Fosamprenavir treatment in a highly active antiretroviral therapy schedule induces a HCV-RNA decrease and a Th1 network boost in HIV/HCV-coinfected patients

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Abstract

HIV/HCV co-infected naïve patients (four females and six males) were evaluated for their response to the following treatment schedule: [(AZT 300 mg + 3TC 300 mg twice daily) + (fosamprenavir 700 mg twice daily) + (RTV 100 mg)]. CD3+/CD4+ T cells, interferon-γ (INF-γ) and interleukin-4 (IL-4) HCV-specific response, viral loads and transaminase levels were evaluated at time 0, and after 1, 3 and 6 months of therapy (T0, T1, T3, and T6 respectively). HIV-RNA, HCV-RNA and transaminases decreased at T1 and T3 compared with T0 (Mann–Whitney $p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). At all time points, CD4+ and HCV-specific INF-γ responses were higher ($p < 0.001$; $p < 0.001$), and IL-4 lower ($p < 0.01$) after treatment. At T6, HCV-RNA was only negative in four out of ten patients whereas all had normal transaminase levels. These findings indicate that HAART treatment including fosamprenavir is able to activate a Th1 network in HIV/HCV co-infected patients. Moreover, these results, to be confirmed by larger cohort follow-up studies, suggest that this protease inhibitor could have potential implications for the treatment of chronic hepatitis C in HIV-positive patients.

Keywords: Coinfection, fosamprenavir, HAART, HCV, HIV, IFN-γ

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It is known that HIV/HCV-coinfected patients have a faster hepatic disease progression with early onset of cirrhosis and end-stage liver disease [1,2], although discordant data have been published about HCV influence on HIV disease progression [3]. HCV antiviral treatment, nonetheless, represents an important strategy in the treatment of these patients. Indeed, chronic hepatitis C treatment should be initiated if the CD4 cell count is >350 cells/L, without the need to begin antiretroviral therapy. On the other hand, an antiretroviral treatment (ART) should be commenced in patients showing a lower CD4+ cell count (<200 cells/L) before embarking on anti-HCV therapy with interferon and ribavirin [4]. The treatment choice in this latter subset of patients is represented by highly active antiretroviral therapy (HAART) with particular attention to possible influences on liver function. Fosamprenavir (FA), an oral prodrug of amprenavir, has been included in the HAART schedule and has been shown to have an improved bioavailability with a reduced daily pill load for HIV patients [5]. We evaluated the response to treatment of ten naïve HIV/HCV-coinfected patients (four female and six male), from October 2006 to June 2008, undergoing the following HAART schedule: [(AZT 300 mg + 3TC 300 mg twice daily) + (fosamprenavir 700 mg twice daily) + (RTV 100 mg)]. Patients had at least a 1-year history of histologically proven, persistent HCV infection and were either interferon and ribavirin naïve or had been previously treated (at least 18 months before enrolment). After obtaining written informed consent, we evaluated immunological, biochemical and virological parameters at the following time points: Time 0 (T0), after 1 month (T1), 3 months (T3) and 6 months (T6). HCV-specific immune response [interferon-γ (IFN-γ) and interleuking-4 (IL-4)] was evaluated by ELISpot assay at T0, T1, T3 and T6 on peripheral blood mononuclear cells (PBMCs). Cytokine production was assessed after stimulation with 2 μg/mL of pooled HCV core peptides (35–48—YLLPRRGPRL; 132–40—DLMGYIPLV—DLMGYIPAV; 41–49 GPRLGVRAT) (Proimmune, Oxford, UK) on 40-mL blood samples (taken on two different days) with at least 150 000 cells per well; all experiments were carried out in triplicate and were expressed as spot forming colonies (SFCs). Serum level transaminases were assayed in our centralized laboratory and flow cytometry was used to enumerate lymphocytes according to protocols previously described [6]. Plasma HCV and HIV viral loads were measured using the Cobas Amplicor

TABLE 1. Data were evaluated at Time 0, after 1 month (T1) and 3 months (T3)

Patient parameters	Time 0	Time 1	Time 3	p
CD3+/CD4+	186 ± 23	414 ± 63	486 ± 48	<0.001
ALT level (IU/L < 40)	121 ± 44	22 ± 9	30 ± 4	<0.01
AST level (IU/L < 40)	93 ± 31	25 ± 8	28 ± 6	<0.01
HCV-RNA (IU/mL mean ± SD)	596 ± 236 × 10^3	13 ± 30 × 10^3	Negative	<0.01
HIV-RNA (IU/mL mean ± SD)	90 ± 19 × 10^3	209 ± 42	Negative	<0.001
ELISpot assay[a]				
IFN-γ	69 ± 13	112 ± 14	122 ± 8	<0.001
IL-4	88 ± 19	48 ± 16	55 ± 16	<0.01

[a]ELISpot assay, expressed as spot forming colonies (SFCs); CD4+ expressed as cells per cubic millimetre. Data expressed as mean plus standard deviation. Statistical analysis to assess differences was performed using the Mann–Whitney *U*-test.

Monitor assay with a detection limit of 50 copies/mL for HIV and 60 IU/mL for HCV (Roche Diagnostics, West Sussex, UK). All patients were HBsAg negative and did not show any other acute infection (IgM-HAV; IgM-EBV; IgM-CMV; IgM-HSV) or autoimmune illness (negative ANA, ASMA and anti-LKM). Results were expressed as mean and standard deviation (SD); however, given the small number of subjects, the nonparametric Mann–Whitney *U*-test was used. All hypothesis tests were two-tailed and statistical significance was assessed at p 0.05 using GraphPad Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, USA).

HIV-RNA was found to be statistically significantly decreased at T1 and was negative at T3, whereas CD4+ T cells increased within the first month and were stable by T3 (Mann–Whitney test p <0.001) (Table 1). HCV viral load showed a statistically significant decrease at T1 compared with T0 (Mann–Whitney p <0.01) and was negative at T3 (Table 1). Serum transaminase levels were found to be under the normal limit value at T1 and T3 (Table 1). HCV-specific IFN-γ SFCs were higher at T1 and T3 than T0, whereas IL-4 SFCs were lower (Mann–Whitney *U*-test p <0.001 and <0.01 respectively) (Table 1). At the 6 months follow-up, patients scored negative for HIV viral load, with a stable mean level of CD4+ (412 ± 32 cells/mm^3). On the other hand, six out of ten patients scored positive for HCV-RNA, with a low titre (HCV-RNA 75 ± 12 × 10^3) although serum transaminase levels were under the normal limit value (ALT 32 ± 4 IU/mL; AST 24 ± 4 IU/mL N.V. <40 IU/mL). A stable and strong IFN-γ HCV-specific immune response was also confirmed at T6 (131 ± 12 SFCs) in all patients, whereas IL-4 was stably lower than T0 (55 ± 7 SFCs). Subjects still positive for HCV-RNA were enrolled for HCV antiviral treatment.

The efficacy of the HAART schedule including fosamprenavir against HIV was confirmed in our ten naïve patients and the treatment was also found to have an impact on HCV, leading to a statistically significant decrease in viral load and inducing a HCV-specific pro-inflammatory network shift within 3 months. Indeed, it has been reported that HAART treatment influences T cell function recovery, possibly having direct immunomodulatory activity, independent of their specific antiretroviral effects, explaining the disconnection between CD4+ cell counts and viral load in some treated patients who develop resistant viral mutants [7]. Moreover, spontaneous HCV-RNA clearance during HAART treatment has also been described [8]. The mechanisms related to our findings still remain to be clarified but we could speculate that the IFN-γ immune network shift, probably related to the immunoreconstitution, might have a role in this viral clearance as has already been suggested [9]. However, it is of note that this viral clearance seems to persist only in about 40% of treated patients at the sixth month follow-up in spite of the Th1 network boost and normal transaminase serum levels observed in all patients. These features could suggest possible HCV viral escape, eluding the immune response, and that specific HCV antiviral treatment is necessary to achieve sustained HCV clearance. In conclusion, despite the small group, this is the first report, to our knowledge, of a significant drop in HCV viral load, with a Th1 boost and transaminase reduction, during HAART treatment including fosamprenavir. In the light of recent reports addressing the important role of a rapid virological response (RVR) in coinfected patients [10] and based on our preliminary results, we would propose that the use of fosamprenavir in an HAART schedule, may, through a yet undetermined immunological and virological pathway, have an impact on HCV infection in coinfected patients. However, a pegylated interferon plus ribavirin therapy should be started to achieve a stable virological response. Future large cohort follow-up studies should be undertaken to confirm these data and to gain a better understanding of fosamprenavir activity on the immune system during HIV/HCV coinfection, as well as determining the possible therapeutic implications in this subset of patients.

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Transparency Declaration

The authors have no conflict of interest to declare. There was no external support for this research.

References

1. UNAIDS/WHO *AIDS Epidemic Update: December 2005*. Available at: http://www.unaids.org/epi/2005/doc/report.asp (last accessed: 6 March 2006).
2. Koziel MJ, Peters MG. Viral hepatitis in HIV infection. *N Engl J Med* 2007; 356: 1445–1454.
3. Rockstroh JK. Influence of viral hepatitis on HIV infection. *J Hepatol* 2006; 44: 525–527.
4. Salmon D, Robain M, Rockstroh JK, Benhamou Y. Therapeutic management of hepatitis and HIV infection in co-infected patients: results of a survey performed before the 2005 Consensus Conference. *J Hepatol* 2006; 44: S2–S5.
5. Hester EK, Chandler HV, Sims KM. Fosamprenavir: drug development for adherence. *Ann Pharmacother* 2006; 40: 1301–1310.
6. Perrella A, Grattacaso S, Gnarini MR *et al.* Increase of granzyme B-positive cells in ascitic fluid of patients with spontaneous bacterial peritonitis. *Hepatol Res* 2008; 38: 410–414.
7. Delmonte OM, Bertolotto G, Ricotti E, Tovo PA. Immunomodulatory effects of two HIV protease inhibitors, Saquinavir and Ritonavir, on lymphocytes from healthy seronegative individuals. *Immunol Lett* 2007; 15: 111–115.
8. Ribeiro RM. Dynamics of CD4+ T cells in HIV-1 infection. *Immunol Cell Biol* 2007; 85: 287–294.
9. Koziel MJ. Cellular immune responses against hepatitis C virus. *Clin Infect Dis* 2005; 41 (suppl 1): S25–S31.
10. Martin-Carbonero L, Nuñez M, Mariño A *et al.* Undetectable hepatitis C virus RNA at week 4 as predictor of sustained virological response in HIV patients with chronic hepatitis C. *AIDS* 2008; 22: 15–21.

Primary investigation of 31 infants with suspected congenital rubella syndrome in Sudan

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Abstract

Between 2005 and 2006, clinical specimens were collected from 31 infants with suspected congenital rubella syndrome (CRS) who presented at six hospitals in Khartoum, Sudan. Eleven (35.5%) were laboratory confirmed as CRS cases by testing for anti-rubella IgM, IgG and viral genome. For the first time in Sudan, the rubella virus genome was directly detected in clinical specimens of six CRS cases and two viruses were isolated in cell culture. Phylogenetic analysis suggested that three genotypes of rubella virus (RV; 1E, 2B and 1G) were co-circulating in Sudan. The study introduced the methodology for CRS confirmation and surveillance in Sudan and provides preliminary data.

Keywords: Congenital rubella syndrome, genotyping, rubella virus, Sudan, virus isolation

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Surveillance for measles/rubella is conducted in Sudan, but rubella vaccination is not included in the national immunization programme. Rubella infection was found to be the most frequent cause of non-measles rash in Khartoum [1]. In 1996, a sero-epidemiological study in Khartoum revealed that 20.5% of the pregnant women were susceptible to rubella [2]. However, no surveillance data for the congenital rubella syndrome (CRS) are available in Sudan. This study was conducted to provide information on the CRS burden and characteristics of its causative agent, which may contribute to the establishment of CRS control strategies in Sudan.

Between June 2005 and May 2006, 31 infants under 1 year of age who presented at six hospitals in Khartoum with suspected CRS [3] were investigated, and 62 specimens including 27 throat swabs (TS), five nasal swabs (NS), six oral fluids (OF) and 24 sera were collected. TS and NS were placed in 2 mL of medium [4] and kept in liquid nitrogen. OF were collected and extracted as previously described [5] and kept at −20°C, as were the sera, prior to testing [6]. Data on the clinical findings in the infants and the medical history of the mothers were recorded (Table 1).

The study protocol was reviewed and approved by the Ethical Review Committee, Federal Ministry of Health, Sudan. Parents of infants meeting the CRS case definition were informed about the study and consent was obtained before enrolment.

Sera and OF were tested using a commercial ELISA for rubella IgM (Microimmune Limited, Brentford, UK), and