

High HIV-DNA Intrahepatic Content is Associated with a More Severe liver disease in HIV/HCV Co-Infected Patients with high HIV viremia compared to aviremic HIV/HCV Co-Infected Patients

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Abstract

Background: The recruitment of HIV infected T cells and/or the accumulation of HIV within non-parenchymal cells of the liver could represent the source of additional viral antigens that may favor the immunologically mediated events responsible for the fast progression of liver disease observed in HIV/HCV patients. The aim of this study was to determine HIV-DNA intrahepatic content and to relate these findings to immunological and pathological correlates of liver disease.

Methods: We randomly selected 56 HIV/HCV chronically infected patients, with similar age and risk factors, among those who underwent liver biopsy for diagnostic purposes. The patients were divided into two groups based on levels of HIV viremia: 31 HIV/HCV co-infected patients had undetectable HIV viremia (Group 1) and 25 HIV/HCV co-infected patients had HIV viremia greater than 25000 copies/ml (Group 2). All patients in Group 1 were treated with HAART, whereas 14 patients in Group 2 were in structured treatment interruptions before liver biopsy. 5 patients in HAART failure and 6 naive to HAART. Total intrahepatic HIV-DNA was determined by using a quantitative real time PCR. The present study was approved by Ethic Committee of San Raffaele Scientific Institute and performed according to the Helsinki declaration.

Results: The two groups of patients had comparable time of HIV/HCV co-infection (Group 1, 17.9 (14-21) years vs 19.9 (11-22) years in Group 2, p=ns). HCV genotypes were similarly distributed and there was no difference in HCV-RNA viremia [Group 1, 766 x10³ (404-1665) copies/ml vs 915 x10³ (667-1820) in Group 2, p=ns]. Intrahepatic HIV-DNA was detected in 20 out of 31 Group 1 patients and in 23 out of 25 Group 2 patients. The median intrahepatic content of HIV-DNA was 40.4 ± 12.5 copies/ug DNA and 120 ± 37 copies/ug DNA in Groups 1 and 2 respectively (p<0.001). The prevalence of moderate to severe grading of hepatic histology was 58% in group 1 and 76% in HIV-viremic subjects (p=0.015), with higher median ALT levels in Group 2 patients (Group1 vs 2= 64 (41-117) IU/L vs 95 (75-135) IU/L, p=0.012).

Conclusions: This data suggest that in HIV/HCV coinfecting patients uncontrolled HIV viremia is associated with the accumulation of HIV infected cells within non-parenchymal cells of the liver. The accumulation of intrahepatic HIV-DNA represents the source of additional viral antigens that may favor the immunologically mediated events, responsible for the fast progression of liver disease in HCV co-infected patients.

Introduction

Liver disease due to HIV/HCV co-infection has emerged a important cause of increased morbidity and mortality in HIV infected patients, the pathogenetic mechanisms responsible for this effect remain elusive.

It is widely believed that HCV is noncytopathic and immunological related events (including the recruitment of HCV specific T-cells and the production of pro-inflammatory and pro-fibrotic mediators) play a crucial role in promoting both liver damage and viral clearance. Furthermore, several recent publications indicate that HIV/HCV progressive liver disease, is associated with increased intrahepatic production/release of several pro-inflammatory mediators that accelerate disease progression. In addition to HCV related immunological events, the recruitment of HIV infected T cells and/or the accumulation of HIV within non-parenchymal cells of the liver could represent the source of additional viral antigens that may favor the immunologically mediated events (intrahepatic cytokine production), responsible for the fast progression of liver disease observed in HIV/HCV patients.

Objective

The aim of this study was to determine HIV-DNA intrahepatic content in two groups of HIV/HCV co-infected patients and to relate these findings to immunological and pathological correlates of liver disease.

Patients

A total of 56 patients were selected between the 288 HIV/HCV patients that underwent liver biopsy for diagnostic purposes between 2003 and 2007.

◆ 31 HIV/HCV co-infected patients with HIV viremia less than 1000 cp/ml (Group 1)

◆ 25 HIV/HCV co-infected patients with HIV viremia greater than 25000 cp/ml (Group 2)

Group 1 - All patients were treated with HAART

Group 2 - 14 patients were in structured treatment interruptions

- 5 patients in HAART failure

- 6 naive to HAART

Inclusion criteria:

- Increased transaminases at least in 2 occasions in the previous year
- Absence of any prior HCV therapy
- Absence of circulating HBSAg
- Availability of a frozen liver biopsy specimen that was not necessary for diagnostic purposes
- For the HIV positive Pz. absence of any opportunistic infection

Table 1. Characteristics of patients at the time of liver biopsies

	Group 1 (n=31)	Group 2 (n=25)	p
Age, years [median ± (IQR)]	42.9 (42-45)	42.6 (41-45)	ns
Gender, female/male	13/18	5/20	ns
Risk factors, % IDU	58	72	ns
Time of HIV infection [median ± (IQR)]	17.9 (14-21)	19.9 (11-22)	ns

IQR, interquartile range; IDU: Former intravenous drug user.

Methods

- ◆ HIV viremia was measured by means of the branched chain DNA (b-DNA) technique (Bayer)
- ◆ HCV viremia was quantified by means of the COBAS Amplicor HCV Monitor test (Roche)
- ◆ HCV Genotype was characterized by 5'UTR amplification, sequencing and phylogenetics analysis.
- ◆ The Histological Activity Index (HAI) and the degree of liver fibrosis was determined on liver biopsies by the method described by Ishak et al. (1995).
- ◆ Intrahepatic HIV-DNA quantification was performed following the SIVIM study group for DNA test standardization protocol. The following primers and probe were used to amplify a conserved region of the HIV-1 pol gene:

GAGFW - TTAAGTGTTTCAATTGGCAAAAGA

GAGRW - AAAAAAATGAGCTGCTCTCAGTACAATC

GAGProbe FAM-CCCCTAGGAAAAGGGCTGTGGAAATG-BHQ1

The 9700 RT-PCR system (AppliedBiosystems) and the ITAQ SPRMX were used (BioRad) for the real time quantification.

Results

Table 2. No difference in serum HCV-RNA levels and distribution of HCV genotypes in the two groups of patients

	Group 1 (n=31)	Group 2 (n=25)	p
HCV-RNA (IU/ml) [median ± (IQR)]	766 x 10 ³ (404-1665)	915 x 10 ³ (667-1820)	ns
HCV-Genotype n, (%)			ns
1a, 1b	14 (45.2)	9 (36)	
2a/c, 2b	1 (3.2)	1 (4)	
3a	7 (22.6)	6 (24)	
4a, 4a/c	9 (29)	9 (36)	

IQR, interquartile range

Table 3. HIV-RNA quantitation and peripheral CD4+ and CD8+ T cells counts

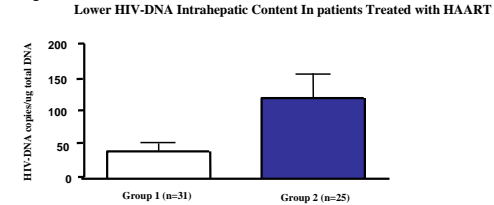
	Group 1 (n=31)	Group 2 (n=25)	p
HIV-RNA (copies/ml) [median ± (IQR)]	< 50	45970 (38000-73000)	< 0.0001
CD4 [median ± (IQR)]	507 (412-746)	384 (238-135)	0.0005
CD8 [median ± (IQR)]	843 (535-1167)	1166 (870-1556)	0.01

Table 4. HIV/HCV Co-Infected Patients treated with Effective HAART have Lower HIV-DNA Intrahepatic Content and Reduced Liver Disease Compared to Co-Infected Patients with High HIV Viremia

	Group 1 (n=31)	Group 2 (n=25)	p
HIV-DNA (copies x ug DNA) [median ± (IQR)]	8.6 (0-44)	49 (22-163)	0.006
ALT [median ± (IQR)]	64 (41-117)	95 (75-135)	0.012
AST [median ± (IQR)]	45 (36-64)	78 (45-116)	0.025
Hepatic Histology: Grading			0.015
Minimal to Mild, n (%)	18 (42)	6 (24)	
Moderate to Severe, n (%)	13 (58)	19 (76)	
Hepatic Histology: Staging			
F1	15	5	
F2	12	15	
F3	3	4	
F4	1	1	

IQR, interquartile range; IDU: Former intravenous drug user; F1, no fibrosis or few portal tracts expansion; F2, portal fibrosis with expansion of most of the portal tracts; F3, bridge fibrosis; F4, cirrhosis.

Figure 1.



Conclusions

- In HIV/HCV coinfecting patients uncontrolled HIV viremia is associated with the accumulation of HIV infected cells within non-parenchymal cells of the liver.
- Effective HAART reduces the accumulation of HIV infected cells in the liver and is associated with a reduced liver disease.
- This effect seems independent of HCV, since similar levels of HCV-RNA are present in the two groups of patients.
- The accumulation of intrahepatic HIV-DNA may represent the source of additional viral antigens that may favor the immunologically mediated events (i.e., increased production of pro-inflammatory cytokines), responsible for the fast progression of liver disease in HCV co-infected patients.

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