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Study of changes in proteolytic systems of rats under conditions of experimental chronic prostatitis

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Abstract

The aim of our research – to analyze the mechanisms of regulation of the body's proteolytic systems during inflammation, detection of inflammation markers in blood and prostate secretions in the simulation of chronic prostatitis (CP) in rats. 1. The leading link in the pathogenesis of many diseases is a change in the structure and functions of biological membranes – membranopathy, the consequence of which is a violation of histochemical barriers, which is manifested in an increase in the excretion of certain metabolites in the extracellular fluid. In the case of experimental CP in rats, the appearance in the secretion of the prostate gland of the components of KKS, which are determined in the secretion of the normal prostate in trace amounts, was noted. Inflammatory damage to the prostate was also evidenced by an increase in the proteolytic potential of prostate secretion due to an increase in kallikrein activity on the 21st day by 807.1% ($p<0.05$), which leads to massive kininogenesis.

Depletion of the adaptive and compensatory potential of the prostate gland was evidenced by a decrease in prekallikrein by 21.7% ($p<0.05$) compared to the intact group of animals. A sharp increase in the activity of kallikrein in CP is probably compensated by an increase in the activity of its specific inhibitor – α2-macroglobulin, the level of which in the secretion of the CP increased on 225 % on the 21st day of the experiment compared to intact rats, and the increase in proteolytic potential – by an increase of 125 % in the inhibitory activity of α1-PI, a protein of the acute phase of inflammation. An increase in the content and activity of KKS components in the secretion of the prostate testifies to its inflammatory damage and violation of the permeability of the hematoprostatic barrier, which can be an important diagnostic criterion.

Keywords*:* chronic prostatitis; proteolytic systems; kallikrein-kinin system; pathogenetic predictors.

Introduction. The activation of proteolysis and one of the most important proteolytic systems of the body – the kallikrein-kinin system (KKS) becomes of key importance during inflammation. The coordinated action of proteases and their inhibitors is one of the forms of maintaining homeostasis in the body, while a complex and multicomponent sequence of reactions is considered as a universal non-specific response to injury. Kallikrein is a multifunctional proteinase that controls many biological processes, including converting the precursor protein kininogen into bradykinin, a "mediator" of pain and inflammation. In addition, kallikrein causes chemotaxis and aggregation of neutrophils, releases elastase, activates the latent form of neutrophil collagenase. The activity of kallikrein and other proteinases is regulated with the help of special proteins - inhibitors of serine proteinases, among which the 1-proteinase inhibitor, whose main function is the inactivation of neutrophil elastase, and α2-macroglobulin, which binds thrombin, plasmin, kallikrein, are present in the largest amount in the blood plasma, elastase and other proteolytic enzymes [4, 7, 10, 11].

In the works of domestic and foreign scientists, there are practically no research results on the mechanisms of formation of an inflammatory reaction in the prostate involving proteolytic systems, as well as protective factors that limit the damage process.

The aim of work – to analyse the mechanisms of regulation of the body's proteolytic systems during inflammation, detection of inflammation markers in blood and prostate secretions in the simulation of chronic prostatitis (CP) in rats.

Materials and methods. The study was conducted on 30 white, sexually mature male rats, which were divided into 2 groups:

1st group (n=6) – intact animals (rats that were on a standard water diet and food);

2nd group (n=24) – rats with modelling CP.

An informative and easy-to-implement model based on a single rectal injection of a mixture of dimexide, and turpentine was used to study CP in rats. Animals were injected rectally with 1 ml (0.75–1.25 ml) of a mixture of 0.5% turpentine and 5% dimexide in a volume ratio of 1:4. Subsequently, hypokinetic stress was reproduced for 10 days by placing males alone in special pen cells [1].

Rats were taken out of the experiment on the 1st day after the end of CP modeling (on the 12th day of the study), on the 7th day (on the 18th day from the beginning of the entire study), on the 14th (on the 25th day from the beginning of the study) and the 21st day of the development of experimental CP (on the 32nd day from the beginning of the simulation) [1].

In order to study the role of the body's proteolytic systems in the pathogenesis of CP, the following indicators were studied both in the blood serum and in the secretion of PZ (after its dilution in a ratio of 1:9 with physiological solution) [5].

The activity of kallikrein and the content of prekallikrein after separation from other serine proteinases were determined using ion exchange chromatography by the rate of hydrolysis of N-benzoyl-1-arginine ethyl ether [5].

The inhibitory activity of α1-PI and α2-macroglobulin was studied using a unified enzymatic method [5].

During the working with animals, the International Code of Medical Ethics (Venice, 1983), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and the Law of Ukraine "On the Protection of Animals from Cruelty" No. 440 (dated 14.01.2020) were used [3]

Statistical processing of the obtained results was carried out with the help of the "Statistica 10.0" program. The probability of differences between the indicators of the control and experimental groups was determined by Student's and Fisher's tests. The level of reliability was accepted at $p<0.05$ [2].

Results of study and their discussion. Were observed in the blood serum of rats a probable increase in the level of kallikrein and a decrease in the content of its precursor – prekallikrein (Table 1). In the group of control pathology animals, this indicator increased already on the first day on 55.3 % and amounted to 23.6±3.6 nmol/min/ml ($p<0.05$), on the 7th day – on 138.2 % ($p<0.05$) (36.2±4.4 nmol/min/ml), on the 14th day – on 202 % ($p<0.05$) (45.9±5.24 nmol/min/ ml), on the 21st day – on 220.4 % ($p<0.05$) (48.7±6.0 nmol/min/ml) compared to intact animals.

Table 1 – The level of proteolytic enzymes in the blood serum of rats against the background of experimental chronic prostatitis (X±Sx, n=6)

Indicator	Intact animals	Control pathology			
		1st day	7th day	14th day	21st day
Kallikrein, nmol/min/ml	15,2±3,1	23,6±3,6*	36,2±4,4*	45,9±5,2*	48,7±6,0*
Prekallikrein, nmol/min/ml	357,8±25,8	320,3±30,2*	315,8±28,4*	298,1±25,3*	264,2±26,4*
Inhibitory activity of α1-PI, IU/ml	24,9±2,7	30,2±3,0*	33,7±3,1*	42,4±3,8*	48,7±4,4*
Inhibitory activity of α_2-macroglobuline, IU/ml	4,2±0,75	3,6±0,68	3,7±0,65	4,0±0,58	3,9±0,7

Notes:

1. * - $p<0,05$ compared to the group of intact animals;
2. n – the number of animals in the group.

The content of prekallikrein decreased sharply: already on the first day of the experiment, its level was 320.3±30.2 nmol/min/ml, which is 10.5 % ($p<0.05$) lower than the similar indicator of intact animals. On the 7th day, the prekallikrein level decreased on 11.8 % ($p<0.05$) (it was 315.8±28.4 nmol/min/ml); on the 14th day – on 16.7% ($p<0.05$) (298.1±25.3 nmol/min/ml); on the 21st day – on 26.2% ($p<0.05$) (264.2±26.4 nmol/min/ml) compared to the intact group of animals.

The index of inhibitory activity of α1-PI probably increased in the group of animals with simulated CP pathology. On the first day, its level increased on 21.3 % ($p<0.05$) (equal to 30.2±3.0 IU/ml), on the 7th day – on 35.3 % ($p<0.05$) (33.7±3.1 IU/ml), on the 14th day – on 70.3 % ($p<0.05$) (42.4±3.8 IU/ml), on the 21st day – on 95.6 % ($p<0.05$) (48.7±4.4 IU/ml) relative to the similar indicator in the group of intact rats. Studying the level of inhibitory activity of α2-macroglobulin in the group of control pathology, no probable changes were found compared to intact animals.

Table 2 – The level of proteolytic enzymes in the secretion of the prostate gland of rats against the background of experimental chronic prostatitis (X±Sx, n=6)

Indicator	Intact animals	Control pathology			
		1st day	7th day	14th day	1st day
Kallikrein, nmol/min/ml	5,6±0,48	27,2±3,1*	37,6±3,9*	45,3±4,6*	50,8±5,2*
Prekallikrein, nmol/min/ml	273,4±22,3	265,3±25,1	247,1±21,4*	225,3±22,9*	214,2±24,2*
Inhibitory activity of α1-PI, IU/ml	2,4±0,68	3,1±0,72*	3,8±0,85*	4,7±1,2*	5,4±1,1*
Inhibitory activity of α_2-macroglobuline, IU/ml	0,16±0,02	0,21±0,02	0,32±0,03*	0,46±0,05*	0,52±0,5*

Notes:

1. * - p<0,05 compared to the group of intact animals;
2. n – the number of animals in the group.

It was established during the studying of these indicators in the secretion of the CP a similar trend in the blood serum of experimental animals. In particular, the level of kallikrein in the group of animals with CP on the first day of the experiment increased on 385.7 % (p<0.05) and amounted to 27.2±3.1 nmol/min/ml, on the 7th day – on 571, 4% (p<0.05) (37.6±3.9 nmol/min/ml), on the 14th day – on 708.9% (p<0.05) (45.3±4.6 nmol/min/ml) and on the 21st day – on 807.1% (p<0.05) (50.8±5.2 nmol/min/ml) compared to intact animals (Table 2).

In the control group of rats, the level of prekallikrein decreased compared to the level of the intact group of animals: on the first day – on 2.9 % (and amounted to 265.3±25.1 nmol/min/ml), on the 7th day – on 9.6 % (p<0.05) (247.1±21.4 nmol/min/ml); on the 14th day – on 17.6% (p<0.05) (225.3±22.9 nmol/min/ml); on the 21st day – on 21.7% (p<0.05) (214.2±24.2 nmol/min/ml).

The level of inhibitory activity of α1-PI probably increased in the group of animals with simulated CP pathology. On the first day its level increased on 29.2 % (p<0.05) (equal to 3.1±0.72 mcg/ml), on the 7th day – on 58.3 % (p<0.05) (3.8±0.85 IU/ml), on the 14th day –

by 95.3 % ($p<0.05$) (4.7±1.2 IU/ml), on the 21st day – on 125 % ($p<0.05$) (5.4±1.1 IU/ml) relative to the similar indicator in the group of intact rats.

The inhibitory activity of α2-macroglobulin in the control group of animals on the first day of observation probably did not differ from intact animals, but on the 7th day its activity increased on 100 % ($p<0.05$) (0.32±0.03 IU/ml), on the 14th day – on 187.5 % ($p<0.05$) (0.46±0.05 IU/ml), on the 21st day – on 225 % ($p<0.05$) (0.52±0.5 IU/ml).

The results of the study showed that in experimental CP there is an increase in the activity of kallikrein in blood serum ($p<0.05$) compared to a similar indicator in intact animals. The intensity of the inflammatory process in the prostate in CP is indicated by a sharp increase in the inhibitory activity of α1-PI ($p<0.05$) – the "protein of the acute phase of inflammation", both in the blood serum and in the secretion of the prostate gland. That is, the activation of the KKS is noted, which leads to the accumulation of bradykinin – one of the main "mediators" of pain and inflammation. As a result of massive kininogenesis, a complex of pathophysiological disorders develops, which are key in the development of the clinical manifestation of CP: pain, hemodynamic disorders, microcirculation disorders, increased vascular permeability [4, 6, 8].

We have shown that the inhibitory potential of prostate secretion increases dramatically in rats with experimental CP. α1-proteinase inhibitor – a protein of the acute phase of the inflammatory process, is the main endogenous regulator of the elastotic activity of the prostate secretion and is secreted during inflammation, thereby reducing the proteolytic activity of leukocyte elastase in the focus of inflammation and thereby preventing excessive tissue damage in the target organs in this pathological process [8, 9, 11].

Thus, α1-proteinase inhibitor plays an important regulatory role in the anti-inflammatory response in CP.

It is worth noting that high inhibitory activity of α2-macroglobulin has been established in the prostate secretion with CP, which indicates damage to the hematoprostatic barrier and the development of membranopathy. α2-macroglobulin plays an important role in the regulation of inflammatory processes, as it limits the substrate specificity of most proteolytic enzymes, turning proteases into peptidases that hydrolyze low-molecular peptides that are mediators of inflammatory processes. In addition, α2-macroglobulin is the main transporter of regulatory cytokines to cells, participates in signal transmission to the cell and the initiation of a cascade of intracellular reactions that affects the formation of antibodies [8, 11, 12].

Conclusions:

1. The leading link in the pathogenesis of many diseases is a change in the structure and functions of biological membranes – membranopathy, the consequence of which is a violation of hematoprostatic barriers, which is manifested in an increase in the excretion of certain metabolites in the extracellular fluid. In the case of experimental CP in rats, the appearance in the secretion of the prostate gland of the components of KKS, which are determined in the secretion of the normal prostate in trace amounts, was noted.

2. Inflammatory damage to the prostate was also evidenced by an increase in the proteolytic potential of prostate secretion due to an increase in kallikrein activity on the 21st day by 807.1% ($p<0.05$), which leads to massive kininogenesis. Depletion of the adaptive and compensatory potential of the prostate gland was evidenced by a decrease in prekallikrein by 21.7% ($p<0.05$) compared to the intact group of animals.

3. A sharp increase in the activity of kallikrein in CP is probably compensated by an increase in the activity of its specific inhibitor – α2-macroglobulin, the level of which in the secretion of the CP increased on 225 % on the 21st day of the experiment compared to intact rats, and the increase in proteolytic potential – by an increase of 125 % in the inhibitory activity of α1-PI, a protein of the acute phase of inflammation.

4. An increase in the content and activity of KKS components in the secretion of the prostate testifies to its inflammatory damage and violation of the permeability of the hematoprostatic barrier, which can be an important diagnostic criterion.

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