The role of TRPM2 channels in oxidative stress-induced liver damage

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Contents

List of Abbreviations ..................................................................................................................... i
Abstract ........................................................................................................................................v
Declaration of Originality ............................................................................................................. vii
Acknowledgements ..................................................................................................................... x
Chapter 1: Research Background ............................................................................................... 1
  1.1 Introduction ........................................................................................................................... 1
  1.2 Oxidative Stress ..................................................................................................................... 4
    1.2.1 Systems Eliminating Oxidative Compounds ...................................................................... 5
  1.2.2 Cellular and Molecular Targets of Oxidative Stress .......................................................... 6
  1.2.3 Mechanisms of Oxidative Stress Mediated Cellular Damage ........................................... 8
  1.3 Oxidative Damage and Ca$^{2+}$ Signalling ............................................................................ 11
  1.4 TRP Channels in Oxidative Stress ......................................................................................... 12
  1.5 TRPM2 Channels .................................................................................................................. 15
    1.5.1 History ............................................................................................................................ 15
    1.5.2 TRPM Subfamily ............................................................................................................ 16
    1.5.3 TRPM2 Channel Isoforms and Variants ........................................................................... 16
      1.5.3.1 TRMP2-S .................................................................................................................. 18
      1.5.3.2 SSF-TRPM2 ............................................................................................................. 18
      1.5.3.3 TRPM2-ΔN ............................................................................................................... 19
      1.5.3.4 TRPM2-ΔC ............................................................................................................... 19
      1.5.3.5 TRPM2-ΔNΔC ........................................................................................................... 19
    1.5.4 TRPM2 Structure ............................................................................................................. 19
      1.5.4.1 TRPM2 Channel Topology ....................................................................................... 19
      1.5.4.2 Nudix Box and NUDT9-H ...................................................................................... 22
    1.5.5 Cellular localizations of TRPM2 channel ....................................................................... 23
    1.5.6 TRPM2 Channel Activation ............................................................................................. 24
      1.5.6.1 TRPM2 Gating ......................................................................................................... 24
      1.5.6.2 TRPM2 Channel Activators ...................................................................................... 24
      1.5.6.3 Direct TRPM2 Activators ......................................................................................... 25
      1.5.6.4 Indirect TRPM2 Channel Activators ......................................................................... 28
    1.5.7 TRPM2 Channel Blockers ................................................................................................. 30
      1.5.7.1 ACA ........................................................................................................................ 31
      1.5.7.2 Fenamates .............................................................................................................. 31
      1.5.7.3 Clotrimazole and other Azoles ................................................................................ 32
      1.5.7.4 2-APB ...................................................................................................................... 33
    1.5.8 The Role of TRPM2 Channels in oxidative stress-related pathologies ............................. 33
  1.6 Oxidative Stress and Liver Diseases ..................................................................................... 37
  1.7 Acetaminophen ..................................................................................................................... 40
    1.7.1 History of discovery ....................................................................................................... 40
    1.7.2 Acetaminophen Pharmacokinetics .................................................................................. 41
    1.7.3 Acetaminophen Pharmacodynamics ............................................................................. 42
    1.7.4 Acetaminophen Overdose and Liver Damage ................................................................. 44
    1.7.5 Oxidative Damage in Acetaminophen Toxicity ............................................................... 46
Chapter 2: The Role of the TRPM2 Channel in Acetaminophen-mediated [Ca^{2+}]_{i} rise in rat hepatocytes

2.1 Introduction .................................................................................................................. 56
2.2 Methods and Materials .................................................................................................. 56
  2.2.1 Chemicals .................................................................................................................. 56
  2.2.2 Animals .................................................................................................................... 56
  2.2.3 Solutions .................................................................................................................. 56
  2.2.4 Hepatocyte Isolation and Culture ............................................................................ 56
  2.2.5 Calcium Imaging ..................................................................................................... 56
  2.2.6 Immunofluorescence Imaging .................................................................................. 56
  2.2.7 Western Blotting .................................................................................................... 56
  2.2.8 Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Quantitative RT-PCR .................................................................................................................. 56
  2.2.9 Patch-clamp Recording ............................................................................................ 56
  2.2.10 TRPM2 Knocked Down (KD) Hepatocytes .............................................................. 56
  2.2.11 HEK 293T Cells Culture and Transfection ............................................................. 56
  2.2.12 Statistical Analysis ................................................................................................ 56
2.3 Results .......................................................................................................................... 56
  2.3.1 Expression of functional TRPM2 channel in rat hepatocytes ................................. 56
  2.3.2 Activation of Ca^{2+} entry and non-selective cation current in rat hepatocytes in response to treatment by H_{2}O_{2} and acetaminophen .......................................................... 56
  2.3.3 siRNA-mediated knock down of TRPM2 protein attenuates H_{2}O_{2} and acetaminophen-induced Ca^{2+} entry and the cation current ......................................................... 56
  2.3.4 H_{2}O_{2} and acetaminophen-induced Ca^{2+} entry requires ADPR.............................. 56
  2.4 Discussion ................................................................................................................... 56

Chapter 3: The role of TRPM2 Channel in acetaminophen-induced Hepatocellular Damage

3.1 Introduction ................................................................................................................... 88
3.2 Methods and Materials .................................................................................................. 88
  3.2.1 Chemicals ................................................................................................................ 88
  3.2.2 Animals .................................................................................................................... 88
  3.2.3 Solutions .................................................................................................................. 88
  3.2.4 Estimation of the number of dead cells using Trypan blue ....................................... 88
  3.2.5 Induction of In Vivo Acetaminophen Toxicity in Mice .............................................. 88
  3.2.6 Blood Liver Enzymes Assay .................................................................................... 88
  3.2.7 Histopathology ...................................................................................................... 88
  3.2.8 Hepatocyte Isolation and Culture .......................................................................... 88
  3.2.9 Calcium Imaging ..................................................................................................... 88
  3.2.10 Western Blotting .................................................................................................... 88
  3.2.11 RT-PCR .................................................................................................................. 88
  3.2.12 Patch-clamp Recording ......................................................................................... 88
3.3 Results .......................................................................................................................... 88
3.3.1 TRPM2 channel inhibitor, ACA, attenuates acetaminophen-induced hepatocellular death in culture .......................................................... 97
3.3.2 TRPM2 expression in mouse hepatocytes ......................................... 97
3.3.3 The effect of knocking-out TRPM2 channel on Ca$_{2+}$ entry and non-selective cation current in H$_2$O$_2$- or acetaminophen-treated mouse hepatocytes ............................................................... 98
3.3.3 The effect of TRPM2 channel knock-out on acetaminophen-induced liver damage ........................................................................... 103
3.4 Discussion ......................................................................................... 104

Chapter 4: H$_2$O$_2$- and Acetaminophen-induced Oxidative Stress Initiates
TRPM2 Channel Trafficking to the Plasma Membrane in Rat Hepatocytes ...... 110
4.1 Introduction ...................................................................................... 110
4.2 Methods and Materials ...................................................................... 112
  4.2.1 Chemicals ................................................................................. 112
  4.2.2 Animals .................................................................................... 112
  4.2.3 Solutions ................................................................................. 112
  4.2.4 Hepatocyte Isolation and Culture ............................................... 113
  4.2.5 Detection of TRPM2 Protein on the Plasma Membrane Using Cell Surface Biotinylation ................................................................. 113
  4.2.6 Confocal Microscope Imaging ..................................................... 114
  4.2.7 Statistical Analysis ................................................................... 115
4.3 Results .............................................................................................. 115
4.4 Discussion ......................................................................................... 116

Chapter 5: TRPM2 Channel Inhibition—A Novel Property of Chlorpromazine and Curcumin ........................................................................ 121
5.1 Introduction ...................................................................................... 121
5.2 Methods and Materials ...................................................................... 123
  5.2.1 Chemicals ................................................................................. 123
  5.2.2 Animals .................................................................................... 123
  5.2.3 Solutions ................................................................................. 123
  5.2.4 Hepatocyte Isolation and Culture ............................................... 124
  5.2.5 HEK 293T Cell Line Culture and Transfection ................................ 124
  5.2.6 Calcium Imaging ....................................................................... 125
  5.2.7 Patch-clamp Recording ............................................................... 125
  5.2.8 Statistical Analysis ................................................................... 125
5.3 Results .............................................................................................. 126
5.4 Discussion ......................................................................................... 135

Chapter 6: General Discussion ................................................................ 139

Appendix ............................................................................................... 146

References ............................................................................................ 152
List of Figures

Figure 1.1 TRP channels superfamily .............................................................. 14
Figure 1.2: TRPM2 isoforms ........................................................................ 17
Figure 1.3: TRPM2 channel ......................................................................... 21
Figure 1.4: Acetaminophen excretion and metabolism ............................... 43
Figure 2.1: Calibration of Ca^{2+} imaging system using ionomycin and EGTA 61
Figure 2.2: Validation of $2^{\Delta \Delta C_T}$ method .............................................. 65
Figure 2.3: TRPM2 expression in hepatocytes .............................................. 69
Figure 2.4: TRPM2 current in rat hepatocytes .............................................. 70
Figure 2.5: Inhibition of TRPM2 current by ACA and clotrimazole .............. 71
Figure 2.6: Activation of TRPM2 current in response to H_{2}O_{2} in hepatocytes 72
Figure 2.7: Acetaminophen and H_{2}O_{2} activate Ca^{2+} entry in rat hepatocytes 74
Figure 2.8: The effect of NAPQI on $[Ca^{2+}]_c$ in hepatocytes ..................... 75
Figure 2.9: H_{2}O_{2} and acetaminophen activate a non-selective cation current in rat hepatocytes .......................................................... 77
Figure 2.10: siRNA-mediated knockdown of TRPM2 expression ................. 78
Figure 2.11: The effect of TRPM2 knockdown on hepatocyte Ca^{2+} entry ....... 81
Figure 2.12: The effect of TRPM2 knockdown on ADPR-, H_{2}O_{2}- and acetaminophen-induced current in rat hepatocytes ................................................ 82
Figure 2.13: The role of ADPR in activation of Ca^{2+} entry in hepatocytes .... 83
Figure 2.14: The effect of PARP inhibitor on Ca^{2+} entry in hepatocytes ......... 84
Figure 3.1: Area selection in mouse liver sections using CellSense software .... 93
Figure 3.2: TRPM2 channel expression in mice .......................................... 99
Figure 3.3: H_{2}O_{2}- and acetaminophen-activated Ca^{2+} entry is attenuated in TRPM2 KO mouse hepatocytes ................................................................. 101
Figure 3.4: Acetaminophen and H_{2}O_{2} activate a nonselective cation current in mouse hepatocytes ................................................................. 102
Figure 3.5: The acetaminophen effect on AST and ALT in the experimental groups 105
Figure 3.6: Histopathology of the liver slices obtained from acetaminophen-treated WT and TRPM2 KO mice ................................................................. 106
Figure 3.7: The acetaminophen-induced necrotic areas in liver sections ....... 107
Figure 4.1: The effect of H₂O₂ on TRPM2 channel expression on hepatocyte plasma membrane ................................................................. 116
Figure 4.2: Effect of H₂O₂ and acetaminophen on TRPM2 channel trafficking in hepatocytes ........................................................................ 118
Figure 5.1: Chlorpromazine blocks ADPR- and acetaminophen-activated TRPM2 current in rat hepatocytes and HEK293T cells ............................................................................................................ 127
Figure 5.2: The effect of curcumin on [Ca²⁺]ᵢ in acetaminophen- and H₂O₂-treated hepatocytes .............................................................................................................................................. 130
Figure 5.3: The effect of curcumin on membrane current of acetaminophen- and H₂O₂-treated hepatocytes ............................................................................................................................................... 131
Figure 5.4: The effect of curcumin and NAC on the ADPR-activated TRPM2 current in hepatocytes ...................................................................................................................................................... 133
Figure 5.5: The effect of curcumin on ADPR-activated current in HEK 293T cells expressing TRPM2 ...................................................................................................................................................... 134
Figure 5.6: The wash-out the effect of curcumin on ADPR-activated current in TRPM2-expressing HEK 293T cells ...................................................................................................................................................... 136
List of Tables

Table 1.1: Drugs that induce liver damage through oxidative stress and the corresponding pathologies .......................................................... 39
Table 2.1: The primers used in RT-PCR and quantitative RT-PCR experiments ........ 64
Table 3.1: List of primers used to detect TRPM2 in mouse hepatocytes ................. 96
Table 3.2: The protective effect of ACA against acetaminophen-induced hepatocellular damage ................................................................. 97
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>Ab</td>
<td>antibody</td>
</tr>
<tr>
<td>ACA</td>
<td>anthranilic acid</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ADPR</td>
<td>adenosine diphosphate ribose</td>
</tr>
<tr>
<td>ALT</td>
<td>alcoholic liver disease</td>
</tr>
<tr>
<td>AMAP</td>
<td>acetyl-m-aminophenol</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>AP</td>
<td>apurinic/apyrimidinic</td>
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<tr>
<td>APECED</td>
<td>autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy</td>
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<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>BER</td>
<td>base excision repair</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>cADPR</td>
<td>cyclic-ADPR</td>
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<tr>
<td>CaM</td>
<td>calmodulin</td>
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<tr>
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<td>catalases</td>
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<td>central nervous system</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
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<tr>
<td>DAG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>DCDPC</td>
<td>dichlorodiphenylamine-2-carboxylica acid</td>
</tr>
<tr>
<td>DDW</td>
<td>double distilled water</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>DPQ</td>
<td>3,4-Dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone</td>
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<tr>
<td>--------------</td>
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<tr>
<td>DRG</td>
<td>dorsal root ganglia</td>
</tr>
<tr>
<td>DSB</td>
<td>double-strand break</td>
</tr>
<tr>
<td>ECL</td>
<td>enhanced chemiluminescence</td>
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<tr>
<td>EGTA</td>
<td>ethylene glycol tetraacetic acid</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FBS</td>
<td>foetal bovine serum</td>
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<td>FFA</td>
<td>Flufenamic acid</td>
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<td>FITC</td>
<td>fluorescein isothiocyanate</td>
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<tr>
<td>G6PD</td>
<td>glucose 6 phosphate dehydrogenase</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
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<tr>
<td>H$_2$O$_2$</td>
<td>hydrogen peroxide</td>
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<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>iNOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>ip</td>
<td>intra peritoneal</td>
</tr>
<tr>
<td>IR</td>
<td>ionising radiation</td>
</tr>
<tr>
<td>IVC</td>
<td>inferior vena cava</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinases</td>
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<tr>
<td>KD hepatocytes</td>
<td>knocked down hepatocytes</td>
</tr>
<tr>
<td>KRH</td>
<td>Krebs-Ringer-Hepes</td>
</tr>
<tr>
<td>KO</td>
<td>knocked out</td>
</tr>
<tr>
<td>LOO$^\cdot$</td>
<td>peroxy radical</td>
</tr>
<tr>
<td>LPO</td>
<td>lipid peroxidation</td>
</tr>
<tr>
<td>LTRPC</td>
<td>long TRP channels</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MOMPP</td>
<td>mitochondrial outer membrane permeabilisation</td>
</tr>
<tr>
<td>MPT</td>
<td>mitochondrial permeability transition</td>
</tr>
<tr>
<td>mPTP</td>
<td>mitochondrial permeability transition pores</td>
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<tr>
<td>NAC</td>
<td>N-Acetyl Cysteine</td>
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<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
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<tr>
<td>NAADP</td>
<td>nicotinic acid adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADP</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
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</table>
NADPH  nicotinamide adenine dinucleotide phosphate
NAPQI  N-acetyl-p-benzoquinone imine
NFW    nuclear free water
NIF    niflumic acid
NK     natural killer
NMDG   N-Methyl-D-glucamin
NSAID  non-steroidal anti-inflammatory drug
NO     nitric oxide
NOS    nitric oxide synthases
NUDT9-H Nudix-type motif 9 homology
O₂⁻    superoxide anion
OH⁻    hydroxyl radical
OTRPC  Osm TRP Channels
PBP    para-bromophenacyl bromide
PBS    phosphate buffered saline
PCR    polymerase chain reaction
PGs    prostaglandins
PMN    poly-morphonuclear
RNA    ribonucleic acid
RNS    reactive nitrogen species
ROS    reactive oxygen species
RT     reverse transcriptase
RT-PCR reverse transcription polymerase chain reaction
SDS    sodium dodecyl sulfate
SEM    standard error of the mean
SOD    superoxide dismutase
SPP    short-patch pathway
SR     sarcoplasmic reticulum
SSB    single-strand break
STRPC  short TRP channels
TBS    Tris-buffered saline
TEMED  tetramethylethylenediamine
TM     transmembrane
<table>
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<tr>
<td>TRP</td>
<td>transient receptor potential</td>
</tr>
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<td>TRPML</td>
<td>transient receptor potential mucolipin</td>
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<td>TRPP</td>
<td>transient receptor potential polycystin</td>
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<tr>
<td>UV</td>
<td>ultra-violet</td>
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<td>WT</td>
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Abstract

The increased production of highly reactive oxygen and nitrogen species plays a significant role in development of a number of liver disorders associated with hepatocellular death and impaired cell regeneration. Liver injury induced by drug toxicity, ischemia-reperfusion, excessive alcohol consumption and different types of viral hepatitis is in large part mediated by oxidative stress. Liver damage due to oxidative stress induced by drugs, including acetaminophen, accounts for 5% of all hospital admissions and for almost half of all acute liver failures.

One of the features of hepatocellular death mediated by oxidative stress is Ca\(^{2+}\) overload due its release from intracellular organelles and activation of ion channels on the plasma membrane. Ca\(^{2+}\) is fundamental for normal cellular functioning. Ca\(^{2+}\) signalling, mediated by the rise in free cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_c\)]), regulates many cellular events. However, a sustained rise in [Ca\(^{2+}\)\(_c\)] can be detrimental, leading to mitochondrial dysfunction and cell death through apoptosis and necrosis.

Although it is well recognised that Ca\(^{2+}\) plays a significant role in oxidative stress-induced liver damage, the molecular identities of the ion channels that provide a pathway for Ca\(^{2+}\) entry in hepatocytes remain unidentified.

One of the potential candidates that could be responsible for such Ca\(^{2+}\) entry pathway in hepatocytes is Transient Receptor Potential Melastatin 2 (TRPM2) channel. TRPM2 is a non-selective cation channel permeable to Na\(^+\) and Ca\(^{2+}\). The main physiological activator of TRPM2 channel is ADP-ribose, which binding to NUDT9-H motif in the TRPM2 C-terminus leads to the opening of the channel pore. It is known that oxidative stress promotes generation and release of ADPR from mitochondria and nuclei into the cytoplasmic space, thus promoting activation of TRPM2-mediated Ca\(^{2+}\) entry.

In this thesis, we hypothesised that oxidative stress-induced Ca\(^{2+}\) entry in hepatocytes is mediated by TRPM2 channels, and used acetaminophen overdose as a model of oxidative stress-induced liver damage. We show that hepatocytes express long isoform of TRPM2, which mediates ADPR- and H\(_2\)O\(_2\)-induced Ca\(^{2+}\) entry and the cation current in these cells. Furthermore, we show that TRPM2 channels are activated in hepatocytes treated with high concentrations of acetaminophen and are responsible for Ca\(^{2+}\) overload in acetaminophen-induced liver toxicity. Experiments using TRPM2 KO mice provide first evidence of a pivotal role of TRPM2 channels in acetaminophen-induced
liver injury, showing that lack of TRPM2 expression largely protects liver from acetaminophen overdose.

An important finding that TRPM2 channels translocate from intracellular compartments to the plasma membrane provides explanation for a slow development of Ca$^{2+}$ entry in response to H$_2$O$_2$ and acetaminophen.

Finally, we show that substances previously known to protect liver from acetaminophen-induced damage are, in fact, inhibitors of TRPM2 current. Chlorpromazine, an antipsychotic drug, reversibly blocks TRPM2 channel pore, and curcumin, a chemical found in common spice, potently blocks activation of TRPM2 current by ADPR.

The results presented in this thesis provide a fundamental knowledge about the role of TRPM2 channels in oxidative stress-induced liver injury, but also open a new chapter in search for the new drugs and drug targets for the treatment of a number of oxidative stress-related liver pathologies.
Declaration of Originality

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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AUTHOR STATEMENTS
Chapter 2 & 3:

“The Role of the TRPM2 Channel in Acetaminophen-induced Hepatocellular Damage”

“The Lack of TRPM2 Channel–prevented Acetaminophen-induced Hepatocellular Damage in Mice”

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The main results of these chapter were published as a part of manuscript: “TRPM2 channels mediate acetaminophen-induced liver damage” Proceedings of the National Academy of Sciences of the USA, vol. 111, pp. 3176-3181 (Appendix).

The authors’ responsibilities were as follows:

Ehsan Kheradpezhouh was responsible for the conception and design of the study, collection and assembly of data, data analysis and interpretation, and writing and preparation of the manuscript.

Linlin Ma contributed to design and collection western blot and PCR blot data, and data analysis and interpretation.

Arthur Morphett contributed to design of histopathologic examination of liver tissue samples.
Greg Barritt contributed to the conception and design of the study, data interpretation and preparation of the manuscript.

Grigori Rychkov was responsible for the conception and design of the study, collection of data, data analysis and interpretation, writing and preparation of the manuscript, and acted as the corresponding author.

Authors Signatures:
I agree with the author contributions for the manuscript “TRPM2 channels mediate acetaminophen-induced liver damage”, and give permission for the use of this manuscript in the thesis.

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Linlin Ma ..............................................................................

Arthur Morphett .................................................................

Greg Barritt ...........................................................................

Grigori Rychkov .....................................................................
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I also want to acknowledge Dr David Wilson for his precious comments during my study. A great thank you to Elite Editing Company for proof reading of my thesis.

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