HCV Virological Response During Treatment of Chronic Hepatitis C is Associated With Liver Histological Improvement in Patients With HCV/HIV Co-Infection

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Liver histological improvement after treatment for chronic hepatitis C in patients co-infected with human immunodeficiency virus-1 (HIV-1) has been described. Paired liver biopsies in twenty six HCV/HIV co-infected patients were compared to determine factors possibly associated with histological improvement. The patients were submitted to a liver biopsy before treatment for hepatitis C and 25 months after the end of treatment. Fragments of the liver biopsy obtained before and after treatment were compared regarding the following parameters: histological activity index (HAI) and degree of fibrosis (Knodell); intensity of collagen deposits (Sirius Red staining) and degree of stellate cell activation (alpha-smooth muscle actin labeling). The ratios of the post and pre-treatment variables were related through logistic regression to body mass index (BMI), alcohol ingestion, HCV genotype, HCV viremia, presence of hepatic iron and pre-treatment hepatic steatosis. A negative RNA test in the 24th week of treatment was associated with improvement in fibrosis, collagen deposits and stellate cell numbers. The other variables analyzed did not correlate to an improvement in hepatic histology after hepatitis C treatment. Reduction in HCV viremia during treatment may result in reduced hepatic fibrosis even in patients without a sustained virological response.

Key-Words: Chronic hepatitis C, hepatic stellate cell, histological response, human immunodeficiency virus, paired liver biopsies.

It is known that human immunodeficiency virus (HIV) may modify the natural history of hepatitis C virus infection (HCV) by accelerating the progression of hepatic fibrosis and its complications, especially in severely immunocompromised patients [1-3]. PEGylated or non-PEGylated interferon-alpha in combination with ribavirin, the recommended treatment for chronic hepatitis C, can promote a sustained virological response (SVR) in a considerable number of patients and, in some cases reduce the liver inflammatory and fibrotic processes [4,5]. In addition, some studies have shown that treatment with interferon-alpha leads to improved liver histology, mainly reducing inflammation and having a lesser effect on fibrosis [6], especially in patients who show a sustained virological response [7,8]. On the other hand, the influence of the treatment on the improvement of hepatic histology in patients who do not have SVR has shown controversial results [9-13].

Progression of hepatic fibrosis has been associated in HCV/HIV co-infected patients with some factors like gender (males), more advanced age when HCV infected, higher alcohol ingestion, lymphocytes CD_4^+ cells < 500/mm^3 and HAART non-users [14,15]. In mono-infected patients, iron overload and hepatic steatosis may also be associated with a rapid fibrotic progression [16,17].

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The objective of the present study was to evaluate factors possibly associated with improvement in liver histology after the treatment of chronic hepatitis C in patients co-infected with HIV.

Material and Methods

Patients

Twenty six adult patients (19 males) with chronic hepatitis C co-infected with HIV-1 were included in the study.

The inclusion criteria were, to be older than 18 years, to have confirmed HCV and HIV infections, to have been in treatment for chronic hepatitis C for at least 24 weeks and to accept participation in the study by signing the informed consent form. The exclusion criteria were, to have other associated hepatic diseases, to be infected with hepatitis B virus, to have any active opportunistic disease and to present any contraindication for a liver biopsy or for treatment of hepatitis C. The study was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil.

Table 1 shows the general, demographic virological and histological characteristics of the patients included in the study, obtained before hepatitis C treatment.

Treatment of Chronic HCV Infection

Patients were treated, in standard regimens, with interferon-alpha and ribavirin (RBV) in 70% of cases, with PEGylated interferon-alpha and RBV in 25% of cases and with interferon-alpha monotherapy in 5% of the patients over a mean period of 34.4 ±9.03 weeks.

HCV RNA

HCV RNA was determined by a qualitative test, having an inferior limit of detection of 50 copies/mL (COBAS

Table 1. Baseline characteristics of patients (n=26) with chronic hepatitis C co-infected with HIV.

Characteristics			N	
Male (%)			19.0	(73.0)
Age (years) mean (SD)			37.3	(5.3)
Body-mass index (kg/m^2) mean (SD)			24.2	(4.0)
Use of antiretroviral drugs, nº (%)			22.0	(84.6)
CD_4^+T lymphocytes in blood, mean (SD)			687.5	(254.8)
200 - 500 cells/μL nº (%)			4.0	(15.3)
≥ 500 cells/μL nº (%)			22.0	(84.7)
HIV RNA				
≤50 copies/mL nº (%)			10.0	(41.7)
>50 copies/mL nº (%)			14.0	(58.3)
Log_{10} of HIV RNA, mean (SD)			3.8	(0.7)
HCV Genotype 1 nº (%)			21.0	(80.8)
Genotype 2			1.0	(3.8)
Genotype 3			4.0	(15.4)
Histological parameters of liver biopsies				
HAI	nº (%)	1-3 (minimal)	0.0	(0)
		4-8 (mild)	8.0	(30.8)
		9-12 (moderate)	17.0	(65.4)
		13-18 (severe)	1.0	(3.8)
Fibrosis	nº (%)	F0 (absent)	0.0	(0)
		F1 (mild)	8.0	(30.8)
		F2 (moderate)	17.0	(65.4)
		F3 (severe)	1.0	(3.8)
		F4 (cirrhosis)	0.0	(0)
Collagen deposits, mean (SD)			26.2	(9.3)
Number of HSC alpha-SMA positive per field, mean (SD)			24.7	(7.8)
Iron deposits nº (%)		Absent	15.0	(57.7)
		Present	11.0	(42.3)
Steatosis nº (%)		Absent	10.0	(38.5)
		Present	16.0	(61.5)
ALT nº (%)		≥ 2 ULN	13.0	(50.0)
		< 2 ULN	13.0	(50.0)

HSC alpha-SMA=hepatic stellate cells positive for smooth muscle alpha actin; HAI=histological activity index; ALT=alanine aminotransferase; ULN=upper limit of normality.

AMPLICOR®, Roche Diagnostics GmbH, Mannheim, Germany), carried out before the beginning of treatment and, afterwards, in the 24th week, in the 48th week and 6 months after the end of treatment (72nd week). A negative HCV RNA by the 72nd week was considered as a sustained virological response (SVR).

Histological Evaluation

Patients were submitted to an ultrasound-guided percutaneous liver biopsy with a 14 G Tru-Cut needle under local anesthesia before the beginning of treatment and about 25 months after the end of treatment for hepatitis C. Liver histology and immunohistochemical analysis were carried out by two independent pathologists in a blinded fashion. Fibrosis degree and the histological activity index (HAI) were assessed according to the classification of Knodell et al. modified by Desmet et al. [18]. Semi quantitative analysis of collagen deposition was performed by Sirius Red staining, using a point-counting procedure at 400x magnification. A total of 1,000 points per liver were counted for each patient and each point was classified as positive or negative for collagen. The results were reported as percentage of points stained by Sirius Red [19].

Histological semi quantitative analysis of iron deposits was made by Perl's staining according to the classification by Sciot et al. [20]. The grade of hepatic steatosis was evaluated by the method of Patton et al. [21].

Hepatic stellate cells (HSC) were evaluated by immunohistochemical staining for α-smooth muscle actin (α-SMA, 1A 4, DAKO A/S, Denmark, 1:100 dilution), using an avidin-biotin-peroxidase complex system (Novostain Super ABC Kit, Universal, Novocastra Laboratories Ltd., UK), counterstaining with hematoxylin and by comparison with

positive and negative controls. The number of α-SMA-positive cells per microscopic field was evaluated, blindly, at high power (400x) magnification in 10 fields chosen at random [22].

Paired Analysis of the Biopsies

The parameters were expressed numerically as follows: for HAI and fibrosis, scores of 1 to 18 and from 0 to 4, respectively. Collagen deposit was expressed as percentage of points stained by Sirius Red and HSC, as the number of alpha-SMA-positive cells per microscopic field. Histological improvement, evaluated separately for each parameter, was considered when the numerical ratio of results obtained for the paired biopsies (post-/pre-treatment) was 1 or less.

Statistical Analysis

A model of logistic regression was used to analyze the association of liver histological improvement with the following independent variables: body mass index (BMI, < or ≥ 25 kg/cm^2); alcohol ingestion (yes/no); HCV genotype (1/non-1); RNA HCV at the 24th week after the beginning of treatment (positive/negative); hepatic iron in the pre-treatment biopsy (positive/negative); hepatic steatosis in the pre-treatment biopsy (absent/present) and the pre-treatment alanine aminotransferase (ALT) values (< or ≥ twice the upper limit of normality (ULN). The dependent, response variables, were the fibrosis grade, the HAI index, the grade of collagen deposits and the number of HSC-α-SMA positive cells. Results were expressed as odds-ratios (OR), with the respective 95% confidence intervals (CI).

Results

The analyses of associations of the independent variables with the improvement of fibrosis, HAI, collagen deposits and number of HSC-α-SMA positive cells in paired biopsies, before and after hepatitis C treatment are summarized in Tables 2-5.

In relation to HAI there was no association between variables and improvement of this histological parameter (Table 2). On the other hand, there was an association between the variable RNA HCV negative at the 24th week of treatment and improvement in fibrosis (OR, 11.94; CI, 11.41:...), collagen deposition (OR 11.48; CI, 1.024-214.86) and the grade of activated hepatic stellate cells (OR, 11.48;CI, 1.044- 214.86).

Discussion

Sustained virological response (SVR) has been obtained in about 50% of patients after treatment for chronic hepatitis C depending on the type of treatment employed and the HCV genotype [4]. Lower rates of SVR are obtained in patients co-infected with HIV [24,25], indicating that a high percentage of these patients continue to be at risk of developing cirrhosis and its complications. Thus, analysis of histological evolution in these patients, especially in those without SVR, is important for a better understanding of the disease course.

In the present study, a negative HCV RNA at the 24th week of treatment was related to improvement in fibrosis and collagen deposits and to reduced activation of hepatic stellate cells. This means that viremia control during treatment might be determinant in reducing disease progression, even in HCV/HIV co-infected individuals. It is interesting to note, however, that while the histological improvement was observed in 4 SVR patients, it was also present in three patients who did not show a sustained virological response (data not shown).

There are few studies on the course of hepatic histology after treatment for hepatitis C in patients co-infected with HIV. In some of these, non-responders to treatment with interferon-alpha also had histological improvement or stabilization of fibrosis after treatment [25,26]. Association of virological and histological responses in HCV/HIV co-infected patients was also observed by Lissen et al. [27]. These authors reported improvements in hepatic histology in 62% to 74% of SVR patients and in 32% to 43% of non-responders, according to the type of treatment they used.

Liver histology, evaluated by means of paired biopsies in HCV mono-infected patients, demonstrated an improvement in necroinflammation and even in fibrosis in those presenting SVR [7,8,13]. Stabilization of fibrosis has also been observed in non-responder patients after treatment with interferon-alpha [28], a fact that could be indicative of histological response since progression of fibrosis is expected to be the natural course of chronic hepatitis. Histological improvement was also observed in non-responders, even in the patients presenting a reduction of HCV viremia during treatment [29].

Physiopathological mechanisms implicated in the progression of hepatic fibrosis during chronic HCV infection are multiple, but HSC (perisinusoidal cells, Ito cells or fat-storing cells) activation is known to play a key role in the pathogenesis of hepatic fibrosis [30,31]. Under the action of mediators released by the inflammatory process in the liver, HSCs undergo activation and trans-differentiation to a "myofibroblast-like" phenotype, which express membrane a-SMA. These cells loose the ability to store retinoids, proliferate vigorously and proceed to contract and produce cytokines [32], which result in the increased production and degradation of the extracellular matrix, originating collagen and adhesive proteoglycans and glycoproteins. Increased fibrosis and, consequently, evolution to cirrhosis, are the final steps of these phenotypic and functional changes [33].

HSC were also evaluated in paired biopsies, obtained before and after treatment in mono-infected patients. Sakaida et al. [34], observed an association between a reduction in the number of these activated cells and an improvement in fibrosis, both in patients presenting and not presenting sustained virological responses.

The present study suggests that the control of HCV viremia during treatment of hepatitis C can modify the natural history of the chronic disease in patients co-infected with HIV and this beneficial effect is accompanied by the reduced activation of HSC cells. However, the long-term benefit of this histological improvement will need further evaluation.

Table 2. Analysis of variable values associated to HAI results (Improved and Not improved) in paired biopsies, taken before and after chronic hepatitis treatment in patients co-infected with HIV. Results of logistic regression (OR).

Variables	HAI		OR (CI 95%)
	Improved	Not Improved	
BMI			
< 25	9 (81.8)	10 (66.7)	
≥ 25	2 (18.2)	5 (33.3)	0.45 (0.03; 3.73)
Alcohol ingestion			
No	1 (9.1)	4 (26.7)	
Yes	10 (90.9)	11 (73.3)	3.47 (0.28; 196.66)
HCV genotype			
Non-1	2 (18.2)	3 (20.0)	
1	9 (81.8)	12 (80.0)	1.12 (0.10; 16.09)
RNA HCV (24th week)			
Positive	5 (45.5)	8 (88.9)	
Negative	6 (54.5)	1 (11.1)	8.53 (0.71; 495.38)
Iron (pre-treatment)			
Absent	7 (63.6)	8 (53.3)	
Present	4 (36.4)	7 (46.7)	0.66 (0.09; 4.11)
Steatosis (pre-treatment)			
Absent	3 (27.3)	7 (46.7)	
Present	8 (72.7)	8 (53.3)	2.26 (0.34; 18.61)
ALT (pre-treatment)			
< 2 ULN	4 (36.4)	9 (60.0)	
≥ 2 ULN	7 (63.6)	6 (40.0)	2.53 (0.41; 17.79)

HAI=histological activity index; BMI=body mass index; ALT=alanine aminotransferase; ULN=.upper limit of normality.

Table 3. Analysis of variable values associated to results of fibrosis (Improved and Not improved) in paired biopsies, taken before and after chronic hepatitis C treatment in patients co-infected with HIV. Results of logistic regression (OR).

Variables	Fibrosis		OR (CI 95%)
	Improved	Not improved	
BMI			
< 25	13 (86.7)	6 (54.6)	
³ 25	2 (13.3)	5 (45.4)	0.198 (0.01; 1.64)
Alcohol ingestion			
No	2 (13.3)	3 (27.3)	
Yes	13 (86.7)	8 (72.7)	2.352 (0.22; 34.07)
HCV genotype			
Not1	3 (20.0)	2 (18.2)	
1	12 (80.0)	9 (81.8)	0.893 (0.06; 9.63)
RNA HCV (24th week)			
Positive	5 (41.7)	8 (100.0)	
Negative	7 (58.3)	0 (0.0)	11.936 (1.41;...)
Iron (pre-treatment)			
Absent	9 (60.0)	6 (54.6)	
Present	6 (40.0)	5 (45.4)	0.807 (0.13; 5.09)
Steatosis (pre-treatment)			
Absent	5 (33.3)	5 (45.4)	
Present	10 (66.7)	6 (54.6)	1.634 (0.25; 10.89)
ALT (pre-treatment)			
< 2 ULN	7 (46.7)	6 (54.6)	
³ 2 ULN	8 (53.3)	5 (45.4)	1.355 (0.22; 8.59)

BMI=body mass index; ALT=alanine aminotransferase; ULN=upper limit of normality.

Table 4. Analysis of variable values associated to results of collagen deposits (Improved and Not improved) in paired biopsies, taken before and after chronic hepatitis C treatment in patients co-infected with HIV. Results of logistic regression (OR).

Variables	Collagen deposit		OR (CI 95%)
	Improved	Not improved	
BMI			
< 25	7 (100.0)	12 (63.2)	
³ 25	0 (0.0)	7 (36.8)	0.205 (...; 1.68)
Alcohol ingestion			
No	1 (14.3)	4 (21.1)	
Yes	6 (85.7)	15 (78.9)	1.573 (0.12; 91.75)
HCV genotype			
Not1	2 (28.6)	3 (15.8)	
1	5 (71.4)	16 (84.2)	0.484 (0.04; 7.34)
RNA HCV (24th week)			
Positive	2 (28.6)	11 (84.6)	
Negative	5 (71.4)	2 (15.4)	11.480 (1.04; 214.86)
Iron (pre-treatment)			
Absent	5 (71.4)	10 (52.6)	
Present	2 (28.6)	9 (47.4)	0.458 (0.03; 3.73)
Steatosis (pre-treatment)			
Absent	1 (14.3)	9 (47.4)	
Present	6 (85.7)	10 (52.6)	5.094 (0.47; 275.43)
ALT (pre-treatment)			
< 2 ULN	3 (42.9)	10 (52.6)	
³ 2 ULN	4 (57.1)	9 (47.4)	1.459 (0.18; 12.85)

BMI, Body mass index; ALT, alanine aminotransferase; ULN, upper limit of normality.

Table 5. Analysis of variable values associated to the results of HSC activation (Improved and Not improved) in paired biopsies, taken before and after chronic hepatitis C treatment of patients co-infected with HIV. Results of logistic regression (OR),

Variables	HSC activation		OR (CI 95%)
	Improved	Not improved	
BMI			
< 25	8(100.0)	11 (61.1)	
³ 25	0 (0.0)	7 (38.9)	0.16 (0; 1.33)
Alcohol ingestion			
No	2 (25.0)	3 (16.7)	
Yes	6 (75.0)	15 (83.3)	0.61 (0.05; 9.08)
HCV genotype			
Not1	2 (25.0)	3 (16.7)	
1	6 (75.0)	15 (83.3)	0.61 (0.05; 9.08)
RNA HCV (24th week)			
Positive	2 (28.6)	11 (84.6)	
Negative	5 (71.4)	2 (15.4)	11.48 (1.04; 214.86)
Iron (pre-treatment)			
Absent	5 (62.5)	10 (55.6)	
Present	3 (37.5)	8 (44.4)	0.76 (0.09; 5.42)
Steatosis (pre-treatment)			
Absent	1 (12.5)	9 (50.0)	
Present	7 (87.5)	9 (50.0)	6.53 (0.62; 349.80)
ALT (pre-treatment)			
< 2 ULN	4 (50.0)	9 (50.0)	
³ 2 ULN	4 (50.0)	9 (50.0)	1.00 (0.14; 7.28)

HSC, hepatic stellate cells; BMI, Body Mass Index; ALT, alanine aminotransferase; ULN, upper limit of normality.

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