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Short Communication

No reduction of HCV viral load in HIV patients co-infected with HCV genotype 1 during a 30 days course of nitazoxanide monotherapy

N. Laufer [a,b,c,*], L. Abusamra [b,d], F. Bolcic [a,c], A. Gun [d], M.J. Rolón [b], H. Pérez [b], A. Krolewiecki [d], H. Salomón [a,c], J. Quarleri [a,c], P. Cahn [b,d]

[a] Centro Nacional de Referencia para el SIDA, Universidad de Buenos Aires, Argentina
[b] Hospital Fernández, División Enfermedades Infecciosas, Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[d] Fundación Huésped, Buenos Aires, Argentina

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ABSTRACT

There are two new drugs approved and several in development for treatment of chronic HCV; among them nitazoxanide (NTZ). Twelve HIV/HCV genotype 1 co-infected patients were enrolled prospectively to receive a 30 days course of oral NTZ 500 mg bid. This therapy was well tolerated in this group of HIV patients co-infected with HCV genotype 1. Nevertheless no changes in HCV viral load were observed during treatment in none of the patients evaluated. This data suggests that despite the promising results reported for HCV genotype 4 mono-infected patients, NTZ exhibit poor activity as monotherapy in HIV/HCV co-infected patients with genotype 1.

The World Health Organization (WHO) estimates that about 3% of the world's population has been infected with hepatitis C virus (HCV) and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer (NIH, 2002). About 20% of the people living with HIV/AIDS worldwide are co-infected with HCV (7 million persons) (Soriano et al., 2010). In Argentina, a study showed a HIV/HCV coinfection rate of approximately 21% (Laufer et al., 2010).

In the context of highly active antiretroviral therapy (HAART), chronic hepatitis C has emerged as one of the leading causes of morbidity and mortality in HIV patients, mainly in developed countries (Rockstroh et al., 2005; Weber et al., 2006). The combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) is the best available treatment for chronic HCV infection in patients co-infected with HIV and HCV (Chung et al., 2004) and presents rates of sustained virological response (SVR, ie: undetectable HCV-RNA 6 months after completion of treatment) of 14–36% in HIV patients co-infected with HCV genotypes 1 and 4 (Chung et al., 2004). New drugs (boceprevir and telaprevir) have been recently approved in the US as components of combination regimens for HCV treatment in monoinfected patients but they are not yet licensed for HIV co-infected subjects. Several other drugs are currently been tested for this infection.

Nitazoxanide (NTZ) is a thiazolide oral prodrug approved for the treatment of protozoal infections (Anderson and Curran, 2007). NTZ has been reported as a drug able to inhibit HCV replication *in vitro* (Korba et al., 2008b). Of note, it has also been suggested that NTZ selectively induces PKR phosphorylation, leading to increased cell concentration of phosphorylated eIF2 (Elazar et al., 2009) which is an important factor in the innate immune response. There is also *in vitro* evidence that both NTZ and its metabolite, tizoxanide, inhibit HCV replication in the replicon system for HCV genotypes 1a and 1b (Korba et al., 2008a).

Monotherapy with NTZ has been shown, in a phase II study, to yield a 17% SVR rate for HCV genotype 4 (Rossignol et al., 2008). Another study that evaluated its efficacy as 12 weeks lead-in therapy prior to PEG-IFN/RBV reported a modest but significant decrease in HCV RNA genotype 4 with NTZ monotherapy (Rossignol et al., 2009). There is only one report of three patients monoinfected with HCV genotype 1 and 1 with genotype 2 that received NTZ as lead-in therapy follow by PEG-IFN/RBV that reached SVR (Rossignol et al., 2010). The ACTG 5269 is an ongoing trial that is studying NTZ in combination with PEG-IFN/RBV for HIV/HCV genotype 1 co-infected patients.

The aim of this study was to evaluate in HIV/HCV co-infected with HCV genotype 1 the activity of NTZ as monotherapy during

* Corresponding author at: Centro Nacional de Referencia para el SIDA, Departamento de Microbiología, Facultad de Medicina – UBA CONICET, Paraguay 2155, Piso 11, C1121ABG Buenos Aires, Argentina. Tel.: +54 45083689; fax: +54 45083705.
E-mail address: natalialaufer@gmail.com (N. Laufer).

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a course of 30 days and as a secondary objective to assess its tolerance and safety.

Part of the results of this study have been previously presented at the 18th Conference on Retroviruses and Opportunistic Infections (CROI), held in Boston, Massachusetts (February 27–March 2, 2011).

This was an open-label pilot study conducted at Fundación Huésped (Buenos Aires, Argentina) from April to August 2010. All patients provided written informed consent, and the study was carried out in accordance with the principles of the Declaration of Helsinki.

Male and female adult patients infected with HIV and chronically co-infected with HCV genotype 1 were eligible for enrollment. HCV Genotype was determined by Versant HCV Genotype 2.0 Assay (LiPA). Chronic HCV infection was defined as the presence of anti-HCV antibodies and detectable HCV RNA for more than 6 months. Patients had to be on HAART, with undetectable HIV viral load, CD4 cell counts >200 cells/μL for at least 6 months. The protocol was approved by the Institutional Review Board (IRB) at Fundación Huésped, Buenos Aires, Argentina. Exclusion criteria included: presence of other causes of liver disease, decompensated cirrhosis, active opportunistic infection, pregnancy, active drug use within the last 12 months and use of any investigational drug or interferon within the previous 30 days.

Three patients were previously treated for HCV, one with interferon and two with PEG-IFN/RBV without response.

A total of 12 patients were included. All patients received oral nitazoxanide (500 mg tablets, Roemmers Laboratories, Argentina) b.i.d with food for 4 weeks.

Patients were evaluated and blood samples were drawn before beginning treatment, at 24, 48, 72, 96, 120 h, weeks 1, 2, 3 and 4 after starting therapy. HCV-VL (Bayer VERSANT® HCV RNA 3.0 Assay) was evaluated at each time point. Plasma samples were frozen at −80 °C until use.

Adverse events were categorized as mild, moderate, severe and life-threatening according the AIDS Clinical Trials Group (ACTG) (NIH-NIAID, 2004) and were considered to be probably, possible, unlikely or not related to nitazoxanide.

HCV viral load was use to evaluate HCV kinetics in response to nitazoxanide monotherapy. Changes in alanine aminotransferase (ALT) were assessed at baseline, of 120 h, and at weeks 1, 2, 3 and 4 blood sample, and so were safety and laboratory parameters.

A descriptive analysis of baseline variables was conducted looking at the central tendency and dispersion. Fisher's Exact Test was used to analyze qualitative variables; Mann–Whitney U test and repeated measures Anova to analyze quantitative variables. The significance level was set at 5% and all tests were 2-tailed. Statistical analyses were performed using SPSS v.12.0 (SPSS Corporation, Chicago, IL).

Adherence to NTZ was evaluated by patients self-report. Two patients discontinued treatment prematurely; one for personal reasons and one due to adverse events (moderate diarrhea and nausea) probably related to the study medication.

Demographic, virological, immunological and clinical characteristics are described in Table 1. Fifty percent of the patients were infected with HCV genotype 1a, 25% genotype 1b, and in 25% the genotype 1 was not possible to be sub-typified by LIPA. Liver biopsy was available in half of the patients; exhibiting 3 patients METAVIR F1, 1 F2 and 2 F3 (two biopsies were performed 3 years before the study was initiated and the other 4 within the previous 6 months). One patient was an insulin-requiring diabetic and one had hypertension, both of them with good control of its comorbidities.

Mean baseline HCV viral load was 5.98 log10 IU/mL (SD 0.79), and only two patients presented HCV viral load <600,000 IU/mL at baseline. Mean HCV VL (log10 IU/mL) at 24 h: 6.11 (SD 0.63), 48 h: 5.97 (SD 0.78), 72 h: 6.12 (SD 0.48), 96 h: 5.96 (SD 0.65), 120 h: 6.20 (SD 0.43), week 1: 6.07 (SD 0.66), week 2: 6.08 (SD 0.55), week 3: 6.24 (SD 0.44) and week 4: 6.26 (SD 0.40); with no statistical differences ($p = 0.10$) (Fig. 1).

Table 1
Demographics and clinical characteristics of patients and safety laboratory parameters at baseline and week 4.

Parameter	Baseline ($N = 12$)	Week 4	p
Age (years)[A]	41.9(6.2)		
Baseline weight (kg)[A]	72.3(11.8)		
Baseline ALT (IU/mL)[A]	73.4(31.3)		
Baseline CD4 TL (cell/μL)[A]	466(148)		
Baseline CD4 TL (%)[A]	26.2(5.3)		
Male[B]	10(83)		
Duration of HCV infection(years)[A]	13.2(6.2)		
Duration of HIV infection(years)[A]	13.3(6.3)		
Baseline HIV VL < 50 cp/mL[B]	12(100)		
Baseline HCV VL log10 IU/ml[B]	6.1(0.47)		
Fibrosis METAVIR 3–4 score[B]	0(0)		
IDU[B]	5(41.7)		
HAART with abacavir[B]	6(50)		
HAART with tenofovir[B]	4(33)		
HAART with efavirenz[B]	9(75)		
Hemoglobin (gr/dL)[A]	15.8(1.25)	15.9(1.6)	0.57
White blood cells (cells/dL)[A]	5958(1304)	6480(1488)	0.18
Platelets (103/mL)[A]	229(54)	238(78)	0.57
Creatinine (mg/dL)[A]	0.98(0.17)	0.91(0.2)	0.82
Amylase (mg/dL)[A]	91.6(64.1)	93(64)	0.91
ALT (IU/mL)[A]	84(56)	89(75)	0.56
AST (IU/mL)[A]	54(28)	52(36)	0.55
Total cholesterol (mg/dL)[A]	173(37)	169(33)	0.11

[A] Mean (Std. Desv).
[B] Number (%). IDU: intravenous drug user, VL: viral load, HAART: highly active antiretroviral therapy.

There were no statistically significant changes in ALT and AST levels during the 4 weeks ($p = 0.56$ and 0.55, respectively) (Table 1).

Thirty percent of the patients presented mild gastrointestinal symptoms (nausea, vomiting, and diarrhea) that were considered by the investigator as probably related with the study medication. There were no abnormalities in the safety laboratories parameters (Table 1).

This is the first report of the nitazoxanide activity for HCV in HIV co-infected patients. In summary, in this pilot study of a 30 day course of oral NTZ 500 mg twice daily in HIV patients co-infected with HCV genotype 1 there were only mild adverse events in 30% of the patients, leading to treatment discontinuation in one case. Nevertheless, no changes in HCV RNA levels were observed during treatment in any of the patients. Even though the activity of NTZ against HCV is probably related to an immunodulator stimulus to host cells defense, in HCV monoinfected patients

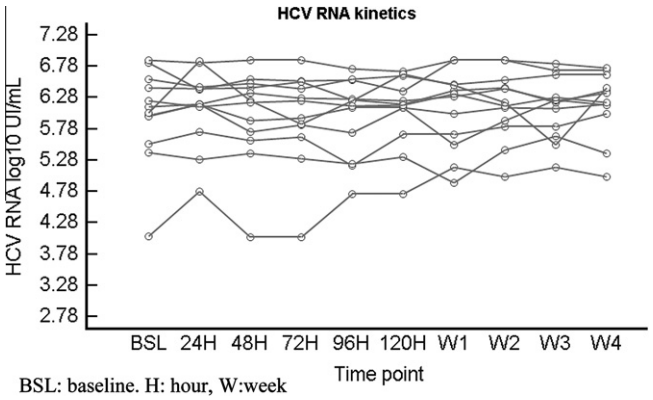

Fig. 1. HCV RNA kinetics.

has been reported a modest to significant decline of HCV RNA with NTZ monotherapy. This was not observed in our study including HIV/HCV-coinfected patients.

One of the possible reasons for the lack of decline in HCV RNA observed could be that patients presented very high viral loads at baseline, a factor associated with poor response in previous studies with nitazoxanide (Rossignol et al., 2008).

Another possible explanation is the long history of both HCV and HIV infection among subjects that could have impacted the specific anti-HCV immunity despite the preserved absolute number of CD4 T lymphocytes. There are reports of different responses to NTZ treatment for cryptosporidiosis between HIV negative and positive individuals, mainly related to more advanced immunodeficiency (Amadi et al., 2002; Pantenburg et al., 2009). Some authors have suggested that a higher dose of NTZ can overcome this lower activity (Amadi et al., 2009; Rossignol, 2006). In this regard it could be hypothesized that the dose of NTZ used in this study could be insufficient to exert anti-HCV activity in the setting of HIV infection.

In this study half of the patients were infected with HCV genotype 1a. Increasing data is becoming available about the different patterns of response for boceprevir and telaprevir for genotypes 1a and 1b (Nelson, 2011); such difference could not be ruled out for NTZ, as well.

Interactions between nitazoxanide and antiretrovirals drugs have not been described, also NTZ is a widely used drug in HIV positive individuals so it seems unlikely that drug–drug interactions might have influenced our results. Liver biopsy was available in half of patients and in those that were available mild fibrosis was present.

Future studies in HIV/HCV co-infected patients including patients with different HCV genotypes, increased NTZ dosing and particularly NTZ combined with PEG-IFN/RBV are necessary to define if nitazoxanide could be an option for HCV treatment in this population.

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