Expression of interferon-alpha receptor mRNA in the liver in chronic liver diseases associated with hepatitis C virus: Relation to effectiveness of interferon therapy

RYO FUKUDA, NORIHISA ISHIMURA, SHUNJI ISHIHARA, ALEJANDRO TOKUDA, SHUICHI SATOH, SHINO SAKAI, SHUJI AKAGI, MAKOTO WATANABE, and SHIRO FUKUMOTO

Second Department of Internal Medicine, Shimane Medical University, 89-1 Enya-cho, Izumo-shi, Shimane 693, Japan

Abstract: To investigate whether interferon-alpha receptor (IFN-αRc) expression was related to the effectiveness of interferon therapy in hepatitis C virus (HCV)-associated chronic liver disease (CLD), IFN-αRc mRNA was investigated by reverse transcription polymerase chain reaction (RT-PCR) in liver biopsies and peripheral blood mononuclear cells (PBMCs) from 40 patients with HCV-associated CLD who subsequently received IFN-α therapy. IFN-αRc mRNA in the liver was detected in 18 of 20 (90%) responders to IFN and in 5 of 20 (25%) non-responders (P < 0.01). In PBMCs, IFN-αRc mRNA was detected in all patients regardless of response to IFN. Increased histological hepatitis activity and liver fibrosis were significantly related to the absence of IFN-αRc mRNA. The HCV-RNA genotype showed no significant relationship to IFN-αRc mRNA expression. Our results suggest that IFN-αRc mRNA expression in the liver, but not in PBMCs, is closely associated with the effectiveness of IFN-α therapy in HCV-associated CLD.

Key words: Chronic hepatitis C, interferon-α receptor mRNA, interferon therapy

Introduction

Various factors predictive of response to interferon (IFN) therapy in chronic hepatitis C (CHC) have been reported; two important steps are necessary for the induction of an anti-viral state by IFN: the binding of IFN to specific receptors and intracellular signal transduction after IFN receptor (IFN-Rc) binding. The number of IFN-Rc on the cell surface is less (10^2 to, at most, 10^3 per cell) than the number of receptors for other substances; for example, the number of receptors for adrenocorticotropic hormone (ACTH) is 10^4-10^6 per cell. IFN-α and β have a common receptor (type-I IFN receptor), whereas IFN-γ has its own receptor (type-II IFN receptor). Although the major role of IFN-γ is to mediate the immune response, the type-I IFN receptor is important for the induction of the antiviral state. Accordingly, the efficacy of IFN therapy may be closely related to the expression of type-I IFN-Rc mRNA.

We have already shown that transcript of IFN-αRc gene, which had been cloned by Uze et al., decreased with progress to the advanced stage of HCV-associated chronic liver disease (CLD), a phenomenon which may explain the poor effect of IFN therapy in liver cirrhosis (LC). In the same report, we also demonstrated that IFN-αRc mRNA expression was significantly correlated with the amount of HCV-RNA and with histological activity in the liver, both of which are major predictors of IFN response. However, we did not investigate the relationship between IFN-αRc mRNA expression and the efficacy of IFN in that study. In the present study, to clarify whether the expression of IFN-αRc mRNA is related to the clinical effectiveness of IFN therapy, we investigated the relationship between the expression of IFN-αRc mRNA in the liver of 40 patients with HCV-associated CLD and the subsequent response to IFN. We also investigated IFN-αRc mRNA in peripheral blood mononuclear cells (PBMCs), since these cells are the major source of serum 2', 5'-oligoadenylate synthetase (2,5AS), an indicator of response to IFN.

Materials and methods

Patients

Forty patients with CHC were the subjects of this study. All patients had been followed up at the Shimane
Medical University Hospital for at least 6 months (range, 6 months to 4 years). All patients had received IFN therapy after liver biopsy. The study was approved by the Ethics Review Committee of the University.

IFN therapy and response to IFN

IFN therapy was standardized as follows; 6 megaunits (MU) of recombinant IFN-α 2b (Intron-A, Yamamouchi Pharmaceutical, Tokyo, Japan) was injected intramuscularly every day for the first 2 weeks and three times weekly for 22 weeks subsequently (total treatment period, 6 months). Routine blood tests, including liver function tests and blood cell counts were performed every 2 weeks for the 1st month and then every month for the subsequent observation period. During the course of the IFN therapy, there were no severe side effects, except for influenza-like symptoms, in any patient. Patients were divided into two groups according to the in response to IFN; responders and non-responders. Responders were defined as those patients whose serum alanine aminotransferase (ALT) level decreased to the normal range (40IU/l) within 6 months after the administration of the IFN and remained within the normal range for at least 1 year after the discontinuation of the IFN treatment (n = 20). Non-responders were those patients whose ALT did not decrease to the normal level during or after the IFN treatment (n = 20).

Liver tissue

In all patients, liver biopsy was performed, using Silvermann’s biopsy needle, the purpose of the biopsy being to determine whether IFN therapy was indicated in patients with viral hepatitis by evaluating histological progress and activity. The biopsy specimen was immediately divided into two parts, one for histological examination and one for RNA extraction. Informed consent was obtained from all patients before the biopsy.

PBMC

PBMC, separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation, were suspended, at a concentration of 1 x 10⁶ cells/ml, in RPMI-1640; RNA extraction was performed immediately. For the control, PBMC from five healthy volunteers were used.

RNA extraction and preparation of cDNA

Total RNA was extracted by the acid guanidinium phenol/chloroform (AGPC) method, as previously reported. Ten-μg of total RNA was used as a template for reverse transcription (RT), the procedure for RT being the same as we described previously.

PCR

Complementary DNA (cDNA), 250 ng, was used as the template for the PCR. The sequence of primers was: sense, 5’-AGTGTATGTGGCTTTGGATGGTT-TAAGC-3’ (nt 534-563) and anti-sense, 5’-TCTGGTGTTTCAACAAATATACAGTCAGTGG-3’ (nt 1270-1299). The primers were obtained from Clontech Laboratories (Palo Alto, CA, USA) and ideally made a single band of 765 base pairs. The PCR conditions were: denaturing, 95°C for 1 min; annealing, 55°C for 1 min; extension, 72°C for 2 min. In actual PCR, initial heating at 95°C for 5 min was followed by 35 cycles and final extension at 72°C for 15 min. The procedure for PCR was the same as the one we used previously. After PCR, a 5-μl aliquot was electrophoresed on 1% agarose gel. Positive signals were seen at the ideal length. As a control, β-actin mRNA was amplified, using a primer set; sense, 5’-AGTGTTATGTTGGGCTTTGAGTT-TAAGC-3’, and anti-sense, 5’-TCTGGCTTTTCAACAAATATACAGTCAGTGG-3’. The PCR conditions were the same as those for IFN-αRc.

Hybridization of PCR product

To reconfirm that IFN-αRc mRNA was amplified, 5 μl of PCR product was, treated with 1 μl of exonuclease I (1 unit/μl) (USB, Cleveland, OH, USA) at 37°C for 15 min to degrade the single-strand DNA; 1 μl of shrimp alkaline phosphatase (2 units/μl) (USB) was added and the product treated at 37°C for 15 mins to remove the primers. After incubation at 80°C for 15 min, the whole reaction mixture was spotted on a nitrocellulose filter and hybridized with a 32P-labeled synthesized oligonucleotide IFN-αRc probe. The se-