PRO- AND ANTI- INFLAMMATORY CYTOKINES IN PATIENTS WITH CHRONIC VIRAL HEPATITIS ON THERAPY WITH BIOR

Citochinele pro- și antiinflamatoare la pacienți cu hepatitele cronice virale la tratament cu BioR.

Adela Turcanu, Vlada Dumbrava, Lucia Andries, V. Rudic

State Medicine and Pharmaceutics University "Nicolae Testemitanu", Chisinau, Republic of Moldova

Summary

Introduction: T-cell immunoregulatory cytokines influence the persistence of hepatitis C and B virus (HCV, HBV) chronic infection and the extent of liver damage. Th1 cytokines positively correlate with hepatic inflammation in HBV and HCV chronic infection. The pro-inflammatory cytokines are involved in viral clearance and in metabolic and viral hepatic diseases, respectively. **The aim** of this study is to evaluate the biochemical, haematological parameters and profile of Th1/Th2 cytokines in HCV and HBV hepatitis before and after treatment with BioR. **Methods:** The study included 42 patients with chronic viral hepatitis. Following the aim the establishment of the viral hepatitis aetiology it was decided to define the markers of the viral hepatitis, the antibodies antiHCV total, antiHCV IgM, HbsAg, antiHBs, antiHBcor total and IgM, as well as to identify the alcohol consumption through use of different questionnaires. Along with the evaluation of the liver status also the abdominal ecography and the hepatic gamma-scintigraphy or scanning of the liver has been performed. The immunoenzymatic assay has been used to study the interleukins 1, 10 and TNF-alpha. Patients included in the study have been administered the injection solution of BioR, 1.0 ml intramuscular, daily, during 10 days. **Results**: Our study has indicated that BioR causes a number of haematological actions in case of patients with HCV. Thus, patients with HCV show a real amelioration of haemoglobin ($p<0.05$), and of lymphocyte levels ($p<0.05$) compared to patients with HBV. In result of the application of a therapy with BioR an evident diminution of cytolysis (ALT, AST) takes place both in patients with HBV ($p<0.05$) and in the ones with HCV ($p<0.05$). This indicates the fact that BioR plays the role of a hepatic protector. It has been also proven that BioR reduces gamma GTP in patients with HCV ($p<0.05$) compared to patients with HBV ($p>0.05$), indicating to the occurrence of a disintoxication. This study has shown that a treatment with BioR brings about the stimulation of production of IL 10, TNF alpha in patients with HCV and HBV. We consider this action a key mechanism of this preparate with antiviral effect, and we recommend it for treatment of persons with viral hepatitis. **Conclusions:** This study indicates that the use of the BioR preparate in chronic viral B and C hepatitis is justified, given the fact that it acts as a hepatic protector, causes haematological regulation and has the disintoxication and immune-modulation effects.

Actuality. Imbalance between pro-inflammatory and anti-inflammatory cytokines influences the immunopathogenesis of viral B and C hepatitis. A number of studies indicate that the plasmatic concentration of IL 1 varies in case of viral hepatitis it getting involved into the viral clearance. For example, in the study of Haizhen Zhu and Chen Liu (Florida, 2003) the IL-1 is shown to have a role in the effective inhibition of VHC through the inhibition of the VHC subgenomic replication and reduction of the viral protein expression. These authors suggest the idea that IL 1 has a direct antiviral effect. IL -1 has a similar effect to IL -4 in case of acceleration of the B -lymphocytes proliferation and production of antibodies. It induces production of proteins by hepatocytes in an acute inflammatory phase and influences SNC, favouring somnolence and anorexia. It increases creation of E2 prostaglandin and A2 phospholipase, which might lead to fever development. It contributes to the increase of other cytokines production, such as IL-6, IL-8, gamma-IFN and TNF. The suppressor factor IL10 is produced mainly by the T- lymphocyte helper type 2. In causes inhibition of the T-helper type 1 function and the monocytes, reducing production of immunocytokines (gamma IFN, IL-1, IL-8, TNF). It increases proliferation of B-lymphocytes and tissue basophiles. Thus, IL- 10 is one of the most important cytokines with a regulatory function, the action of which causes inhibition of the T-lymphocytary type 1 cellular response and stimulates the humoral response (T- lymphocytes type 2). It is an anti-inflammatory cytokine. The Th1\Th2 disbalance plays an active role in the immunopathogenesis of chronic hepatitis. Studies carried out in this area indicate that the response of cytotoxic T lymphocytes is rather heterogenic in patients with chronic hepatitis. In about 30 - 46% cases of chronic viral hepatitis the obtained response was evident. The response of cytotoxic T lymphocytes varies from person to person and is limited by the HLA spectrum, thus determining the viral epitopes presented in the immune system.

TNF – alpha is produced by different cells: monocytes, macrophages, B- and T-lymphocyte. This is a pro inflammatory cytokine. It is characterised by rather varied effects, which depend, to a great extent, on its concentration. In small concentrations the TNF-alpha increases synthesis of adhesion molecules on the endothelial cells, thus allowing the neutrophils to adhere to the blood vessels at sites of inflammation.

Among other effects are the activation of the respiratory system in neutrophils, stimulation of potential killer cells via phagocytosis, increase of lymphokines caused by

T-lymphocytes and stimulation of maturation of B-lymphocytes. In high concentrations TNF-alpha acts as a mediator able to favour the appearance of the septic juice induced by endotoxins. In high concentrations the TNF-alpha is also called cachexia, given its property to inhibit the lipoprotein lipase of the adipose tissue and consequently to decrease the use of fat acids, thing that can induce development of cachexia. The TNF-alpha favours cell necrosis of certain tumours as a result of development of intravascular thrombosis in the tumour tissue region. It also stimulates the production of IL1 and IL-6 and the expression of the class 1 MCH molecules.

We decided to study the level of these cytokines in patients with HCVC and HCVB and also to assess the dynamics of their concentration following the treatment with BioR. This is a preparate already known in our country for its hepatic protection, anti oxidant and immunomodulation effects.

Goal of the study: investigation of the haematological, biochemical parameters and of the cytokine profile of patients suffering from chronic hepatic diseases, before and after the treatment with BioR, and of the cytokine profile of patients suffering from chronic hepatic diseases.

Objectives of the study:

1. assessment of the haematological indices of patients suffering from chronic hepatic diseases, before and after the treatment with BioR;
2. determination of the biochemical parameters (cytolysis, cholestasis and hepatopriv syndromes) in patients suffering from chronic hepatic diseases, before and after the treatment with BioR;
3. evaluation of the interleukin pro- (IL-1) and anti- (IL-10) inflammatory profiles and of the TNF alpha values of patients suffering from chronic hepatic diseases, before and after the treatment with BioR;

Materials and methods. The study included 42 de patients with chronic viral hepatitis. Following the aim the establishment of the viral hepatitis aetiology the markers of the viral hepatitis, the anti HCV antibodies, HBsAg, anti HBs, anti HBcor total were defined, and the alcohol consumption through use of different questionnaires was identified. Apart from the evaluation of the liver status, the abdominal ecography and the hepatic gamma-scintigraphy or scanning of the liver with Th has been also performed. In order to highlight the accompanying pathology and the complications of the hepatic disease, the esophagogastroscopy, irigoscopie, duodenal evaluation with insemination of the bile investigations have been additionally carried out. The immunoenzymatic assay has been used to study the interleukins 1, 10 and TNF-Alpha.

Patients included in the study have been administered (at the recommendation of the expert) the treatment with BioR in its injection solution, 1.0 ml, intramuscular, daily, during 10 days. BioR is a hepatoprotective agent, obtained via original targeted synthesis technologies of successive extraction from the cyanophilous algae biomass – Spirulina platensis (Nordst) Geitl. BioR contains a large range of free amino acids, saturated and unsaturated fatty acids, polyzaharidies, all liposoluble and hydrosoluble vitamins and microelements.

Obtained results. The clinical and paraclinical investigations have indicated that 20 patients were VHB infected and 22 patients were VHC infected. The average age of patients was 37 ± 3.2 years old and women prevailed as gender (58%). Investigated patients originate from different settlements of the Republic of Moldova where 71.4 % (30) patients come from the rural area and 28.9% (12) – from the urban area. Analysis of the length of the malady showed that 76.1% (32) patients suffer of this disease more than 5 years. The anamnestic investigation made possible the elucidation of a number of aspects dealing with the origin of this affection. Thus, 23.8% (10) of patients have mentioned the fact that they suffered an acute hepatitis, 23.8% (10) of patients had haemotransfusions, 21.4% (9) supported surgery interventions and 11.9% (5) had tattoo or piercing. Depending on the infection phase, patients with the chronic viral B hepatitis have been placed into two categories: 57.8 (11) patients were in the replication phase and 42.2% (8) were patients in the integrative phase of the infection. 81.3% (18) patients with chronic C viral hepatitis fall into the reactivation category, while 18.7% (4) fall into the latent phase. Depending on the degree of activation of the pathologic hepatic process it was stated that 61.8% (26) patients had minimal activation.

Evaluation of the clinical picture has indicated the presence of a diversity of symptoms. Nevertheless, forms with short clinic predominated. Table 1 shows basic symptoms of investigated patients.

Table 1. Characteristics of the patient clinic with chronic hepatitis included in the study

	HBV (n=20)	HCV (n=22)
dolor symptom	**13 (68.4%)**	**12 (54.5%)**
astenic symptom	**15 (78.9%)**	**18 (81.8%)**
dyspeptic symptom	**11 (57.8%)**	**17 (77.2%)**
icteric symptom	**3 (15.7%)**	**4 (18.1%)**
arthralgia	**8 (42.10%)**	**11 (50%)**
cutaneous pruritus	**4 (21.05%)**	**4 (18.1%)**
subfebrility	**3 (15.7%)**	**4 (18.1%)**
hepatomegaly	**11 (57.8%)**	**16 (72.7%)**
splenomegaly	**6 (31.5%)**	**9 (40.9%)**

The investigated patients (n=42) have been administered BioR, 1.0 ml intramuscular, daily, during 10 days. Out of them, 10 days later, 32 patients have been subjected to a repeated control. In case of patients consulted for the second time the evolution of the clinic showed a decrease of the intensity of the asthenoneurotic and dyspeptic syndromes and a reduction of the cutaneous pruritus. In spite of the fact that there was no evident improvement of the clinical symptomatology, the reason being the short treat-

ment period, nevertheless a trend of improvement has been observed.

No substantial deviations of the haematological parameters from the accepted limits have been identified in case of patients with HBV (16), while in case of patients with HCV (16) an evident anaemic symptom has been recorded. Persons with chronic C hepatitis have shown a moderate lymphopenia versus admitted limits and versus patients with HBV. Administration of BioR implies certain modifications of the evolution of these indicators. Thus, there is practically no modification of the investigated haematological parameters in patients with HBV, compared to a significant amelioration of both Hb ($p<0.05$) and lymphocytes ($p<0.05$) in persons with HCV. Consequently, agent BioR acts as an evident haematological modulator.

The biochemical analysis carried out within this study has shown the presence of cytolysis syndrome of different degrees in both patients with HBV and with HCV. 25% of patients have registered a moderate cytolysis, out of which 62.5% are patients with HCV. Nevertheless, 31.2% of patients with chronic hepatitis tolerate the malady with normal transaminases.

Initially, ALT (mmol/l) in HCVC constituted 90.26±6.17 and AST 69.6±6.44 (mmol/l), compared to 66.4±4.33 and AST 54.5±4.22 after the BioR therapy, while in HVB before the therapy ALT 82.6±11.11 and AST 63.2±3.46 have been registered, and after the therapy – ALT 59.3±4.61 and AST 52.22±2.9, this indicating the fact that the agent has been included into the hepatocytary recovery processes.

Statistically veridical results have been obtained after the administration of the BioR agent, these including the evident reduction of cytolysis (ALT, AST) both in patients with HBV ($p<0.05$) and in the ones with HCV ($p<0.05$). Nevertheless, the value of these transaminases continues to remain high compared to the value registered in case of healthy persons, thing suggesting that the duration of the treatment might be too short.

It is interesting to mention that the investigation of the cholestatic syndrome has revealed the presence of a high level of gGTP in patients with HCV (2.5 times bigger than the normal level, in the average) versus patients with HBV (1.2 times more). Under the influence of the BioR therapy the level of gGTP has suffered a veridical decrease in patients with HCV ($p<0.05$) versus patients with HBV ($p>0.05$). Also the level of the cholesterol has undergone a veridical statistic regression in both groups of patients.

Trustworthy data have also been obtained with respect to the dynamics of the hepatopriv syndrome in patients with chronic B and C viral hepatitis subjected to a therapy with BioR. Thus, treatment with BioR has caused a veridical increase of albumin in both patients with HBV ($p<0.05$) and in patients with HCV ($p<0.05$).

The interrelation between the IL1 pro- inflammatory cytokines with the IL10 anti- inflammatory cytokines has also been investigated in this study. We think that the cytokine disbalance has a key role in the progress of the chronic hepatitis. Assessment of their concentration in the blood of patients with chronic hepatitis indirectly offers information about the functional activity of different types of immunocompetent cells, severity of the inflammation process and the latter's transfer to a systemic level process. It also constitutes a forecast of the affection.

Table 2. Levels of IL1, IL10, TNF alpha in patients with HBV, HCV before and after the BioR therapy

	M		m		p
	initial	after therapy	Initial	After therapy	
IL1	0.53	0.57	0.128	0.139	p>0.05, t=0.2116
IL10	7.23	9.03	1.008	1.29	p>0.05, *t=1.0994*
TNF alpha	11.08	14.72	0.64	1.29	p<0.05, t=2.5277

The following events have been observed in this study: a significantly reduced level of IL-1 in patients with HCVB, versus patients with HCVC, in conditions of a smaller IL-10 concentration in HCVC, versus HCVB. This phenomenon suggests the idea that in case of HCVB the ante- inflammatory processes predominate, while in HCVC predominates the pro- inflammatory process. Following the completion of the recommended therapeutic scheme, levels of interleukins have been investigated, which has highlighted an increase of IL-1, non veridical in the two groups, and a veridical increase of IL-10 in both HCVB and HCVC.

This result invokes the idea that BioR contributes to the regression of the inflammatory process, stimulating the concentration of the ante- inflammatory cytokines, in our case of IL-10.

Assessment of the TNF alpha level in patients included in our study has indicated that the initial level of this cytokine falls in the same limits with the ones in patients with HCVB and HCVC. Following the administration of the BioR therapeutic scheme, a similar increase of the TNF alpha concentration has been identified in the two groups of patients.

Discussions: Studies in this area indicate the occurrence of a reduction of the T helpers' concentration in blood in case of C hepatitis, followed by a decrease of IL-1 and IL-2 levels. IL-1 is shown to have an important function in the regulation of the hepatic mechanism and it can also be responsible for the modifications of the protein synthesis, especially synthesis of albumin. Also, IL-1 contributes to an increased synthesis of alpha 2-macroglobuline, an important factor in the evolution of the hepatic fibrosis. In viral hepatitis the Kupfer cells get involved into the IL-1 synthesis and secretion, via which they influence the hepatic regeneration. Along with the increase of the serum viral load, also an increase of IL-10 is registered in persons with VHC, it acting as an important inhibitor of IFN alpha (consequently decreasing the IFN alpha level, thing also identified in our study).

Recent investigations demonstrate that AgHBcor and AgHBe stimulate the secretion of IL 10 by T cells and monocytes. Excessive production of IL-10 can modulate the antiviral response in HBV infection, contributing to the establishment of a protection against severe injuries and viral persistence. At the same time the HbeAg also represents a promoter of the IL-1 secretion by stimulating the IL-1RacP (receptor accessory for the IL-1 protein).

It is necessary to mention that multiple hepatological studies highlight the role of IL-10 in the suppression of the hepatic fibrosis, diminishing, in such a way, the activity of macrophages in their capacity of antigen-presenting and cytokine-producing cell. An opinion has been put forward, according to which the malady length is smaller in patients with HCVC and HCVB, in case of which increased levels of IL-1 and IFN gamma, and reduced levels of IL-10 have been registered. Under the influence of VHC and VHB, along with the process development in the hepatic tissue, the IL-1 level undergoes a gradual decrease and immunodeficiency arises, while the IFN gamma and IL-10 show an increase. Investigation of the level of cytokines in patients included in our study has also included assessment of the TNF alpha, which is a trigger of the antiviral response development in B and C viral hepatitis and which correlates with the inflammation progress at the serum level of the TNF alpha. Assessment of the TNF alpha in patients included in our study has indicated that the initial level of this cytokine falls into the same limits with the ones registered in patients with HCV and HBV. The in vivo and in vitro investigations denote that TNF alpha inhibits the VHB replication via the acceleration of the VHB mRNA degradation mechanism. Additionally, AgHBcor is sensible to TNF alpha, IFN gamma, IFN alpha. Abbas Z et al. in their study in 2005 specify that in chronic C hepatitis high levels of cytokines are registered: IL-2, TNF alpha, IFN alpha. In his turn Mamaev C.N. (Russia, 2006) indicates to a high concentration of TNF alpha and IL-4 in patients with chronic hepatitis, compared to persons from the control group. Also in this study a veridical correlation between TNF alpha and IL-4 with ALT, AST and with the hepatic morphological characteristics (histologic activity index) has been stated. In his research Tokmalev A.K. (Russia, 2006) indicates high levels of TNF and IL2 in patients with chronic C hepatitis (91 persons), which diminish after an antiviral therapy with interferon.

In our case, a similar increase of TNF alpha concentration took place in the two groups of patients following the administration of the therapeutic therapy with BioR.

Consequently, stimulation of the IL-10 TNF alpha production possibly represents one of the key mechanisms of the antiviral activity of BioR. This gives us grounds to recommend it to persons with viral hepatitis. According to our data BioR gets involved into the immune mechanisms at the basis of the B and C chronic hepatitis evolution and causes a modulation of pro- and ante- inflammatory cytokines, regulating in such a way their secretion by the Th1 and Th2 lymphocytes, monocytes.

Conclusions:

1. efficiency of the BioR preparate is proved by the amelioration of the haematological picture (increase of Hb, lymphocytes) in patients with chronic C hepatitis;
2. the BioR preparate is recommended for the therapeutic administration to patients with viral chronic B and C hepatitis, characterised both by increased and by normal transaminases;
3. amelioration of the hepatic synthesis, expressed by an increase of the albumin level, speaks in favour of use of the BioR preparate in the HBV and HCV treatment;
4. a visible diminution of the cholestasis syndrome takes place in patients with HBV and HCV that have been subjected to the therapy with the BioR preparate;
5. increase of the level of pro-inflammatory cytokines (IL-1, TNF alpha) in patients with HBV and HCV that have been subjected to the therapy with the BioR preparate, indicates to an immunoregulatory activity of this preparate;
6. there is an increase of the anti- inflammatory cytokines (IL-10) under the action of the BioR preparate in patients with HBV and HCV, fact which indicated to the immunomodulation effect of the antiviral response;

References

1. Abbas Z, Moatter T, Hussainy A, Jafri W: Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis c genotype 3. World J Gastroenterol 2005; 11(42): 6656- 6661.
2. Tilg H, Kaser A, Moschen AR: How to modulate inflammatory cytokines in liver disease. Liver Int. 2006; 26(9):1029-39.
3. Kusumoto K, Uto H, Hayashi K, et al. Interleukin-10 or tumour necrosis factor-alpha polymorphisms and the natural course of hepatitis C virus infection in a hyperendemic area of Japan. Cytokine 2006; 34(1-2):24-31.
4. Hyodo N, Tajimi M, Ugajin T *et al.* Frequencies of interferon-g and interleukin-10 secreting cells in peripheral blood mononuclear cells and liver infiltrating lymphocytes in chronic hepatitis B virus infection. Hepatol Res 2003; **27**:109–16.
5. Nelson DR, Lauwers GY, Lau JY, et al. Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon non responders. Gastroenterology. 2000; 118:655-660.
6. Haizhen Zhu and Chen Liu Interleukin-1 Inhibits Hepatitis C Virus Subgenomic RNA Replication by Activation of Extracellular Regulated Kinase Pathway Journal of Virology, May 2003, Vol. 77, No. 9, p. 5493-5498