

Effect of IL-2 on hepatitis C virus RNA levels in patients co-infected with human immunodeficiency virus receiving HAART

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SUMMARY. The effect of interleukin-2 (IL-2) on the plasma levels of hepatitis C RNA (HCV-RNA) has varied in published reports. We measured the impact of IL-2 on plasma HCV RNA levels in 54 human immunodeficiency virus (HIV)/HCV coinfecting patients enrolled in a randomized trial of 512 participants designed to compare the virologic and immunologic effects of cycled IL-2 plus antiretroviral therapy (ART) vs ART alone in the treatment of HIV in patients with CD4 cell counts ≥ 300 cells/mm³. The mean decreases in average HCV RNA levels (copies/mL, log₁₀) were 0.28 log in the IL-2 group ($n = 26$) and 0.04 log in the ART alone group ($n = 28$) at 12 months ($P = 0.18$). The changes in HCV RNA level were not associated with baseline or nadir

CD4 cell counts, baseline aspartate aminotransferase, CD4 cell response to IL-2, or changes in plasma HIV RNA values. Compared with those participants who only had HIV, the HIV/HCV co-infected patients did not have a significantly different CD4 cell response to IL-2 therapy. Intermittent IL-2 therapy does not produce a significant sustained decrease in plasma HCV RNA levels among patients co-infected with HIV/HCV who are on highly active ART.

Keywords: bDNA Assay, Co-infection, hepatitis C, human immunodeficiency virus, interleukin-2, transcription-mediated amplification assay.

INTRODUCTION

The immunological determinants that orchestrate the spontaneous clearance of hepatitis C virus (HCV) and the resolution of disease are incompletely understood. However, brisk intrahepatic CD4 cell responses particularly to nonstructural HCV proteins, specific CD8 cell cytolytic action, and high level local gamma-interferon production are believed to be important [1–5]. Interleukin-2 (IL-2) may have potential for the treatment of hepatitis C because it can expand the T-lymphocyte pool, preserve the immunological repertoire, functionally enhance T-lymphocyte and natural killer cell

activity, and promote immunoglobulin synthesis [6, 7]. There are few published reports concerning the use of IL-2 for the treatment of chronic HCV infection [8–13]. While the majority of studies have demonstrated no effect of IL-2 on HCV viraemia, most have shown an improvement in alanine aminotransferase (ALT) values in IL-2 treated persons [8–13]. Clearance of HCV during or after IL-2 therapy was described in three of 36 coinfecting individuals enrolled in these studies. However, only two of six trials included IL-2 untreated hepatitis C patients as controls [11–13]. Furthermore, IL-2 dose and frequency of administration and mode of administration were different across studies [5–12].

As the impact of IL-2 therapy on hepatitis C infection remains uncertain, and because the number of human immunodeficiency virus (HIV)/HCV coinfecting participants considered in this report was larger than those included in other studies, we undertook an evaluation of the variations of HCV RNA levels among those HIV/HCV coinfecting recipients of cycled IL-2 compared with those who received antiretroviral therapy (ART) alone, in the context of a large randomized trial designed to evaluate the virologic and immunologic effects of IL-2 on HIV disease [14].

*Study group members are listed in the Appendix.

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-2, interleukin-2; TMA, transcription-mediated amplification.

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PATIENTS AND METHODS

Study design

The Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA) is an established clinical trials programme that conducts research through a network of community-based clinical units. The CPCRA 059 trial was a large, multi-centre, phase II, randomized, open-label clinical trial that compared the effects of ART vs antiretroviral plus IL-2 (Chiron Corporation, Emeryville, CA, USA) on plasma HIV RNA levels and CD4 cell counts [14]. Participants were randomized to receive one of two doses of IL-2 (4.5 or 7.5 MIU SQ twice daily) or no IL-2 in a 1:1:2 allocation. Administration of IL-2 (five consecutive days every 8 weeks) began immediately following randomization and was to be continued for at least the required three cycles. Protocol guidelines also stated that additional cycles of therapy should be given in order to maintain CD4 cell counts at twice baseline CD4 level or >1000 cells/mm³. Subjects were followed for at least 12 months. The trial opened for enrolment on 7 September 1998 and closed on 3 July 2000 and was approved by a local Institutional Review Board at each site.

Patient selection

The HIV infected patients who were at least 18 years of age, who gave informed consent, were taking combination ART and had CD4 cell counts of at least 300 cells/mm³ were eligible for CPCRA 059. Patients were excluded for the following reasons: prior therapy with IL-2; any central nervous system disorder that required the use of antiepileptic medication; use of corticosteroids or cytotoxic agents (including hydroxyurea) within 4 weeks prior to randomization; history of an AIDS defining illness; history of Crohn's disease, psoriasis, or other autoimmune disease; pregnancy or breast feeding. There were no specific criteria related to stage of liver disease in the study criteria except for those just described.

Data collection

Prior to randomization, a baseline evaluation was performed which included a targeted health and treatment history, physical examination, hepatitis B serostatus and anti-HCV antibody. Plasma HIV RNA levels (VERSANT HIV-1 RNA 3.0 bDNA assay; Bayer Diagnostics, Tarrytown, NY, USA) were collected at baseline and monthly for all patients. Measurements of CD4 cell counts were performed at baseline, and subsequently every 4 months for all study participants.

For patients whose hepatitis C antibodies were positive at baseline, plasma HCV RNA assays were performed. The HCV RNA was assayed by bDNA [VERSANT HCV RNA 3.0 bDNA assay, Bayer Diagnostics; dynamic range 3200–40 million HCV copies/mL (640–8 million IU/mL)] from samples stored

at -70 °C that were collected at baseline and months 1, 2, 3, 6 and 12.

If HCV RNA was undetectable at any time point, transcription-mediated amplification [TMA, VERSANT HCV RNA Qualitative Assay, Bayer Diagnostics; lower limit of detection 50 HCV copies/mL (9.6 HCV IU/mL)] was performed on both baseline and month 6 specimens [15]. If both TMA tests failed to demonstrate virus, the patient was considered aviraemic. Any discordance in the ability to detect HCV RNA by either bDNA or TMA assay triggered a repeat test using both the bDNA and TMA assay at all study time points. If the TMA tests failed to demonstrate virus, the patient was considered aviraemic. Bayer Corporation supplied the bDNA and TMA assays. Chiron Corporation supplied recombinant IL-2 medication.

Statistical methods

For patients with detectable HCV RNA over the time points tested, changes in plasma HCV RNA level, CD4 cell count, and HIV RNA level between baseline and follow-up were compared for the IL-2 group (both dose groups combined) and the ART only group with a linear model, adjusted for baseline value. The groups were also compared by using longitudinal regression models for the average change over follow-up. In plasma HCV RNA analyses, a value of 3199 copies/mL was assigned to results with HCV RNA of <3200 copies/mL (the lower limit of detection), and a value of 40 000 001 was assigned to results with HCV RNA >40 million copies/mL (the upper limit of detection). In plasma HIV RNA analyses, a value of 49 copies/mL was assigned to results with plasma HIV RNA <50 copies/mL (the lower limit of detection). Multiple regression models were performed to study the association between changes in HCV RNA levels and CD4 cell count changes over follow-up adjusting for baseline HCV RNA level. Among the patients who received IL-2, similar analyses were performed on changes in HCV RNA levels and cumulative dose of IL-2 received over follow-up.

RESULTS

Study population

Overall, 63 patients (12.3%) out of the 511 individuals enrolled in CPCRA 059 tested positive for HCV antibody. In nine of 63 patients testing positive for HCV antibody (14.3%), HCV RNA was undetectable by both bDNA and TMA testing for all time points. Of the remaining 54 patients with detectable plasma HCV RNA levels, 26 were assigned to receive IL-2 (10 in the high dose group, 16 in the low dose group) and 28 to the ART only arm. Among the 26 patients who received IL-2, 20 finished at least three cycles of IL-2 treatment, and another three persons finished at least two cycles.

Compared with the patients with HIV infection only, the co-infected group was older, had a higher percent of Latinos

or African Americans, had a higher percent of injection drug users, and a higher baseline aspartate aminotransferase (AST) level (Table 1). For the 54 patients with detectable HCV RNA levels, baseline characteristics were similar between the IL-2 groups vs the ART only group (Table 1). The average age was 42 years, 48% were nonwhite, and 57% of the patients had a history of injection drug use. Patients had been on antiretroviral treatment for a mean of 47 months. The mean CD4 cell count was 570 cells/mm³, the mean log₁₀ HIV RNA was 2.3 copies/mL, and the mean HCV RNA level was 6.7 log₁₀ copies/mL. No significant differences between the two groups were noted for AST, bilirubin and creatinine levels. One patient in the ART only group was hepatitis B surface antigen positive and HCV antibody positive.

HCV RNA Changes

Plasma HCV RNA levels decreased 0.22 log₁₀ copies/mL 1 month after the initiation of IL-2 in the IL-2 group and

increased 0.1 log in the ART only group (95% CI for difference, 0.06–0.53, *P* = 0.02, Fig. 1). Although this difference was statistically significant, the difference in HCV RNA levels between the two treatment arms was not statistically significant at 12 months (*P* = 0.18). However, results of the longitudinal regression analysis showed the average HCV RNA level over follow-up for the IL-2 group was 0.26 log less compared with the ART only group (95% CI for difference, 0.04–0.48, *P* = .02).

The changes in HCV RNA from baseline to month 12 were not associated with baseline AST level (*P* = 0.19) or change from baseline to month 12 in either CD4 cell count (*P* = 0.20) or HIV RNA (*P* = 0.12). No relationship was seen between cumulative dose of IL-2 (average of 169 MIU) and change in HCV RNA for the 26 patients receiving IL-2 (*P* = 0.56).

CD4 cell count changes

When HIV/HCV co-infected patients who were recipients of IL-2 or ART only were compared with the corresponding

Characteristic	CPCRA 059 cohort		HIV/HCV coinfectd	
	HIV infected (<i>n</i> = 459)	HIV/HCV coinfectd (<i>n</i> = 54)	SC rIL-2 (<i>n</i> = 26)	ART Only (<i>n</i> = 28)
Age (mean years)*	38.9	42.7	42.2	43.3
Female (%)	10.7	18.5	26.9	10.7
Race/ethnicity (%)*				
White	71.5	51.9	61.5	42.9
African American	18.1	27.8	19.2	35.7
Hispanic	7.6	18.5	19.2	17.9
Other	2.8	1.9	0	3.6
Prior IV drug use (%)*	6.8	57.4	65.4	50.0
Hepatitis B surface antigen positive (%)	6.8	1.9	0	3.6
CD4 cell count (mean cells/mm ³)	594	570	540	599
Nadir CD4 cell count (mean cells/mm ³)	322	326	327	325
HIV viral load (mean log ₁₀ copies/mL)	2.2	2.3	2.3	2.3
HCV viral load (mean log ₁₀ copies/mL)	–	6.7	6.8	6.5
SGOT/AST (mean U/L)*	30.1	49.5	47.8	51.1
Bilirubin (mean mg/dL)	1.3	1.3	1.3	1.3
Creatinine (mean mg/dL)	1.0	1.0	1.0	1.0
Antiretroviral treatment (months)	42	47	50	45

Table 1 Baseline characteristics by hepatitis C (HCV) status and drug treatment

HCV, hepatitis C virus; HIV, human immunodeficiency virus; ART, antiretroviral therapy; AST, aspartate aminotransferase; SGOT, serum glutamic oxaloacetic transaminase/aspartate aminotransferase.

**P*-value < 0.01 for comparisons between the HIV infected group and the HIV/HCV coinfectd group.

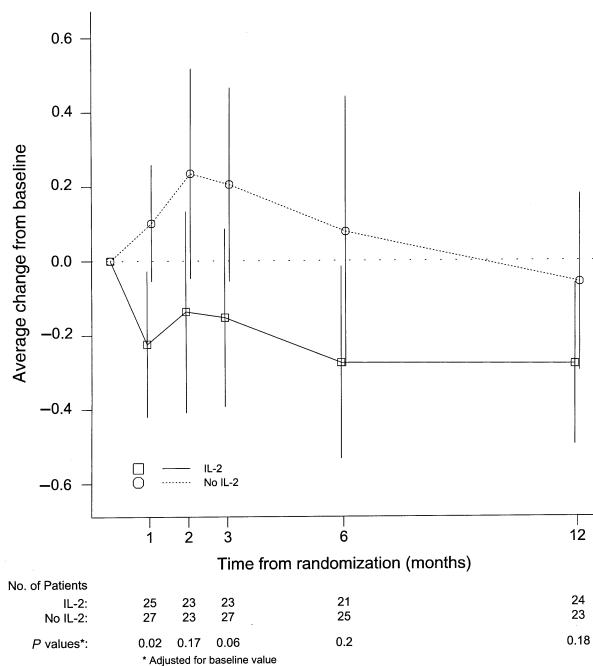


Fig. 1 Mean changes (+, -2SE) from Baseline in log₁₀(HCV-RNA).

groups infected with HIV only, CD4 cell count changes were similar at months 4, 8 and 12 (Table 2). Among the 54 patients co-infected with HIV/HCV, CD4 cell counts rose an average of 300 cells/mm³ from baseline for the 26 patients in the IL-2 group and declined 21 cells/mm³ for the 28 patients in the ART only group (95% CI for difference, -470 to -189, *P* < 0.001) after 4 months of follow-up. At months 8 and 12, the changes in CD4 cell count from baseline were also significantly greater in the IL-2

group than in the ART only group (*P* < 0.001 at both months 8 and 12). Therefore, HCV infection did not appear to blunt the CD4 response.

HIV RNA changes

The average changes in HIV RNA levels did not differ between the IL-2 and the ART only groups over follow-up. At 12 month, the change in log₁₀ HIV RNA level for the IL-2 and ART only groups was -0.15 and 0.19, respectively (95% CI for difference, -0.17 to 0.81, *P* = .21).

DISCUSSION

In this randomized comparison of antiretroviral treated HIV infected patients with baseline CD4 cell counts of at least 300 cells/mm³, cycled IL-2 produced a statistically significant reduction in plasma HCV RNA levels at one month (*P* = 0.02) but no differences (*P* = 0.18) by month 12. This early decrease in HCV RNA may be artifactual, because of chance in the setting of fluctuating plasma levels of HCV coupled with the inherent variability of the assay. However, a limited effect of IL-2 cannot be excluded, as the average HCV RNA levels over time were 0.26 log less in the IL-2 group compared with the group which received ART alone (*P* = .02). This difference is small and unlikely to have clinical significance. These results are not attributable to differences in HIV disease status. The groups were comparable in baseline and nadir CD4 cell counts, pretreatment HIV and HCV levels, and baseline characteristics.

Many factors that may have potentially affected our results cannot be evaluated. These include HCV genotype and species diversity, duration of HCV infection, age when first infected, severity of hepatitis by histologic analysis of

Table 2 CD4 cell count (cells/mm³) during follow-up by hepatitis C status and IL-2 treatment arm

	HIV/HCV coinfectd		HIV infected only		Difference*	SE	P-value
	n	Mean	n	Mean			
IL-2 arm							
Baseline	26	540.0	231	598.3	-58.3	43.4	0.18
Month 4	22	858.4	217	962.6	-60.5	91.8	0.51
Month 8	21	851.0	211	891.1	27.4	72.3	0.70
Month 12	24	846.2	221	871.9	29.7	65.7	0.65
ART only arm							
Baseline	28	598.7	228	590.3	8.3	43.5	.85
Month 4	27	584.9	206	595.2	-22.0	29.8	.46
Month 8	28	590.0	206	606.4	-21.2	37.6	.57
Month 12	26	618.9	211	618.4	-11.9	36.5	.75

SE, standard error of the difference between the HIV/HCV coinfectd group and the HIV infected group.

*Difference of means adjusted for baseline CD4 cell count, IL-2, subcutaneous recombinant IL-2.

liver biopsies, and intra and extra-hepatic functional differences in HCV specific immunity [16–19]. IL-2 may have little effect on HCV dynamics, even among patients who demonstrate a beneficial clinical effect in the absence of significant changes in HCV RNA. The duration of IL-2 therapy may be another potential confounder in the response of HCV RNA levels. In one uncontrolled study of IL-2 and HAART, the two patients that cleared HCV RNA received daily IL-2 therapy as compared with the cycling methods utilized in most of the other reports [11].

While some of the other IL-2 studies have shown a decrease in ALT levels [9–13], this decrease may or may not represent a clinically relevant improvement in hepatic inflammation or disease progression. HCV viral load and ALT have not been shown to be surrogate markers of the extent of liver injury nor of its rate of progression. In some coinfection cases, ALT decline may simply represent a return to baseline value after an antiretroviral flare [20]. However, in an uncontrolled trial of IL-2 administered to 33 HIV-uninfected HCV patients, there was no significant improvement in hepatic histology, despite a statistically significant decline in ALT, among the 20 individuals who underwent follow-up liver biopsy [8]. Neither ALT value nor liver biopsy information were routinely collected in CPCRA 059.

Despite a significant rise in mean CD4 cell count of IL-2 recipients, this response did not translate into improved HCV clearance. This is not surprising since the magnitude and specificity of the peripheral CD4 cell response to HCV may not mimic the intrahepatic response [3]. While the evidence remains inconclusive [21], it is the local response that appears to promote the clearance of virus and resolution of disease in acute infection [3]. In contrast to the attenuated CD4 cell response to ART reported among HIV/HCV coinfecting patients [22–24], there were no differences in the CD4 response to either IL-2 plus ART or to ART only when coinfecting individuals were compared with HCV uninfected individuals within their respective randomized treatment groups (i.e. CD4 cell count changes were similar). However, the sample size was small for the HIV/HCV group, and the analysis did not control for the prior duration of ART.

Antiretroviral therapy given to controls had no apparent impact on hepatitis C viraemia. Of note, ART induced neither an increase [25] nor a decrease in HCV RNA. The viral load trajectory reported here is remarkably similar to that reported by Chung [20] for coinfecting individuals with pre-therapy CD4 cell counts above 350 cells/mm³. However, as participants in our study had a mean of 47 months of prior therapy, early effects of HCV RNA levels would not have been detected. Evaluation of specific antiretroviral regimens could not be evaluated in this study because of sample size constraints.

To date, this analysis of CPCRA 059 represents the largest sample of HIV/HCV coinfecting persons who have received cycled IL-2 in a randomized trial [14]. In this study, the effects of IL-2 were examined in the context of a

larger trial designed to measure the effects of IL-2 on HIV RNA and CD4 cell count responses. Persons having more severe liver disease were preferentially excluded by protocol eligibility criteria. Patients who were HCV antibody negative but HCV RNA positive were also excluded from our analyses, since HCV RNA testing was not performed on all participants in CPCRA 059. This may have omitted approximately 2.8% of the available HIV/HCV population [26]. In addition, approximately 14% of hepatitis C antibody positive patients in this study had no detectable HCV RNA levels at baseline. Chronicity of hepatitis C infection as characterized by the persistence of viraemia is reported in 70–85% of individuals [27, 28]. The effect of IL-2 in this group of patients who presumably cleared hepatitis C was not part of this analysis, although rebound hepatitis C viraemia was not detected by TMA assays in any of these individuals. As biopsies were not performed the severity of HCV-related liver disease was not characterized and could not be evaluated. Finally, long-term outcome information beyond the study's closure date is currently unavailable. However, CPCRA 059 participants are undergoing follow-up as part of the larger international IL-2 trial, the Evaluation of Subcutaneous Proleukin in a Randomized International Trial (the ESPRIT Study) [29].

In agreement with the findings of most prior studies, we found that cycled IL-2 has little to no effect on plasma HCV RNA levels. While the absence of an antiviral response does not exclude the possibility of clinical benefit, cycled IL-2 administered by itself or in combination with ART does not appear to impact clearance of hepatitis C in coinfecting patients. Future studies specifically designed to address these issues may ultimately determine if IL-2 is beneficial to patients with HCV disease, particularly if prescribed in conjunction with specific anti-HCV therapies [30, 31].

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APPENDIX

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