - Glycogen synthase kinase-3
- h - Hour
- H1 - Histone-1
- H_2O - Dihydrogen monoxide (Water)
- HL-60 - Human promyelocytic leukaemia cells
- HRP - Horseradish peroxidase
- I-(1-2) - Inhibitor (heat-stable inhibitor protein)
- IC_{50} - Half maximal inhibitory concentration
- IgG - Immunoglobulin G
- IP - Inositol monophosphate
- IP3 - Inositol 1,4,5-triphosphate
- K^+ - Potassium ion
- kDA - Kilodalton
- K_i - Concentration of inhibition which doubles the observed K_m of the enzyme
- K_m - Michaelis constant
- Li^+ - Lithium ion
- LiCl - Lithium chloride
- mA - Milliamperes
- MDM2 - Murine double minute 2
- ml - Millilitres
- min - Minute

- mM - Millimolar
- MOPS - 3-(N-morpholino) propanesulfonic acid
- MPF - Mitotic phase promoting factor/ Maturation promoting factor
- M-phase - Mitotic phase
- mRNA - messenger RNA
- MuLV - Murine leukaemia virus
- Na^{+} - Sodium ion
- NaCl - Sodium chloride
- Nek 2 - NIMA-related protein kinase 2
- NIMA - Never in mitosis gene A
- nM - Nanomolar
- NP-40 - Non-ionic detergent P-40
- PBS - Phosphate-buffered saline
- PCR - Polymerase chain reaction
- PI - Polyphosphoinositol
- PIP2 - Phosphatidylinositol bisphosphate
- PKB - Protein kinase B
- PLC - Phospholipase C
- PMSF - Phenylmethylsulfonyl fluoride
- PP1 - Protein phosphatase type-1
- PP2A - Protein phosphatase type-2A
- PP2B - Protein phosphatase type-2B
- PP2C - Protein phosphatase type-2C
- PPM - Mg^{2+}-dependent protein phosphatase
- PPP - Phosphoprotein phosphatase
- pRB - Retinoblastoma protein
- PSN - Penicillin/streptomycin/neomycin cocktail
- PTP - Protein tyrosine phosphatase
- R - Restriction point (cell cycle)

- RPMI - Roswell Park Memorial Institute
- RNA - Ribose nucleic acid
- RNase - Ribonuclease
- rpm - Revolutions per minute
- rRNA - Ribosomal RNA
- RT-PCR - Reverse transcription-polymerase chain reaction
- SDS - Sodium dodecyl sulphate
- SDS-PAGE - Sodium dodecylsulphate-polyacrylamide gel electrophoresis
- sec - Second
- S-phase - DNA synthesis phase
- Ser - Serine
- TBS - Tris-buffered saline
- TEMED - Tetramethylethylenediamine
- Thr - Threonine
- TNF - Tumour necrosis factor
- TRIS - Trishydroxymethylaminomethane
- Tyr - Tyrosine
- UV - Ultraviolet
- µl - Microliter

ABSTRACT

The biochemical mechanism of apoptosis induced by lithium remains unclear, although there is evidence suggesting the involvement of Bax and Bcl-2. Bcl-2 family of proteins play a critical role in the regulation of apoptosis in various tumour cell lines. This pathway may be altered in cancer cells. We have used calyculin-A (CL-A), an inhibitor of protein phosphatase 2A (PP2A), to investigate the mechanism by which lithium induces apoptosis in HL-60 cells. Previous studies in our laboratory established that lithium induces apoptosis of HL-60 cells at 10 mM and above; while CL-A induces apoptosis at 1 nM and above. The observed apoptotic effects were additive. These observations led to the hypothesis that lithium and CL-A exert their biological effects by acting on a similar target. It was, therefore, the aim of this study to establish whether lithium would also exert similar inhibitory effects on the apoptotic and cell cycle regulatory genes. We further aimed at delineating the effects of both lithium and CL-A on the expression profiles of apoptotic and cell cycle regulatory genes. In this study, HL-60 cells were treated with lithium, CL-A and the combination of both. This was followed by the assessment of cell proliferation and viability at specific time points, using Coulter Counter and trypan blue dye exclusion assay, respectively. Concentrations of lithium at 10 mM and 20 mM were found to inhibit cell proliferation and exerted modest effects on cell viability in a time- and dose-dependent manner. Likewise, CL-A inhibited cell proliferation and viability in a time- and dose-dependent fashion. The combination of lithium and CL-A showed additive inhibitory effects on the growth of HL-60 cells.

Further, semi-quantitative RT-PCR analyses of apoptotic (*bax* and *bcl-2*) and cell cycle regulatory genes (*cdc2* and *cyclin-B1*) were determined. Our data revealed an under-expression of *bcl*-2 mRNA and an up-regulation of *bax* mRNA in HL-60 cells treated with lithium, CL-A and the combination of both. In addition, the expression levels of *cdc2* mRNA remained constant, while *cyclin-B1* mRNA expression levels were up-regulated after 24 h in HL-60 cells that were treated with cytotoxic concentrations of lithium and CL-A alone. Furthermore, the combination of lithium and CL-A showed an

up-regulation of *cyclin-B1* mRNA while *cdc2* mRNA levels remained constant in both treated and untreated HL-60 cells.

To corroborate the RT-PCR data, we present evidence by Western blot analysis that Bcl-2 family of proteins and cell cycle regulatory genes indeed play a critical role in the regulation of apoptosis in HL-60 cells. Western blot analysis revealed a down-regulation of Bcl-2 under all treatment conditions. However, lithium and CL-A alone failed to show any detectable expression levels of both Bax and cyclin-B1 proteins. In contrast, the combination of both lithium and CL-A showed an up-regulation of Bax and Cdc2 proteins in HL-60 cells. These findings suggest that the molecular mechanism elicited by lithium, CL-A and the combination of both on the growth inhibition of HL-60 cells involves an aberrant expression of apoptotic and cell cycle regulatory genes. In addition, these observations may allude to a notion that both lithium and CL-A may be used and administered successfully as positive alternative anticancer drugs.