POSSIBLE ANTIFIBROTIC EFFECTS of FENOFIBRATE a PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-α AGONIST ALONE or COMBINED with PENTOXIFYLLINE in CONCANAVALIN A- INDUCED CHRONIC HEPATITIS in RATS

A Thesis

Submitted for partial fulfillment of M.D.
in Pharmacology and Therapeutics

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2013

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List of Abbreviations

AGEs	Advanced glycation end products
ALT	Alanine transaminase
APCs	Antigen presenting cells
AST	Aspartate transaminase
Con A	Concanavalin A
COX	Cyclooxygenase
CTL	Cytotoxic T lymphocyte
DAAs	Direct acting antivirals
ECM	Extracellular matrix
ET1	Endothelin 1
FF	Fenofibrate
GSH	Glutathione
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immune deficiency virus
HSCs	Hepatic stellate cells
ICAM	Intercellular adhesion molecule
IFN-γ	Interferon-γ
IL	Interleukin
IR	Insulin resistance
KCs	Kupffer cells
LDL	low density lipoproteins
MDA	Malondialdehyde
MFB	Myofibroblast
MHC class II	Major histochompitability complex class II
MMP	Matrix Metalloproteinases
n LDL	Native low-density lipoprotein
NASH	Non alcoholic steatohepatitis

List of Abbreviations (Cont...)

NFκB.....	Nuclear factor kappa B
NKT	Natural killer T
NO	Nitric oxide
OS.....	Oxidative stress
PDE	Phosphodiesterase
PDGF.....	Platelet derived growth factor
PGE2.....	Prostaglandin E2
PKA	Protein kinase-A
PP	Portal pressure
PPARs	Peroxisome proliferator-activated receptors
PPRE	Peroxisome proliferator response element
PTX.....	Pentoxifylline
RBV	Ribavirin
RES	Reticuloendothelial system
ROS.....	Reactive oxygen species
SOD.....	Superoxide dismutase
SVR.....	Sustained virologic response
TGFβ1	Transforming growth factor beta
Th cells.....	T helper cells
TIMP	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor alpha
VCAM.....	Vascular cell adhesion molecule
α SMA.....	α smooth muscle alpha-actin

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ABSTRACT

Background&aim: Peroxisome proliferator-activated receptor-α (PPARα) are physiologically highly expressed by hepatocytes, where they play a pivotal anti-inflammatory and metabolic role. The decreased expression and functional activity of PPARα in hepatocytes and increased TNF- α level during hepatitis C virus infection may contribute to the pathogenesis of diseases in humans. This study aims at evaluating the effects of PPARα activation with fenofibrate (FF) and TNF- α inhibition with pentoxifylline (PTX) either as a monotherapy or in combination on liver inflammation, fibrosis and portal pressure (PP) in concanavalin A (Con A)-induced chronic hepatitis in rats. **Methods:** The rats were randomly divided to 5 groups; control group recieved (1ml saline iv/wk), con A treated group recieved (20mg/kg .iv/wk), Con A+ FF pretreated group recieved (100 mg/kg/day p.o), Con A +PTX preteated group recieved (200 mg/kg/day p.o) and (Con A+FF-PTX) pretreated group with the above doses. Measurement of PP was performed by the end of the 8^{th} week. Blood samples and livers were collected at the end of the 1^{st}, 2^{nd}, $4^{th}$and 8^{th} weeks for biochemical, histopathological and immunohistochemistry studies for α-smooth muscle actins (α SMA). **Results:** FF and PTX pretreated groups produced significant ($p<0.05$) decrease in PP and serum ALT, AST, hepatic TNF α and MDA compared to Con A treated group. Histopathological examination revealed that FF pretreatment started early to improve the histopathological and immunohistochemical studies (anti-inflammatory, anti-fibrogenic effects through suppressing HSC activation, and apoptosis of nonparenchymal cells) in comparison to PTX pretreatment. On the other hand, chronic pretreatment with FF and PTX as combination did not add a significant additive beneficial effect. **In conclusion**: FF or PTX as a monotherapy significantly reduced PP, liver inflammation and fibrosis in Con A model of chronic hepatitis that may represent a new therapeutic strategy for hepatitis and its complications and the combination of both drugs did not add an additive beneficial effect.

Key words: PPARα; fenofibrate; pentoxifylline; concanavalin A; portal pressure; Malondialdehyde; tumor necrosis factor alpha.

INTRODUCTION

Viral hepatitis is a major public health issue worldwide. It not only carries a high morbidity, but also stresses medical resources and can have severe economic consequences ***(Welsch et al., 2012)***.

Sequence analysis performed on isolates from different geographical areas around the world has revealed that it is possible to classify hepatitis C virus (HCV) into six different genotypes, labeled with numbers (1 to 6). Moreover, a seventh genotype was recently reported. Molecular and epidemiological studies have shown that HCV genotypes display significant differences in their global distribution and prevalence. Genotype 4 is largely confined to the Middle East, Egypt, and Central Africa. Thus, genotyping has become a useful method to determine the response to treatment and the source of HCV transmission in an infected localized population ***(Lavanchy, 2011).***

About 10 million HCV infected patients live in Egypt, giving Egypt the distinction of having the highest prevalence of HCV in the world and it infects almost 500,000 people in the country each year ***(Centers for Disease Control and Prevention Egypt, 2012).*** Chronic HCV is the main cause of liver cirrhosis and liver cancer in Egypt, and indeed, one of the top five leading causes of death ***(Abd El-bary et al., 2012).***

The precise mechanisms underlying HCV-related liver injury are not well understood but involve a cell-mediated immune response with lymphocytic infiltration leading to a chronic inflammatory response and progressive fibrosis which are dependent on proinflammatory and fibrogenic mediators ***(Dharancy et al., 2009).***

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily. These receptors contribute to the great diversity of physiological processes in the liver, such as control of lipid and glucose metabolisms, inflammatory responses, and cellular differentiation and proliferation. PPAR activation has been associated with anti-inflammatory and antifibrotic functions in different liver models like NASH. In light of their multiple activities, PPARs quickly became considered as therapeutic targets of the most widespread human metabolic diseases (obesity, diabetes, and atherosclerosis). The development of new non hepatotoxic ligands made it possible to use PPARs as therapeutic targets in hepatology ***(Cheng et al., 2005).*** For these reasons, recent discoveries in the field of PPARs are of intense interest not only to fundamental researchers but to clinicians as well.

PPARα exert catabolic function through peroxisomal, microsomal, and mitochondrial β-oxidation pathways, and therefore allow the use of fatty acids by hepatocytes as energetic substrate. Thus, it degrades several lipid inflammatory

mediators (prostaglandins, leukotriens) using these metabolic pathways. There are numerous experimental and clinical evidence in favor of anti-inflammatory activity of PPAR in the liver ***(Colville-Nash et al., 2004).***

T-lymphocytes, natural killer T cells (NKT), and monocytes/macrophages express significant amount of PPARα when activated. PPARα inhibit the production of inflammatory and immunomodulatory cytokines activity by these cells ***(Dharancy et al., 2009).***

Liver inflammation, hepatocyte fat accumulation, and diabetes are three pivotal hallmarks in the natural history of chronic HCV infection which are at least in part controlled by PPARs. Thus it might therefore play a role in the pathophysiology of HCV infection. Numerous studies have demonstrated that PPARα were physiologically highly expressed by parenchymal hepatic cells, where they play a pivotal anti-inflammatory and metabolic role. Several teams have demonstrated a decreased expression and functional activity of PPARα in hepatocytes during HCV infection ***(Gottardi et al., 2006).***

Hepatic stellate cells (HSCs) are the main cells responsible for liver modulation, by producing the protein of the ECM (***Friedman, 2003*).** In response to liver injury, HSC changes from a quiescent to an activated phenotype. This activation process includes a phenotypic change to a myofibroblast-like cell (MFB),

with increased proliferation rate, contractility, loss of retinoid stores and increased production of ECM proteins and several cytokines. Interestingly, HSC activation is associated with a decrease in binding to the peroxisome proliferator response element (PPRE) in vivo. PPARα suppression was associated with increased tumor necrosis factor alpha (TNF- α) secreation. So, PPARα ligands may have rescue effects on hepatic fibrosis probably due to an antioxidant and an anti-iflammatory effect in the liver ***(Toyama et al., 2004).***

Tumor necrosis factor alpha (TNF- α) has been recognized as key regulatory cytokine in liver damage through its direct role in the activation of HSCs ***(Zhou et al., 2010).*** It is known as a mediator of hepatocyte death. It also induces other cytokines and enhances cytotoxicity of lymphocytes, macrophages and neutrophils ***(González et al., 2009).***

The concanavalin A (Con A)-induced hepatitis model has been widely used to study immune system-mediated liver injury. Con A is a lectin, which when injected intravenously in rats, induces hepatic activation of T cells, NKT cells and Kupffer cells (KCs) which secrete large amounts of hepatotoxic cytokines such as TNF-α and interferon-γ (IFN-γ) ***(Erhardt et al., 2007)***. Reactive oxygen species (ROS) also play a role in Con A-induced hepatitis probably secondary to immune-mediated liver damage ***(Shirin et al., 2010***). Bolus administration of Con A induces acute hepatitis, while repeated

administrations induce a chronic form. In the latter, hepatic fibrosis occurs as a result of the chronic inflammation and the process of repairing the tissue damage ***(Yang et al., 2004).***

Now the idea that hepatic fibrosis is reversible is taking root, many clinicians are beginning to ask why, if fibrosis is reversible, is there so little progress in the clinical setting, and will patients ever really benefit from antifibrotic therapies? Underlying such questions is a subtle cynicism that the reversibility of fibrosis and cirrhosis has been discussed ***(Friedman, 2007).***

PPAR α is broadly involved in lipid metabolism, inflammation and fibrogenesis ***(Rizzo and Fiorucci, 2006)*** however, the importance of PPAR α itself for fibrosis associated with the autoimmune hepatitis has not been described. The present study was undertaken to explore the therapeutic potential of administration of a PPAR α ligand, fenofibrate (FF) and pentoxyfilline (PTX) anti TNF-α during Con A mediated chronic hepatitis in rats as regard changes in liver biochemical, histological and immunohistochemistry markers. An attempt will be made to evaluate the effects and the underlying mechanisms of PPARα agonist, fenofibrate and pentoxyfilline on HSCs activity (the major effectors of hepatic fibrogenesis). In addition the beneficial effects of their combination on the above measurements will be studied.

PATHOPHYSIOLOGY OF HCV INFECTION

HCV infection is characterized by its propensity to chronicity. Because of its high genetic variability, HCV has the capability to escape the immune response of the host. HCV is not directly cytopathic and liver lesions are mainly related to immune-mediated mechanisms. Co-factors influencing the outcome of the disease including age, gender and alcohol consumption are poorly understood and other factors such as immunologic and genetic factors may play an important role ***(Briana et al., 2012).***

The pathophysiological basis of HCV is a disturbed cooperation between parenchymal and non-parenchymal cells. The parenchymal cells commonly referred to the hepatocytes while non-parenchymal cells constitute 40% of the total number of liver cells and 6.5% of its volume. HSCs, kupffer cells (KCs) and sinusoidal endothelial cells are some of the non-parenchymal cells that line the hepatic sinusoid ***(Kogure et al., 2007*)**.

T helper cells (Th cells) activation are essential for humoral and cell mediated immune response in HCV infection.It palys a role in the activation and growth of cytotoxic T lymphocytes (CTL) and in maximizing bactericidal activity of phagocytes such as macrophages. Th cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen presenting cells (APCs).

Once activated, they divide rapidly and secrete cytokines that regulate or assist in the active immune response. T cells can differentiate into one of several subtypes, including Th1, Th2, which secrete different cytokines to facilitate a different type of immune response ***(Dienes and Drebber, 2010).***

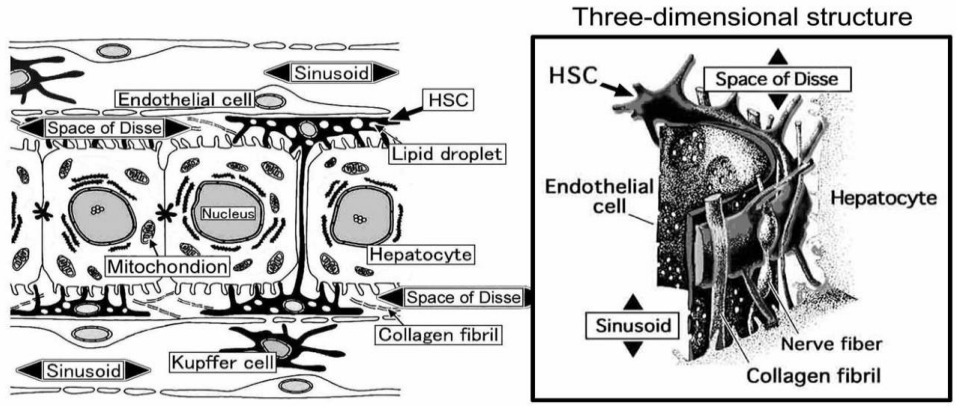

Fig. (1): Schema of the sinusoidal wall of the liver.Schematic representation of hepatic stellate cells (HSCs). Kupffer cells (hepatic resident macrophages) rest on fenestrated endothelial cells. HSCs are located in the space of Disse in close contact with endothelial cells and hepatocytes, functioning as the primary retinoid storage area. Collagen fibrils course through the space of Disse between endothelial cells and the cords of hepatocytes ***(Keiko et al., 2012).***

KCs are liver resident macrophages, one of the non parynchymal cells which are activated on HCV infection. Several observations suggested that KCs play an important role as an initial cytotoxic cell type and are likely to be a source of various products including ROS and proinflammatory cytokines particularly TNF-α ***(Boshra and Moustafa, 2011).*** These secreted cytokine play an important role in the inflammatory response of the body against infection and increase the transmigration of leukocytes to the site of injury ***(Lichtman et al., 1988).***