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INFLUENCE OF COMPLEX TREATMENT WITH QUERCETIN INCLUSION ON CYTOKINE PROFILE INDICATORS IN CHRONIC NONVIRAL HEPATITIS PATIENTS

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Abstract

The influence of treatment with quercetin inclusion on the clinical and biochemical course and the cytokine profile of chronic nonviral hepatitis patients have been studied. In addition to the basic therapeutic treatment quercetin intake led to the decrease in the cytolytic and cholestatic syndromes activities which were manifested by a faster regression of clinical signs of the disease. During two weeks of treatment chronic hepatitis patients who received quercetin in addition to standard treatment, showed decrease in proinflammatory tumor necrosis factor-α by 61,9% ($p = 0,02$) and a marker of early cardiovascular injury atrial natriuretic propeptide in the blood by 53,8% ($p = 0,04$). However, according to the data obtained, it is clear that for a complete correction of clinical manifestations of the disease, biochemical changes and the cytokine profile two-week appointment of complex treatment with quercetin inclusion is not enough, which requires longer administration of the chosen treatment course before the onset of persistent remission at the out-patient stage.

Key words: chronic hepatitis, quercetin, tumor necrosis factor-α, interleukin-10, atrial natriuretic propeptide.

Introduction. Cytokines are the factors of apoptosis and necrosis of hepatocytes, playing an important role in the processes of inflammation and fibrogenesis of the liver tissue [8, 11]. Proinflammatory cytokines are important factors in the development of liver disease, while anti-inflammatory cytokines are involved in its regeneration [7]. Sustained hyperproduction of proinflammatory cytokines, as a result of chronic inflammation, is one of the factors that lead to the development of such diseases as chronic hepatitis (CH) [14]. However, the participation of each cytokine in the processes of inflammation in the liver tissue requires further study. An important role among proinflammatory cytokines is played by tumor necrosis factor-α (TNF-α), which is a pleiotropic cytokine with a wide range of biological effects. In particular, it affects the processes of proliferation of hepatocytes, detects proinflammatory effects through the nuclear factor kappa B pathways and regulates liver cell apoptosis by activating caspase-8 [1, 10]. In the experiment on mice liver damage in animals with a decreased number of type I receptors to TNF-α was not found to develop after a prolonged administration of ethanol [11].

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays a protective role against damaging the liver from a number of factors, in particular inhibits the development of alcohol-mediated liver damage by activating STAT3 cellular messengers (signaling sensors and transcription activators) in Kupffer cells. At the same time, IL-10 inhibition stimulates the expression of cytokines involved in inflammation of the liver tissue [5, 6].

Current issue of modern hepatology is the improvement of existing treatment regimens for CH patients. Among the significant spectrum of substances possessing hepatoprotective properties, our attention has attracted by the flavonoid quercetin. Quercetin belongs to the group of flavonols - compounds of plant origin, the main structural element of which are two aromatic rings A and B, connected by a three-carbon bridge, which forms a pyranic or pyron (in the presence of double bonds) cycle. Flavonoids have high antioxidant activity, which is most pronounced in quercetin, stimulate the synthesis of proteins, regulate the exchange of phospholipids and have membrane-stabilizing properties [2]. Quercetin has pronounced anti-inflammatory properties due to a decrease in the activity of 5-lipoxygenase, which reduces the synthesis of leukotrienes from arachidonic acid. The indicated flavonoid also reduces the activity of protein kinase C and calmodulin-dependent protein kinase [3, 9]. L.M. Sheremeta have showed that the use of quercetin contributes to the reduction of the activity of cytolytic and cholestatic syndromes in patients with chronic diffuse liver disease [13]. Further investigations

require learning the mechanisms of quercetin action in chronic nonviral hepatitis patients, in particular its effect on the cytokine profile of blood.

The **aim** of the study was to investigate the effect of additional to the basic treatment administration of quercetin on the clinical course, biochemical parameters and indicators of the cytokine profile in chronic nonviral hepatitis patients.

Material and methods. 55 patients with active nonviral CH were involved in the study, who according to the administered treatment were divided into two groups. The main group consisted of 25 patients with active CH who in addition to the standard treatment received pills of Qvertin (Quercetin) (PJSC SIC "Borshchahivskiy CPP", Ukraine) at the dose of 40 mg (1 tablet) three times daily 30 minutes before taking meals within 14-16 days.

The comparison group consisted of 30 patients with active nonviral CH who received the standard basic treatment comparable to those of the main group by age and gender distribution. The control group consisted of 45 practically healthy individuals, of the correlative age and gender of the patients.

Blood samples were obtained in the morning on empty stomach from the antecubital vein in the first day of hospitalization before the treatment was administered. The investigation was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and the Recommendations of the Committee on Bioethics of the Ministry of Public Health of Ukraine, and was approved by the Biomedical Ethics Commission of the Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (Chernivtsi, Ukraine). Written informed consents were obtained from all the participants.

All of the observed patients and healthy individuals underwent comprehensive clinical, laboratory and instrumental diagnostic investigations. In order to exclude a viral etiology of the liver disease all of the patients were tested on possible hepatitis B and C infections with the help of polymerase chain reaction method. Biochemical studies were performed on the blood biochemical analyzer "Accent-200" ("Cormay SA", Poland). The range of indicators of biochemical blood analysis included: total bilirubin and its fractions, total protein and albumin, urea, creatinine, plasma enzyme activity (aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDG), gamma-glutamyl transferase (GGT), alkaline phosphatase (AP)).

Investigation of cytokine profile was performed on immunoenzyme analyzer "Statfax 303/Plus" ("Awareness Technology Inc.", USA). The plasma levels of TNF-α ("Bender MedSystems GmbH", Austria), IL-10 ("Bender MedSystems GmbH", Austria), atrial natriuretic propeptide (1-98) (proANP) (“Biomedica”, Austria) were investigated both in the examined patients and healthy individuals.

Results and Discussion. Faster improvement of general condition, more effective reduction of general weakness and sensation of heaviness in the right hypochondrium, decreased discomfort in the heart area and shortness of breath, increased tolerance to physical activity were seen in patients who in addition to basic treatment received quercetin. The dynamics of the investigated biochemical parameters is presented in Table 1. According to these data significant decrease in the total bilirubin plasma concentration during treatment was observed in patients of both groups: by 33,5% (p= 0,008) in the main group and by 26,6% (p = 0,02) in the comparison group as compared to the indicators before the treatment. More effective decrease of the total bilirubin and its plasma fractions levels, combined with faster regression of skin and mucosal icterity were tyical for young patients, in comparison with mature and elderly people.

Investigation of the enzymes reflecting cytolytic processes showed higher activity of ALT and AST in the blood of all patients. ALT activity in patients of the main group decreased by 43,7% (p = 0,02), in patients of the comparison group – by 28,1% (p = 0,03) (Table 1) after the treatment. Significant decrease of AST activity was achieved only in patients of the main group – by 27,8% (p = 0,03) (Table 1). It is indicative of more effective reduction of cytolysis in these patients as compared to those who received only standart therapy. The obtained biochemical data correlated with the dynamics of clinical symptoms reflecting the general-somatic status and condition of the cardiovascular system. However, in spite of this, in both groups of patients, AST and ALT activities in the blood were still higher after the treatment in comparison with the mentioned parameters in practically healthy persons, indicating the need for continued therapy at the out-patient stage.

There was a significant decrease in LDG activity by 16,9% (p = 0,02), as compared to appropriate rates before treatment in patients of the main group. Meanwhile in patients of the comparison group, this enzyme activity did not significantly decrease during the treatment (Table 1). While investigating cholestasis processes higher AP activity was found in most CH patients.

Table1

Biochemical blood indicators in chronic hepatitis patients in the dynamics of treatment (M±m, n, p)

Plasma level	Healthy volunteers, n = 45	Comparison group, n = 30		Main group, n = 25	
		Before the treatment	After the treatment	Before the treatment	After the treatment
Total bilirubin, mkmol/L	11,1 ± 0,79	29,0 ± 4,73 $p_1 < 0,001$	22,9 ± 5,01 $p_1 = 0,001$, $p_2 = 0,02$	34,3 ± 7,15 $p_1 < 0,001$	25,7 ± 3,32 $p_1 < 0,001$, $p_2 = 0,008$
Direct bilirubin, mkmol/L	3,1 ± 0,31	11,6 ± 3,58 $p_1 < 0,001$	7,0 ± 1,02 $p_1 < 0,001$, $p_2 = 0,009$	15,5 ± 3,25 $p_1 < 0,001$	10,3 ± 2,18 $p_1 < 0,001$, $p_2 = 0,01$
Albumin, g/L	45,0 ± 0,41	43,7 ± 1,07	41,7 ± 1,11 $p_1 = 0,001$, $p_2 = 0,004$	43,0 ± 1,25	42,5 ± 1,57
Total protein, g/L	69,3 ± 0,62	71,8 ± 1,26	70,8 ± 1,16	69,8 ± 1,93	70,2 ± 1,87
Urea, mmol/L	4,2 ± 0,23	4,7 ± 0,47	4,4 ± 0,35	5,1 ± 0,39	4,3 ± 0,47
Creatinine, mkmol/L	82,6 ± 1,80	80,0 ± 3,52	78,8 ± 3,24	83,7 ± 5,27	79,2 ± 3,87
Aspartate aminotransferase, units of action/L	22,6 ± 1,37	57,6 ± 7,02 $p_1 < 0,001$	49,6 ± 5,91 $p_1 < 0,001$	62,5 ± 7,38 $p_1 < 0,001$	48,9 ± 5,31 $p_1 < 0,001$ $p_2 = 0,03$
Alanine aminotransferase, units of action/L	18,5 ± 1,46	70,6 ± 10,01 $p_1 < 0,001$	55,1 ± 6,76 $p_1 < 0,001$, $p_2 = 0,03$	68,7 ± 5,43 $p_1 < 0,001$	47,8 ± 5,29 $p_1 < 0,001$, $p_2 = 0,02$
Lactate dehydrogenase, units of action/L	387,0 ± 13,59	438,1 ± 29,07	401,0 ± 25,49	445,9 ± 31,92 $p_1 = 0,01$	381,6 ± 30,82 $p_2 = 0,02$
Alkaline phosphatase, units of action/L	80,3 ± 3,20	112,8 ± 9,41 $p_1 = 0,01$	105,6 ± 8,34 $p_1 = 0,005$	119,5 ± 14,19 $p_1 = 0,02$	91,3 ± 11,57 $p_2 = 0,03$
Gamma-glutamyl transferase, units of action/L	21,9 ± 1,62	107,0 ± 17,57 $p_1 < 0,001$	80,4 ± 11,65 $p_1 < 0,001$, $p_2 = 0,03$	110,5 ± 11,25 $p_1 < 0,001$	71,3 ± 7,95 $p_1 < 0,001$, $p_2 = 0,009$

p_1 – significance of differences as compared to the indicators in the group of healthy people; p_2 – significance of differences as compared to the indicators before the treatment.

Significant decrease of AP plasma activity (by 30,9% (p = 0,03) was observed only in patients who received quercetin in addition to the standart treatment (Table 1).

Similar dynamics was characteristic for GGT activity in both groups of patients, however, in patients of the main group, this decrease was 55,0% (p = 0,009), in the comparison group – 33,1% (p = 0,03) (Table 2). These findings reflect the reduction of intoxication and cholestatic syndromes in the examined patients. These changes of biochemical parameters of blood were associated with clinical symptoms of the disease: improving general condition and decreasing dyspeptic disorders. More pronounce decrease in GGT plasma activity and a faster regression of clinical manifestations of intoxication and cholestatic syndromes were observed in CH patients of young age, as compared to older ones.

S. Chirumbolo and G.S. Kelly repoted about quercetin anti-inflammatory properties [3, 9]. According to this we consider the investigation of its effect on cytokine regulation of inflammatory and immune processes in CH patients to be rather essential. Patients of the main group showed a significant decrease in TNF-α content in the blood by 61,9% (p = 0,02) (Table 2), while patients of the comparison group demonstrated only the tendency to reduce this proinflammatory cytokine. The content of IL-10 before the initiation of treatment was elevated in the blood of the observed patients as compared to practically healthy people. During the treatment only the tendency towards normalization of its blood level was determined, which may be indicative of the need for a longer course of the chosen therapeutic treatment in order to achieve the normal values of the indicated anti-inflammatory cytokine.

According to C. Montoliu et al. and U. Cillo et al. investigations proANP is not only a reliable predictor of cardiovascular risk in cardiological patients but also can serve as a marker for the risk of hemoynamic complications development in patients with chronic diffuse liver disease [4, 12]. Higher content of proANP in blood was noted in the examined patients as compared to practically healthy individuals, which can indicate the development of subclinical cardio-vascular insufficiency. Additional to the standard treatment quercetin prescription caused significant decrease of proANP level in patients of the main group by 53,8% (p = 0,04) (Table 2), which was accompanied by a regression of cardiac complaints and improvement of patient’s general condition.

Table 2

Indicators of cytokine profile in the blood of chronic hepatitis patients in the dynamics of treatment (M±m, n, p)

Plasma level	Healthy volunteers, n = 20	Comparison group, n = 15		Main group, n = 15	
		Before the treatment	After the treatment	Before the treatment	After the treatment
Interleukin-10, pg/ml	3,9 ± 0,34	6,3 ± 0,37 $p_1 = 0,02$	5,3 ± 0,49	6,5 ± 1,87 $p_1 = 0,03$	5,1 ± 0,79
Tumor necrosis factor-α, pg/ml	15,3 ± 0,95	29,8 ± 4,24 $p_1 = 0,004$	21,5 ± 4,77 $p_1 = 0,01$	30,6 ± 5,96 $p_1 = 0,01$	18,9 ± 4,88 $p_2 = 0,02$
Atrial natriuretic propeptide, nmol/l	1,2 ± 0,23	1,8 ± 0,26	1,5 ± 0,37	2,0 ± 0,32 $p_1 = 0,02$	1,3 ± 0,29 $p_2 = 0,04$

p_1 – significance of differences as compared to the indicators in the group of healthy people; p_2 – significance of differences as compared to the indicators before the treatment.

Conclusions

1. During two weeks of treatment clinical symptoms, functional liver parameters accompanied by a decrease in tumor necrosis factor-α and atrial natriuretic propeptide blood levels were more effectively corrected in chronic hepatitis patients who in addition to the standard treatment received quercetin.

2. For a complete correction of clinical manifestations of the disease, biochemical changes and the cytokine profile two-week complex treatment with quercetin inclusion is not enough, which requires longer administration of the chosen treatment course before the onset of persistent remission at the out-patient stage.

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