International Journal of Infectious Diseases 33 (2015) 15–21

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Pretreatment serum macrophage inflammatory protein (MIP)-1 levels predict sustained virological responses to re-treatment in patients with chronic hepatitis C virus infection

Shibin Zhang [a], Yan Zhao [a], Huiping Yan [a], Hao Wu [a], Lai Wei [b], Yonghong Zhang [a,*], Xinyue Chen [a,*]

[a] Department of Hepatology, Beijing You'an Hospital, Capital Medical University, 100000, Beijing, China
[b] Hepatology Institute, Peking University, China

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 26 August 2014
Accepted 28 August 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
MIP-1
Sustained virological responses
Chronic hepatitis C virus
Infection

SUMMARY

Background: There are few predictors of the virological response in patients who are re-treated with antiviral therapies. In this study, we evaluated the levels of chemokines that bind to C-C chemokine receptor type 5 (CCR5) and their impact on combination therapy in both treatment-naïve and treatment-experienced patients chronically infected with hepatitis C virus (HCV).

Methods: Longitudinal analysis of CCR5 chemokines was performed using the multiplex Bio-Rad 27-plex assay in 56 treatment-naïve and 24 treatment-experienced patients with chronic HCV infection during combination therapy with peginterferon alfa and ribavirin. A group of healthy donors was included as the control ($n = 11$).

Results: The pretreatment level of macrophage inflammatory protein 1 (MIP-1) was determined to be an independent predictor, with an ideal predictive threshold for sustained virological response of 95.23 pg/ml. A rapid decline in HCV RNA was observed in patients with a pretreatment MIP-1 level of <95.23 pg/ml, while a slow reduction was measured in patients with levels of ≥95.23 pg/ml ($p = 0.014$). Of note, the dynamics of MIP-1 further indicated that a lower level at baseline and at treatment week 12 was significantly associated with a favorable outcome of antiviral therapy ($p = 0.014$), especially in treatment-experienced patients ($p = 0.04$), while a higher level of MIP-1beta correlated with the elevation of transaminases.

Conclusions: Serum MIP-1 is an independent and effective predictor of early and sustained virological response in chronically HCV-infected patients undergoing re-treatment.

1. Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease and infects approximately 170 million people worldwide.[1,2] Upon HCV infection, 70–85% of individuals do not spontaneously resolve acute HCV infection, resulting in chronic infection with the potential to develop cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[3] Combination therapy with peginterferon alfa and ribavirin is currently the recommended antiviral therapy for chronic HCV-infected patients, leading to long-term resolution of infection in only 45–80% of individuals depending on the viral genotype.[4–6] Despite great improvements in treatment efficacy, non-virological responders constitute a large part of the population, and re-treatment of patients is becoming a new challenge in HCV management due to the lack of optimal therapies and predictors of sustained virological response (SVR).

The factors that determine the outcome of therapy have been well documented in naïve patients undergoing combination therapy, while there are only a few studies focusing on therapy in previously treated patients. The CC genotype of interleukin (IL)-28B, HCV genotypes other than type 1, and low viral loads have been identified as factors associated with a favorable treatment outcome in naïve patients on combination therapy with peginterferon alfa and ribavirin.[7–9] However, in published studies, these factors were rarely able to predict the outcomes of treatment-experienced patients because the majority were infected with HCV genotype 1,

* Corresponding authors. Tel.: +86 133 01030203; fax: +86 10 63054847.
E-mail addresses: zhangyonghong_1980@163.com (Y. Zhang), chenxydoc@163.com (X. Chen).

http://dx.doi.org/10.1016/j.ijid.2014.08.021

had high levels of HCV RNA, and carried the CT/TT genotype of the IL-28B gene.[10]

Chemokines, a subgroup of small cytokines, direct leukocyte trafficking and positioning within tissues,[11] thus playing an important role in spontaneous clearance of HCV infection and long-term resolution with antiviral therapy.[12] The levels of chemokines in peripheral blood have been found to be critical predictors of responsiveness to antiviral therapy with peginterferon alfa and ribavirin. For example, levels of monokine induced by interferon gamma (IFN-γ) (MIG), IFN-γ-inducible protein 10 (IP-10), and IFN-inducible T-cell α chemoattractant (I-TAC) could be used to predict the efficacy of treatment.[13–16] In particular, ligands of C-C chemokine receptor type 5 (CCR5), including chemokines CCL3 (macrophage inflammatory protein-1 alpha, MIP-1alpha), CCL4 (MIP-1beta), and CCL5 (regulated upon activation normal T-cell expressed and secreted, RANTES) play an important role in predicting antiviral outcome, since they regulate T cell functions by mediating recruitment, polarization, activation, and differentiation of antiviral type 1 cytokine secreting T helper and cytotoxic T cells.[12,17–19] While most studies have focused on treatment-naïve patients, data on treatment-experienced patients who have been re-treated with peginterferon alfa and ribavirin are scarce.

Understanding the difference in chemokine levels between treatment-naïve and treatment-experienced patients before, during, and after antiviral therapy is of great importance in the analysis of effectiveness and the optimization of current treatment strategies. In order to address this issue, we performed a prospective longitudinal study to distinguish the peripheral blood levels of CCR5-associated chemokines MIP-1alpha and MIP-1beta before therapy and at treatment weeks 4 and 12, between treatment-naïve and treatment-experienced patients treated with peginterferon alfa and ribavirin, using the multiplex Bio-Rad 27-plex assay.

2. Materials and methods

2.1. Patients

This study was approved by the Human Subjects Protection Committees of Beijing You'an Hospital. Written informed consent was obtained from all study participants. Eighty HCV-infected patients (56 treatment-naïve and 24 treatment-experienced who were null responders to standard interferon and ribavirin), who were participating in a clinical study designed to assess the efficacy of the treatment regimen (described in detail below) were enrolled in the study. All patients were negative for HIV and hepatitis B virus antibodies and underwent combination therapy with peginterferon alfa 2a (180 μg) and ribavirin (10.6–15 mg/kg/day) for a total of 48 weeks.[20] No cirrhosis or fibrosis was detected on routine examination of all patients by B ultrasound and abdominal computed tomography examination. Control subjects included 11 self-reported healthy volunteers, who were HCV, HBV, and HIV antibody negative. HCV genotypes were determined using the Abbott RealTime HCV Genotype II assay kit (Abbott Molecular). Clinical and demographic characteristics of these patients are detailed in Table 1. Serial blood samples were collected before treatment (week 0) and at weeks 4 and 12 after the start of treatment. Serum was frozen at −20 °C.

2.2. Viral load

HCV RNA was measured in serum using the qualitative Roche COBAS Amplicor assay (version 2.0; Roche Molecular Systems; lower limit of detection 50 IU/ml).

2.3. Clinical definitions

Clinical definitions are based on viral clearance. Super rapid virological response (SRVR) was defined as an undetectable serum HCV RNA level (<50 IU/ml) at week 2 of therapy.[21] Rapid virological response (RVR) was defined as an undetectable serum HCV RNA level (<50 IU/ml) at week 4 of therapy. Early virological response (EVR) was defined as an undetectable serum HCV RNA level at treatment week 12 (cEVR) or an at least 2-log reduction in serum HCV RNA level from baseline to week 12 of antiviral therapy (partial EVR). SVR was defined as an undetectable serum HCV RNA level at the end of the 24-week follow-up period.[8]

2.4. Measurement of CCR5-associated chemokines

Serum CCR5-associated chemokine levels were measured using a Human Cytokine 27-plex assay kit (Bio-Rad, Hercules, CA, USA) with Bio-Plex Manager software version 6.0 in the Bio-Plex 200 System (Bio-Rad). This system allows quantitative measurement of 27 different chemokines, cytokines, growth factors, and immune mediators, including MIP-1alpha and MIP-1beta, while consuming 12.5 μl volumes of samples. Chemokines were evaluated according to the manufacturer's instructions.

Table 1
Summary of clinical and demographic characteristics[a]

Parameter	Naïve patients	Experienced patients	Healthy controls
Total number	56	24	11
Gender			
Male, *n* (%)	29 (51.8%)	17 (70.8%)	6 (54.6%)
Female, *n* (%)	27 (48.2%)	7 (29.2%)	5 (45.5%)
Age, years	37.82 ± 15.34	39.09 ± 16.93	38.12 ± 14.31
HCV genotype, 1b/2a/others, *n*	49/7/0	21/3/0	NA
HCV-RNA load, $\log_{10}$ IU/ml	6.18 ± 1.11	6.25 ± 0.98	NA
IL-28B (rs12979860), CC/CT/TT, *n*	45/11/0	19/4/1	ND
ALT, IU/l	64.84 ± 53.76	52.93 ± 47.87	ND
AST, IU/l	55.88 ± 37.03	47.31 ± 39.80	ND
Virological response			
SRVR[b]	14 (27.5%)	2 (10.5%)	NA
RVR	26 (46.4%)	6 (25.0%)	NA
EVR	46 (82.1%)	12 (50.0%)	NA
SVR	45 (80.4%)	12 (50.0%)	NA

HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SRVR, super rapid virological response; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response; NA, not available; ND, not detected.

[a] Results are presented as the mean ± standard deviation, or *n* (%), unless stated otherwise.

[b] Total number of patients enrolled in week 2 = 70, with 10 samples missing.