Potential anti-fibrotic effect of deferoxamine in concanavalin A-induced liver fibrosis.

Thesis presented by

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بسم الله الرحمن الرحيم
نَرْفَعُ دَرَجَٰتٍ مَّن نَّشَآءُ ۗ
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Samar Fathy Darwish

Abstract

ABSTRACT

Background & Aims: Iron-overload is a well-known factor of hepatotoxicity and liver fibrosis, which was found to be a common finding among hepatitis C virus patients and related to interferon resistance. Here, the potential anti-fibrotic effect of deferoxamine; the main iron chelator, was elucidated and its additional usefulness to interferon-based therapy in an immunological model of liver fibrosis.

Methods: Liver fibrosis was induced by concanavalin A (15 mg/kg/week, i.v.). Rats were co-treated with deferoxamine (300 mg/kg, 3times/week, i.p.) and/or pegylated interferon-α (1.5 µg/kg/week, s.c.) for 6 consecutive weeks. Hepatotoxicity indices, oxidative stress, inflammatory and liver fibrosis markers were assessed.

Results: Concanavalin A induced a significant increase in the hepatotoxicity indices and lipid peroxidation accompanied with a significant depletion of the total antioxidant capacity, glutathione level and superoxide dismutase activity. Besides, it increased the expression of $CD4^+$ T-cells, NF-κB as well as all the downstream inflammatory cascades. Further, α-SMA, TGF-β1 and hydroxyproline were increased markedly, which were confirmed by histopathology. Co-treatment with either deferoxamine or pegylated interferon-α alone reduced liver fibrosis markers significantly, and improved liver histology. However, some of the hepatotoxicity indices and oxidative stress markers didn't improve upon pegylated interferon-α alone, besides the remarkable increase in interleukin-6 liver content. Combination therapy of deferoxamine with pegylated interferon-α further improved all previous markers, ameliorated interleukin-6 elevation, as well as increased hepcidin gene expression.

Conclusion: This study provides evidences for the potent anti-fibrotic effects of deferoxamine and the underlying mechanisms that involved attenuating oxidative stress, subsequent inflammatory cascade, as well as the production of profibrogenic factors. Addition of deferoxamine to interferon regimen for hepatitis C virus patients may offer a promising adjuvant modality to enhance therapeutic response.

Keywords: Liver fibrosis; Iron; Deferoxamine; Interferon; Hepcidin

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List of Abbreviations

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
CCl_4	Carbon tetrachloride
Con A	Concanavalin A
COX-2	Cyclooxygenase-2 enzyme
DFO	Deferoxamine
dNTPs	Deoxynucleotide triphosphates
ECM	Extracellular matrix
ELISA	Enzyme-linked Immunosorbent Assay
ET-1	Endothelin-1
GSH	Reduced glutathione
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis delta virus
H&E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
HSCs	Hepatic stellate cells
IFN	Interferon
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IL	Interleukin
iNOS	Inducible nitric oxide synthase enzyme
i.p.	Intraperitoneal
i.v.	Intravenous
KCs	Kupffer cells
MCP-1	Monocyte chemotactic protein-1
MDA	Malondialdehyde as an index for lipid peroxides
MMPs	Metalloproteinases
NASH	Non-alcoholic steatohepatitis
NF-κB	Nuclear factor kappa-B
NKs	Natural killer cells

NO_2^+	Nitrogen dioxide radical
$O_2^{\bullet}$	Superoxide
$OH^{\bullet}$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
peg IFN-α	Pegylated interferon-alpha
RBV	Ribavirin
RNase	Ribonuclease
ROS	Reactive oxygen species
RQ	Relative quantitation
s.c.	Subcutaneous
SECs	Sinusoidal endothelial cells
α-SMA	Alpha-smooth muscle actin
SOD	Superoxide dismutase
STAT	Signal transducer and activator of transcription
SVR	Sustained virological response
$t_{1/2}$	Half-life
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TC	Total cholesterol
TG	Triglycerides
TGF-β1	Transforming growth factor beta-1
TIMPs	Tissue inhibitors of the metalloproteinase
TNF-α	Tumor necrosis factor-alpha

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