Mechanistic Study of Potential Hepatoprotective Effect of Liraglutide in an Experimental Model of Liver Toxicity

Thesis presented by

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Aya Ismail Abdelazi

Table of Contents

List of Abbreviations

7-OH-MTX	7-Hydroxymethotrexate
AICAR	5-Aminoimidazole-4-Carboxamide Ribonucleotide
AIF	Apoptosis Inducing Factor
ALT	Alanine Aminotransferase
ANP	Atrial Natriuretic Peptide
APAF-1	Apoptotic protease activating factor-1
ARE	Antioxidant Response Elements
AST	Aspartate Aminotransferase
ATIC	5−aminoimidazole-4−carboxamide ribonucleotide (AICAR) transformylase
ATP	Adenosine Triphosphate
BAFF	B cell-activating factor
BAT	Brown Adipose Tissue
BMI	Body Mass Index
cAMP-GEFII	Camp-Regulated Guanine Nucleotide Exchange Factor II
CBP	CREB-Binding Protein
cGMP	Cyclic Guanosine 3′,5′-Monophosphate
CNS	Central Nervous System
COX-2	Cycloxygenase-2
CPG2	Glucarpidase
CrCl	Creatinine Clearance
CREB	Cyclic AMP Response Element Binding Protein
DAMPA	2,4-Diamino-N^{10}-Methylpteroic Acid
DHFR	Dihydrofolate Reductase
DILI	Disease Induced Liver Injury
DISC	Death-Inducing Signaling Complex
DISC	death inducing signaling complex

DKD	Diabetic Kidney Disease
DMARD	Disease-Modifying Anti-Rheumatic Drugs
DPP-4	Dipeptidyl Peptidase-4
DTNB	5,5'-Dithiobis (2- Nitrobenzoic Acid)
eNOS	Endothelial Nitric Oxide Synthase
ENT	Equilibrative nucleoside transporter
ER	Endoplasmic Reticulum
FADD	Fas-Associated Protein With Death Domain
FADD	Fas-associated death domain
FasL	Fas Ligand
FDA	Food And Drug Administration
FGAR	N2-formyl N1-(5−phospho-D−ribosyl) glycinamide
FIG21	Fibroblast Growth Factor 21
GAR	N1-(5−phospho-D−ribosyl) glycinamide
GLP-1	Glucagon-Like Peptide-1
GLP-1 R	GLP-1 Receptor
GPx	Glutathione Peroxidase
GSH	Glutathione
GTP	Guanosine-5'-Triphosphate
GTP	Guanosine triphosphate
GTPCH	GTP Cyclohydrolase
HIV	Human Immunodeficiency Virus
HO-1	Heme Oxygenase-1
IKK	IkB kinase
IL-1	Interleukin-1
IL-12	Interleukin-12
IL-8	Interleukin-8
IMP	Inosine monophosphate

iNKT	Invariant Natural Killer T
IP	Intraperitoneal
Keap1	Kelch-Like ECH-Associated Protein1
LTβ	Lymphotoxin Beta
MAPK	Mitogen-Activated Protein Kinase
MCL-1	Myeloid Cell leukemia-1
MDA	Malondialdehyde
MILI	Methotrexate Induced Liver Injury
MTHFR	Methylene-Tetrahydro Folate Reductase
MTX	Methotrexate
NADP	Nicotinamide Adenosine Diphosphate
NADPH	Nicotinamide Adenosine Diphosphate Hydrogen
NAFLD	Non-Alcoholic Fatty Liver Disease
NF-κB	Nuclear Factor Kappa B
NIK	NF-κB inducing kinase
NODCAR	National Organization Of Drug Control And Research
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
NSAID	Non-Steroidal Anti-Inflammatory Drugs
PDE	Phosphodiesterases
PI3K	Phosphoinositide 3-Kinase
PKA	Protein Kinase A
PKC	Protein Kinase C
PKG	Protein Kinase G
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
PUMA	p53 upregulated modulator of apoptosis
RIP	Receptor Interacting Protein
ROS	Reactive Oxygen Species
SAM	S-Adenosyl Methionine

SDS	Sodium Dodecyl Sulfate
SOD	Superoxide Dismutase
TBARS	Thiobarbituric Acid Reactive Substances
TBS	Tris Buffer Saline
TCA	Trichloroacetic Acid
TEMD	Tetramethylethylenediamine
TGFβ1	Transforming Growth Factor B1
THF	Tetrahydrofolate
TNF-α	Tumor Necrosis Factor-A
TRADD	TNF-R1 associated death domain
TRAF	TNF receptor-associated factor
TRAIL	TNF-Related Apoptosis-Inducing Ligand
TS	Thymidylate Synthase
TYMS	Thymidylate synthetase.
UMP	Uracil Monophosphate
WAT	White Adipose Tissue
XIAP	X-Linked Inhibitor of Apoptosis;,
β-TrCP	β-transducin repeat-containing protein

List of tables

List of Figures

Abstract

Drug-induced liver injury (DILI) is one of the main clinical problems and the leading cause of acute liver failure. Methotrexate (MTX) is commonly used to treat several types of cancer and autoimmune diseases. Along with MTX clinical uses, however, there is increasing concern over its organ toxicities particularly liver toxicity. Liraglutide, glucagon like peptide-1 agonist, approved to treat type-2 diabetes, possesses antioxidant and anti-inflammatory features. The purpose of the present study is to explore the potential protective effect of pre-treatment liraglutide in ameliorating MTX-induced hepatotoxicity and to further investigate the underlying molecular mechanism. Male Sprague-Dawley rats received 1.2 mg/kg liraglutide intraperitoneal twice daily for 7 days before exposed to MTX injection. The results revealed that liraglutide significantly decreased activities of liver enzymes and oxidative stress in hepatocytes. Furthermore, NF-kB expression and the related inflammatory markers (TNF-α, COX-2, and IL-6) were reduced in the pre-treatment group of liraglutide. These data validate the advantageous effects of liraglutide on MTX hepatotoxic animals. In addition, novel data was found that liraglutide increased the expression of the antioxidant transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf-2), along with the transcription of downstream phosphorylated cAMP response element-binding protein (pCREB) which increases the activity of Nrf-2. Additionally, caspase-3 expression and BAX/Bcl-2 ratio were decreased following liraglutide pre-treatment. In conclusion, it was proposed that liraglutide may enhance the antioxidant activity of liver cells by activating the Nrf-2 and pCREB signaling, thereby, reducing liver cell inflammation and apoptosis induced by MTX.

Keywords:

Methotrexate; Hepatotoxicity; Liraglutide; NF-kB; Apoptosis; Nrf-2; pCREB; HO-1