

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

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List of Abbreviations

| | |
|--------|---|
| aa | amino acid |
| Ab | antibody |
| ACA | anthranilic acid |
| ADP | adenosine diphosphate |
| ADPR | adenosine diphosphate ribose |
| ALD | alcoholic liver disease |
| ALT | alanine aminotransferase |
| AMAP | acetyl-m-aminophenol |
| AMP | adenosine monophosphate |
| ANOVA | analysis of variance |
| AP | apurinic/apyrimidinic |
| APECED | autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy |
| AST | aspartate aminotransferase |
| ATP | adenosine triphosphate |
| BER | base excision repair |
| BSA | bovine serum albumin |
| cADPR | cyclic-ADPR |
| CaM | calmodulin |
| CATs | catalases |
| CNS | central nervous system |
| COPD | chronic obstructive pulmonary disease |
| COX | cyclooxygenase |
| DAG | diacylglycerol |
| DCDPC | dichlorodiphenylamine-2-carboxylic acid |
| DDW | double distilled water |
| DM | diabetes mellitus |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | dimethyl sulfoxide |
| DPQ | 3,4-Dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone |

| | |
|-------------------------------|---|
| DRG | dorsal root ganglia |
| DSB | double-strand break |
| ECL | enhanced chemiluminescence |
| EGTA | ethylene glycol tetraacetic acid |
| ER | endoplasmic reticulum |
| FBS | foetal bovine serum |
| FFA | Flufenamic acid |
| FITC | fluorescein isothiocyanate |
| G6PD | glucose 6 phosphate dehydrogenase |
| GI | gastrointestinal |
| GSH | glutathione |
| H ₂ O ₂ | hydrogen peroxide |
| H&E | hematoxylin and eosin |
| HRP | horseradish peroxidase |
| iNOS | nitric oxide synthase |
| ip | intra peritoneal |
| IR | ionising radiation |
| IVC | inferior vena cava |
| JNK | c-Jun N-terminal kinases |
| KD hepatocytes | knocked down hepatocytes |
| KRH | Krebs-Ringer-Hepes |
| KO | knocked out |
| LOO [·] | peroxyl radical |
| LPO | lipid peroxidation |
| LTRPC | long TRP channels |
| MAPK | mitogen-activated protein kinase |
| MOMP | mitochondrial outer membrane permeabilisation |
| MPT | mitochondrial permeability transition |
| mPTP | mitochondrial permeability transition pores |
| NAC | N-Acetyl Cysteine |
| NADH | nicotinamide adenine dinucleotide |
| NAADP | nicotinic acid adenine dinucleotide phosphate |
| NADP | nicotinamide adenine dinucleotide phosphate |

| | |
|------------------------------|---|
| NADPH | nicotinamide adenine dinucleotide phosphate |
| NAPQI | N-acetyl-p-benzoquinone imine |
| NFW | nuclear free water |
| NIF | niflumic acid |
| NK | natural killer |
| NMDG | <i>N</i> -Methyl-D-glucamin |
| NSAID | non-steroidal anti-inflammatory drug |
| NO | nitric oxide |
| NOS | nitric oxide synthases |
| NUDT9-H | Nudix-type motif 9 homology |
| O ₂ ^{•-} | 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 |

| | |
|-------|---|
| TRP | transient receptor potential |
| TRPML | transient receptor potential mucolipin |
| TRPP | transient receptor potential polycystin |
| UK | United Kingdom |
| US | United States |
| UV | ultra-violet |
| WT | wild-type |

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 ($[\text{Ca}^{2+}]_c$), regulates many cellular events. However, a sustained rise in $[\text{Ca}^{2+}]_c$ 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_2O_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_2O_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 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.

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