PROTEOLYTIC SYSTEM ACTIVITY DYSFUNCTION IN CASE OF EXPERIMENTAL LIVER CIRRHOSIS

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Experimental data presented are devoted to influence of the alpha-lipoic acid and tivortin on activity of proteolytic enzymes in conditions of experimental liver cirrhosis in rats. Experimental model of CCl4-induced liver cirrhosis was shown to be accompanied by the proteolysis system activation. Both pharmacological compounds were shown to reveal the similar normalising effect resulting in the normalisation of proteolytic activity starting 6 hours after the disease induction. The therapeutic efficiency of the alpha-lipoic acid is higher than that of tivortin in case of their single administration in the modelled condition. The data obtained showed the pathogenetic importance of the proteolytic system activation in case of the experimental liver cirrhosis. The data is also an experimental background for prospects of using the tested pharmacons in the pathogenetically oriented complex correction of liver functioning in clinical conditions.

Keywords: proteolysis, liver cirrhosis, alpha-lipoic acid, tivortin, pathogenetic mechanisms, pharmacological treatment, pathogenetically oriented complex correction.

Statistical indices of cirrhosis morbidity in the population, as well as other forms of destructive inflammatory affections of the liver parenchyma, are disappointing, considering the growth of patients with this pathology [1, 2]. Given this, this medical problem is important for the entire field of gastroenterology. It is clear that the topic chosen for the research is also of economic and social significance, taking into account considerable complications in this group of patients [3], rapid development of viral hepatic parenchyma affections, tumors and other common forms of the liver disease in patients of predominantly working age [4-6].

When considering the basic concepts of schemes chosen for treatment of liver cirrhosis and prevention of its complications, we proceeded from pathogenic substantiation of perspective therapeutic and preventive schemes. Based on the pathophysiological mechanisms of the general inflammatory cell response (meaning hepatocyte, of course), we know that any traumatic effect on it causes the development of a chain of biochemical, morphological and other reactions which lead to the development of inflammation with some manifestation of the inflammatory stage for some time, meaning the alternative component either exudative or proliferative one of the inflammatory process [7]. Taking into consideration the general pathophysiological mechanisms of the typical pathological inflammation process, it is clear that one of the pathogeneses of inflammatory

and destructive forms of affection of the hepatic parenchyma is activation of the blood lysosomal enzymes due to dysfunction of lysosomal membranes, resulting in the powerful and active "release" of enzymes from lysosomes [8].

Important therapeutic effect in the complex treatment of inflammatory affections of the abdominal organs is caused by pharmacological compounds with anti-inflammatory properties due to their antioxidant, membrane-stimulating, energy-efficient and other effects. One of such compounds, which has these and anti-inflammatory properties is tivortin (TV) and alpha-lipoic acid (ALA), which are also characterised by hepatoprotective effects [9, 10].

Objective. Comparative study of anti-inflammatory efficacy of TV and ALA in experimental liver cirrhosis (ELC) with a focus on the dynamics of the blood proteolysis activity under their influence.

Materials and methods. The studies were conducted under conditions of chronic experiment with 180 Wister male rats. Work with laboratory animals was carried out in compliance with fundamental ethical and regulatory requirements, and provided by the European Commission for Supervision of laboratory and other experiments involving experimental animals of different species.

The model of ELC was reproduced in rats, in a toxic liver affection with a hepatotropic poison - carbon tetrachloride, which caused a direct

cytolytic effect on the hepatic parenchyma [11]. CCl_4 solution was prepared from the pure (99.99% purity) drug by adding the refined sunflower oil (a final solution concentration was 50%) and administered orally using a plastic probe twice a week during 10 weeks. The animals of the comparison group (intact rats, n = 9) were administered 0.5 ml of 0.9% saline solution of NaCl orally in the similar conditions. The ELC formation was controlled laparoscopically with biopsy and subsequent histological examination of the biopsy samples in animals of both experimental and control groups.

When modelling the ELC, 45 of 171 rats died from acute liver failure (mortality rate was 26.3%). 126 rats that remained alive were divided into seven groups - 18 animals each. They were subjected to euthanasia by overdosage of aethaminalum-natrium (100 mg/kg, intraperitoneally) in 1 hour (group N1), in 6 hours (group N2), in 12 hours (group N3), in 24 hours (group N4), in 3 days (Group N5), in 5 days (group N6) and in 7 days (group N7) after the development of ELC. Blood samples were taken from all animals. In blood the activity of cathepsin D, cathepsin L, cathepsin B, trypsyn-like proteinase (trypsin), metalloproteinase carboxypeptidase A and carboxypeptidase B were determined by the conventional way [12]. But further experiments were conducted in two sessions: in the first session each group had 6 rats, which were examined using the markers of the proteolysis activity

in the blood in order to determine their perspective significance in the dynamics of ELC. In the second session each group had 12 rats, which were equally administered ALA (50mg kg/i.p.) and TV (100 mg/kg/i.p.). After this the activity of proteolysis markers was determined in blood.

The data obtained was statistically calculated using the criterion ANOVA, which in case of reliability was accompanied by Newman-Keuls criterion. The minimum expected reliability was in $p < 0.05$.

Results and discussion. In the dynamics of the ELC development in the rat blood in 1 hour of reproduction of the pathological condition the acidic protease activity – cathepsins of the type D, L and B - exceeded the initial data by 67%, 75% and 41% ($p < 0.01$; Table. 1). Hereafter, the investigated enzyme activity continued to grow, reaching the maximum values in 12 hours of ELC modeling ($p < 0.001$). Changes in the activity of neutral proteases (trypsin) also gained statistical significance, starting 1 hour after the ELC induction when its activity exceeded the initial value by 44% ($p < 0.01$), and finishing by the 7th day of the pathological process, when the trypsin activity was higher than the corresponding control indices by 56% ($p < 0.01$). In ELC rats without treatment, dynamics of the increased

activity of metalloproteinase was less pronounced being 1.5 times higher than the corresponding reference data in 6 hours of the experiment ($p < 0.05$), just like carboxypeptidase A and B, which were higher than the corresponding initial data by 40% ($p < 0.05$) and 73% ($p < 0.001$) in 6 hours after inducing the pathological condition, and acquired maximum activity in 24 hours after the ELC induction (Table. 1).

In the second session of studies attempts were made to identify the ALA therapeutic activity, the introduction of which caused a pronounced decrease in the activity of proteolytic and lysosomal enzymes (Table. 2). Over the course of 6 hours of ELC, ALA reduced the activity of acidic protease of the type D, L and B by 34%, 47% and 48%, respectively, as compared to such indices in rats with ELC without treatment ($p < 0.01$) in the same period of time. Such absolute numerical values of the acidic protease activity were recorded during 5 days of the induced pathological condition. Approximately in the same way ALA affected the activity of trypsyn-like proteases, actual figures of which, according to the modelled conditions, were lower starting in 6 hours (by 51% less as compared to those before ALA introduction) and ending on the 5th day of the pathological process (by 36% less than without introducing ALA, in both

cases $p < 0.01$). To the lesser extent ALA affected indices of the metalloproteinase activity for 12 hours - 5 days (average 19% - 41%, $p < 0.05$), carboxypeptidase A and B - for 12 hours - 5 days (on an average 23-41% $p < 0.05$; Table. 2).

Tivortin also brought about the normalization effect on the ELC course. The data on effectiveness of TV concerning normalization of the proteolytic lysosomal enzymes during the ELC are shown in Table 3. There has been approximately the same but less when compared to the ALA impact of TV on the absolute figures, covering the activity of the proteolysis system in the blood of rats with ELC.

Thus, the course of liver cirrhosis is accompanied by activation of acidic, trypsyn-like proteases and lysosomal enzymes in the rat blood serum, indicating the spread of the inflammatory process from hepatocytes to the entire body - the generalization of the process. Activation of cathepsin D indicates the presence of inflammatory and destructive changes in the liver, which reflects the presence of systemic inflammation under the conditions of ELC.

The results obtained suggest that activation of the proteolysis system in the blood of rats is probably one of the pathogenesis links of the disease, resulting in amplified intensity of lipid peroxidation due to initiation of the

Table 1.

Dynamics of changes in proteolysis enzyme activity indices in the rat blood with reproduced experimental liver cirrhosis

Studied enzymes	Proteolysis enzyme activity in the rat blood in different periods after reproduced ELC							
	Initial data	1 hr	6 hr	12 hr	24 hr	3 days	5 days	7 days
Cathepsin D, mcmol/mg	0.009±0.001	0.015± 0.001**	0.041± 0.03***	0.048± 0.005***	0.042± 0.004***	0.033± 0.003***	0.016± 0.001***	0.012± 0.01**
Cathepsin L, mcmol/mg	0.48±0.04	0.84± 0.08***	2.20± 0.19***	2.75± 0.24***	2.00± 0.17***	1.44± 0.12***	0.80±0.07**	0.62±0.05*
Cathepsin B, mcmol/mg	0.062±0.005	0.088± 0.008*	0.276± 0.023***	0.286± 0.026***	0.198± 0.016***	0.144± 0.013***	0.112± 0.009***	0.086± 0.007*
Trypsin-like proteinases, mcmol/mg	0.350±0.027	0.500± 0.044**	1.100± 0.09***	1.600± 0.135***	1.900± 0.174***	1.200± 0.114***	0.900± 0.085***	0.550± 0.035**
Metalloproteinases, mcmol/mg	0.06±0.01	0.06±0.01	0.09±0.01*	0.12±0.01**	0.18± 0.01***	0.16± 0.01***	0.10±0.01**	0.07±0.01
Carboxipeptidase A, mcmol/mg	70.5±.6.6	82.7±.7.7	118±9 ***	200±17 ***	176±14 ***	134±11 ***	108±9 **	74±6
Carboxipeptidase B, mcmol/mg	770±67	920±82	1520±144 ***	1770±163 ***	1671±154 ***	1430±132 ***	1200±114 **	960±82

Note: * - P<0.05, ** - P<0.01, *** - P<0.001 –reliable discrepancies of the studied indices compared with the corresponding initial data (criterion of ANOVA, which was accompanied by a post hoc Student –Newman-Keuls).

Table 2.

Influence of alpha-lipoic acid on the dynamics of changes in proteolysis enzyme activity indices in the rat blood with reproduced ELC

Studied enzymes	Proteolysis enzyme activity in the rat blood in different periods after reproduced ELC						
	1 hr	6 hr	12 hr	24 hr	3 доби	5 діб	7 діб
Cathepsin D, mcmol/mg	0.012±0.001	0.031±0.002**	0.033±0.003**	0.022±0.002**	0.017±0.001**	0.010±0.001**	0.010±0.001
Cathepsin L, mcmol/mg	0.56±0.04	1.06±0.09**	1.00±0.09**	0.88±0.08**	0.70±0.07**	0.56±0.05**	0.52±0.04
Cathepsin B, mcmol/mg	0.068±0.005	0.106±0.009**	0.110±0.009**	0.108±0.008**	0.094±0.007**	0.080±0.007**	0.072±0.006
Trypsin-like proteinases, mcmol/mg	0.370±0.033	0.550±0.045**	0.750±0.065**	0.800±0.072**	0.700±0.065**	0.550±0.045**	0.400±0.035
Metalloproteinases, mcmol/mg	0.06±0.01	0.07±0.01	0.10±0.01**	0.12±0.01**	0.10±0.01*	0.07±0.01*	0.06±0.01
Carboxipeptidase A, mcmol/mg	73±6	98±8**	120±10**	109±10**	94±8**	87±7**	72±7
Carboxipeptidase B, mcmol/mg	810±7	1000±98*	1100±102**	1170±105**	1030±99*	920±89*	830±76

Note: * - $P<0.05$, ** - $P<0.01$ – reliable discrepancies of the indices under study compared with corresponding initial data (criterion of ANOVA, which was accompanied by a post hoc Student –Newman-Keuls).

Table 3.

Impact of tivortin on the dynamics of changes in proteolysis enzyme activity indices in the rat blood with reproduced ELC

Studied enzymes	Proteolysis enzyme activity in the rat blood in different periods after reproduced ELC						
	1 hr	6 hr	12 hr	24 hr	3	5 діб	7 діб
Cathepsin D, mcmol/mg	0.014±0.001	0.037±0.002**	0.036±0.003**	0.028±0.003**	0.022±0.002**	0.012±0.001**	0.010±0.001
Cathepsin L, mcmol/mg	0.62±0.05	1.17±0.09**	1.16±0.09**	0.96±0.08**	0.77±0.07**	0.59±0.05*	0.52±0.04
Cathepsin B, mcmol/mg	0.071±0.006	0.115±0.009**	0.118±0.009**	0.116±0.009**	0.109±0.009**	0.088±0.008**	0.073±0.006
Trypsin-like proteinases, mcmol/mg	0.370±0.035	0.590±0.045**	0.820±0.075**	0.970±0.088**	0.810±0.077**	0.670±0.059*	0.440±0.040
Metalloproteinases, mcmol/mg	0.06±0.01	0.09±0.01	0.13±0.01	0.18±0.01	0.15±0.01	0.09±0.01	0.06±0.011
Carboxipeptidase A, mcmol/mg	75±6	102±9**	126±11**	114±10**	102±9**	91±8**	76±6
Carboxipeptidase B, mcmol/mg	820±77	1080±98*	1200±113**	1210±116**	1180±101**	1000±98*	900±83

Note: * - $P<0.05$, ** - $P<0.01$ – reliable discrepancies of the indices under study compared with corresponding initial data in rats with ELC (criterion of ANOVA, which was accompanied by a post hoc Student –Newman-Keuls).

so-called pathological "vicious circle" that strengthens presence of lysosomal enzymes in blood, characterized by powerful destructive potential. If this happens, autocatalytic activation of the kinin–kallikrein system will occur. It will promote vasodilation, further ischemia of the liver parenchyma and the rest of the abdominal cavity [7.12]. All of the aforesaid fundamentally explains the development of the corresponding complications in the body in the course of development of cirrhosis.

We consider clinical implementation of results, explaining the normalization effect of ALA and tivortin on the proteolytic enzyme system activity in ELC, to be useful in future. Their hepatoprotective effect (as to inhibition of the proteolysis system activity) was virtually identical in the manifestation with slight advantage in ALA, and developed in 6 hours of the ELC course. Of interest may be the determined

hepatoprotective effects of tivortin, the active substance of which is the amino acid L-arginine, which is a major participant of the ornithine cycle (by which the toxic ammonia binds and is converted into the non-toxic urea). Besides, L-arginine is a substrate for NO synthase - a key enzyme of NO synthesis in the endothelial cells [13].

Mechanism of action of Tivortin is associated with increasing concentrations of nitric oxide in the body. It activates guanylate cyclase, increases the level of cyclic guanidine monophsphate in the endothelium of the blood vessels, reduces activation and adhesion of leukocytes and platelets to the endothelium, inhibits protein synthesis of adhesion of VCAM-1 (vascular cell adhesion molecule-1) and MCP-1 (monocyte chemoattractant protein-1), thereby causing a general anti-inflammatory effect. The drug has hepatoprotective properties due to antioxidant, membrane stabilizing activity and, as a result, there is positive impact on energy supply processes in hepatocytes. Therefore, this drug may be promising as to further study of its hepatoprotective effects by modelling.

In our opinion, the data obtained is an experimental substantiation of expediency of the clinical testing of ALA and TV hepatoprotective effects in case of their separate application or in terms of combined administration in patients with cirrhosis. Our results allow us to expand the boundaries of experimental research in order to determine the prospects of clinical or prophylactic use of pharmaceutical compounds tested in conditions of experimental liver cirrhosis, and in similar clinical conditions.

Conclusions.

1. The course of liver cirrhosis in the rat blood serum is accompanied by activation of the acidic trypsin-like proteases and lysosomal enzymes in the rat blood serum indicating the generalization of the inflammatory process.

2. Activation of the proteolysis system in the rat blood is probably one of the pathogenetic links of proteolysis activation in the rat blood, and is probably one of the pathogeneses of the disease - liver cirrhosis.

3. Normalization of alpha-lipoic acid and tivortin impacts on activity of the proteolysis system are promising for further clinical implementation of the results.

4. The data obtained is an experimental substantiation of expediency of the clinical testing of hepatoprotective effects of alpha-lipoic acid and tivortin in cases of individual application or in combined administration in patients with liver cirrhosis.

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