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Short Communication

Daclatasvir plasma level and resistance selection in HIV patients with hepatitis C virus cirrhosis treated with daclatasvir, sofosbuvir, and ribavirin

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SUMMARY

Objectives: Effective treatment with direct-acting antiviral drugs against hepatitis C virus (HCV) is a medical need in cirrhotic HIV–HCV co-infected patients.
Methods: This study investigated the plasma levels of daclatasvir (DCV) and ribavirin (RBV) in HIV–HCV co-infected subjects treated with DCV, sofosbuvir, and RBV. Drug concentrations were quantified using validated high-performance liquid chromatography methods with ultraviolet detection. The HCV non-structural protein 5A and non-structural protein 5B coding regions were analyzed by population-based sequencing.
Results: DCV was dosed at week 4 and at week 8 of treatment, and RBV at week 8. One patient had the lowest DCV level, corresponding to 32.7% of the overall median value of the other patients at week 4 and about 40% at week 8. The Y93H variant was detected in this subject at weeks 8, 16, and 20 of treatment, but not before treatment or at day 2, and the patient experienced virological failure. Another subject with the Y93H variant at baseline and appropriate DCV levels had HCV RNA <12 IU/ml at week 12 and undetectable at week 16.
Conclusions: Sub-optimal DCV drug levels allow the selection of resistance-associated variants and fail to contribute to antiviral activity. No definite reason for the low DCV level was found. Quantifying the drug is suggested in difficult-to-treat patients.

1. Introduction

Patients with HIV infection and hepatitis C virus (HCV)-induced cirrhosis are at high risk of liver failure-related morbidity and mortality; thus, effective anti-HCV treatment represents a medical need.[1] The cases of eight HIV-positive patients on highly active antiretroviral therapy (HAART) with HCV cirrhosis, all naïve to direct-acting antivirals for HCV (DAA) and treated with daclatasvir (DCV), sofosbuvir (SOF), and ribavirin (RBV), are reported herein.

2. Patients and methods

Eight HIV-positive patients with Child–Pugh A HCV-related (genotype 3) cirrhosis were treated with SOF (400 mg once daily), DCV (60 mg once daily), and RBV (fixed dose, 800 mg daily) planned for 24 weeks. The successful HAART was modified in all patients because of potential drug–drug interactions (Table 1). The HCV non-structural protein 5A (NS5A) and non-structural protein 5B (NS5B) coding regions were analyzed by population-based sequencing at baseline in six patients. DCV and RBV concentrations were quantified using validated high-performance liquid chromatography methods with ultraviolet detection (unpublished results) in plasma collected before taking drugs (C_{trough}).[2,3] The Ethics Committee for Clinical Experimentation, Padova Province, was notified of the study (27602/16).

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Table 1
Main characteristics of the HIV–HCV-infected patients at baseline

Patient	Sex	Age, years	Previous anti-HCV treatment[a]	ART at baseline	CD4+ cell count (cells/mm^3)	CD4+ cell percentage	HIV RNA (copies/ml)	NS5A	NS5B
1	F	49	No	TDF + FTC + RGV	320	18	UND	30S	WT
2	M	52	Yes	ATV	320	19	UND	WT	WT
3	M	64	No	3TC + RGV	380	40	UND	WT	WT
4	M	46	No	ABC + 3TC + RGV	200	10	59	ND	ND
5	M	57	No	TDF + FTC + RPV	640	41	UND	WT	WT
6	M	50	Yes	ABC + 3TC + RGV	430	35	UND	WT	WT
7	M	56	No	TDF + FTC + RPV	1080	40	UND	WT	ND
8	M	52	No	TDF + FTC + RPV	610	31	UND	Y93H	WT

ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; F, female; FTC, emtricitabine; HCV, hepatitis C virus; M, male; ND, not determined; RGV, raltegravir; RPV, rilpivirine; TDF, tenofovir; UND, undetectable (lower limit of quantification 40 copies/ml); WT, wild type; 3TC, lamivudine.
[a] Interferon-based.

Table 2
HCV RNA kinetics during antiviral treatment, ribavirin dosing, and daclatasvir dosing[a]

Patient	HCV RNA[b] Baseline	HCV RNA[b] Week 4	DCV[c] Week 4	HCV RNA[b] Week 8 and RAV	DCV[c] Week 8 (a)	DCV[c] Week 8 (b)	RBV[c] Week 8	HCV RNA[b] Week 12	HCV RNA[b] Week 16 and RAV	HCV RNA[b] Week 20 and RAV	HCV RNA[b] Week 24
1	1746	UND	0.071	UND	0.124	0.095	4.01	UND	UND	UND	UND
2	9 768 530	25	0.327	<12	0.415	0.469	1.012	UND	UND	UND	UND
3	3 123 663	26	0.04	<12	0.088	0.092	1.847	<12	UND	UND	UND
4	2 170 547	248	0.152	47	0.113	0.114	1.509	<12	<12	UND	UND
5	926 162	UND	0.153	UND	0.191	0.153	1.915	UND	UND	UND	UND
6	4 746 034	213	0.061/0.056	231 Y93H/ND	0.046	0.05	1.74	331	3128 Y93H/ND	843 Y93H/ND	1986
7	155 869	20	0.185	<12	0.124	0.128	1.212	<12	UND	UND	UND
8	6 847 316	509	0.313	61	0.5	NA	NA	<12	UND	N/AP	N/AP

DCV, daclatasvir; HCV, hepatitis C virus; NA, not available; N/AP, not applicable (patient was still on therapy and did not achieve the specific week); ND, not detectable; RAV, resistance-associated variant; RBV, ribavirin; UND, undetectable (Lower limit of quantification 12 IU/ml).
[a] Daclatasvir was tested twice in patient 6 at week 4 to confirm plasma levels and it was tested twice in seven of the eight patients at week 8 (all results reported, (a) and (b)). Patient 8 had no second daclatasvir determination at week 8 and no ribavirin dosing because no more samples were available. Resistance-associated variants at weeks 8, 16, and 20 are described as NS5A-related/NS5B-related.
[b] IU/ml.
[c] C_{trough}, μg/ml.

3. Results

At the start of treatment with DAA, no naturally occurring resistance-associated variant (RAV) was found in six subjects; however the Y93H variant was found in one (patient 8). Six patients had undetectable HCV RNA at week 24. Patient 8 was still on therapy but with HCV RNA <12 IU/ml at week 12 and undetectable at week 16, while patient 6 continued to have detectable HCV RNA up to week 24. Patient 4 had consistently low levels of plasma HIV RNA; all of the other patients had HIV RNA fully suppressed throughout the study period.

DCV was dosed at week 4 and at week 8 of treatment, and RBV at week 8. Patient 6 had a DCV level corresponding to 32.7% of the overall median value of the other patients at week 4 and corresponding to 37.1% and 41% in two repeat controls at week 8 (Table 2). The Y93H variant was identified in patient 6 at weeks 8, 16, and 20 of treatment, but not on day 2 of treatment. RBV levels were within the normal range in all seven patients tested. All patients had a 100% self-reported adherence to the treatment. Patient 6 was questioned regarding his adherence, but he confirmed taking the drugs in a timely manner each day; in keeping with this, his plasma HIV RNA was successfully suppressed.

4. Discussion

The relationship between the DCV plasma level and the role of the resistance mutant Y93H in determining the outcome of a regimen containing DCV is reported here . Two therapy time points were selected to monitor the bioavailability of DCV in HIV–HCV-infected patients (underexposure has been described previously for RBV).[4] The lowest DCV concentrations were observed in a subject who experienced virological failure (patient 6). Also patients 1 and 3 had low levels of DCV at week 4, but they reached higher concentrations at week 8; their better outcomes may relate to these different drug kinetics, or to the absence of 93H quasispecies, or to the activity of the other two components of the therapy. All patients had their successful HAART modified. These data underline the strong need to treat all co-infected patients regardless of the severity of liver fibrosis in order to avoid the risk of missing the chance to modify HAART in the case of resistance to antiretrovirals .

For whatever reason, HCV RNA was always detectable in patient 6 and an NS5A resistance variant was identified at weeks 8, 16, and 20; NS5B sequences were not available due to the lower efficiency of the NS5A sequencing procedure at low HCV RNA copies. The patient's RBV plasma concentration was in the therapeutic range. Unfortunately, the patient's SOF plasma level was not measured and this is a limitation of the work. The failure to detect the Y93H mutant at baseline in patient 6 underlines the utility of deep sequencing to detect minor variants at baseline in difficult-to-treat subjects, such as HCV genotype 3 infected patients, to correctly address the therapeutic strategy. The Y93H substitution is reported to compromise the treatment with DCV,[5] to confer high-level resistance to DCV in genotype 3a patients, and to reduce the binding affinity of DCV to NS5A.[6]

Patient 8 achieved HCV RNA suppression despite the presence of the Y93H mutation at baseline; of note, his DCV levels were

much higher than those found in patient 6. This result highlights the importance of residual drug activity even in the presence of RAVs and the importance of a therapeutic drug level to overcome the role of RAVs in determining therapeutic failure when present as either a minor or prevalent quasispecies. On the other hand, sub-optimal drug levels allow the selection of RAVs and fail to contribute to antiviral activity, as seen in patient 6. However, other factors need to be taken into account, such as the baseline HCV plasma level, body mass index, interleukin 28 (IL28) genotype, and co-administered drug activity.[7]

The HAART regimen of patient 6 was compatible with the anti-HCV therapy and no other drug with possible known interactions was co-administered,[8,9] so no definite reason for his low DCV level was found. This suggests some difficulties in predicting DCV therapeutic levels in HIV–HCV-infected cirrhotic patients on HAART.

5. Author contributions

SGP designed and coordinated the study, supervised the virological laboratory experiments, collected the data, interpreted the findings, and wrote the paper. AL supervised the pharmacokinetic laboratory experiments, interpreted the findings, and wrote the paper. SA performed the laboratory experiments. GN performed the laboratory experiments. SC managed the patients. MB helped to interpret the findings and wrote the paper. RS managed the patients and helped to interpret the findings. FDB performed the laboratory experiments. LM performed the laboratory experiments. AMC managed the patients and wrote the paper. GP interpreted the findings and wrote the paper.

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Conflict of interest: None.

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