BIOSPECIFIC HEMOSORBENTS. THE SUCCESSES AND PROBLEMS

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ABSTRACT	Understanding the leading role of the endogenous proteolysis activation in a number of serious diseases has existed for a long time, the large group of antifermental drugs has been created and widely used. The hemosorbent, capable of selectively removing from the blood the most significant class of serine proteases in purulent septic pathology, was developed and tested in the clinic. The nature of the hemosorbents` therapeutic effect and the status of the proteins metabolism certain parts in connection with the application for haemosorption with biospecific antiproteinase hemosorbents has been studied in patients with diffuse purulent peritonitis of appendicular origin.

Keywords:	hemosorbent, peritonitis, appendicitis, antiproteinase hemosorbent, detoxification.

ABP — arterial blood pressure
CVP — central venous pressure
DPP — diffuse purulent peritonitis
HR — heart rate
RR — respiratory rate
TLA — trypsin-like activity

BACKGROUND

A popular expression of the founder of medicine Hippocrates is widely known: medicine is the art of adding and subtracting, adding what is missing, and subtracting what is excessive, and the best doctor knows how to do it, but if he doesn't, then he is far from the art of healing. The development of "adding what is missing" is traditionally performed by the best minds of mankind. Today, thousands of new forms of drugs are developed and put into production. Its irrational use leads to severe forms of polypharmacy. Moreover, considering "advances" of modern food industry, populations consume a huge number of xenobiotics which metabolism is not always sufficiently understood.

In this context, the development of devices and methods for extraction of pathogenetic compounds froom blood, is extremely urgent. The widespread adoption of methods, promoting its bonding and accelerated transport to biotransformation system and excretion, helped dramatically expand possibilities of modern medicine in the treatment of various pathological conditions [1]. Its main pathogenetic mechanism is excessive formation and accumulation or entry from the outside of biologically active or toxic substances. This is the way a new field of "efferent medicine" was established [2, 3].

Leaving aside methodological aspects of other methods that make up this line of efferent medicine, we would like to concentrate on the techniques which ensure the direct extraction of pathogenetically meaningful compounds with various hemosorbents. So, in the late 70-ies in the Soviet Union, a group of authors under the leadership of Y.M. Lopukhin developed and applied methods of rehabilitation with the help of blood uncovered coal hemosorbents [4, 5]. However, with all the advantages of this technique allowing to extract comounds of ulimited molecular weight including water-insoluble substances, it has several disadvantages. Also, it was clear that the main therapeutic action was extraction of compounds, indirectly related to the end metabolites such as bilirubin, urea, creatinine, etc. Y.M. Lopukhin suggested to develop new-generation hemosorbents which could remove specific pathogenetic metabolites or a group of such metabolites. This process was called "metabolic amputation" [1].

While studying possible causes of severe functional and metabolic abnormalities in a number of serious diseases, including organs for natural detoxification (liver and kidneys), it was found that unlimited activation of endogenous proteolysis system and exhaustion of anti-proteolytic resourses were major causes not only of severe degeneration of parenchymal organs due to the collapse of their structural proteins, but also the appearance of abnormally high concentrations of protein metabolism intermediate products and its derivatives in fluid environments. These substances, according to experts, dealing with this issue, have a wide variety of pathobiological and toxic properties [2, 3].

Understanding the leading role of endogenous proteolytic system activation in a number of serious diseases has existed for a long time. Numerous antifermental drugs were developed and applied. However, an analysis of the results of its application allowed one of the domestic medicine giants to make an unambiguous conclusion that the hopes for high efficiency of antifermental drugs were not justified [5].

Considering this, we developed and tested a hemosorbent capable of selective removal of the most significant serine proteases from blood in septic diseases in collaboration with Institute of Petrochemical Synthesis and Moscow State University of M.V. Lomonosov. The concept was the use of ovomucoid covalently bonded to

polyacrylamide gel matrix (a protein of duck eggs) [6, 7].

The polyacrylamide hydrogel does not dissolve in water, but has hydrophilic properties and swells. It is similar to living tissues: elastic, permeable to many molecules and contains a large amount of water. The blood interacts with quite a biocompatible surface under the perfusion through this hemosorbent. The polyacrylamide hydrogel has pores which provide effective transport of extractable substrates and binding with ovomucoid (Fig. 1).

Ovomucoid immobilized on the matrix acts as a ligand selectively binding serine proteases and forming a strong complex, having a dissociation constant of about 10^{-10} [6].

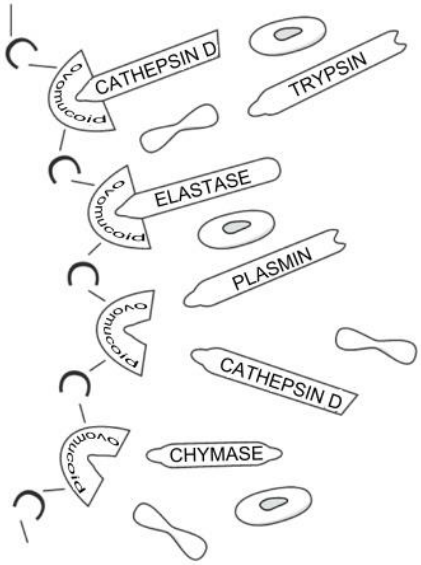

Fig. 1. The concept scheme of the biospecific antiproteinase hemosorbent

MATERIAL AND METHODS

The therapeutic effect of biospecific antiproteinase hemosorbent and the status of certain sraged of the protein metabolism were studied in patients with diffuse purulent peritonitis (DPP) of appendicular origin.

The study included 104 patients (18–89 years) with this complication. All patients underwent medical therapy in intensive care units. Patients had significant impairment of consciousness even up to delirium, respiratory failure, severe tachycardia, unstable blood pressure with a tendency to hypotension, intestinal paresis manifesting as dynamic ileus and reduced renal excretory function. As shown in the Table 1, some patients also had leukocytosis, accelerated erythrocyte sedimentation rate, and toxic granularity of neutrophils.

Table 1

Changes of biochemical parameters of blood plasma in patients with diffuse purulent peritonitis of appendicular origin in the complex therapy including biospecific antiproteinase hemosorbent

Index (normal value)	Before hemosorption	After hemosorption	24 hours after hemosorption
Total protein (64-83 g/l)	55.4±2.2	54.4±2.2	55.2±2.0
Albumin (35-50 g/l)	25.1±1.1	25.8±1.3	23.8±1.4
Fibrinogen (2-4 g/l)	4.8±0.8	4.7±0.9	4.6±0.7
Trypsin-like activity (16-67 nmol/(s · l))	28.3±9.4	14.5±2.9*	28.7±16.1*
Plasminogen (80-135%)	185.9±13.5	190.0±16.0	194.0±14.0
Alpha-1-proteinase inhibitor (5-10 μmol/(s · l))	14.9±0.1	15.1±0.8	14.2±2.1
Alpha-2-macroglobulin (0.7-1.2 μmol/(s · l))	0.33±0.04	0.34±0.08	0.35±0.06
Products of fibrin degradation (<100 mcg/ml)	155.0±3.0	130.0±1.0*	112.0±2.0*
Middle molecules (0.35±0.01 g/l)	1.7±0.15	1.79±0.07	1.28±0.06
Urea (4-8.3 mmol/l)	8.58±0.94	8.28±1.04	7.9±0.9

Note: * — significant differences in parameters

Prior to manipulation, one of central veins was punctured and cannulated with a dual-lumen catheter. Bolus heparinization included 5,000±2,500 IU of heparin. Upon hemosorption, the rate of perfusion through mass exchanger averaged 60±10 ml/min within a group, the procedure duration averaged 90±15 min. The perfused blood volume per 1 procedure was 5,400±120 ml.

The following parameters were measured before and after hemosorption: heart rate, arterial blood pressure, central venous pressure, respiratory rate, as well as the number of red blood cells, white blood cells, band neutrophils, lymphocytes, platelets, body temperature, erythrocyte sedimentation rate, hourly diuresis. Simultaneously, we measured the level of total protein in blood, albumin, fibrinogen, urea and trypsin-like activity of plasma, cathepsin *D,* plasminogen, prekallikrein, kallikrein, alpha-1-proteinase inhibitor, alpha-2-macroglobulin, fibrin degradation products and middle molecules.

The statistical analysis of findings was performed using the Student's test and non-parametric Mann-Whitney test, 2x2 contingency tables and χ^2 criterion.

RESULTS

The condition of patients before hemosorption was assessed as critical or extremely critical.

As shown in the Table 1, immediately after a session of Ovosorb hemosorption, a statistically significant decrease in proteasemia was observed, while trypsin-like activity (TPA) of plasma reduced by 2 times.

A day after the procedure, the level of TPA in plasma grew up to previous values. The change in the proteinase inhibitors concentration was not statistically significant. The study of cathepsin *D* activity in the blood plasma of these patients showed that its concentration change was not statistically significant by the end of the 1st hemosorption (131.8±19.5 U), but significantly diminished the next day (from 153.6±17.2 to 115.0±12.1 U). The downward trend of cathepsin *D* (lysosomal acidic peptid hydrolase not inhibited by the ovomucoid) indicates a decrease in activation of tissue proteolysis system as a result of elimination of serine proteases with Ovosorb. According to findings, either directly after hemosorption or a day after, the level of plasminogen remained unchanged, while the fall of fibrin degradation products was statistically significant. The concentration of α-1-proteinase inhibitor and α-2-macroglobulin remained unchanged.

It should also be noted that this type of sorbent, resulting in normalization of plasma TPA and restoration of blood inhibitory capacity, doesn't significantly affect concentration of total protein, albumin, urea, and MM. However, a day after hemosorption, a decrease and a subsequent gradual normalization of blood levels of urea, MM, total protein and albumin were noted. Changes in parameters characterizing the main trends in the protease-inhibitory status in this disease, are shown in Fig. 2 and 3.

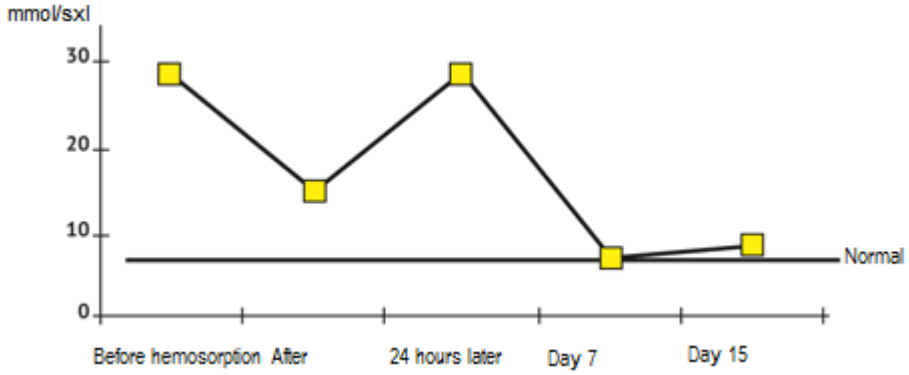

Fig. 2. Changes of trypsin-like activity of blood plasma in patients with diffuse purulent peritonitis of appendicular origin, who underwent hemosorption with "Ovosorb": 1 — the average values before hemosorption; 2 — after hemosorption; 3 — a day after hemosorption; 4 — on day 7; 5 — on day 15.

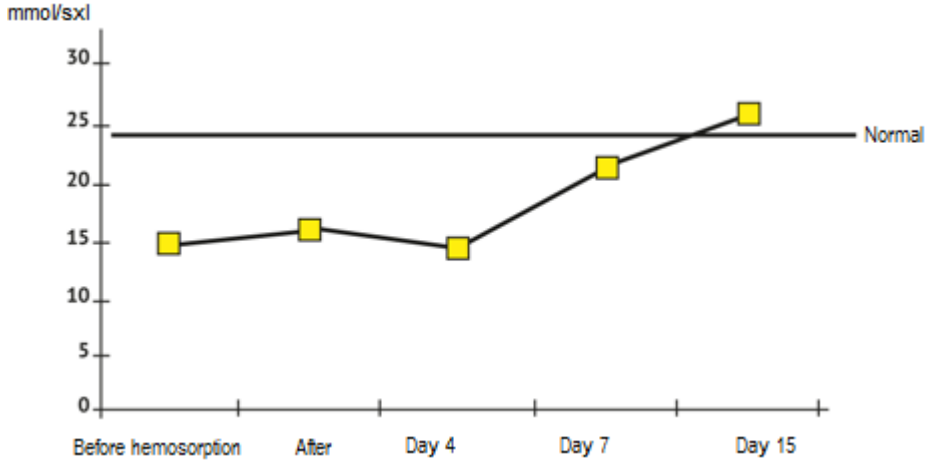

Fig. 3. Changes of alpha-1-proteinase inhibitor concentration in blood plasma in patients with diffuse purulent peritonitis of appendicular origin, who underwent hemosorption with "Ovosorb": 1 — average values before hemosorption; 2 — after hemosorption; 3 — a day after hemosorption; 4 — on day 7; 5 — on day 15.

In abdominal sepsis, the mortality of patients reaches more than 50% according to different authors [8-10]. As shown in the Table 2, the inclusion this type of hemosorbent in the postsorption period, along with the

improvement of some indicators of protein metabolism lead to a marked reduction in the severity of clinical and laboratory signs of endogenous intoxication and clinically detectable intensed of peristalsis. Detoxification effect manifested as reduced or eliminated encephalopathy signs, decreased heart rate, shortness of breath, leukocytosis, increased hourly diuresis. Hemosorption did not result in statistically significant changes in body temperature, arterial blood pressure, hemoglobin levels, erythrocyte sedimentation rate, and lymphocytes.

Table 2

Change of clinical parameters in patients with diffuse purulent peritonitis of appendicular origin after sorption with the biospecific antiproteinase hemosorbent

Index	Before hemosorption	After hemosorption	*p*
Body temperature, °C	37.6±0.3	37.1±0.2	
HR, beats/min	112.6±2.5	100.0 ±1.2	<0.05
CVP, mmAq	+19.0±1.7	+17±1.0	
RR, breaths/min	28.0±4.0	20.0 ± 2.0	<0.05
Hourly diuresis ml/hour	45.0±2.0	56.0±3.1	<0.05
ABP, mmHg			
- max - min	133.0±3.0 80.0±2.1	124.0±2.0 80.0±1.1	
Hemoglobin, g/l (12-14)	13.2±0.7	13.0±0.9	
Leukocytes (10^{9}) (4-9)	13.4±0.4	10.4±0.7	<0.05
Band neutrophils,% (1-6)	10.8±0.4	10.7±0.4	
Lymphocytes,% (19-37	16.1±0.5	20.4±0.8	
ESR, mm/h (1-15)	33.7±1.2	34.5±1.1	

Note. *P* values are indicated in the presence of significant differences of the studied parameters. ABP — arterial blood pressure; CVP — central venous pressure; ESR — erythrocyte sedimentation rate; HR — heart rate; RR — respiratory rate.

Despite the intensive care and early inclusion of hemosorption into complex treatment in the postoperative period, the detoxification effect after the 1st session was noticeable, but unstable. Therefore, repeated procedures were required to achieve a sustained improvement in the condition of patients. According to our research, after 2-3 sessions of hemosorption, stabilization occurs in half of patients in this category. In some cases, 5 or more sessions are required. Every patient in the group underwent 3.4±0.6 hemosorptions, averagely. Long-lasting therapeutic effect, manifesting as elimination or reduction of endogenous intoxication severity, signs of inflammation of the peritoneum, and the recovery of bowel function, was noted in 99 patients. Five patients died with symptoms of chronic heart and cardiopulmonary failure, mainly pre-existing and growing after surgery. The postoperative mortality was 4.8%.

CONCLUSION

These clinical data confirm the high efficiency of antiproteinase biospecific hemosorbent in management for protein metabolism disorders and associated systemic disorders in patients with DPP of appendicular origin. It is evident, that detoxification and other positive effects after perfusion of blood through the antiproteinase hemosorbent is associated with its influence on hyperactivity of endogenous proteolysis systems which is one of the key elements in the pathogenesis of peritonitis. The duration, sequence and frequency of hemosorption is determined in each case individually, depending on the severity of the proteinase inhibitory capacity disorder and related disorders of protein metabolism, as well as the severity of microhemodynamics disorders, paresis of the intestines, signs of liver and kidney failure and others. Even until now, we may conclude on the severity of protease-inhibitor balance disorders basing on indirect signs. Methods for determining TPA, the level of cathepsins, elastase and other important pathogenetic proteases and antiproteinase capacity are not available for adoption in the clinical practice. Therefore, the state of this system is still assessed by the nature of dysproteinemia, level of intermediate and final products of protein metabolism — "middle molecules". As shown by clinical experience, this class of metabolites is most closely correlated with the severity of endogenous intoxication and the progress of the pathological process, and therefore, it must be more widely used to assess the effectiveness of detoxification and changes of postoperative period.

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