"Potential antifibrotic effect of Deferasirox in an experimental model of liver fibrosis"

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وَقَالُوا۟ ٱلْحَمْدُ لِلَّهِ
ٱلَّذِى هَدَىٰنَا لِهَٰذَا
وَمَا كُنَّا لِنَهْتَدِىَ لَوْلَآ أَنْ هَدَىٰنَا ٱللَّهُ ﷺ

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Abstract

Abstract

Hepatic iron overload is one of the main causative factors for liver fibrosis and is observed in chronic hepatitis C patients, hemochromatosis patients, and patients with different blood disorders as myelodysplastic syndrome, and β-thalassemia. In the present study, we aimed to investigate the potential anti-fibrotic effect of the iron chelator; deferasirox (DFX), in an experimental model of immunologically-induced liver fibrosis using concanavalin A (con A). The study was divided into 2 parts. Firstly, the hepatoprotective dose of DFX was screened by giving DFX at doses 25, 50, and 100 mg/kg orally to rats followed by IV injection of con A 2 hours later. Con A induced significant hepatotoxicity as manifested by elevated liver enzymes' activities in serum, in addition to histopathological damage. Only DFX at the dose 100 mg/kg could offer considerable hepatoprotection and restore histopathological integrity.

The second part of the study was carried out to explore the possible molecular mechanisms underlying the hepatoprotective and antifibrotic effect of DFX. Rats were randomly divided into 4 groups. The first group was considered as the control group and received vehicles only. The second group was injected with con A (15 mg/kg, IV) once/week for 6 weeks to induce liver fibrosis. The third group was given DFX (100 mg/kg, P.O.) 3 times/week in addition to con A injection (15 mg/kg, IV) once/week, and the last group was given DFX only (100 mg/kg, P.O.) 3 times/week. Con A injection induced significant hepatotoxicity. Additionally, it caused serum and hepatic iron overload *via* increasing CHOP, and decreasing P-CREB and hepcidin levels in hepatic tissues. This in turn precipitated oxidative stress status as was evident by the upregulated gene expression of NOX4 and $p22^{phox}$, together with marked reduction in anti-oxidant enzymes' activities including superoxide dismutase and catalase. Consequently, the inflammatory cascade was switched on beginning with the upregulation of NF-κB that ultimately induced the transcription of various downstream inflammatory mediators; mainly iNOS, TNF-α, and IFN-γ.

All those events eventually led to activation of HSCs as shown by the elevated expression of α-SMA, resulting in excessive collagen deposition and hence, liver fibrosis. Co-treatment with DFX showed significant hepatoprotective effect, and antifibrotic effect through decreasing iron overload, antioxidant, and anti-inflammatory effects. Collectively, these findings imply that DFX can be a promising candidate for halting the progression of hepatic fibrosis.

Keywords:

Liver fibrosis, Concanavalin A, Deferasirox, Hepcidin, P-CREB, CHOP

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List of Abbreviations

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BCG	Bromocresol green
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
C/EBP	CCAAT enhancer binding protein
CHC	Chronic hepatitis C
CHOP	C/EBP homologous protein
Con A	Concanavalin A
COX-2	Cyclooxygenase-2
CREB	cAMP responsive element binding protein
C_t	Cycle threshold
DFX	Deferasirox
DMSO	Dimethyl sulfoxide
dNTPs	Deoxyribonucleotide triphosphate
DTNB	5,5'-Dithio-bis (2-nitrobenzoic acid) [Ellman's reagent]
ECM	Extracellular matrix
EDTA	Ethylene diamine tetra-acetic acid
EGF	Endothelial growth factor
ELISA	Enzyme-Linked Immunosorbent Assay
EMT	Epithelial mesenchymal transition
ERK	Extracellular signal-regulated kinase

ET-1	Endothelin-1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSH	Reduced glutathione
HAMP	Hepcidin antimicrobial peptide
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCl	Hydrochloric acid
HCV	Hepatitis C virus
H & E	Hematoxylin and eosin
HFE	High Fe
HIF	Hypoxia inducible factor
HIV	Human immunodeficiency virus
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
HSCs	Hepatic Stellate cells
IFN-γ	Interferon gamma
IGF	Insulin-like growth factor
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
I.V.	Intravenous
KCs	Kupffer cells
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein 1
MDA	Malondialdehyde

MMPs	Matrix metalloproteinases
mRNA	Messenger RNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NASH	Non-alcoholic steatohepatitis
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
NO_2^+	Nitronium ion
NOX-4	NADPH oxidase-4
$O_2^{\cdot}$	Superoxide
$OH^{\cdot}$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PI3-K	Phosphatidylinositol 3-kinase
PMS	Phenazine methosulphate
P.O.	Per oral
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PPIs	Protease and phosphatase inhibitors
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RNase	Ribonuclease
ROS	Reactive oxygen species
RQ	Relative quantitation
α-SMA	Alpha-smooth muscle actin
SOD	Superoxide dismutase