International Immunology, Vol. 16, No. 1, pp. 51–54
doi: 10.1093/intimm/dxh024

Paradoxical alteration of acute-phase protein levels in patients with chronic hepatitis C treated with IFN-α2b

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Keywords: chronic hepatitis C, fetuin/α2HS-glycoprotein, IFN-α therapy, orosomucoid, transferrin

Abstract

Previously we observed elevation of the serum concentration of two acute-phase protein (AFP) complement components (C9 and C1-inhibitor) in patients with chronic hepatitis C who responded (R) to IFN-α therapy, but not in non-responders (NR). In the present study we investigated the effect of high-dose IFN-α therapy on serum concentrations of two positive [orosomucoid (OROSO) and C-reactive protein (CRP)] and two negative [transferrin (TF) and fetuin/α2HS-glycoprotein (AHSG)] AFP in an outpatient setting. We investigated blood samples of 40 patients with chronic hepatitis C at the onset and at the end of a 3-month treatment with high-dose IFN-α2b (5 MIU/day for 6 weeks, followed by 5 MIU t.i.w.) and of 52 healthy individuals. Serum concentrations of OROSO, TF and AHSG were measured by radial immunodiffusion; CRP levels were determined by immunotubridimetry. Compared to controls, patients with chronic hepatitis C had significantly lower OROSO and CRP, and higher AHSG levels. By the end of treatment, OROSO concentration increased in R ($P = 0.0054$), but not in NR patients. In contrast, TF levels decreased in R ($P = 0.0040$), but did not change in NR patients. Similarly, in R patients, AHSG levels tended to decrease ($P = 0.0942$) following IFN-α treatment. We conclude that the acute-phase reaction is suppressed in patients with chronic hepatitis C that may be potentially related to the responsiveness to IFN-α therapy.

Introduction

The pathomechanism of hepatic and extrahepatic manifestations of hepatitis C virus (HCV) infection has been reviewed extensively. Little is known, however, of the behavior of the acute-phase reaction during chronic hepatitis caused by HCV. Recently we found significant increase in serum concentrations of C9 and C1-inhibitor (C1-INH), two acute-phase proteins (AFP) belonging to the complement system in patients with chronic hepatitis C who responded to treatment with IFN-α with at least 50% drop of HCV RNA concentration (1). This observation has recently been confirmed by

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Transmitting editor: A. Falus

Received 27 February 2003, *accepted* 26 September 2003

Table 1. Serum concentrations of AFP in patients with chronic hepatitis C and healthy blood donors

	Controls (n = 52)	HCV patients before treatment (n = 40)	P^a	HCV patients after treatment (n = 40)	P^b	P^c
HCV RNA (MEQ/ml)		7.15 ± 1.71		2.07 ± 0.72		<0.0001
OROSO (mg/dl)	80.1 ± 2.9	68.1 ± 1.9	0.0008	73.0 ± 1.7	0.084	0.0058
CRP (mg/l)	2.6 ± 0.3	1.44 ± 0.4 (n = 20)	0.0273	1.9 ± 0.6 (n = 18)	0.0703	0.4668
TF (mg/dl)	257.0 ± 9.1	282.0 ± 10.0	0.3210	261.2 ± 8.0	0.9153	0.0061
AHSG (μg/ml)	591.3 ± 13.3	729.9 ± 29.3	0.0001	698.8 ± 20.8	<0.0001	0.0821

Values are expressed as mean ± SEM.
[a]P controls versus patients before treatment, calculated by the Mann–Whitney test.
[b]P controls versus patients after treatment, calculated by the Mann–Whitney test.
[c]P patients versus before and after treatment, calculated by the Wilcoxon test.

Dumestre-Perard *et al.* (2) who observed a significant increase of the reduced C4 activity in chronic hepatitis C patients who responded to IFN-α2b or IFN-α2b + ribavirin therapy. These observations prompted us to determine serum levels of two positive [orosomucoid (OROSO) and C-reactive protein (CRP)] and two negative [transferrin (TF) and fetuin/α2HS-glycoprotein (AHSG)] AFP in patients with chronic hepatitis C prior to and following IFN-α therapy.

Methods

Forty patients with chronic hepatitis C (20 men and 20 women, age 45.5 ± 6.1 years, mean ± SEM) were investigated. The diagnosis of chronic HCV hepatitis was based on persistent (>6 months) and elevated (>2-fold of the upper normal limit) transaminase activities, and the presence of anti-HCV antibodies and HCV RNA in their serum samples. Each patient had a liver biopsy, which was done within 1 year (6.1 ± 1.3 months, mean ± SEM) before initiation of IFN-α treatment. The mean duration of the disease was 48.5 ± 50.9 months (mean ± SEM). The initial histological activity of hepatitis assessed by the Knodell index was 9.62 ± 0.53 (mean ± SEM). Blood samples of 52 age-matched healthy blood donors (28 men and 24 women, age 46.6 ± 4.3 years, mean ± SEM) were used as controls. Patients received high-dose recombinant IFN-α2b (Schering-Plough, Budapest, Hungary) 5 MIU s.c. daily for 6 weeks followed by 5 MIU s.c. 3 times a week for a further 6 weeks. Blood samples were taken before starting therapy and at the end of the 3-month treatment. Patients were considered as responders (R, n = 18) when a drop at least of 90% occurred in HCV RNA after 3 months of IFN therapy, whereas those without such decrease were defined as non-responders (NR, n = 22).

Serum concentrations of OROSO, TF and AHSG were determined by radial immunodiffusion. Antisera to each glycoprotein were raised in goats (DiaSorin; Stillwater, Minnesota, MN, IgG fraction, cat. no. 81901, 91913 and 81931 for OROSO, TF and AHSG respectively). Variation coefficients were 4.2, 5.0 and 3.6% for OROSO, TF and AHSG measurements, respectively. CRP levels were measured by particle-enhanced immunoturbidimetric assays (Roche Cobas Integra 4000). The detection limit of the assay was 0.07 mg/l and the coefficient of variation was 3.9% at 108 mg/l mean value.

The concentration of HCV RNA was determined by the branched-chain DNA method using the Quantiplex HCV bDNA 2.0 kit (Bayer-Chiron, Emeryville, CA).

Non-parametric tests were used for the statistical analysis, which was performed using GraphPad Prism version 3.0 (San Diego, CA) and SPSS version 10 (Chicago, IL) for Windows.

Results

Serum concentrations of positive AFP OROSO and CRP and negative AFP TF and AHSG in healthy controls and patients with chronic hepatitis C before treatment with IFN-α

Serum concentrations of the AFP measured in patients before treatment and healthy controls are shown in Table 1. Serum OROSO levels were significantly lower in patients with chronic hepatitis C compared to those measured in control sera. Serum CRP levels in patients' sera were within the reference range (0–6 mg/l), although significantly lower than those measured in the controls. In contrast to the positive AFP, concentration of the negative AFP AHSG was markedly higher in patients with chronic hepatitis C than in healthy controls and the mean concentration of the negative protein TF was higher in patients than in controls.

Knodell index values exhibited significant negative correlation (r = –0.398, P = 0.0190) to the OROSO levels, whereas no correlation with other AFP was found.

Serum AFP and HCV RNA levels in patients with chronic hepatitis C before and after treatment with IFN-α

When all patients were considered, at the end of the therapy OROSO levels were slightly, but significantly, increased compared to pre-treatment values, while no significant change in CRP levels was observed (Table 1). IFN-α therapy resulted in a significant decrease in TF levels and a decrease in the AHSG concentrations (Table 1). Serum HCV RNA decreased significantly from initial values following treatment.

Changes in serum concentrations of the positive AFP OROSO and CRP and the negative AFP TF and AHSG in R and NR patients

Only R patients showed a marked decrease in transaminase activities (Table 2). Significant differences in the changes of the levels of some AFP tested were found between the R and

Table 2. Serum concentration of HCV RNA and AFP in patients with chronic hepatitis C prior to and following IFN-α treatment

	R (n = 18)			NR (n = 22)		
	Before treatment	After treatment	P	Before treatment	After treatment	P
HCV RNA (MEQ/ml)	8.05 ± 3.2	0.06 ± 0.05	0.0002	6.40 ± 1.75	3.72 ± 1.22	0.0749
OROSO (mg/dl)	67.0 ± 2.6	75.2 ± 1.8	0.0054	69.2 ± 3.6	68.9 ± 3.1	0.2617
CRP (mg/l)	1.3 ± 0.5 (n = 10)	2.7 ± 1.3 (n = 11)	0.8125	1.5 ± 0.7 (n = 10)	1.4 ± 0.4 (n = 17)	0.9102
TF (mg/dl)	296.1 ± 17.8	262.2 ± 13.7	0.0040	270.3 ± 10.7	260.3 ± 9.5	0.3176
AHSG (μg/ml)	717.4 ± 45.9	690.6 ± 35.5	0.0942	740.1 ± 38.8	704.7 ± 25.6	0.3349
ASAT/SGOT (U/l)	109.1 ± 28.0	25.9 ± 2.6	0.0156	77.4 ± 9.10	63.1 ± 12.1	0.9375
ALAT/SGPT (U/l)	138.3 ± 19.6	43.9 ± 8.1	0.0006	101.4 ± 13.2	71.6 ± 6.5	0.0175

Values are expressed as mean ± SEM. Calculated by the Wilcoxon test.

the NR groups (Table 2). In the R patients, serum OROSO levels significantly increased and returned to normal values (Mann–Whitney test, P = 0.5017, 3-month values compared to healthy controls), whereas in sera of NR patients no change was observed and OROSO levels remained low by the end of therapy (P = 0.0391, compared to controls). In contrast, no changes in the CRP levels occurred either in the R or the NR patients during therapy. There was a marked drop in the concentration of TF during IFN-α treatment in the R patients, whereas in NR patients no significant changes in the TF levels occurred. Serum AHSG levels decreased both in the R and NR groups, but the difference was not statistically significant (Table 2).

Discussion

The chronic activation of the acute-phase reaction in liver cirrhosis is well known (3). However, the behavior of the acute-phase reaction in chronic HCV hepatitis has not been clarified. In one study, no abnormalities in CRP, fibrinogen and albumin levels were observed, whereas low C3 and C4 concentrations were found that returned to normal in patients responsive to IFN-α therapy (4). Similar results were reported for the C9 and C1-INH levels by our group (1), and for C4 activity by Dumestre-Perard *et al.* (2). Japanese authors did not find any correlation between CRP concentration and liver histological activity (5). Serum TF levels have been studied only in terms of hepatic iron load; the latter has been considered as a risk factor contributing to severity of the disease and poor response to IFN-α therapy (6,7). Although there was no difference between TF concentrations in R and NR individuals, a higher than normal iron saturation in the latter group has been found (7).

Our results merit discussion in two aspects: (i) the difference between healthy controls and patients with chronic hepatitis C, and (ii) the difference in the changes of AFP levels between R and NR to IFN-α therapy.

The comparison of values of healthy controls and those of patients prior to therapy demonstrates decreased concentration of the positive AFP OROSO and increased levels of the negative protein AHSG in chronic hepatitis patients prior to treatment. Given its lower limit of zero, one cannot expect a difference in the levels of the positive protein CRP that is both biologically relevant and statistically significant. However, the fact that only seven out of 19 patients had CRP values >1 mg/l and only two >6 mg/l gives the impression that CRP levels are also decreased compared to controls. These findings, in accordance with the previous findings of our group (1) and others (2,4), indicate that in chronic hepatitis C the levels of at least some AFP change to a direction that is opposite to that observed in the acute-phase response.

In our patients, the consistent changes of the positive and negative proteins suggest altered regulation in synthesis. Altered removal from the circulation would affect both positive and negative AFP levels to the same direction. Since we observed different directions of change in serum concentration this mechanism does not seem to be significant in our study.

A decreased level of AHSG has been found in several chronic liver diseases including alcoholic cirrhosis and hepatocellular cancer, and proved to be a good predictor of short-term mortality (8). Hepatitis B and Epstein–Barr virus-induced acute hepatitis did not alter AHSG levels compared to controls. The marked elevation of AHSG in chronic hepatitis C patients is of particular interest in this respect. We think that out of the four proteins we examined, AHSG is the best indicator of altered control of liver protein synthesis in that disease. Apart from osteogenesis imperfecta (9) and pregnancy (10,11), elevated AHSG levels have not been described. It is important to remember that recently it has been demonstrated that AHSG was in fact the product of two different genes, which arose by tandem duplication (12). In this respect, it would be of interest to study whether the increase reported here is due to the expression of one or both gene products, which substantially differ from each other.

The difference between the levels of the AFP of healthy controls and chronic hepatitis C patients levels can be explained in to ways: (i) the altered immune status of the host induced by the acute viral infection and (ii) the direct immunosuppressive effect of the virus. The infection induces a T_h1 cytokine profile dominance (IL-1 and IFN-γ) that may depress the synthesis of the AFP. However, this does not seem to be a thorough explanation since positive AFP levels are increased in many diseases that are characterized by T_h1 dominance (e.g. rheumatoid arthritis or sarcoidosis).

There is increasing evidence that HCV has a direct immunosuppressive effect on the host. Peripheral blood mononuclear cells produce less IFN-α in the presence of

HCV (13). The NS3 antigen of the virus has been demonstrated to induce proliferation of T_h cells, but without production of IL-2 (14). The strong negative correlation between the Knodell histological index values and the OROSO levels also indicate that a decrease in OROSO levels in chronic hepatitis C is related to the pathological process occurring in the liver.

The paradoxical alteration of the AFP was reversed in patients who responded well to IFN-α treatment, whereas it persisted in NR. Changes may be explained by the direct immunomodulatory effect of IFN-α. The fact that NR were treated with the same doses of IFN-α, however, strongly argues against this possibility. The reversion of the immunosuppressive effect of HCV resulting in restoration of the regulation of the acute-phase reaction is another possibility. This is supported by the observation that there is a stronger cellular response and IFN production to recombinant HCV core antigens in R than in NR patients (15). It is widely accepted that a dominant T_h1 cytokine profile is required to eliminate HCV and a diminished T_h1, a dominantly T_h2 profile supports the persistence of the virus (16). In accordance with this, high levels of IL-10, a T_h2 cytokine with a depressive effect on T_h1 function, are associated with more aggressive hepatitis and a poor response to IFN-α therapy (13). On the other hand, recent data suggest that the NS5A viral protein may induce the synthesis of IL-8 (17) and a high IL-8 concentration is associated with a poor therapeutic response to IFN-α (18). The induction of IL-8, along with other factors, may be involved in the paradoxical alteration of the AFP concentrations. We plan to address this assumption with comparative measurements of IL-8 and AFP in patients with chronic hepatitis C before and during IFN-α therapy.

It is tempting to speculate that the alteration of the AFP levels we observed, regardless of its origin, could be used as predictors of outcome of antiviral therapy in patients with chronic C hepatitis. Obviously, further studies are needed to determine the clinical value of AFP levels in predicting successful antiviral therapy.

Acknowledgements

Authors thank to N. M. Vers for her skilful technical assistance. This work was supported by Schering-Plough AG (Budapest), and grants ETT 278/2003, FKFP 0138/2001 and ETT 248/2001. Z. P. is a Bolyai research fellow.

Abbreviations

AFP	acute-phase protein
AHSG	fetuin/α2HS-glycoprotein
C1-INH	C1-inhibitor
CRP	C-reactive protein
HCV	hepatitis C virus
NR	non-responder
OROSO	α_1-acid glycoprotein/orosomucoid
R	responder
TF	transferrin

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