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• BRIEF REPORTS •

Monocyte chemotactic protein-1 and soluble adhesion molecules as possible prognostic markers of the efficacy of antiviral treatment in chronic hepatitis C

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Abstract

AIM: To explain the role of Monocyte chemotactic protein-1 (MCP-1) and soluble adhesion molecules in chronic hepatitis C during the treatment of interferon alpha (IFNα) 2 b and ribavirin (RBV).

METHODS: Concentrations of MCP-1, soluble adhesion molecules intercellular adhesion molecule-1 (sICAM-1), sP-selectin, interleukin (IL) 6, and IL10 in serum were estimated in the group of 40 patients with chronic hepatitis C treated with IFNalpha2 b and RBV in 0, 16, 32, 48 wk of the therapy.

RESULTS: In chronic hepatitis C, before and during the treatment, the serum levels of MCP-1 and sP-selectin in responders were similar to those of healthy subjects. In non-responders (NR), MCP-1 increased in the course of IFNα+RBV treatment, differences were statistically significant as compared to responders. MCP-1 correlated statistically with the activity of periportal inflammation ($r = 0.35$, $P<0.05$) but not with staging of liver fibrosis. sICAM-1 positively correlated with inflammatory activity and fibrosis in NR. sP-selectin did not correlate with histological findings in the liver. The MCP-1 correlated with the soluble form of sP-selectin concentrations ($r = 6$, $P<0.001$) and with IL-10 level in NR ($r = 0.4$, $P<0.05$). There was no correlation observed between the concentration of MCP-1 and sICAM-1, IL-6 during the treatment.

CONCLUSION: MCP-1 concentration may be a prognostic marker of the efficacy of IFN+RBV therapy in patients with chronic hepatitis C.

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INTRODUCTION

Alteration of the immune system in chronic hepatitis C patients may underlie their inadequate response to antiviral therapy. The factors responsible for the persistence of HCV infection and low response to interferon alpha treatment are poorly understood. Circulating monocytes/macrophages are important for the host immune responses to HCV. HCV-RNA was detected in mononuclear cells of chronically infected patients[1]. It seems that monocyte functioning impairment is a decisive factor of chronic HCV infection and lack of positive therapeutic response. Monocytes might be a reservoir of the virus and the source of re-infection after IFN treatment[2]. In chronic hepatitis C, IFN-alpha mRNA was decreased in liver tissues and mononuclear cells significantly increased in peripheral blood. It might result in the inhibition of antiviral immune mechanisms in the liver and enable HCV infection persistence in monocytes[3]. Some responders revealed, with negative HCV-RNA in blood serum, the presence of RNA virus in peripheral blood monocytes.

Hepatitis C virus chronic infection is associated with functional impairment of peripheral blood mononuclear cells. The application of IFN alpha could lead to HCV-RNA reduction in monocytes[4]. Elimination of HCV in monocytes after 6-mo-treatment might be a coefficient of efficiency of the therapy[5].

Chronic liver diseases are associated with increased hepatic and monocytic expression of monocyte chemotactic protein 1 (MCP-1). MCP-1 level was higher in hepatic veins than in peripheral blood and occurred in severe cases of liver diseases[6]. Hepatic expression of MCP-1 is up-regulated during chronic HCV infection mainly in activated hepatic stellate cells (HSC). In chronic hepatitis C with advanced fibrosis and inflammation, hepatic MCP-1 mRNA levels were significantly higher[7]. Peripheral blood monocytes and activated HSC are the source of MCP-1. Monocyte chemotactic protein-1 recruits monocytes and lymphocytes to damaged area in the liver tissue. Profibrinogenic properties of MCP-1 could be reflected by the induction of HSC chemotaxis and its transformation to myofibroblasts[8].

Adhesion molecules are proteins expressed on a variety of cells, which mediate the interaction between endothelial cells with lymphocytes, monocytes and leukocytes[9,10]. Intracellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily participates in the immunological system, cell-to-cell communication and in inflammatory responses. Many of proinflammatory proteins (IL-1, TNFα, GM-CSF) generated by leukocytes, monocytes, macrophages could enhance the expression of adhesion molecules on cells and in soluble form in circulation[10]. Activated blood platelets which constitute inflammatory cells are the source of P-selectin[11]. Soluble ICAM-1 and sP-selectin, secreted from activated cells or expressed on the microparticles, transmit signals from inflammation sites to peripheral circulating monocytes, lymphocytes and others cells. In viral hepatitis, cell-mediated responses could lead to accumulation of activated immunocompetent cells into the hepatic parenchyma. This phenomenon could cause viral elimination and/or focal liver damage[12,13].

The aim of the study was to estimate the monocyte chemotactic protein-1 in correlation with adhesion molecules, such as sICAM-1, sP-selectin and with cytokine Th2 (IL-6, IL-10) in chronic hepatitis C during IFN alpha and ribavirin treatment. Correlation of the factors and histological staging and grading in liver tissue was also analyzed. The factor concentrations in responders (R) and non-responders (NR) were analyzed as well.

MATERIALS AND METHODS

Examinations were performed in 40 patients with chronic hepatitis C (16 women, 24 men), aged 36±12 years. Chronic hepatitis was confirmed by HCV infection persisting for longer than 6 mo (HCV-RNA positive) and increased ALT values. Blind liver biopsies were done by means of the Hepafix System (Braun, Melsungen, Germany) before the treatment. Histopathological inflammatory activity (grading, 0-4 scale) and fibrosis grade (staging, 0-4 scale) were evaluated in accordance with the classification of chronic viral hepatitis according to Scheuer classification[14]. Patients were divided into responders (R) and non-responders (NR) according to their sustained response to a course of interferon alpha 2 b (3 MU three times weekly for 48 wk, Rebetron, Schering-Plough Corporation, USA) with ribaviryn (1.2 g/d for 48 wk, Rebetron, Schering-Plough Corporation, USA) treatment.

The levels of monocyte chemotactic protein-1, soluble intracellular adhesion molecule-1, soluble form of P-selectin, and cytokines IL-6 and IL-10 were determined by ELISA method in blood serum in 0-16-32-48 wk of the therapy. Moreover, routine biochemical examinations concerning the grade of liver damage were performed. Ethical approval for research was obtained from local Ethics Committee in Medical University.

Statistical analysis

The results were presented as mean±SD. The statistical analysis was performed using Student's-*t* test for pairs, and the correlation by using parametric Spearman's test.

RESULTS

MCP-1 in chronic hepatitis C

MCP-1 concentrations before and during the treatment in non-responders were higher than those in responders and increased during the treatment (Table 1). MCP-1 concentrations in R were comparable to the values observed in the controls. The second half of the treatment showed the statistically significant increase in MCP-1 in R as compared to the control group. However, it diminished to normal values at the end of the treatment (statistically significant difference to NR, $P<0.05$). There was no correlation between MCP-1 and ALT level, prothrombin index, leukocyte and platelet count. There was a positive correlation between MCP-1 and periportal inflammatory activity in R (Table 2). However, no correlation was observed between MCP-1 and the grade of fibrosis and periportal inflammatory activity. A positive correlation was observed between MCP-1 and sP-selectin and IL-10 in NR in the second half of the therapy (examinations III and IV; $r = 0.6$, $P<0.001$, Spearman test). A correlation between MCP-1 level and ICAM concentration and IL-6 in chronic hepatitis C during treatment was not observed.

Table 2 Correlation of adhesion molecules, cytokines and MCP-1 levels with liver histopathology in responders (R) and non-responders (NR)

		Grading (inflammation)					
		Periportal		Intralobular		Staging (fibrosis)	
		R	NR	R	NR	R	NR
sICAM-1	*r*	*N*	0.36	0.38	0.56	*N*	0.32
	P	*N*	<0.05	<0.05	<0.01	*N*	<0.05
MCP-1	*r*	0.35	*N*	*N*	*N*	*N*	*N*
	P	<0.05	*N*	*N*	*N*	*N*	*N*
IL-6	*r*	*N*	*N*	0.53	*N*	*N*	0.38
	P	*N*	*N*	<0.01	*N*	*N*	<0.05
IL-10	*r*	*N*	*N*	*N*	*N*	*N*	*N*
	P	*N*	*N*	*N*	*N*	*N*	*N*
sP-selectin	*r*	*N*	*N*	*N*	*N*	*N*	*N*
	P	*N*	*N*	*N*	*N*	*N*	*N*

r -statistical correlation, Spearman test, $P<0.05$ statistical significant.

sICAM-1 and sP-selectin in chronic hepatitis C

sICAM-1 concentrations were significantly higher in R than in NR and the controls. During IFNalpha+RBV treatment, sICAM-1 level decreased in both groups. Responders revealed its decrease, which was statistically significant ($P<0.0001$) as compared to non-responders. However, there was a positive correlation between sICAM-1 concentrations and intralobular and periportal inflammatory activities and fibrosis in responders, which was not observed in non-responders. sICAM-1 and ALT levels, leukocytes, and platelet count showed a positive correlation.

Table 1 Levels of MCP-1, sICAM-1, sP-selectin, IL-6 and IL-10 in patients with chronic hepatitis C during IFN+RBV therapy in responders (R) and non-responders (NR)

		0-wk	16-wk	32-wk	48-wk	Control
sICAM-1 (pg/mL)	NR	434±104[ab]	372±111[ab]	404±87[b]	389±77[b]	230±29
	R	527±109[ab]	433±87[ab]	353±98[b]	363±99	
sP-selectin (pg/mL)	NR	240±101[ab]	137±47	145±33	147±41	144±75
	R	144±57[a]	131±68	131±58	23±47	
MCP-1 (pg/mL)	NR	290±91	365±124[ab]	315±61	325±50[a]	268±109
	R	281±84	285±55[a]	335±32[b]	286±59[a]	
IL10 (pg/mL)	NR	0.45±0.46[a]	0.81±0.54	0.77±0.68	0.9±0.63	0.62±0.83
	R	0.88±0.78[a]	0.89±0.74	0.69±0.73	1.01±0.77	
IL6 (pg/mL)	NR	0.65±0.61[ab]	2.21±0.94[ab]	1.07±1.68[b]	1.56±1.32[b]	0.22±0.49
	R	0.16±0.37[ab]	0.76±0.69[ab]	0.77±0.55[b]	1.05±1.21[b]	
ALT(IU/mL)	NR	82±40	26±17	23±10	61±91	32±6
	R	60±30	21±6	24±6	22±6	
Prothrombin index (%)	NR	96±23	97±6	98±11	99±10	98±6
	R	5±7	90±10	83±13	90±7	
Blood platelet (G/L)	NR	196±21	189±45	190±52	179±51	203±45
	R	196±21	189±45	190±52	17±51	

a-statistically significant differences between responders (R) and non-reponders (NR), Student's *t* test, [a]$P<0.05$, *b*-statistically significant differences compared to healthy, Student's *t* test, [b]$P<0.01$.

The concentrations of sP-selectin were significantly higher in non-responders than in responders. During the treatment, sP-selectin concentrations decreased in both groups. Its concentrations in R were comparable to the control group and there was no correlation between histological changes in the liver and sP-selectin concentrations.

Serum Th2 interleukins (IL-6, IL-10) in chronic hepatitis C

The concentrations of IL-6 before and during the treatment were lower in R than in NR. Its levels in R were 8 times higher during the treatment while IL6 concentrations were significantly higher in NR than in R ($P<0.02$) and during the treatment it increased twice. There was a positive statistical correlation between IL-6 concentrations and intralobular inflammatory activity in R, and fibrosis in NR. IL-6 level, leukocyte count, and prothrombin index showed a positive correlation.

IL-10 concentrations before the treatment were statistically higher in R than in NR ($P<0.05$). Although IL-10 values increased during the therapy, there was a temporary decrease in IL-10 concentrations both in R and in NR during the second half of the treatment. There was no statistical correlation between IL-10 concentrations and histological changes in the liver.

DISCUSSION

The results confirmed that MCP-1 was a chemokine reflecting the inflammatory activity in the liver and might be a prognostic factor of the efficacy of the treatment with IFNalpha and RBV. Patients with a persisting long-lasting response to the treatment revealed MCP-1 concentrations statistically lower and during the treatment it did not undergo significant changes. Patients with negative effect of the therapy showed higher MCP-1 levels and increased significantly during IFN+RBV treatment.

Monocyte chemotactic protein-1 is a potent chemokine secreted by monocytes and activated hepatic stellate cells undergoing up-regulation during chronic HCV infection. In normal liver, MCP-1 protein and gene expression detected by immunohistochemistry and *in situ* hybridization showed a modest expression in peri-sinusoidal cells and in bile duct epithelial cells[15]. In chronic hepatitis, MCP-1 expression was directly correlated with the degree of inflammatory infiltrate in the portal tract, activated stellate cells and monocyte/macrophages. In active cirrhosis, MCP-1 expression was present in the portal tract, epithelial cells of regenerating bile ducts, and the active septa surrounding regenerating nodules[15]. There was a direct relationship between MCP-1 expression and monocyte infiltration after acute liver injury. Monocytes stimulated by lipopolysaccharide, T lymphocytes and by IL-2 become mature macrophages and place themselves in the liver tissue. Antioxidants significantly reduced MCP-1 expression and the number of infiltrating monocytes in toxic liver injury[16].

Hepatic tissues are damaged by activated monocytes, which secrete MCP-1, a chemoattractant and activator for circulating monocytes and T lymphocytes. Soo *et al*. in experiment on cultured monocytes infected with HCV showed that secreted proteins could influence the progress and outcome of liver injury[17]. It was shown that HCV NS5A proteins could lead to monocyte activation with MCP-1 secretion[17].

We did not observe any dependence between MCP-1 and Th2 lymphocyte stimulation during the treatment. IL-6 and IL-10 concentrations were higher in chronic hepatitis C than in healthy subjects and their levels increased in the course of IFN+RBV treatment. We showed significant differences in IL-6 and IL-10 concentrations between R and NR before the therapy. Masaki *et al*. noted that non-responders revealed a significantly higher Th1 and Th1/Th2 ratio than responders[18]. The prognostic factors for favorable long-term virological responses were non-1b genotype, low HCV viremia (HCV viral load less than 500 kilocopies/mL), and low Th1/Th2 ratio[18]. In hepatitis C, the predominance of Th2 cytokine IL-10 was observed. Monocytes secreting cytokines could enable cellular immune responses to activate, which decided the outcome of HCV infection[19]. IL-10 and IL-12 secreted by monocytes were higher in asymptomatic HCV carriers than in chronic hepatitis C ones. After IFN+RBV therapy was completed, increased IL-12 was noted in responders and decreased in non-responders[1]. It seemed to confirm that damage of monocyte function could decide chronic HCV infection and lack of positive therapeutic response[1]. The use of IFN alpha had no influence on the production of IFNgamma and IL-10 by monocytes[4].

We presented a significant correlation of MCP-1 in chronic hepatitis C and soluble P-selectin concentration. The main sources of sP-selectin are activated blood platelets. Our previous studies showed that there was a positive correlation between platelet activation and a degree of histological change intensity in the liver[20]. We also noted the inhibition of activated blood platelets by interferon alpha 2 b in chronic hepatitis C (data in press). It may reflect the cooperation of monocytes and platelets in the process of inflammation and fibrosis in chronic hepatitis C. Soluble P-selectin level was significantly lower in responders than in non-responders and its concentration was sustained lower during IFNα+RBV therapy. However, we did not observe any correlation between sP-selectin with histological changes in liver. These results suggest lack of priority of sP-selectin in efficacy of antiviral therapy. It reflects the intensity of inflammation and mobilization of blood platelets in chronic hepatitis C. Activated platelets have been found to play a crucial role in HSC transformation to myofibroblasts and in liver fibrosis[21].

Activated monocytes could also lead to endothelial cell damage[22]. Our studies did not show any correlation between activation of endothelial cells and monocytes. On the other hand, sICAM-1 concentrations correlated positively with periportal and intralobular inflammatory activity as well as staging of liver fibrosis, mainly in non-responders. Decreased sICAM-1 level during the treatment was common in all patients with chronic hepatitis C especially in responders. Our observations were similar to those of researchers who suggested that sICAM-1 might be used as a prognostic marker of efficacy of antiviral therapy in chronic hepatitis C[10,23,24]. Decreased sICAM-1 level could reflect diminished inflammatory processes in the liver due to HCV elimination.

Studies conducted so far have ascribed an important role to monocytes in chronic hepatitis C pathogenesis. However, ways for effective elimination of HCV infection are still to be discovered. Monocytes are the cells that are damaged by HCV and the non-specific immunologic barrier which prevents or removes the infection. Thus, the question why most HCV infected patients were not able to eliminate HCV by their immune system is still to be answered.

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