Role of Heme oxygenase-1 activation and Type-5 Phosphodiesterase inhibition in Hepatic Ischemia Reperfusion Injury of Rat

Thesis

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By

Hend Ashour Ahmed Hassan
(*M.B., B.Ch. Kasr Elini,Cairo University)*

Supervised by

Prof. Dr. Hassan Mohamed Eissa

Head of Physiology department
Faculty of Medicine
Cairo University

Dr. Mohamed E. Saleh

Ass. Professor of Physiology
Faculty of Medicine
Cairo University

Dr. Laila A. Alsayed

Ass. Professor of Physiology
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University
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ALT :alanin transferase

ADP : adenosine diphosphate

AHR : aryl hydrocarbon receptor

ANP : atrial natriuretic peptide

APC : antigen presenting cell

Bcl-2 : B-cell lymphoma 2

BNP : brain/B-type natriuretic peptide

CaM : calmodulin

cAMP : cyclic adenosine monophosphate

CD : cluster differentiation

cGMP :cyclic guanosine monophosphate

cNOS : constitutive NOS

CO : carbon monoxide

CoPP : Cobalt III protoporphyrin IX chloride

CORMs : CO releasing compounds

DAMPs : Danger-Associated Molecular Patterns

ECs : endothelial cells

ED : erectile dysfunction

eNOS : endothelial NO synthase

GP : platelet glycoprotein

HO : heme oxygenase

HOs : Heme oxygenases

hsp : heat-shock proteins

I.P : intraperitoneally

I/R : Ischemia reperfusion

IFN-γ : interferon-gamma

IL : interleukin

iNOS: inducible nitric oxide synthase

IPC : ischemic preconditioning

IRF-3 : interferon regulatory factor-3

IRI : Ischemia reperfusion injury

KATP : adenosine triphosphate-sensitive potassium channels

LDL : low density lipoprotein

LPS : lipopolysaccharide

MAPK : mitogen activated protein kinase

MHC : major histocompatibility complex

MMP : Matrix metalloproteinase

MnP : manganese-porphyrin

MyD88 : myeloid differentiation primary response gene 88

NF-κB : nuclear factor kappa B

NK : natural killer

nNOS : neuronal NO synthase.

NO : nitric oxide

NOS : nitric oxide synthase

OFRs : Oxygen free radicals

PDE : phospho-diesterase

PKC : protein kinase C

PKG : protein kinase G

PMNs : polymorpho neutrophils

PS : phosphatidyl-serine

PSGL-1 : P-selectin glycoprotein ligand

RANTES : regulated upon activation, normal T-cell expressed and secreted

ROS : reactive oxygen species

sGC : soluble guanylate cyclase

SOD : superoxide dismutase

TGF-β : transforming growth factor-beta

TLR : Toll-Like receptors

TNF-α : tumor necrosis factor-α

TUNEL : terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

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My husband

My children Hassan & Omar

My family & my husband's family

ABSTRACT

Ischemia/reperfusion induced injury is one of the major preoperative complications in liver surgery, so that this work provides evidence that HO-1 gene over expression, and type 5 PDE inhibitions perform a protective role against injury and there is a correlation between both pathways. It has been reported that both systems, NO producing nitric oxide synthase (NOS) as well as CO releasing heme oxygenase (HO), are capable of modulating each others activity and demonstrate beneficial effects on the cellular level in a model of hepatocyte cell death in mice **.**

Aim of work : Is to investigate the role of two pathways , HO-1 activattion by use of Cobalt (III) protoporphyrin IX chloride (CoPP) and phosphodi -esterase type **5** inhibition by use of sildenafil,on ischemia reperfusion **(I/R)** injury of rat liver. To achieve this, fifty male adult white albino rats of average weight between 160 to 200 grams were used The rats were randomly divided into five main groups in which each group included 10 rats :

Group I(control group):The rats of this group were subjected to laparotomy and liver exposure was performed with no other further surgical manipulations.

Group II: This group underwent induction of hepatic ischemia reperfusion injury as 45 min of ischemia and 2 hours of reperfusion without injection of drugs.

Group III: Rats in this group were subjected to the same procedures as group Π, but they were previously injected (I.P) with CoPP 24 hours before operation at a dose of 5 mg/kg body weight.

Group IV: This group underwent the same procedure as group Π but Sildenafil citrate {inhibitor of phosphodiesterase type 5 (PDE-5)} administered intravenously at a dose of 10μg/kgbody weight, 15 min after stabilization of surgery

Group V : In this group, the two pathways were examined by injection of Cobalt (III) CoPP I.P and sildenafil citrate dissolved in saline and administered intravenously.

At the end of the experimental period, blood samples were collected to measure serum ALT.The animals were sacrificed and their livers were excised to assess the level of NO and HO-1 gene expression in liver tissues.

Samples were fixed in alcohol–formalin–acetic acid, embedded in paraffin and stained with standard haematoxylin-eosin stain for histological evaluation. Stained sections were examined at ×200 and×400 magnification for severity of hepatic injury to measure the necrotic index.

Results of the present study showed that the serum ALT level and necrotic index were significantly increased in group II(**I/R**) compared to group I (control) denoting the presence of liver injury with I/R. The serum ALT level and necrotic index were significantly decreased in groups III **(I/R** +CoPP) and **Group IV (I/R**+ sildenafil) compared to group II (**I/R**) denoting a protective from liver injury .In **Group V**: (I/R+CoPP+ sildenafil),there was marked decrease in the serum ALT level and necrotic index compared to group II (**I/R**), and no significant change compared to the control) .

Key word: **ischemia reperfusion, heme oxygenase and sildenafil**

Introduction

The insult to an organ after the onset of reperfusion is the result of the interplay between different complex mechanisms. Ischemia reperfusion is a major cause of morbidity and mortality in patients undergoing liver surgery and liver transplantation ***(Taniguchi et al., 2004).***

Ischemia reperfusion injury plays an important role in the quality and function of the graft and is a major cause for increased length of hospitalization and decreased long term graft survival ***(Mehrabi et al., 2007).*** Despite improvements in organ preservation solutions, ischemia- reperfusion damage during the preservation and the implantation of the liver is still a major problem in liver transplantation ***(Chattopadhyay and Kumar, 2009).***

A lengthy period of ischemia is required for a number of liver procedures, especially in the setting of hepatic transplantation ***(Serracino-Inglott et al., 2001).*** Temporary clamping of the portal triad is common strategy to minimize bleeding during liver transplantation. Unfortunately, this method resulted in hepatic ischemia reperfusion and may cause postoperative functional disorder of the liver ***(Gujral et al., 2001).***

On restoring the blood, the liver is subjected to further reperfusion insult. A complex multifactorial, pathphysiological process that induces the release of oxygen free radicals, cytokines and endothelines ***(Serracino-Inglott et al., 2001)***

The heme oxygenase (HO) system is the rate-limiting step in the oxidative degradation of heme into biliverdin, carbon monoxide (CO) and free iron ***(Choi and Alam,1996).*** inducible HO-1, also known as heat shock protein 32. Upregulation of HO-1 is known to be a protective response from cellular stress, following I/R injury, radiation and

inflammation and overexpression of HO-1 exerts a cytoprotective function in a number of I/R injury and liver transplant models ***(Kobayashi et al., 2002).***

Thus, HO-1 is an attractive target for anti-inflammatory therapies and potential candidate responsible for cell injury ***(Zhong et al., 2010).*** Immunochemical studies with specific monoclonal antibodies have revealed the distribution of HO-1 in the rat liver with distinct topographical patterns ***(Kobayashi et al., 2002).*** HO-1 has been shown to be expressed principally in Kupffer cells ***(Kiemer et al., 2003).*** However, exact mechanisms by which HO-1 induction may lead to cytoprotection during I/R injury in organ transplantation have not been fully clarified ***(Zhong et al., 2010).***

Generally, the cellular mechanisms of HO-1- derived protection including regulation of the inflammatory response, improvement of microvascular flow and the antiapoptotic effects. The beneficial effects of HO-1 may be the result of the ability of the end-products of heme degradation . It was reported that exogenous CO treatment suppressed early proinflammatory gene expression and neutrophil infiltration, and efficiently ameliorated hepatic I/R injury***(Kaizu et al., 2005)***.

On the other hand one therapeutic approach, of recent interest, to minimize I-R injury, is the enhancement of nitric oxide (NO) bioavailability. NO is a product of the oxidative conversion of L-arginine to citrulline via the enzyme endothelial NO synthase (eNOS). NO is of physiological importance because of its involvement in cardiovascular homeostasis. NO fulfills many physiological roles including acting as a vasodilator by relaxing vascular smooth muscle cells ***(Ignarro et al., 1987)*** inhibiting platelet aggregation and leuckocyte adhesion ***(Radomskiet al., 1987)*** and general inflammation ***(Laroux et al., 2000).***

Sildenafil is a potent and selective inhibitor of cGMP-phospho-diesterase (PDE) type 5 that exerts its pharmacological effect by increasing