The impact of Cannabinoid Receptor 2-63 variants on the liver histology of 166 patients with HIV infection and chronic hepatitis C

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Introduction

Two types of CB are known, type 1 (CB1) and type 2 (CB2), mainly exerting anti-inflammatory and immunomodulatory actions. Both CB1 and CB2 have been classified as immunosuppressive receptors on CD4+ cells, although antagonism of CB1 and CB2 do not enhance immune activation and knockout mice do not exhibit differences in T cell frequency or increases in autoimmune pathogenesis. The CB2 is predominantly expressed in the cells of the immune system, and at a high extend on CD4+ cells and Kupffer cells, both plaing an essential role in the acute and chronic responses to toxic and infectious agents. CB2 may function as a chemotactic modulator inhibiting CXCR4-induced chemotaxis in transformed lymphocytes chemokine receptor 5 (CCR5), a chemokine receptor that is a co-receptor for HIV-1 entry. In HIV infection endocannabinoids may interact with different pro-inflammatory events and influence HIV-1 pathogenesis.

A polymorphism at codon 63 of the CB2 gene allows the substitution of glutamine, Gln (Q), with arginine, Arg (R), with a consequent differentiation in the protein polarization. These CB2 variants affect differently the ability of the CB2 to perform its inhibitory function. For incidence, T lymphocytes from CB2-RR homozygotes showed an in-vitro a two-fold reduction in the endocannabinoid-induced inhibition of proliferation as compared with T lynphocytes from CB2-QQ homozygotes. The CB2-QQ variant of the rs35761398 polymorphism of the CB2 gene associated with the more severe degrees of liver necroinflammation in HCV-monoinfected CHC patients.

Methods

One-hundred sixty-six consecutive anti-HIV-positive patients with HCV-RNA-positive asymptomatic CHC lasting 18-36 months have been consecutively enrolled from 1993 to 2008 at the time they underwent their first liver biopsy at one of the two participating Units of Infectious Diseases. All patients enrolled were naive for anti-HCV treatment, were HBsAg-negative, declared no active alcohol abuse (≥ 40 g/day for males, ≥ 30 g/day for females for at least 5 years) or statin assumption and had no evidence of autoimmune or genetic disorders that may induce liver disease. Ultrasound or serological signs of HCC were never detected.

At the first observation, each patient signed the informed consent to undergo LB, for collection and storage at –80°C samples of serum and whole blood and for the use in clinical research of the data obtained, as established by the Ethics Committee of Second University of Naples (protocol number 112/15 March 2013). Percutaneous LB was performed for all patients under US guidance. An experienced pathologist unaware of the clinical and laboratory data used the Ishak's scoring system to grade necroinflammation (HAI) and fibrosis. Liver steatosis was assessed as follows: score 1 for a fatty deposition in 1-10% of hepatocytes, score 2 in 11-30%, score 3 in 31-60% and score 4 in >60%.

Genomic DNA was extracted from peripheral whole blood with a DNA extraction kit (Roche Diagnostics, NJ, USA). Molecular screening for the CNR2 rs35761398 polymorphism (CAA/CGG) underlying the CB2 Q63R substitution was performed using a TaqMan Assay (Real Master Mix Probe, 5 PRIME, Germany). Both PCR and post-PCR allelic discrimination were carried out on an ABI PRISM 9600 apparatus (Applied Biosystems, CA). PCR products were analyzed using an ABI PRISM 3100 automated sequencer (Applied Biosystems, CA) and the Big Dye Terminator reaction kit (Applera, CA), according to the manufacturer's instructions. All primers were chosen using Primer3 software (http://primer3.sourceforge.net/).

Results

Of the 166 HIV/HCV-coinfected patients included in the present study, 72.9% were males, 42.5% were infected with HCV-genotype 3 and 38.1% with genotype 1, 74.1% had a history of previous intravenous drug use (IVDU) but no patient stated an active drug addiction. The median age was 40.58 years and the immunological condition quite good: median 507.0 (IQR: 398.0-669.5) $CD4^+$ cells/mm^3 at the time of enrollment and a mean nadir of 258.0 cells/mm^3 (IQR: 163.5-404.5). Thirty-five (21.8%) patients were naive for HAART and 131 (78.9%) on HAART. Untreated patients showed higher mean values of $CD4^+$ cell count than those under ART, both at the time of liver biopsy (581.0 cells/ mm^3 vs. 492.0 cells/ mm^3) and as mean nadir (431.0 cells/ mm^3 vs. 230.5 cells/ mm^3).

Table 1: Demographic, biochemical and histological characteristics in 166 HIV/HCV coinfected patients according to CB2 63 genotype variants.

	CB2 QQ	CB2 QR	CB2 RR	p-value
N° of patients	26	64	76	
Males, N° (%)	23 (88.5)	41 (64.1)	57 (75.0)	0.05
Age, years, median (IQR)	40.6 (36.4-43.4)	40.0 (37.1-42.6)	42.0 (37.98-46.6)	0.06
BMI, m2/kg, median (IQR)	23.3 (21.7-25.4)	22.5 (21.1-24.2)	23.2 (21.5-25.3)	0.07
IVDU, N°(%)	13 (22.4)	43 (76.8)	44 (73.3)	0.27
Nadir of CD4, cell/mmc, median (IQR)	231.0 (82.5-322.5)	261.5 (160.3-427.0)	283.0 (182.5-378.8)	0.20
HIV-RNA cps/mL, median (IQR)	10870.0 (2450.5-44338.0)	3800.0 (1007.0-18687.0)	13301.0 (3991.0-37219.0)	0.37
Negative (<100 cps/mL),N° (%)	15 (57.7)	38 (59.4)	32 (42.1)	0.14
CD4, cell/mmc, median (IQR)	474.0 (369.5-557.3)	570.00 (420.3-725.3)	492.0 (394.8-659.0)	0.56
AST, IU/mL, median (IQR)	58.0 (41.75-71.00)	56.0 (39.5-87.8)	67.0 (40.0-132.0)	0.04
ALT, IU/mL , median (IQR)	89.5 (58.3-127.0)	73.5 (44.0-114.0)	111.5 (49.0-158.8)	0.17
Bilirubin, mg/dL, median (IQR)	0.7 (0.53-0.92)	0.72 (0.5-0.5)	0.7 (0.47-1.0)	0.91
GGT, IU/mL, median (IQR)	87.0 (42.75-157.50)	70.5 (38.0-154.25)	75.0 (36.0-189.0)	0.13
ALP, IU/mL, median (IQR)	174.0 (133.0-240.5)	198.0 (139.0-253.0)	187.5 (136.50-249.8)	0.39
Glucose, mg/dL, median (IQR)	95.0 (80.5-104.50)	85.50 (79.0-92.8)	88.0 (81.0-97.5)	0.13
Creatinine, mg/dL, median (IQR)	0.79 (0.1-0.84)	0.8 (0.7-0.9)	0.8 (0.7.9)	0.83
Triglycerides, mg/dL, median (IQR)	125.5 (98.75-153.0)	122.0 (81.3-153.0)	133.0 (88.5-184.0)	0.77
Total cholesterol, mg/dL, median (IQR)	170.5 (149.75-195.0)	160.0 (129.0-186.0)	162.0 (129.5-194.8)	0.75
HCV RNA, IUx10^3, median (IQR)	445956.0 (115463.3-1575000.0)	452925.0 (104103.0-1771436.5)	730500.0 (336750.0-1411766.5)	0.76
Duration of HIV infection, years, median (IQR)	15.98 (10.95-17.4)	14.4 (7.7-18.1)	11.7 (6.86-18.5)	0.19
HAART, N°(%): treated	22 (76.9)	52 (81.3)	57 (75.0)	
HAART-naïve	4 (15.4)	12 (18.7)	19 (25.0)	0.49
Duration of ART, years, median (IQR)	6.5 (3.4-12.8)	7.7 (5.9-10.4)	8.22 (6.28-11.2)	0.41
HCV genotype, N° (%):1	10 (40.0)	22 (35.5)	29 (39.7)	
2	1 (4.0)	2 (3.3)	5 (6.9)	0.89
3	10 (40.0)	27 (43.55)	31 (42.5)	
4	4 (16.0)	11 (17.7)	8 (10.96)	
missing, N°	1	2	3	
HAI, score (M ± SD)	5.8 ± 2.6	5.3 ± 2.7	6.8 ± 3.4	0.056
Degree of HAI, N° (%): 0-8	23 (88.5)	55 (85.9)	51 (61.1)	
9-18	3 (11.5)	9 (9.4)	25 (35.1)	0.01
Fibrosis, score (M ± SD)	2.4± 1.4	2.2 ± 1.7	2.4 ± 1.6	0.85
Degree of fibrosis, N° (%): 0-3	22 (84.6)	53 (82.8)	61 (80.3)	
4-6	4 (15.3)	11 (17.12)	15 (19.7)	0.86
Steatosis, score (M ± SD)	1.9 ± 1.4	1.7 ± 1.3	1.6 ± 1.3	0.57
Degree of steatosis, N° (%): 0-2	15 (57.7)	45 (60.3)	50 (65.8)	
3- 4	11 (42.3)	19 (29.7)	26 (34.2)	0.51

The CB2-63 RR variant was detected in the 45.8% of the patients, QR in 38.5% and QQ in 15.7%, the minor allele frequency was QR and the genotypes were distributed according to the Hardy–Weinberg equilibrium. The mean degree of HAI, expressed as scores of the histological activity index (HAI 0-18), was 5.4 ± 3.0 (SD), the mean degree of liver fibrosis 2.3 ± 1.6 and the mean degree of liver steatosis 1.7 ± 1.3 (range 0-4).

Table 2: Demographic, biochemical and histological characteristics in 166 HIV/HCV coinfected patients according to necroinflammation score

	Degree of HAI		*P-VALUE*
	0-8	9-18	
N° of patients	129	37	
Males, N° (%)	91 (70.5)	30 (81.1)	0.20
Age, years, median (IQR)	40.9 (37.2-44.2)	41.0 (38.0-44.3)	0.85
BMI, m2/kg, median (IQR)	22.8 (21.2-24.86)	23.6 (21.5-25.4)	0.72
Nadir of CD4, cell/mmc, median (IQR)	258.0 (165.25-422.0)	272.0 (149.0-374.0)	0.82
IVDA, N°(%)	77 (71.96)	23 (76.7)	0.93
HIV-RNA, cps/mL, median (IQR)	8321.0 (1541.5-36329.8)	12000.0 (3183.5-31052.0)	0.19
Negative (< 100 cps /mL), N° (%)	72 (55.8)	13 (35.1)	0.04
CD4, cell/mmc, median (IQR)	509.5 (404.5-691.0)	466.0 (355.0-608.5)	0.37
AST, IU/mL, median (IQR)	54.0 (38.3-77.0)	112.0 (76.0-166.5)	0.000002
ALT, IU/mL, median (IQR)	72.0 (43.5-120.0)	137.0 (87.5-223.5)	0.0004
Bilirubin, mg/dL, median (IQR)	0.7 (0.49-1.05)	0.8 (0.4-1.1)	0.7
GGT, IU/mL, median (IQR)	68.5 (33.0-155.3)	99.0 (60.0-189.0)	0.06
ALP, IU/mL, median (IQR)	179.0 (132.0-238.3)	205.0 (177.0-274.0)	0.008
Glucose, mg/dL, median (IQR)	87.0 (79.0-95.0)	91.0 (83.3-99.8)	0.015
Creatinine, mg/dL, median (IQR)	0.78 (0.7-0.9)	0.8 (0.7-0.8)	0.64
Triglycerides, mg/dL , median (IQR)	127.0 (86.5-169.8)	123.0 (169.75-182.0)	1
Total cholesterol, mg/dL, median (IQR)	163.0 (137.0-194.0)	154.0 (121.5-180.5)	0.53
HCV RNA, IUx10^3 , median (IQR)	511250.0 (115550.0-1432735.0)	732000 (342000.0-1487013.0)	0.303
Duration of HIV infection, years, median (IQR)	14.4 (7.7-18.1)	13.3 (7.5-18.3)	0.37
HAART, N°(%): treated	103 (82.2)	28 (75.7)	0.58
HAART-naive	26 (20.2)	9 (24.3)	
Duration of ART, years, median (IQR)	8.2 (6.2-11.3)	6.79 (4.46-10.3)	0.2
CB2-63, N° (%): QQ	23 (17.8)	3 (8.1)	0.010
QR	55 (42.6)	9 (24.3)	
RR	51 (47.3)	25 (65.6)	
HCV genotype, N° (%):1	47 (38.2)	14 (37.8)	
2	7 (5.7)	1 (2.7)	0.19
3	48 (39.0)	20 (54.0)	
4	21 (17.1)	2 (5.4)	
missing, N°	6	0	
Fibrosis, score (M ± SD)	1.9 ± 1.4	3.6 ± 1.5	<0.000001
Degree of fibrosis, N° (%): 0-3	114 (88.4)	22 (67.6)	0.0001
4-6	15 (11.6)	15 (40.5)	
Steatosis, score (M ± SD)	1.6 ± 1.3	2.0 ± 1.3	0.03
Degree of steatosis, N° (%): 0-2	91 (70.5)	19 (51.5)	0.03
3- 4	38 (29.5)	18 (48.5)	

The 64 subjects with the CB2-QR variant and 76 with the CB2-RR variant more frequently than the 26 patients with the CB2-QQ variant had a history of previous IVDU ($p \leq 0.01$).

Patients with CB2-RR showed a high degree of HAI (score 9-18) more frequently than those with CB2-QQ or CB2-QR than in those (35.1% vs. 11.5% and 9.1%, respectively, $p = 0.01$). (Table 1).

Table 2 reports a comparative analysis of patients' initial characteristics, based on difference in liver necroinflammatin, absent or mild (HAI scores 0-8) vs moderate or severe (HAI score 9-18).

The 37 patients with moderate or severe HAI, compared with the 129 patients with a lower HAI score had more frequently the CB2-RR variant, showed significant higher serum level of AST, ALT, and ALP, higher degree of fibrosis, and higher degree of steatosis. In patients with high degree of HAI (score 9-18) severe fibrosis (grade 3 or 4) were detected more frequently than in those with lower HAI scores ($p = 0.0001$) as also severe steatosis (grade 3 or 4) (48.7% vs. 29.5) (Table 2).

The association between the CB2-RR variants and a HAI score 9-18 was analyzed in a multivariate analysis including as covariants age at liver biopsy (p =0.8838), HIV-RNA (≤ 100 copies/ml vs > 100 copies/ml $p = 0.4883$), CD4+ cell/mL (low than 500 vs. more than 500 cell/mL, $p = 0.7073$), fibrosis score (severa vs non severa), and HAART regimen ($p = 0.6028$). Both the CB2-RR variant ($p = 0.0355$), and the severe fibrosis ($p = 0.0001$) were found to be independently associated with severe necroinflamation.

Of the 26 patients with the CB2-QQ variant enrolled in the present study, 16 were treated with Pegylated Interferon (Peg-IFN) or Peg-IFN+ribavirin, according to the current international guidelines, and 8 (50%) of them achieved a sustained virological response (SVR). The response to Peg-IFN or Peg-IFN+ribavirin treatment was similar with no statisticali significant for the 39 with CB2-QR with a SVR in the 38.5% of cases and for the 53 with CB2-RR variant with a SVR in the 60.4% of cases (p=ns).

Conclusion

The CB2-RR variant was identified as an independent predictor of severe necroinflamation in HIV/HCV coinfected patients with chronic hepatitis.

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