ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 7 July 2019

Research Article

Influence of Proteolytic Enzymes of Donated Blood on the Survival of People with Gunshot Wounds

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Received: May 02, 2019; **Published:** June 03, 2019

DOI: 10.31080/ASMI.2019.02.0261

Abstract

The purpose of the work. Determination of the presence in the donor blood of a person trypsin-like proteinases and their inhibitors and the impact on the survival of people with gunshot wounds.

Keywords: Enzymes; Blood; Gunshot Wounds

Introduction

Enzymes play a significant role in the functioning of biological systems, providing, in fact, their livelihoods. In the tissues of macroorganism are widely represented enzymes possessing proteolytic activity. They are taking the lead in Homeostasis and regulate the activity of many systems of the body [1,2]. The special role of proteolysis plays in the process of inflammation, which initiates a universal nonspecific cascade mechanism of proteolytic enzymes both locally and at the systemic level [3]. The main inhibitors Proteinas human blood serum is glycoproteins: α_1 -inhibitor proteinas and α_2 -macroglobulin, which make up 75-80% of the α-globulin fraction of blood serum. These are synthesized Inhibitors are mainly in hepatocytes and in smaller numbers – in Monocys [2]. The strongest is α_1 – inhibitor proteinase, which provides 90% antiproteinase activity of blood serum. We have established in the experiment and assumed role in the pathogenesis of influenza A and in the proteinase system [4,5], Forced us to study the blood of a healthy person for the presence of trypsin-like proteinases and its inhibitors [6,7]. According to our data, under the action of the virus in the cell there is a violation of the dynamic equilibrium between trypsin-like proteinases and their inhibitors [8,9].

When searching for biological material for the antiviral drug for the treatment of influenza and other SARS, we studied the human donor blood to detect the presence of the components of the proteolytic system.

Materials and Methods

In The work used canned red blood mass of donated blood 30 people, received from the resuscitation Department of the 2nd City Hospital of Odessa; blood plasma of people of four groups (I-IV, 2 series), received from the station from the station Transfusion of the city of Odessa.

In all samples the protease activity was determined according to the method of K. N. Veremeenko, the content inhibitor the method of A. P. Levitsky and the general protein by the method J. Lowry.

Results

In The intensive care unit of the hospital for 6 months.

There were 30 male patients, of whom 15 were with the I-th group of blood, 11 people- with II-th, 4 persons-WITH IV-th, with Group III did not received. All the patient was carried out transfusion of donated blood. In the course of the research it was established that human donor blood contained trypsin-like proteinase and its endogenous inhibitor.

In general, it can be noted quite a wide range of indicators: for the activity of proteinase from 5.31 to 29.45 mmol Arg/min/mg protein, even more it was for the activity of the inhibitor from 4.0 to 186.83 cu. of fluctuations for proteinase amounted to 5.0 times, and for Inhibitor more than 25.0 times. This variability indicates a sufficiently high sensitivity of the proteinase/inhibitor system, as well as its dependence on many factors.

On average, regardless of blood groups, the activity of trypsin-like proteinases and the content of its inhibitor amounted to 21.4 ± 1.96 mmol arg/Min protein mg and 62.7 ± 7.31 cu, respectively. Should be To note that the greatest activity of the enzyme is set in the blood of the SECOND blood group (22.85 ± 2.06 mmol arg/min mg protein). Enzyme activity in the donated blood of the I-th and IV-th blood groups was almost the same and amounted to 20.47 ± 1.89 mmol arg/min mg protein and 20.67 ± 1.84 mmol Arg/min per mg protein, respectively. The activity of inhibitor trypsin-like proteinase was the highest and almost the same in donor blood I-th AND IV-blood groups and amounted to 70.07 ± 6.80u. e and 70.07 ± 6.80u. e, respectively. In donor BLOOD II-th blood group Activity inhibitor wasaleas ther– 51.59 ± 5.06cu, while the activity of trypsin- like proteinase-the highest among the studied blood groups (Figure 1). This imbalance can be explained by the deficiency of the synthesis of endogenous inhibitor trypsin-like proteinase. The existence of hereditary causes of such Imbalances Cannot be ruled out. In addition, it should be emphasized that regulatory influences and additional impacts are likely to be more susceptible to the content of inhibitor, and therefore to the impact on This system is also possible through a change in its content.

Figure 1: Protein content, the activity of trypsin-like proteinase and the content of inhibitor trypsin in donated blood (note: O/I blood group (n = 15); A/II (n = 12); B/III – absent; AB/IV (n = 3)).

However, from the obtained results it is not clear in what component is trypsin-like proteinase: cellular or plasma blood.

As our further studies have shown, the serum of donated blood also possessed a high enough activity of trypsin-like proteinase (Figure 2). The highest activity trypsin-like proteinase possessed IV-i Group of blood against the background of the protein content (Figure 2).

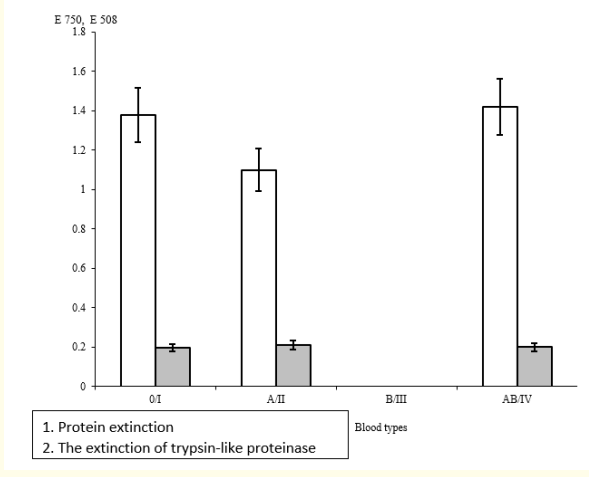

Figure 2: Trypsin-like proteinase activity and protein extinction in the blood serum of donors (Note: O / I blood group (n = 15); A / II (n = 12); B / III - absent; AB / IV (n = 3)).

In the next stage of work we have studied red-cell mass. In the red blood mass of donated blood, on average, the activity of trypsin- like proteinase was 1.5 times higher than in the blood serum (Figure 3).

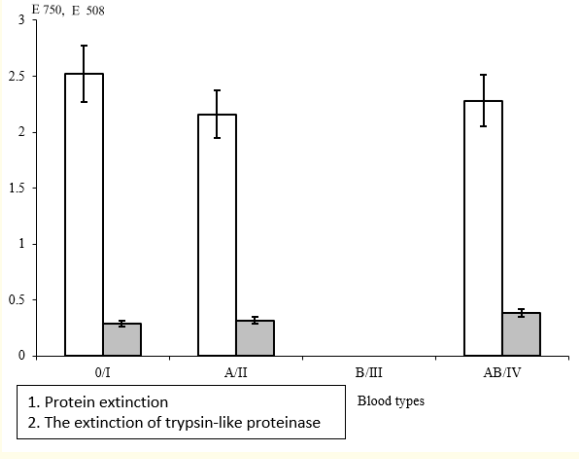

Figure 3: Activity of trypsin- like proteinase and protein exection in the red blood mass of donors (note: O/I blood group (n = 15); A/II (n = 12); B/III – absent; AB/IV (n = 3).

The activity of trypsin-like proteinase in the red blood mass of donated blood of the 4th Group was 2.0 times higher than in the blood serum of donors of the same blood group. Protein content in the red blood mass of all Blood groups was 2.0 times higher than in blood serum donors of the same blood groups.

Proceeding from the fact that trypsin-like proteinase could be either connected with a cell membrane, or is in cytoplasm, the next stage of work is executed. To increase the output of the enzyme was applied the method of hemolysis of red blood cells. For this purpose one part of the red-blood mass of the donation was subjected to 2-fold freezing and thawing, and the second part of the red-cell mass was left unchanged.

As the results of the researches presented in Figure 4, showed 2-fold freezing and thawing did not affect the output of the enzyme. This data suggests that trypsin-like proteinasis is most likely fixed on the membrane of red blood cells, and not in their cytoplasm.

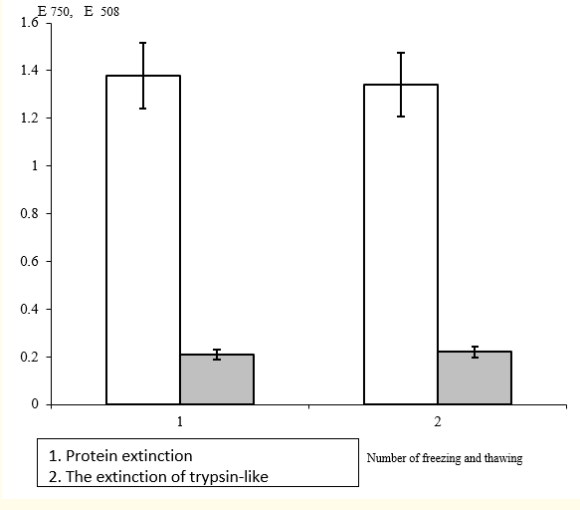

Figure 4: Effect of freezing and thawing on the activity of trypsin-like proteinase and protein content in human donated blood (1 – before freezing; 2 – after 3 multiple freezing).

We analyzed the distribution of proteinase in blood plasma. The high enzyme activity was also found in plasma, while the protein content in the serum and in the blood plasma was n the same (Figure 5).

The most important characteristics of trypsin-like proteinase should be attributed to its stability in storage. Thus, it was found that the duration of blood donation does not affect the activity of the enzyme. Thus, high activity trypsin-like proteinase persisted during 32 day, after the collection of blood (Figure 6). Therefore, donor blood stored more than 21 days (the deadline for the storage of donated blood for transfusion) and Subjected to recycling after this period, can be used for the allocation of trypsin-like proteinase.

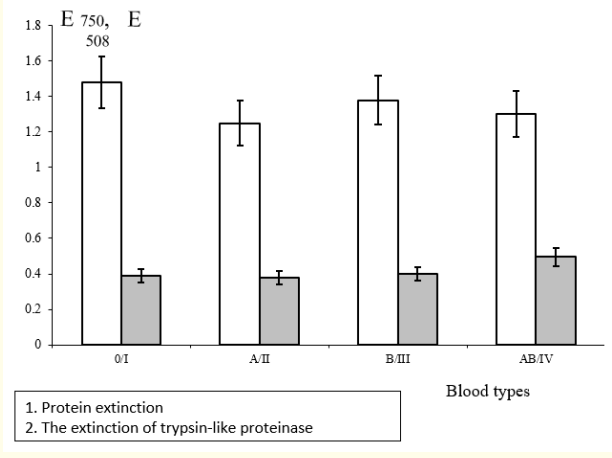

Figure 5: Trypsin-like proteinase activity and protein extinction in donor plasma (Note: O / I blood group (n = 15); A / II (n = 12); B / III - (n = 3); AB / IV (n = 3).

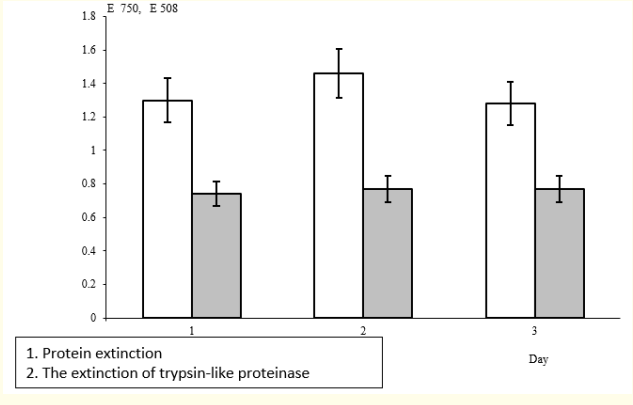

Figure 6: Effect of storage duration on the activity of trypsin- like proteiase and protein content of donated blood (note: 1 – 10 days; 2 – 21 days; 3 – 32 days.

Based on the fact that the participation of trypsin-like proteinase in physiological and pathological reactions depends largely on the relationship with the activity of its inhibitor. In the following, we have defined inhibitor to characterize this system of regulation.

According to the results of our research, fresh canned blood Whseula A large number of not only trypsin-like proteinase, but also inhibitor trypsin-like proteases.

In donor blood plasma activity inhibitor trypsin- like proteinase was unreliable higher in donors with the SECOND Group of Blood (Figure 7). Therefore, the group features of blood are also characteristic for the content of inhibitor. Follow-up Research was aimed at studying the distribution and ingibitor activity in the blood components.

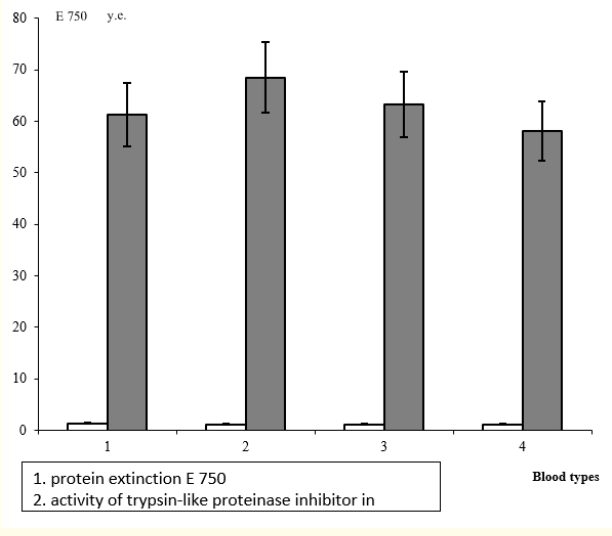

Figure 7: Activity inhibitor trypsin-like proteinase (in Cu) and the action of protein in the blood plasma donors (Note: 1-O/I blood group (n = 15); 2-A/II blood group (n = 12); 3-B/III blood group (n = 3); 4-AB/IV blood group (n = 3); 1 cu corresponds to 1 mg inactivated crystal line trypsin).

Thus, in erythrocytes the activity of inhibitor trypsin-like proteinase was reliably higher in donors with the IV-th group of blood and reliably lower-in donors with the SECOND Group of Blood (Figure 8).

In the storage of the donor of the blood during the year at a temperature of-180with the activity of inhibitor trypsin -like proteinase was 5.0 times (from 450.0 to 70.0 cu).

Thus, the average studied indicators in the donor blood with the I-th group sing were: Activity trypsin-like proteinase-20.47 ± 1.89 mmol/arg/min per 1 mg protein; Content of inhibitor-70,07 ± 6,80 cu; Protein content – 3.90 ± 0.34 mg/ml. The least amount of inhibitor was 8,74-9,36 cu from two donors. Average figures in the donor of the blood for the wounded from the SECOND group of blood were: the activity of trypsin-like proteiase-22,85 ± 2,06 mmol/arg/min per 1 mg protein; Inhibitor Content-51.59 ± 5.06 cu; Protein content – 3.51 ± 0.32 mg/ml. The lowest index of inhibitor content was 10.19 USD in one donor. For the IV-th group of blood indicators were: the activity of trypsin-like proteinase-20.67 ± 1.84 mmol/arg/min per 1 mg protein; Inhibitor content-70.26 ± 8.11 cu; Protein content – 4.45 ± 0.41 mg/ml. The smallest indicator of inhibitor amounted to 4.00 cu in one donor (1 CU corresponded to 1 mg inactivated crystalline trypsin).

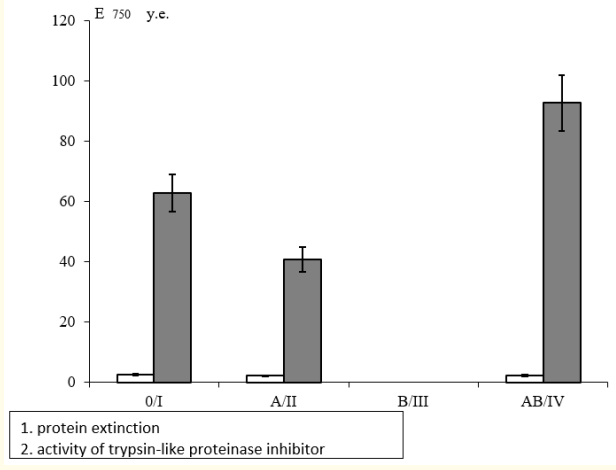

Figure 8: Activity inhibitor trypsin-like proteinase (CU) * And protein exection in the red blood mass of donors (Note: O/I Blood group (n = 13); A/II (n = 12);B/III was absent; Off/IV (n = 5).

After 1 year it was clarified that four patients with gunshot wounds, who had been over flown with very low rates of inhibitor trypsin-like proteinas, died from this group.

Conclusions

The Human Donor's shelter contained a trypsin- like similar (Serine) proteinase and its inhibitor. The presence of donated blood with low levels of inhibitor was not suitable for patients with gunshot wounds. The received results of further research to confirm our assumptions.

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