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UNDERLYING PATHOPHYSIOLOGY OF HCV INFECTION IN HIV-POSITIVE DRUG USERS

Anuradha Balasubramanian, PhD, Jerome E. Groopman, MD, and Ramesh K. Ganju, PhD*
Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Abstract

HCV and HIV infections are very common among injection drug users (IDUs). It is well known that 80–90% of HIV-infected IDUs are also infected with HCV. Furthermore, patients with HCV/HIV co-infection are at a higher risk of progressing to end-stage liver disease, namely cirrhosis. Even though there is increasing global awareness of HCV/HIV co-infection and extended therapeutic programs for this infected population, little is known about the HCV/HIV pathophysiology that mediates the rapid progression to hepatic disease. Liver disease caused by HCV/HIV co-infection is characterized by inflammation and cell-death. Recent reports suggest that the HIV and HCV envelope proteins may induce apoptosis and inflammation in hepatocytes via a novel pathway involving collaborative signaling. Moreover, HCV/HIV co-infection may also alter the cytokine production *in vivo*. Further studies to elucidate the molecular mechanisms of HCV and HIV-mediated pathogenesis will help in the development of therapeutic strategies against HCV/HIV co-infection in these patients.

Keywords

HCV; HIV; drug user; inflammation; cell death

Introduction

Hepatitis C virus (HCV) is an enveloped single-stranded positive sense RNA virus.¹ This non-cytopathic hepatotropic Flaviviridae causes acute and chronic hepatitis and hepatocellular carcinoma (HCC).² Although the liver is the primary target organ, extra-hepatic reservoirs such as peripheral blood lymphocytes,³ epithelial intestinal cells,⁴ and the central nervous system⁵ also harbor HCV. An estimated 3% of the world's population (more than 170 million people) are infected with HCV, and approximately 38,000 new infections occur annually in United States alone.⁶ Chronically-infected patients have viral loads that usually range from 10^3 – 10^7 genomes/ml of serum and mathematical derivations show about 10^{12} viruses produced/day in a typical infected person.⁷ This is about 100-fold greater than the rate reported for HIV. Current medical options are limited, and around 10,000 to 20,000 deaths per year in the US are attributed to HCV infection. The worldwide prevalence of HCV among various population categories is illustrated in Figure 1.

HCV is primarily transmitted percutaneously⁸ and through blood and related products,⁹ hemodialysis,¹⁰ and organ transplantation.¹¹ An estimated incidence of HCV among injection drug users is 30–90%.¹² HCV is dominant among drug users who share drug-using equipment,

*Address Correspondence to: Ramesh K. Ganju, PhD, BIDMC, Harvard Institutes of Medicine Room # 342, 4 Blackfan Circle, Boston, MA 02115, Tel: (617) 667-0060, Fax: (617) 975-5243, E-mail: E-mail: rganju@bidmc.harvard.edu.

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practice front-loading and/or frequently inject cocaine, heroin, opium and poly-drugs.^{13,14} Liver, the major *in vivo* detoxification site for drugs, is strained due to alcohol and drug abuse in addition to HCV infection.¹⁵ Hepatotoxicity due to drug interaction is a common problem encountered in patients who undergo therapeutic programs. However, years of illicit drug use likely aggravates the irreversible liver damage caused by HCV infection.^{16,17}

Since the vast majority of HIV-positive injection drug users have been exposed to HCV, the prevalence of HIV/HCV co-infection mirrors that of HIV. HCV infection is likely to be predominant in 80–90% of HIV-infected injection drug users.¹⁸ Co-infection causes higher HCV titers and an accelerated progression to liver cirrhosis.¹⁹ There is a high co-morbidity in IDUs due to this co-infection, which is approximately 33% in the United States alone.²⁰

Progressive liver disease caused by HCV and HCV/HIV co-infection

Chronic hepatitis is a complex clinico-pathological syndrome with multiple causes, varying stages of necro-inflammatory and sclerosing liver damage, different prognoses and responses to treatment.²¹ Liver disease caused by HCV can be clinically categorized into three stages: (i) early or active hepatitis, (ii) cirrhosis and (iii) liver failure and HCC.^{22,23}

During active hepatitis, increased inflammation of the liver results in elevated liver enzymes.²⁴ Approximately 45% of HCV-infected drug users admitted for treatment demonstrate elevation of one or more liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).^{24,25} Patients are more responsive to antiviral therapy at this stage,^{22,23} which may last indefinitely or until cirrhosis develops.²⁶

During the cirrhotic phase, fibrosis and scarring of the liver may occur. The liver may be initially enlarged due to sequestration of fluid, scarring and fatty infiltration. Later, the liver becomes shrunken and shrinks to a smaller size.²⁷ There may be disturbances in bile secretion, and back up blood flow into the spleen causing hypersplenism and thrombocytopenia.²⁸ Protein abnormalities are very prominent and observed as elevated total globulins, lowered albumin and a low albumin/globulin ratio.^{22,23,29} In contrast, liver enzymes in serum are within the normal range.²⁵

Liver failure may be due to the lack of viable liver tissue resulting in jaundice or its inability to deactivate ammonia and other compounds or to adequately excrete bile. Major signs of failure include edema, stasis changes, and discoloration and ulceration of the feet and ankles. Ascites or right-sided pulmonary congestion may also occur.²⁷ HCV-associated cirrhosis leads to liver failure and death in about 20–25% of cirrhotic cases.¹

Chronic HCV infection appears to be associated with the development of HCC in 1–5% of infected individuals,¹ and is commonly observed among drug users.^{26,30} Nodular liver is a characteristic feature of HCC and is rare in HCV patients without cirrhosis.³¹ Although cirrhosis is increasingly seen in HCV/HIV co-infected individuals, reports of HCC among HIV-infected patients are scarce.³²

As compared to people infected with HCV, those with HCV/HIV co-infection have an approximately 2 to 6 fold increased risk of end-stage liver disease.^{33,34} This leads to the hypothesis that HCV load may predict the risk of HCV-associated end-stage liver disease with or without HIV infection.³⁵ Most recent studies have involved determining the epidemiological profiles and evaluating promising therapies for HCV/HIV co-infected IDUs.^{36,37} The clearance of HCV infection either alone or in the presence of HIV infection and drug addiction is complex.³⁸ Therefore, recent studies have mainly focused on host immune response and/or the therapeutic response to HCV/HIV co-infection.^{39,40} Others have reported on the clinical

consequences of HCV/HIV co-infection such as neuropsychiatric complications,⁴¹ neurocognitive impairments,⁴² liver toxicity and hepatocarcinoma.^{43,44}

Pathophysiology of HCV/HIV co-infection

Inflammation and cell death are the synergistic pathways that determine the underlying pathogenesis of HCV. Numerous attempts have been made to outline HCV pathogenesis in the liver. Despite the cloning of HCV genome in 1989,⁴⁵ not much has been deciphered about the modulation of host cellular processes in the HCV-induced liver disease due to the lack of appropriate *in vitro* and small animal model systems. Likewise, reports on the pathology of HCV/HIV co-infection are greatly lacking.

Elevated serum levels of the adhesion molecules, E-selectin, IL-8 and TNF- α have been reported in HCV patients with liver inflammation and fibrosis.^{46,47} The level of chemokines present in plasma or inflammatory biological fluids is often correlated with the severity of the pathology and/or the outcome of these patients.^{48,49} Furthermore, greater degrees of portal, periportal and lobular inflammation (centrilobular fibrosis, cholestasis and granulocytic cholangiolitis) are seen in patients with HCV/HIV co-infection than in those with HCV alone.⁵⁰

Previously, apoptosis of hepatocytes has been demonstrated in the liver biopsies of HCV infected patients.⁵¹ Similarly, apoptosis has been observed in long-term HIV infected hemophilia patients.⁵² Specifically, the HIV envelope protein gp120, has been reported to induce apoptotic cell death of lymphocytes, neurons, myocardiocytes and hepatocytes.⁵³ Recent studies have shown Fas expression on hepatocytes and irreversible hepatic apoptosis in HCV patients with or without HIV infection. However, in presence of HIV, Fas expression and the hepatic apoptosis took on an accelerated course in the co-infected patients.⁵⁴

Mechanisms of hepatic inflammatory response and cell death

Several mechanisms have been proposed to elucidate the pathophysiology of liver disease caused by HCV/HIV co-infection. Inflammation is characterized by the invasion of activated leukocytes into the injured tissue and is critically dependent upon the rapid expression of chemokines.⁵⁵ Some researchers have hypothesized that the pathogenesis of the liver during HCV infection might be due to cellular immune responses against the virus, specifically involving CD8⁺ T cells which activate hepatic stellate cells leading to liver inflammation and fibrosis.^{56,57} However, the progression of liver disease seen in immunocompromised hosts, such as patients with HIV infection,⁵⁸ defies the above hypothesis. Several studies have shown that both HCV-specific CD4⁺ and CD8⁺ cell responses are less frequent than HIV-specific responses in the peripheral blood of co-infected individuals.^{59,60} Moreover, in HCV/HIV co-infected patients, the vigor of the HCV-specific CD8⁺ cytotoxic T cell responses is directly related to the CD4⁺ cell count.⁶¹

Others have hypothesized that pathogenesis of the liver during HCV infection might be due to the responses of infected and uninfected liver cells against the viral proteins during infection. Recently, cellular responses to individual HCV proteins namely core, E2 and nonstructural proteins have been increasingly reported.^{62–66}

A new report by Blackard et al has shown that suppression of intrahepatic cytokines during HCV/HIV co-infection leads to an imbalance between pro-fibrogenic and anti-fibrogenic cytokines, thus favoring liver fibrosis and HCV replication in the liver.⁶⁷ An enhanced progression of hepatic fibrosis has been observed in co-infected as opposed to monoinfected patients.^{68,69} However, there have been controversial reports on the association of steatosis

and fibrosis in co-infected patients,^{70,71} even though steatosis was correlated with fibrosis in mono-infected patients.^{58,72,73}

Recently, it was reported that HCV-E2 and HIV-gp120 co-operatively induced apoptosis and the pro-inflammatory cytokine IL-8 in HepG2 cells and primary hepatocytes. Figure 2 shows the time-dependent induction of cell death and IL-8 production upon HCV-E2 and HIV-gp120 co-stimulation. HCV-E2 and HIV-gp120 might trigger downstream signaling pathways that contribute to inflammation⁷⁴ and apoptosis^{75–77} in uninfected hepatocytes via an ‘innocent bystander’ mechanism. This effect may be due to the binding of these viral envelope proteins to the cell surface independent of direct viral infection. A similar induction of these signaling pathways by HCV envelope proteins was also recently reported in human endothelial cells.⁷⁸

Inflammation is predominant during the early stage of HCV infection when the therapeutic response rate is high.^{79–81} Even though inflammation persists throughout the process of liver damage, the liver is inclined towards cirrhosis and cell death during the end stage.^{82,83} Findings with HCV and HIV envelope proteins also seem to point towards the co-existence of both hepatic inflammation and cell death during HCV/HIV co-infection. Chemokines have been known to play an important role in inflammation.⁸⁴ Chemokine (IL-8) production was reported to be enhanced when hepatocytes were stimulated with HCV-E2 and HIV-gp120. Moreover, IL-8 expression was found to be positively regulated by p38 MAP kinase and SHP2, a protein tyrosine phosphatase. In particular, p38 MAP kinase was reported to upregulate AP-1 mediated IL-8 production independent of NFκB activation. In parallel, the enhanced activities of non-receptor mediated tyrosine kinases, serine kinases and p38 MAP kinase observed during HCV-E2 and HIV-gp120 co-stimulation may also induce the downstream phosphorylation of transcriptional factors causing upregulation of FasL. Altered mitochondrial membrane potential followed by cytochrome C release and caspase-3 activation, appears to be the mode of HCV-E2/HIV-gp120 induced Fas-mediated cell death in hepatic cells (Figure 3).

Summary

Drug use is a frequent complicating factor associated with HCV infection in patients both with and without AIDS. Since the liver is the site of *in vivo* drug detoxification, studies on HCV and HIV should be conducted in an environment of impaired drug metabolism. Moreover, such studies would give advantageous insights into adverse drug interactions within the cell during antiviral therapy. While clinical research and therapeutic studies done on HCV/HIV co-infected IDUs with liver disease are essential, liver disease can be completely understood and controlled only when more pathophysiological reports become available. In recent years, research on both of these complicated viruses has advanced to a promising degree. However, investigation of host cell responses is a necessary step towards identifying the specific cellular proteins responsible for disease pathology in HCV/HIV co-infection. In summary, modulation of such cellular proteins might be used as a potential therapeutic strategy to evaluate and eradicate HCV and HIV infections as well as greatly assist in the treatment of drug using populations.

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Abbreviations

AP-1, Activator protein-1; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HCC, Hepatocellular carcinoma; IDUs, Injection drug users; IFN α , Interferon α ; IL-8, Interleukin 8; NS, Nonstructural protein; NF κ B, Nuclear factor-kappa B; TNF- α , Tumor necrosis factor α .

Figure 1. The worldwide prevalence of HCV among various population categories

Figure 2. Time-dependent apoptotic and inflammatory responses to HCV-E2 and HIV-gp120 envelope proteins in HepG2 cells

HepG2 cells were unstimulated [UN] or stimulated with HCV-E2 [1.5 nM], HIV-gp120 [0.8 nM] or their combination [HCV-E2 + HIV-gp120] for a period of 6, 12 and 24 h. [A] Cells were labeled with propidium iodide and the cell death was quantified by flow cytometry. After stimulation for the time periods indicated above, the culture media supernatants were collected and assayed for the production of IL-8. Data represent one of three independent experiments performed in quadruplicates.

Figure 3. Diagrammatic representation of the mechanism of cell death and inflammatory responses induced by HCV-E2 and HIV-gp120 envelope proteins