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Research paper

Endogenous antimicrobial peptides as markers of immune status

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Abstract

The aim of this work was to study the role of endogenous peptides in toxification processes, as well as their relationship with the immune system indicators in the development of chronic viral hepatitis C. For this purpose, endotoxins and lipopolysaccharide-binding protein (LSP) were determined by enzyme-linked immunosorbent assay (ELISA). Moreover, lymphocyte subpopulation estimated by an indirect immunofluorescent reaction (NIFR) and whereas reaction of rat monoclonal antibodies to lymphocytic antigens was determined. In connection with the data obtained, we consider it expedient to use the endotoxin determination method as an additional marker of the severity of liver damage in patients with chronic viral hepatitis C. It seems also possible to identify endotoxins, defensin and lipopolysaccharide-binding protein as markers of viral and bacterial infections.

Keywords: defensins, endogenous antimicrobial peptides, ALT, AsAT, non-specific immunity, lymphocytes, circulating immune complexes

Introduction

Worldwide more than 500 million people suffer from viral hepatitis C. The disease is characterized by progressive liver damage, increase of fibrosis processes and usually ends with cirrhosis. The structure of the hepatitis C virus is determined, which allowed to shed light on the mechanisms of damage to the liver, as well as its malignant transformation. However, it should be noted that many other aspects of the interaction of hepatocytes and the HVC virus remain unexplored [1]. Thus, aspects concerning the body's immune defense during the development and chronic hepatitis C remain still unstudied [2]. Endogenous antimicrobial peptides are non-specific factors of the body's immune defense, help to neutralize endotoxins, as well as stimulate immune-protective mechanisms. At the same time, they protect the body from a wide range of gram-positive and gram-negative microorganisms, fungi and viruses [3]. Taking into account that in the pathogenesis of hepatitis caused by HVC, the leading role belongs to endotoxins of gram-negative bacteria [4],

one can easily get how important is to perform set by this work task to study the role and interaction of endotoxemia markers (defensin, endotoxin, lipopoly-saccharide-binding protein) in the development of chronic hepatitis caused by HVC.

Material and methods

In order to achieve the aim, the blood of 87 patients aged 17-38 was examined. All patients divided into two groups. The first group of patients consisted of 45 people in whose blood chronic hepatitis C virus detected, the second group consisted of patients in whose blood the HVC was complicated by a bacterial infection (pneumonia). The control group consisted of 20 healthy people. The diagnosis of hepatitis C made using the classification proposed by the World Congress of Gastro-Enterologists (Los Angeles, 1994). As the biochemical markers determined by the Yendrashík method, bound and free bilirubin, determined by the Reitman-Frenkel method AlAT and AcAT, as well as the total protein concentration and determined by the German “Diasys” set gamma-glutamyl transpeptidase were used.

Determination of endotoxins and lipopolysaccharide-binding protein (LSP) performed by enzyme-linked immunosorbent assay (ELISA) using a set of "HyCult Biotechnology" (Holland) based on the solid-phase "sandwich" principle.

In order to study the cellular and humoral aspects of the immunity, the composition of the lymphocyte subpopulation by an indirect immunofluorescent reaction (NIFR) was determined. For this purpose, the reaction of rat monoclonal antibodies to lymphocytic antigens CD3+, CD4+, CD8+, CD14+, CD16+, CD25 was determined. The determination of circulating immune complexes (CIC) performed using a precipitation with a 3.5% solution of polyethylene glycol method.

Statistical processing of the obtained results carried out by determining the indicator -U of Wilkinson (Manna-Uitni). $P < 0.05$ was taken as reliable.

Results and discussion

All the data obtained, as well as the data on the second group represented in Table 1. As one can get from the table, the main factors affecting the liver, namely the total blood bilirubin and its fraction, AlAT, AsAT are increased. Moreover, these data correlated with the severity of hepatitis and its clinical manifestations. As can be seen from the table, in the first group the activity of AlAT

and AsAT is still within the normal range, whereas in the second group this indicator has already been increased, and with reliability P <0,01.

Indicators AlAt and AsAT, as well as gamma-glutamyl transpeptidase were used as typical indicators of the severity of the disease, and a noticeable increase in them as an unfavorable prognosis of the disease development. As is known, the AST indicator characterizes the degree of mitochondrial damage, and complication of disease up to the necrosis of hepatocytes. Note that in the second group, the activity of gamma-glutamyl transpeptidase increased by 1.9 times, which indicates the activation of the process of damage to hepatocytes. Gamma-glutamyl transpeptidase is a membrane enzyme of hepatocytes, so that its increase means damage to the cytoplasmic membrane of liver cells with subsequent release of cytoplasm content, namely, AST into the circulating blood. When the liver is damaged, the accession of a bacterial infection constitutes the most widespread type of complication of the course of the disease [5].

The appearance of the most noticeable changes in the second group confirms the fact of additional damage of the liver by the attached infection. Moreover, as indicated in table 2, in comparison with the control group, noticeable changes of the indices of the immune system were observed in I and II groups.

Table 1: Biochemical indicators of the control group and patients with chronic hepatitis

Parameters	Control group (n=20)	I group (n=45)	II group (n=42)
Total bilirubin, mkmol/l	16.3± 0.7	18.3± 0.7	17.2± 0.4
Direct bilirubin, mkmol/l	3.94 ±0.15	4.92 ±0.25*	4.52 ±0.36
Free bilirubin, mkmol/l	12.74 ±0.62	15.92 ±0.68**	14.91 ±0.76
AlAT, IU/l	5.1 ±0.3	11.6 ±0.12***	71.6 ±1.07***
AsAT, IU/l	4.1 ±0.2	4.7 ±1.03	67.4 ±1.08***
γ -glutamyltransferase, İU/l	15.24 ±1.16	17.24 ±0.83	28.61 ±1.07***

Note: *p <0.05;- p <0.01;***-p < 0.001 statistically significant difference compared to control group**

Table 2: Immunological parameters of the control group and patients with chronic hepatitis

Parameters	Control group (n=20)	I group (n=45)	II group (n=42)
Cd3, %	62.4 ± 1.52	58.07 ± 1.36	59.71 ± 1.41
Cd4, %	32.10 ± 1.38	26.65 ± 1.49*	27.71 ± 0.92**
Cd8, %	29.75 ± 7.10	28.34 ± 1.59	27.39 ± 1.62
Cd14, %	16.05 ± 0.62	17.06 ± 0.54	18.16 ± 0.54*
Cd16, %	13.25 ± 1.08	12.85 ± 0.73	12.26 ± 0.72
Cd25, %	20.3 ± 1.4	10.67 ± 1.08***	7.64 ± 0.89***
Cd4 / Cd8, %	1.09 ± 0.03	0.86 ± 0.02***	0.82 ± 0.02***
B-lymphocyte, %	33.35 ± 2.74	19.84 ± 1.70***	29.52 ± 1.4
T-lymphocyte, %	52,42 ± 1.93	61.54 ± 1.83**	57.6 ± 1.82
DİK, İU	103.40 ± 7.00	130.40 ± 8.68	169.63 ± 9.15***

Note: *p <0.05;- p <0.01;***-p < 0.001 statistically significant difference compared to control group**

The deficiency of the T-lymphocytic protection system determined by a significant increase in the patient's blood indicators CD3+, CD4+, as well as ratio CD4+/CD8+ and decline of CD8+. Thus, a decline in indicators CD4+ and CD8+ and ratio CD4+ /CD8+ indicates a deficiency in the immune system , namely the insufficiency in the helper suppressor immunity support [6].

In both groups of patients, a decrease in the number of B-lymphocytes also noted. A parallel decrease in CD16+ and CD25+ with a simultaneous increase in the number of T-lymphocytes indicates an imbalance in a specific cell-mediated immunity. According to the majority of scientists, the decisive factor in the development of the disease is a violation of the T-cell component of the immune defense [7]. Thus, it is known that the main function of B-lymphocytes is to enter into antigen-antibody type reactions, the production of corresponding antibodies and their subsequent transformation into cell plasma. However, the implementation of the above processes requires the presence of a CD4+ cell component. We have already noted that in the examined patients there is a marked decrease in the level of CD4+. Thus, it becomes clear that in patients with HVC specific antibodies, even being produced, are not able to bind the required amount of virus, because CD4+ in these patients is reduced [8].

Table 3: Levels of antimicrobial peptides of the control group and patients with chronic hepatitis.

Parameters	Control group (n=20)	I group (n=45)	II group (n=42)
Defensin, nq/ml	38.6 ± 3.8	297.0 ± 17.5***	1467.3 ± 27.7***
Endotoxin, İU/ml	0.10 ± 0.01	24.4 ± 2.3***	57.7 ± 5.2***
Lipopolysaccharide-binding protein, mg|ml	23.6 ± 4.2	138.7 ± 8.9***	443.6 ± 29.6***

Note:* - p < 0.001 statistically significant difference compared to control group**

Determination of the level of the CEC allowed to establish their increase in both groups of patients, especially in the second group (control = 103.40 ± 7.00 IU, while in group I this indicator was 130.40 ± 8.68 IU, in II 169.63 ± 9.15 IU). It is likely that an increase in CIC indicates an increase in the absorption of antigens by specific antibodies. This may be an indicator of blocking immunoglobulin-synthesizing receptors of lymphatic cells and complement, and therefore the persistence of the virus, which in turn leads to prolongation of the chronic process in hepatocytes [9].

In general, the activity of the pathological process in the liver directly influenced on the level of antimicrobial peptides determined in blood plasma, and this indicator deteriorated most sharply in the group of patients with chronic pneumonia. When HVC, the content of endotoxin in the first group was 24.4 ± 2.3 IU / ml, in group II 57.7 ± 5.2 IU / ml, whereas in the control group this indicator was 0.10 ± 0.01 IU / ml (See Table 3).

It is known that in chronic viral hepatitis C the endotoxin content in the blood can increase several times, so it can be said that endotoxin aggression increases in the body, which is primarily associated with impaired hepatic neutralizing function, increased intestinal permeability and the syndrome of bacterial infection [10].

The excitation and progression of the immunological inflammatory process through endotoxin occurs via the inclusion of various mechanisms. It is necessary to consider that endotoxin stimulates the appearance of defensins, which are informative markers of the severity of the inflammatory process [11]. So, compared with the control (38.6 ± 3.8 ng / ml), in the I group the level of defensin increased by 7.7 times (297.0 ± 17.5 ng / ml), whereas in the II group compared

to the I group this indicator increased already 5 times (1467.3 ± 27.7 ng / ml). On the one hand, defensins show obvious bactericidal effect, on the other hand they are able to deepen the alteration in the inflammatory focus by damaging there the cells of the body [12]. Defensins play the role of opsonins, even chemokines. Thus, they cause accumulation of certain substances, such as immature dendrites, monocytes and T-lymphocytes, in the infection focus. Defensin effects such as increased proliferation and maturation of a number of cells of the immune system, expression from cells of adhesive molecules and molecules of the histocompatibility complex, and stimulation of cytokines causing the inflammatory process in any cell have been also established [13].

In order to determine the degree of activity of the inflammatory process in the liver, we also determined the content of lipopolysaccharide-binding protein (LBP). It was noted, that the level of LBP correlated with the severity of the infectious process in the liver. Thus, the highest level of this indicator noted in the second group. Compared with the control in this group, the level of LBP increased by 18.8 times (443.6 ± 29.6 ng / ml). For comparison, we say that in I group, the same indicator increased 5.9 times (138.7 ± 8.9ng / ml). On the one hand, an increase in the level of LBP is a marker of the degree of endotoxemia, but on the other hand, this may indicate an increase in the immune defense against endotoxins. Thus, it is known, that with the development of a bacterial infection, the level of lipopolysaccharide-binding protein in the blood begins to grow rapidly. This fact may in the future allow the use of the definition of lipopolysaccharide-binding protein in the blood to quantify endotoxemia and as a valuable marker of bacterial infection in the body [14, 15,16].

Summarizing the above mentioned and taking into account the results of the obtained tests, we concluded that the indicators of immune status and changes in the level of antimicrobial peptides directly related to the activity of the pathological process occurring in the liver in viral hepatitis C. In this regard, we propose the use of the above indicators as additional markers for assessing and predicting the degree of the inflammatory process, as well as immune deficiency in this kind of liver disease.

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