DIRECTLY ACTING ANTI-VIRAL DRUGS AND ITS METABOLIC SIDE EFFECTS IN PATIENTS WITH CHRONIC HCV

Thesis

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List of Contents

List of Abbreviations

Abb.	Full term
ALP	*Alkaline Phosphatase*
ASSLD	*American Association for the Study of Liver Diseases*
CHC	*Chronic hepatitis C*
CLD	*Chronic Liver Disease*
DAAs	*Direct Acting Antivirals*
DM	*Diabetes Mellitus*
EASL	*European Association for the Study of the Liver*
ECM	*Extra Cellular Matrix*
HBsAg	*Hepatitis B Surface Antigen*
HBV	*Hepatitis B Virus*
HCC	*Hepatocellular Carcinoma*
HCV RNA	*Hepatitis CVirus Ribonucleic Acid*
HCV	*Hepatitis C Virus*
HDL	*High density Lipoprotin*
INF	*Interferon*
LDL	*Low Density Lipoprotin*
LT	*Liver Transplantation*
NASH	*Non Alcoholic Steato-Hepatitis*
NIH	*National Institute Of Health*
OVs	*Oesophegeal Varices*
PDGF	*Platelet Derived Growth Factor*
peg-IFN	*Pegylated Interferon*
RBV	*Ribavirin*
SVR	*Sustained Virological Response*
T2DM	*Type 2 Diabetes Mellitus*
TG	*Triglyceride*
TNF	*Tumer Necrotizing Factor*

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million people infected worldwide (Shepard C et al., 2005).

Treatment of hepatitis C virus has traditionally been difficult because of low rates of treatment success and high rates of treatment discontinuation due to side effects (Seeff LB et al., 2002).

Current standard therapy consists of pegylated interferon and ribavirin, both of which have nonspecific and largely unknown mechanisms of action (Shiffman RN et al., 2003).

Directly acting antivirals (DAA) for the treatment of chronic hepatitis C virus (HCV) infection represents a major breakthrough for the 180 million persons infected worldwide (Lawitz E et al., 2013).

Paradoxically, hepatitis C is the only human chronic viral disease that can be cured (Lawitz E et al., 2013).

Treatment of chronic hepatitis C consisted of the combination of peg -interferon plus ribavirin, which provided limited rates of cure and was associated with frequent side effects (Lawitz E et al., 2013).

Several DAA have been identified that inhibit the NS3 protease, the NS5B polymerase or the NS5A replication complex.

Daclatasvir (DAKLINZA) is a hepatitis C virus (HCV) NS5A inhibitor indicated for use with sofosbuvir, with or without ribavirin , for the treatment of chronic HCV genotype 1 or 3 infection (Foster GR et al.,2015).

Many new therapies in preclinical or clinical development act on targets in the viral life cycle to directly inhibit viral production (Alter MJ et al., 2003).

These drugs, which are referred to as specifically targeted antiviral therapy for hepatitis C or direct-acting antiviral (DAA) agents treatment of patients with chronic hepatitis C who are naive to any type of therapy(Zeuzem S et al.,2015) .

The recent and rapid advances in the treatment of hepatitis c have completely changed and discover they have many side effects and drug drug interactions (EASL, 2016).

It is suggested that direct-acting antiviral (DAA) drugs must be used with caution and may have effect on liver function, lipid profile, and renal function (urea and creatinin) and may also effect on blood sugar level.

Aim of the Work

The aim of this work is to study side effects of directly acting anti HCV drug (DAA) and its progression in metabolism of lipid profile, blood sugar level, and renal function.

HEPATITIS C VIRUS INFECTION AND PROGRESSION OF LIVER DISEASE

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide **(Lavanchy., 2009).** HCV, a blood born pathogen that infects hepatocytes and was discovered in 1989. The long-term hepatic impact of HCV infection is highly variable, from minimal changes to chronic hepatitis, extensive fibrosis, and cirrhosis with or without hepatocellular carcinoma **(Idrees et al., 2009).**

- ***Epidemiology:***

It is estimated that approximately 130–210 million individuals, i.e. 3% of the world population, are chronically infected with HCV **(Lavanchy., 2009).** In Western Europe, HCV prevalence ranges from 0.4% to 3%. It is higher in Eastern Europe and the Middle East,

where the numbers are not precisely known **(Esteban et al., 2008).** Egypt has the highest worldwide prevalence, with 9% countrywide and up to 50% in certain rural areas, due to specific modes of infection **(Kamal et al., 2008).**

- ***Genotyping of the HCV:***

Six HCV genotypes, numbered 1–6, and a large number of subtypes have been described **(Simmonds et al., 2005).** HCV genotype 4 is the most common variant in the Middle

East and Africa and is increasing in prevalence in Western countries **(Moucari et al., 2009).**

- ***Routes of transmission:***

Prior to the 1990's, the principal routes of HCV infection were via blood transfusion, blood products, unsafe injection procedures, and intravenous drug use. These modes of acquisition are estimated to account for approximately 70% of cases in industrialized countries. Currently, new HCV infections are primarily due to intra-venous or nasal drug use, and to a lesser degree to unsafe medical or surgical procedures. Parenteral transmission via tattooing or acupuncture with unsafe materials is also implicated in occasional transmissions. The risk of perinatal and of heterosexual transmission is low, while recent data indicate that promiscuous male homosexual activity is related to HCV infection **(Van de Laar et al., 2010).**

- ***Clinical Manifestation of HCV Infection:***

The incubation period of HCV, though ranging up to several months, averages 6-8 weeks. HCV infection is often asymptomatic. Therefore, hepatitis C is often referred to as a "silent disease" (**Wakita et al., 2005).**

1) Acute hepatitis C:

Acute HCV infection is asymptomatic in 50–90% of cases **(Antantonio et al., 2008).** Children and adults who

acquire the infection usually are asymptomatic, or have a non-specific clinical illness characterized by fatigue, malaise, and anorexia and weight loss. Only 25-30% of adults that acquire HCV infection may suffer from jaundice **(Ozaras et al., 2009).**

2) **Chronic Hepatitis C:**

At least 85% of individuals acutely infected with HCV develop chronic HCV infection. Chronic infection with hepatitis C virus (HCV) is the leading cause of cirrhosis. The majority of persons with chronic hepatitis C virus (HCV) infection develop liver cirrhosis ***(*****Kamal et al., 2008).**

Hepatitis C virus (HCV) is an enveloped virus with a ~ 9.6 kb single-stranded RNA genome **,** a member of the

Flaviviridae family and genus Hepacivirus. HCV genome encodes a single polyprotein which is processed co-translationally into three structural and seven nonstructural (NS) polypeptides ***(Ali et al., 2011).***

HCV core protein forms the capsid, which is surrounded by a lipid bilayer containing the envelope glycoproteins, E1 and E2 on the external surface. These envelope glycoproteins are responsible for initiation of infection in a host cell. The nonstructural (NS) proteins coordinate the intracellular processes of the virus life cycle. HCV is a major cause of chronic liver disease, with an estimated 180 million people infected worldwide. An important therapeutic advancement was

achieved with the recent discovery of potent direct acting antiviral agents (DAAs) against HCV **(*Au JS et al*., 2014).**

Several clinical trials have shown various combinations of agents, including interferon-free regimens, to be highly effective in the clearance or sustained viral response (SVR) of chronic hepatitis C infection. However, significant challenges remain in deploying modern antivirals for patients with asymptomatic HCV infection and must be sought through screening programs. HCV infection particularly affects persons of low socioeconomic status who have less access to health care. The very high cost of HCV treatment may also contribute to delays in patients being treated. Majority of the infected patients (approximately 80 %) develop chronic infection and are at high risk for end stage liver disease progression to cirrhosis and hepatocellular carcinoma (HCC). HCC is a common cancer worldwide and accounts for ~5.6 % of all cancers. It is the fifth common cancer in the world and the third common cause of cancer death ***(Sherman et al., 2011).***

The incidence of HCC is rising precipitously, primarily as a result of the increasing prevalence of chronic HCV infection and fatty liver disease.

Liver fibrosis is strongly associated with HCC, since approximately 80-90 % of HCC cases are arising in cirrhotic livers ***(Lok AS et al., 2012)***.

HCC development is also linked to alcoholic cirrhosis . HCV does not integrate into its host genome and has a cytoplasmic life cycle ***(Moradpour et al., 2007).***

HCC, therefore, must involve several indirect mechanisms including the interplay between HCV and host cell genes/proteins for pathological consequences. In addition, HCV induces epithelial to mesenchymal transition (EMT) state that is known as important element in cancer progression ***(Bose et al., 2012).***

EVASION OF INNATE/ADAPTIVE IMMUNE RESPONSES BY HCV:

IFN response HCV infection is sensed by multiple innate immune pathways, but often not cleared by immune responses, resulting in a chronic infection. HCV blocks the IFN response pathway by several mechanisms. HCV NS3/4A utilizes its protease domain to cleave key innate immune signaling adaptor proteins, effectively inactivating viral RNA detection program ***(***Höner Zu ***et al., 2014).***

Hepatocytes persistently infected with HCV and treated with IFN-α, PKR kinase is activated for translational suppression of host mRNAs, including ISGs, and antiviral functions of IFN . Several HCV proteins have been implicated as regulators of the IFN response pathway.

Expression of HCV proteins blocks IFN signaling at the level of the JAK/STAT pathway and impairs IRF-7 nuclear localization through its NS5A protein ***(Chowdhury et al., 2014).***

IFI6 is a type I ISG and plays a critical role in the regulation of apoptosis. IFI6 is strongly associated with the immune system, but its antiviral effects are not well known. Our recent (unpublished) experimental findings suggest co-localization of HCV co receptors during HCV entry are compromised by IFI6 mediated disruption of kinase function, thereby inhibiting HCV at the point of entry.

Cytokine response

A relationship between the activation of genes involved in the IL-6 signaling pathway and the development of HCC has been observed.

An increase of the β-2 microglobulin in serum level as well as IL-6 level was observed among HCV infected HCC patients. Weakening of the immune system, due to IL-6, may be responsible for a more severe progression of HCC and the hyperexpression of β-2 microglobulin ***(Tang et al., 2008).***

HCV core protein attenuates IL-6 stimulated acute-phase response, and contributes to impaired innate immunity for viral persistence . TNF-α plays diverse roles, including in the inflammatory processes, in HCV infection . HCV may actively

contribute to the fibrogenic process via the paracrine effect of IL-8 secreted by infected hepatocytes ***(Koike et al., 2005).***

Autophagy :

Autophagy is a process of degradation of cytoplasmic materials,including damaged organelles and long-lived proteins, in the cells for the maintenance of cellular homeostasis. During autophagy, the double membrane vesicles, called autophagosome, engulf the cytoplasmic materials and fuse with the lysosome for degradation. Autophagy has been identified as a component of the innate immune system against viral infection. We were the first to demonstrate that HCV induces autophagy in immortalized human hepatocyte ***(Ait-Goughoulte et al., 2008).***

Subsequently, HCV subgenomic replicon and infection were shown to induce autophagy in hepatoma cell**.** Autophagy proteins (Beclin-1, Atg4B, Atg5 and Atg12) are required for initiation of HCV replication and contribute contribute to the effective production of virus particles ***(Todorovic V et al., 2***012***).***

Autophagy proteins in HCV infected hepatocytes enhance interferon signaling pathway and induces apoptosis **.** HCV mediated autophagy may promote infectious virus particle production and evade innate immune response for establishment of persistent infection ***(Shin JY et al., 2005).***

Complement:

The complement system is one of the vital effectors in the innate immune system for targeting and eliminating infected cells and invading microorganisms, including free virus particles . HCV escapes the complement response by regulating complement components. HCV proteins suppress C3/C4 complement expression , and attenuates membrane attack complex (MAC)- mediated microbicidal activity by suppressing C9 expression ***(Kim et al., 2014)***.

To avert damage from excessive complement activation and MAC formation, host cells express membrane-bound regulators of complement activation (RCA) proteins, including CD46, CD55 and CD59, to limit these processes ***(Pangburn et al., 2008).***

HCV core protein enhances transcription and surface expression of DAF/CD55 in infected hepatocytes and promotes incorporation onto mature HCV particles**.**

HCV also incorporates CD59 and protects against complement mediated lysis . DAF/CD55 expression has been associated with complement dependent cytolysis (CDC), antibody dependent cell cytolysis (ADCC), and NK cell function ***(Kim et al., 2014).***