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Original Research Article

Dynamics Changes of Proteolytic Balance in Blood Plasma under Experimental Chemical Burns of Esophageal Development in Rats

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ABSTRACT

Background: Proteinases control all metabolic processes involved in neurotransmitter receptor signalling in coordinating the process of fibrinolysis, hemocoagulation, activation of the complement system. In critical conditions there is an infringement of dynamic equilibrium between the proteases and their inhibitors, which is an important link in the pathogenesis of many diseases. In literature there is no data on patterns of change proteases and their inhibitors in the bloodstream after esophagus burning injuries. These experimental data would allow to assess the nature of reparation process in case of esophagus burning, and accordingly develop effective methods of proteolysis targeted correction.
Aim: To investigate features of proteolytic enzymes system and their inhibitors in the pathogenesis post burning process.
Methods: Chemical burns in animals were experimentally modeled in the following way: alkaline esophageal burn was caused by 20% NaOH and acid esophageal burn was caused by 30% CCl_3COOH. The total proteolytic activity analyzed by method of caseinolytic activity with modifications. The level of α1-AT and α2-MG were determined using the method Nartykova.
Results: The result of the research it's shown that the development of alkali burn there was observed increased activity of MMP and serine proteases on 15th and 21st day against a background of increased levels of α2-MG. However when modelling acid burn, decrease of proteolytic enzymes on 15th and 21st day and increased α1-AT level during experiment was observed.
Conclusion: As a result of the research there were showed different dynamics of changes in alkaline and acid burns of the esophagus. Thus, the results may indicate inflammation at the latest experiment terms in modelling the esophagus alkali burns.

Keywords: esophagus burn, MMP, serine protease, protease inhibitor.

INTRODUCTION

According to the World Health Organization reports, a steady increase in the number of chemical burns of the upper gastrointestinal tract is observed. It is associated with the increasing production of new chemicals, the continuing production of the concentrated acetic acid and a careless storage of chemicals in daily (domestic) life. According to statistics, the largest number of chemical poisoning (from 77.2 to 85.0%) occurs in children from 1 to 3 years old. [1, 2]

Proteolytic enzymes are involved in the functioning of various organs and systems in regulation of biological processes. Proteinases control all metabolic processes involved in neurotransmitter receptor signalling in coordinating the process of fibrinolysis, hemocoagulation, activation of the complement system. [3,4] Proteases activity depends usually on the

rate of its formation from inactive precursors and inactivates specific inhibitors that are present in cells, tissues and blood plasma and form the so-called antiproteolytic potential. [5] In critical conditions there is an infringement of dynamic equilibrium between the proteases and their inhibitors, which is an important link in the pathogenesis of many diseases (pancreatitis, fibrosis, cancer etc.). [6,7] However, in literature there are no data on the activity of proteolytic enzymes and inhibitory potential in the bloodstream in case of esophagus burning injuries. Research of the proteolytic enzymes system features of and their inhibitors is essential for the pathogenesis of post burning process.

MATERIALS AND METHODS

White wild rats (1-month, 90-110 g body weight) were used in experiments in compliance with provisions for the use of animals in biomedical experiments approved by the First Ukrainian National Congress on Bioethics (September 2001) and other international agreements and national legislation in this area. Studied animals received a standard diet. Chemical burns in animals were experimentally modeled in the following way: alkaline esophageal burn was caused by 20% NaOH and acid esophageal burn was caused by 30% CCl_3COOH. [8] Materials for research were collected at 1st, 7th, 15th and 21st day.

The total proteolytic activity analyzed by method of caseinolytic activity with modifications. For determination selective activity of MMP and serine proteases to the reaction mixture (final concentration) was added 0.2 mol/l EDTA or PMSF, respectively. [9] The level of α1-AT and α2-MG were determined using the method Nartykova. [10]

The statistical analysis of the obtained results was performed using the methods of variation statistics and correlation analysis using the computer program Excel. To determine the reliability of the differences between the two samples we used the Student test (t). Whereby differences $P < 0.05$ were deemed reliable.

RESULTS

We have determined the overall activity of proteolytic enzymes in the blood plasma of rats in case of alkaline and acid esophagus burning (Fig. 1). It was found that the overall proteolytic activity increased by 50% in animals with alkaline esophageal burn and by 41% in animals with acid esophageal burn on the 1st day.

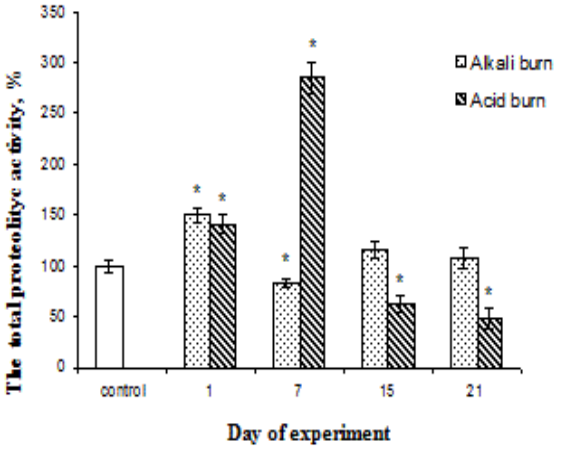

Figure 1. The total proteolytic activity in plasma (caseinolytic units/mg protein) after experimental burn (M±m, n=8) (*p<0,05 compared with control value).

We observed the tendency to decreased the overall proteolytic activity on the 7th day in animals with alkali esophageal burn. Instead, in case of acid esophagus burn development, overall proteolytic activity was increased by 185% compared with control values. In the later stages of the experiment there was no seen significant changes in the overall activity of proteolytic enzymes for alkali esophagus burn. Instead, there was shown decrease in overall proteolytic activity of acid esophagus burn on 21st and 15th day by 37% and 51% respectively.

It was observed increase the activity of MMP by 20% on the 1st day in animals with alkali esophageal burn (Fig.2). On the 7th day of experiment it was shown decrease MMP by 60%, compared with control values. On 15th and 21st day, MMP activity was increased by 60% and 50%, respectively, which may indicate an active

process of the wound interface epithelialisation. When acid esophagus burn development, maximal increase MMP activity was observed on the 7th day of the experiment. On 15th and 21st day mentioned protease activity was reduced by 57% and 71%, respectively, compared with control values.

We have also studied the activity of serine proteases in case of chemical burns development (Fig. 3).

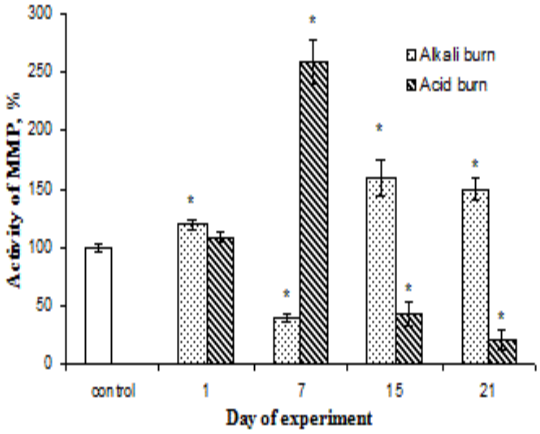

Figure 2. Activity of MMP in plasma (caseinolytic units/mg protein) after experimental burn (M±m, n=8)
(*p<0,05 compared with control value).

There was shown increase the activity of serine proteases during 1 day in case of alkali and acid esophagus burn development by 20% and 44%, respectively, compared with control values. On the 7th day of the experiment there was established decrease in activity of these proteases when alkali esophagus burn development by 60% compared to the control values. Instead of this, in case of acid burn development there was shown sharp increase in these proteases by 98%. At 15th and 21st day of alkali esophagus burn development, a tendency to increase this indicator was shown. When acid burn development, serine proteins activity was reduced to 15th and 21st day.

We have investigated the level of α_2-macroglobulin (α_2-MG) and α_1- antitrypsin (α_1-AT) in research blood plasma for development of esophagus burn (Fig. 4 and 5).

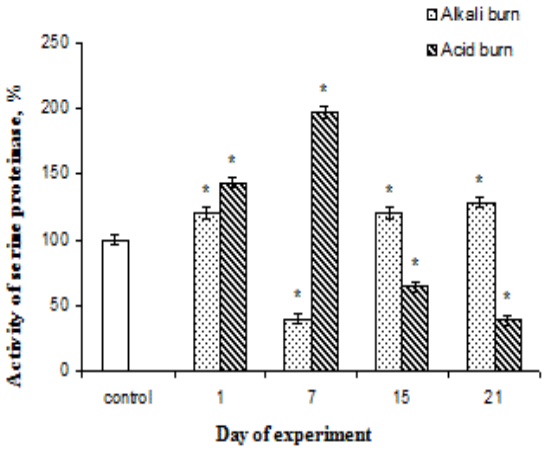

Figure 3. Activity of serine proteinase in plasma (caseinolytic units/mg protein) after experimental burn (M±m, n=8)
(*p<0,05 compared with control value).

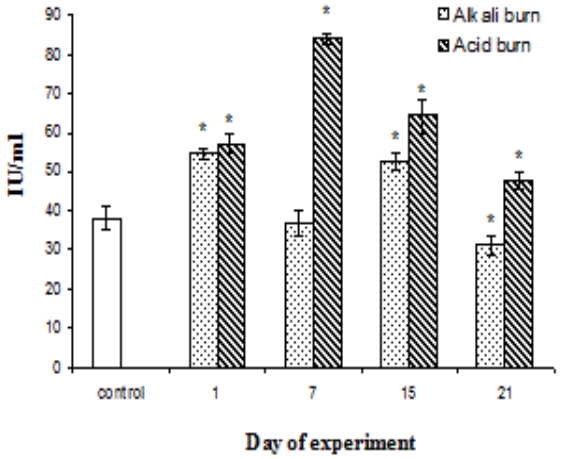

Figure.4. The level of α1-antitrypsin (IU/ml) in the blood serum after experimental burn, (M±m, n=8)
(*p<0,05 compared with control value).

In experimental animals when modelling alkali esophagus burn, α1-AT level was increased on the 1st and 15th day by 43% and 38%, respectively. At 7th and 21st day, significant changes in this indicator were not observed. Whereas in the acid burn development, said inhibitor level was increased throughout the experiment. It should be noted, that maximum level of α_1-AT was measured on 7th day and exceeded the reference value by 120%, that may be the result of compensatory increase of proteolytic enzymes, mainly serine proteases.

When alkali burn development, progressive increase of α2-MG during the experiment was determined. It should be noted, that the level of this inhibitor was

highest at 21st days and exceeded the reference value by 88%. Another trend was observed when acid burn development, level of α2-MG was increased only on 7th and 15th day by 34% and 54%, respectively, compared with control values. Among proteolytic enzymes, metalloproteinases and serine draw particular interest.

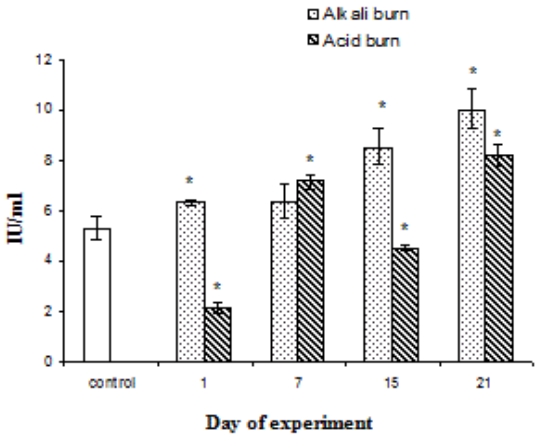

Figure. 5. The level of α2-macroglobulin (IU/ml) in the blood serum after experimental burn (M±m, n=8) (*p<0,05 compared with control value).

DISCUSSION

The progress of the wound process after chemical burns is characterized by cooperation of different metabolic systems of the body as well as contacting tissue, and is controlled by different types of biochemical homeostasis support. A very important role in the wound process belongs to the proteolytic enzymes catalysing protein molecules breakdown. [11,12] Thus the enzymes through a nonspecific proteolysis take part in elimination of necrotized tissues of damaged proteins from the wound. The essential role in physiological balancing of synthesis and proteolysis belongs to the protease inhibitors. These specific proteins prevent abnormal destruction of protein compounds allowing to timely slow down or stop any biological process. [13,14] A disorder in protease-antiprotease system may be a reason as well as a consequence of a pathologic condition. But an abnormal activation of proteolysis leads to damaging of native ("undamaged") tissue proteins, favours to the inflammation processes, the progress if which is connected to an intensive destruction of extracellular matrix and migration processes in cells. On the other hand, the insufficient activity of proteinases accompanied by a prolonged and abnormal build-up of matrix components leads to slowing down of healing of the wound and nascence of a coarse tissue of the scar due to abnormal build-up of collagen. [15-17]

The most typical of serine proteases are chymotrypsin, trypsin, plasmin, elastase, urokinase, kininogenase plasma and some coagulation factors. Thus, increased activity of serine proteases in the extracellular environment is considered as the main link for the development of many pathologies in which tissue infiltration by neutrophils is that, according accompanied by inflammatory reaction. [4] It is known from the literature that the main feature of MMP is degradation of ECM components. However, new impetus for more detailed research of MMP functions have become information that besides destructive one, the indicated proteases can perform a regulatory function, making the processing of biologically active substances. MMP are involved and control numerous processes: proliferation, adhesion, migration, differentiation and apoptosis. [18,19]

As for the results, increasing the proteolytic activity on the 1st day probably is connected to the fact that during burn shock there is significant activation of proteolytic enzymes under the influence of catecholamine, Ca2 +, H +, and other factors. Also as a result of hypoxia and metabolic acidosis damage of cell membranes, including lysosomal ones is happening. Thus, proteases resulting in damage to cell membranes, enter the vascular bed.

Reduced activity of proteolytic enzymes on 7th day of alkali development may confirm formation of complexes with inhibitors and their elimination from the bloodstream. The opposite trend data when acid burn development may be due to the fact that during the first two weeks after

burning in the area of injury is a significant increase in activity of proteolytic enzymes, due to the rejection of necrotic tissue.

Activation of proteases in serum on 21st day of alkali burn development maybe is associated with inflammation, and reflects vascular disorders and abnormal connective tissue. Degranulation of neutrophils and transition MMP and serine proteases in the blood flow is a mandatory part of inflammation. [20] Also MMP are produced by phagocytes that in the interaction of neutrophils play a key role in inflammation. [21] Raising these proteases in the bloodstream may be associated with excessive production by activated monocytes of blood and formation of excessive MMP precursors under oxidative stress influence. [22]

On the one hand, in the process of inflammation, as fast as possible mobilization of biochemical and immunological reactions occurs. However, the other - a long-term process of inflammation is one of the causes of pathological changes in organs and tissues. When hypoxia and microcirculatory disorders inflammatory process becomes pathological, cells actively synthesize collagen. As a result, formation of collagen predominates over its collapse, resulting powerful fibrosis tissue in the form of scars can develop. [16]

From the literature it is known that increasing the activity of α2-MG occurs in some liver diseases, diabetes, cancers, subacute and chronic inflammatory diseases, inflammation in the stage after burns. [23] Increasing the total proteolytic activity in blood plasma of rats during the experiment leads to a compensatory increase in activity of serum inhibitors. Increased α2-MG level can also indicate inflammation process.

Thus, as a result of the research it's shown that the development of alkali burn there was observed increased activity of MMP and serine proteases on 15th and 21st day against a background of increased levels of α2-MG. However when modelling acid burn, decrease of proteolytic enzymes on 15th and 21st day and increased α1-AT level during experiment was observed.

CONCLUSION

So, multidirectional change and proteases and their inhibitors on development of alkaline and acid burns was shown. Thus, when the affected esophageal by alkali there was increased activity of melato- and serine proteases, increased α2-MG level in the last term of research. Instead, when acid burn development, maximum increase of proteolytic enzymes on 7th day of the experiment on the background of elevated α1-AT level during experiment was observed.

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