Role of Autophagy in Ischemic Preconditioning Prior to Liver Ischemic Reperfusion in Aged Rats

Thesis

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Contents

List of Abbreviations

Abbrev.	Meaning
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AMPK	AMP-activated protein kinase
AST	Aspartate aminotransferase
Atg	Autophagy-related genes
ATP	Adenosine triphosphate
Bax	Bcl-2 associated X protein
Bcl	B-Cell Leukemia/Lymphoma
CMA	Chaperone-mediated autophagy
DNA	Deoxyribonucleic acid
d.f.	Degree of freedom
DPX	Distyrene, plasticizer and xylene
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
GOT	Glutamate oxaloacetate transaminase
GPx	Glutathione peroxidase
GPT	Glutamate pyruvate transaminase
GSH	Glutathione
H&E	Hematoxylin and eosin
HGF	Hepatocyte growth factor
HIF-1	Hypoxia inducible factor-1
HO-1	Heme oxygenase-1
HRP	Horseradish peroxidase

H2S	Hydrogen sulphide
IFN-γ	Interferon-gamma
IL	Interleukin
iNOS	Inducible nitric oxide synthase
I.P	Intra-peritoneal
IPC	Ischemic Preconditioning
IR	Ischemia Reperfusion
LC3	Microtubule-associated protein light chain 3
LC3-PE	Light chain 3-phosphatidylethanolamine
LDH	Lactate dehydrogenase
LSD	Least Significant Difference
3MA	3-methyladenine
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
MDH	Malate dehydrogenase
MPO	Myeloperoxidase
MPT	Mitochondrial permeability transition
mRNA	Messenger Ribonucleic acid
mTOR	mammalian target of rapamycin
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NS	Non-significant
OD	Optical density
PBS	Phosphate buffer solution
PH	Potential of hydrogen
PI3K	Phosphatidylinositol-3-kinase-
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species

rpm	Revolutions per minute
SD	Standard deviation
SEM	Standard error of mean
SOD	Superoxide dismutase
SPSS	Statistical Program for Social Science
SQSTM1	Sequestosome 1
SWOP	Second window of protection
TBA	Thiobarbituric acid
TEM	Transmission electron microscope
TLR4	Toll like receptor 4
TNF-α	Tumor necrosis factor-alpha
Ulk	Unc-51-like kinase
VEGF	Vascular endothelial growth factor

List of Figures

List of Tables

Introduction

With the rise in acute and chronic liver disorders, liver transplantation has become an important therapeutic strategy for patients with many end-stage liver diseases ***(Liu et al., 2015).*** Moreover, with advancing age, patients are more likely to acquire hepatic malignancies that are amenable to surgical resection and transplantation **(*Wang et al., 2011).*** However, the mortality rate is still high, and primary graft dysfunction is a major cause of morbidity and mortality that occurs early in the post-transplantation period due to ischemia-reperfusion (IR) injury ***(De Rougemont et al., 2009*)**. Thus searching for an effective preventive and treating method is mandatory ***(Suyavaran and Thirunavukkarasu, 2017).***

Liver IR injury is a phenomenon in which cellular damage due to hypoxia is exacerbated following the return of blood flow and the restoration of oxygen delivery **(*Xue et al., 2016*).** Oxidative stress and inflammation are the main causes that mediate IR induced liver injury ***(Ge et al., 2015)*** and hepatocyte apoptosis ***(Pantazi et al., 2016).***

Ischemic Preconditioning (IPC), which involves brief periods of ischemia and reperfusion before prolonged ischemia, was claimed to play a protective role against liver

IR injury **(*Chu et al., 2015*).** Many studies have demonstrated that IPC may be the most potent innate protective mechanism against IR injury and can attenuate the sustained IR injury in several organs such as heart ***(Iliodromitis et al., 2007),*** kidney ***(Salehipour et al., 2007),*** retina ***(Fan et al., 2016),*** and brain ***(Churchill et al., 2010).*** Studies have claimed that IPC has protective effects against hepatic IR injury ***(Montalvo-Jave et al., 2009).*** Meanwhile, ***Xue et al. (2016)*** stated that the mechanism by which IPC reduces liver IR injury remains to be fully elucidated.

Autophagy emerged as an intracellular self-digesting pathway in which abnormal proteins and damaged organelles are sequestrated into autophagosomes and degraded by lysosomes ***(Cursio et al., 2015).*** Many studies have shown that autophagy can be induced by various cellular conditions in IR, including energy starvation, oxidative stress, and inflammation ***(Huang et al., 2010).***

Evankovich et al. (2012) claimed that increasing beclin-1, the autophagic protein, reduced liver IR injury. Also, ***Sun et al. (2013)*** reported that inhibition of autophagy increased mitochondrial oxidative stress and hepatocellular necrosis following liver IR in rats.

On the other hand, ***Gotoh et al. (2009)*** reported increased levels of autophagy in hepatocytes following

anoxia/re-oxygenation triggers graft dysfunction and that autophagy suppression reduced liver damage and mortality in rats. ***Kang et al. (2014)*** reported that autophagy downregulation improved liver IR injury. Therefore, whether autophagy protects from or promotes liver injury following ischemia-reperfusion injury remains to be elucidated ***(Curiso et al., 2015).***

Emerging evidence shows that autophagy is associated with the protective effect of IPC; however, the relation between IPC and autophagy in protection against IR is not fully understood **(*Wang et al., 2014*).**

Autophagy decrease is a physiological consequence of aging ***(Cuervo, 2008). Wang et al. (2014)*** claimed that aged livers are more susceptible to mitochondria-dependent hepatic IR injury due to impaired autophagy which does not occur in young livers. The role of autophagy in the age dependence of sensitivity to IR injury is a matter of debate ***(Wang et al., 2011).***

From this point of view and for clinical applicability, autophagy induction may be a new potential therapeutic target for liver IR injury.