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

Ehsan Kheradpezhouh

Discipline of Physiology, School of Medical Sciences

University of Adelaide

Submitted for the degree of

Doctor of Philosophy

December 2014

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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-carboxylica 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_20_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
$LOO^{\cdot}$	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	*N*-Methyl-D-glucamin
NSAID	non-steroidal anti-inflammatory drug
NO	nitric oxide
NOS	nitric oxide synthases
NUDT9-H	Nudix-type motif 9 homology
$O_2^{-\cdot}$	superoxide anion
$OH^{\cdot}$	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 ($[Ca^{2+}]_c$), 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_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

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Kheradpezhouh E, Ma L, Morphett A, Barritt GJ, Rychkov GY 2014, 'TRPM2 channels mediate acetaminophen-induced liver damage', *Proceedings of the National Academy of Sciences of the USA*, vol. 111, pp. 3176-3181 (Appendix).

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"

Kheradpezhouh E[1], Ma L[1], Morphett A[2], Barritt GJ[2] & Rychkov GY[1]

[1]Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia.

[2]Department of Medical Biochemistry, School of Medicine, Flinders University, Adelaide, South Australia 5001, Australia

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.

Ehsan Kheradpezhouh ……

Linlin Ma ……..

Arthur Morphett …….

Greg Barritt …….

Grigori Rychkov …….

Acknowledgements

First and foremost, I would like to acknowledge my primary supervisor, A/Professor Grigori Rychkov, for his support, expertise and advice in all moments of my PhD candidature. Without doubt, his mentorship has greatly attributed in forming me as a scientist. In addition, I deeply want to appreciate my co-supervisor, Professor Greg Barritt for his knowledge and advices.

I would like to dedicate my especial thanks to my colleagues in the Cellular Physiology Laboratory, Dr Linlin Ma and Dr Nathan Scrimgeour for their help. In addition, I want to thank my colleagues in Medical Biochemistry department of Flinders University including Dr Yabin Zhoe, Mrs Jin Hua, Mr Eunus Ali and especially Dr John Phillips for advice on the preparation of mouse hepatocytes and Dr Arthur Morphett for his valuable advice on histopathologic evaluation of liver slices.

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.

I want to thank Adelaide University for financial support with providing me a scholarship and project funding.

At last, I would like to thank all my family members especially Mana for her great support in all ups and downs of my study.

To Mana for her unconditional love and support