Potential Beneficial Effects of Verapamil on Liver fibrosis in Rats

Thesis

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Presented by

Mohamed El-Sayed Mahmoud

M.B., B.Ch.- Master Degree in Anesthesia
Clinical Demonstrator of Pharmacology
Faculty of Medicine
Cairo University

Under Supervision of

Prof. Dr. Nadia Ali Gouda
Professor of Pharmacology
Faculty of Medicine
Cairo University

Dr. Amani Nabil Shafik
Assistant Professor of Pharmacology
Faculty of Medicine
Cairo University

Dr. Mostafa Mahmoud Khodeir
Lecturer of Pathology
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University

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Contents

List of Abbreviations

ALT	Alanine aminotransferase
Apo A1	Apolipoprotein A1
As	Arsenic
ASMA	Alpha-smooth muscle actin
AST	Aspartate aminotransferase
Bcl-xL	B-cell lymphoma-extra large
CBDL	Common bile duct ligation
CCL21	C-C chemokine ligand
CCl_4	Carbon tetrachloride
CINC	Cytokine-induced neutrophil chemoattractant
CTGF	Connective tissue growth factor
DILI	Drug-induced liver injury
DMN	Dimethylnitrosamine
ECM	Extracellular matrix proteins
EGF	Epidermal growth factor
ET-1	Endothelin-1
FGD	Fibroblast growth factor
GSH	Glutathione
HA	Hyalouronic acid
Hap	Haptoglobin
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
HSCs	Hepatic stellate cells
ICAM-1	Intercellular adhesion molecule 1
IFN-γ	Interferon-γ
IGF-I & II	Insulin growth factors I & II
IL-6	Interleukin-6
LBP	Lipopolysaccharide-binding protein
mAb	Monoclonal anti-alpha-actinin antibody
MCP-1	Monocyte chemoattractant protein-1
MIP-2	Macrophage inflammatory protein-2
MMP	Matrix-metalloproteinases
NAPQI	N-acetyl-ρ-benzoquinone imine
NCAM	Neural cell adhesion molecule
NS3	Nonstructural protein 3
NS5	Nonstructural protein 5
ODNs	Oligodeoxynuleotide
PAF	Platelet-activating factor
PBHF	Primary biliary hepativ fibrosis
PDGF	Platelet derived growth factor
PIIINP	N-terminal propeptide of type III collagen
PPARγ	Peroxisome proliferators-activated nuclear receptors-γ
ROS	Reactive oxygen species
SOCs	Store-operated calcium channels
SREBP-1c	Sterol regulatory element binding protein-**1c**
TAA	Thioacetamide
TFOs	Triplex-forming oligonuleotide
TGF-α	Transforming growth factor-α
TGF-β_1	Transforming growth factor-β_1
TIMPs	Tissue inhibitor of metalloproteinases
TNF-α	Tumor necrosis factor-α
VCAM	Vascular cell adhesion
α -2-M	α -2-Macroglobulin

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Introduction

Hepatic fibrosis, or scarring of the liver, is the final stage of a wound healing response that invariably follows chronic liver injury of any etiology. The point that the response to recurrent injury in the liver is similar in multiple different types of liver disease underscores the value of identifying common regulatory components of the fibrotic response because such components theoretically could be targeted without respect to cause of disease. However, it is important to emphasize that, at least in theory; different pathologic patterns of fibrosis (biliary, perisinusoidal, pericentral) can occur and thus may merit different types of therapies. Fibrosis develops with different spatial patterns and is a consequence of different prevalent mechanisms according to the diverse causes of parenchymal damage. Indeed, fibrosis, observed as a consequence of chronic viral infection is initially concentrated within and around the portal tract, while fibrosis secondary to toxic/metabolic damage is located mainly in the centrolobular areas. In addition, it is increasingly evident that different cell types are involved in the deposition of fibrillar extracellular matrix during active hepatic fibrogenesis: hepatic stellate cells are mainly involved when hepatocellular damage is limited or concentrated within the liver lobule, whereas portal myofibroblasts and fibroblasts provide a predominant contribution when the damage is located in the proximity of the portal tracts. Progressive fibrosis ultimately leads to a gross disruption of the normal liver architecture (cirrhosis) and subsequent impairment of liver function.

The ‘activated’ hepatic stellate cell (HSC) is the pivotal cell type involved in the development of liver fibrosis, simultaneously secreting excessive scar proteins (collagens) and potent inhibitors of collagen degradation –

tissue inhibitors of matrix metalloproteinases (TIMPs). Resolution of fibrosis is characterized by matrix degradation and is associated with HSC apoptosis and the increased activity of matrix metalloproteinase enzymes (MMPs).

Activation of stellate cells is accompanied by a marked increase in proteins that are characteristic of contractile cells (i.e. such as smooth muscle actin and smooth muscle myosins). Stellate cell contraction is important in the injured liver because it may contribute to the collapse and shrunken state of cirrhotic livers, and because it also appears to play a role in portal hypertension. Thus, stellate cell contractility, although not directly related to fibrosis, is an important physiologic target.

Currently, the most effective treatment for hepatic fibrosis is removal of the causative agent.

Since the HSC plays a central role in the development of liver fibrosis, this cell is a major target.

Verapamil, a phenylalkylamine calcium channel blocker, can prevent energy metabolism disorder in hepatocytes induced by calcium overloading through blockage of voltage-dependent calcium channels and Ca^{2+} inflow. Verapamil as an angiotenic, can increase local blood circulation and oxygen supply, inhibit lipid peroxidation, antioxidation, and thereby limit hepatonecrosis *in vivo*.

The pig serum model induces liver fibrosis without severe inflammation. Because we would like to mainly focus on the effect of verapamil on activated HSC, we employed this model in the present study.

Aim of the Work

The aim of the present work is to:

1- To investigate the effect of variable doses of a calcium channel blocker 'VERAPAMIL" as a prophylactic and therapeutic antifibrotic drug in experimental liver fibrosis induced in rats by pig serum.

2- To compare verapamil to standard prophylactic therapy silymarin.

3- To demonstrate the beneficial effect of combining verapamil to the standard prophylactic therapy silymarin.

LIVER FIBROSIS

Liver fibrosis

Hepatic fibrosis is the wound-healing response of the liver to repeated injury **(Iredale, 2003)**. Liver fibrosis results from chronic damage to the liver in conjunction with the accumulation of extracellular matrix protein (ECM) proteins, which is a characteristic of most types of chronic liver diseases . The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension, respectively **(Gines et al., 2004).**

Liver fibrosis was thought to be a passive and irreversible process due to the collapse of the hepatic parenchyma and its substitution with a collagen-rich tissue **(Schaffner and Klion, 1968; Popper and Uenfriend, 1970).** Currently, it is considered a model of the wound-healing response to chronic liver injury **(Albanis and Friedman, 2001**). Early clinical reports in the 1970s suggested that advanced liver fibrosis is potentially reversible **(Soyer et al., 1976).** However, liver fibrosis received little attention until the 1980s, when hepatic stellate cells (HSCs), formerly known as lipocytes, Ito cells, or perisinusoidal cells, were identified as the main collagen-producing cells in the liver **(Friedman et al., 1985).** This cell type, first described by von Kupffer in 1876, undergoes a dramatic phenotypic activation in chronic liver diseases with the acquisition of fibrogenic properties **(Geerts, 2001).** Methods to obtain HSCs from both rodent and human livers were rapidly standardized in the 1980s **(Otto and Veech, 1980; Friedman et al., 1992),** and prolonged culture of HSCs on plastic was widely accepted as a model for the study of activated HSCs **(Rockey et al., 1992).** Key

signals that modulate HSCs' fibrogenic actions besides HSCs, are portal myofibroblasts and cells of bone marrow **(Forbes et al., 2004; Ramadori and Saile, 2004).** Rapid and slower fibrosers are identified, and genetic and environmental factors influencing fibrosis progression are known **(Bataller et al., 2003).** Since the demonstration, in the 1990s, that even advanced liver fibrosis is reversible, However, the most effective therapy for treating hepatic fibrosis to date is still to remove the causative agent **(Bataller and Brenner, 2001).** A number of drugs are able to reduce the accumulation of fibrous tissue in experimental models of chronic liver injury. Renin-angiotensin system blockers and antioxidants are the most promising drugs, although their efficacy has not been tested in humans.

The onset of liver fibrosis is usually insidious, and most of the related morbidity and mortality occur after the development of cirrhosis (**Poynard et al., 2000**). In the majority of patients, progression to cirrhosis occurs after an interval of 15–20 years. Cirrhosis is also a risk factor for developing hepatocellular carcinoma. Liver fibrosis progresses rapidly to cirrhosis in several clinical settings, including repeated episodes of severe acute alcoholic hepatitis, subfulminant hepatitis, and fibrosing cholestasis in patients with HCV reinfection after liver transplantation (**Berenguer et al., 2003**).

Classifications of Liver Fibrosis:

There are 2 main types, congenital and acquired liver fibrosis. The congenital type is a genetic disorder, which causes polycystic liver diseases, while the acquired type has many different categories and is mainly caused by liver cell injuries **(Desmet, 1992; Friedman, 2000; Hui and Friedman, 2003)**.

Causatively, liver fibrosis can be classified as:

1.Viral hepatitis fibrosis: Usually caused by chronic hepatitis B, C, and D. About 15% of HBV and 85% of HCV infected persons will develop chronic hepatitis and lead to fibrosis. In which, the liver shows peri-portal area inflammation, piecemeal necrosis and fibrosis. With such large population being affected, this is the most important category of the liver fibrosis **(Arias and Irwin, 1994)**

2. Parasitic infestation fibrosis: This kind of liver fibrosis is mainly happening in developing countries and is caused by schistosomiasis. The recurrent infection and the eggs of schistosoma accumulated in the liver can cause liver fibrosis and cirrhosis **(Rockey, 2005).**

3. Alcoholic fibrosis: It is mainly caused by the oxidized metabolite of alcohol, acetaldehyde. Its incidence is positively related to the amount of alcohol consumped. Alcoholic fibrosis causes two morphological changes in the liver: fatty liver and cellular organelles deterioration. The fibrosis first appears around the center veins (central hyaline sclerosis) associated with liver parenchymal inflammation and the fibrosis gradually expands to the whole liver **(Arias and Irwin, 1994)**.

4. Biliary fibrosis: There is primary and secondary biliary fibrosis. Primary biliary hepatic fibrosis (PBHF) is an autoimmune disorder in which chronic bile retention caused the liver fibrosis **(Dufour et al., 1997)**. It affects female around the age 40 to 60. In serum tests there is elevated gamma globulin and serum is positive for the anti-mitochondria antibody. Pathological studies found that the fibrosis mainly around the micro-bile ducts and peri-portal area fibrosis and inflammation. Secondary biliary fibrosis happens following the obstruction of the bile ducts, which causes peri-portal inflammation and progressive fibrosis **(Arias and Irwin, 1994)**.

5. Metabolic fibrosis: It is not common. Wilson's disease and hemochromatosis are the main disorders that cause metabolic fibrosis. The former is a genetic disorder and causes copper metabolism disorder and deposits in the liver. The latter is an iron metabolic disorder and causes hemoglobin deposits in the liver **(Rockey, 2005).**

6. Intoxication fibrosis: When long-term contact with liver-toxic substances, such as carbon tetrachloride, organophosphorus, dimethyl nitrosamine, thioacetamide, or taking liver toxic medications, such as isoniazid, thiooxidizing pyrimidine, wintermin, tetracycline, acetaminophen etc. can all cause various degrees of liver cell injuries, necrosis, bile retention, or allergic inflammation and cause liver fibrosis **(Rockey, 2005).**

7. Malnutritional fibrosis: This type is mainly caused by insufficient or imbalanced nutritional intake **(Rockey, 2005).**

8. Cardiogenic fibrosis: Chronic congestive heart failure can cause long lasting liver vein stagnation. Which causes ischemic degeneration of the

liver cells. In this type of liver fibrosis, the connective tissue hypertrophy starts at the center of the liver lobule and gradually expands to rest of the lobule **(Giallourakis et al., 2002)**.

Pathologically, Liver fibrosis can be classified as:

1. Portal area fibrosis: There is fibroblasts proliferation and fibers expansion from the portal areas to the lobule. Finally, these fibers connected to form bridging septa. This kind of fibrosis is mainly seen in viral hepatitis and malnutritional liver fibrosis (Figure 1) **(Klatt, 2006)**.

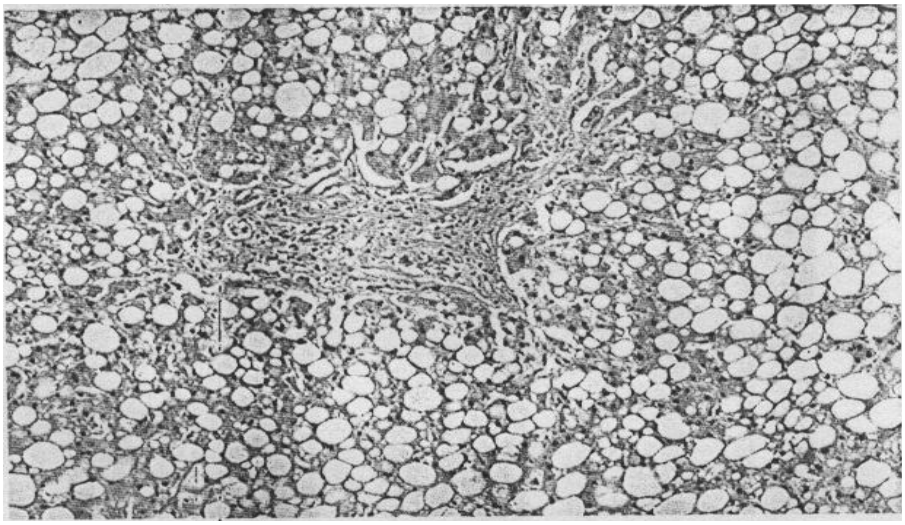

Figure 1: Portal fibrosis:Fibrous expansion of portal fields with fibrosis extending along the terminal centracinar portal veins assuming the appearance of "stellate" fibrosis. Expansion and branching of normal portal fields must not be confused with pathological portal fibrosis which contains inflammatory cell **(JANIS AND FRIENDS, 2008).**

2. Intra-lobular fibrosis: In which, there is almost no fibroblast found in normal lobule. When large numbers of liver cells degenerate and undergo necrosis, the reticular fiber frame collapses and becomes thick collagen fibers. At the same time, intra lobule fibrotic tissue proliferates and surrounds the liver cells (Figure 2) **(Klatt, 2006)**.