The use of immunosorbtion for treatment of critical care conditions due to sepsis

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We have the new method of treatment the critically ill patients by using extra corporal immunosorbtion, while preparing immunosorbment it was fixed antistaphylococus immunuglobulin molecules by glutare aldehide at the activated biogel granules. Immunosorbtion went well for treating the critical ill patients, their clinical picture was complicated by staphylococus sepsis. The usage of selective sorbment prepared with this rule was announced.

Kay words: Critical care, Sepsis, Immunosorbcion, extracorporal prfusion, selective sorbent.

Actuality

In the last years cases of sepsis are very frequent. This is evidenced by the fact that annually 1,5 millions of people all over the world get sick with this pathology. As well index of death cases due to sepsis is high and it reaches up to 30% in the clinics of critical care. Much money is spent for treatment of sepsis, as the treatment costs in critical care clinics amounts USD 70000-90000 and the same amount is spent for rehabilitation of survived patients (N. Kajaia and other 2006).

So sepsis problem use to be one of the main problems of critical care medicine.

In sepsis pathogens the leading factor is an infectious agent, egzotoxins and endotoxines which are circulated in organism via blood or lymph and cause damages of different types of their tissues and cellular.

In sepsis development the condition of macro organism, especially of its immune system, is as well important.

By its help we divine infectious agent having unknown antigen structure and immune response is originated against it. This is frequently followed by disorder of cetaceans and development of anti-inflammatory system reaction, which either plays a big role in sepsis formation (Z. S. Kheladze, 2005). Hence, the most important in sepsis treatment is suppression of pathologic effect of micro organism incorporated in macro organism. Presently it is done by directly operating on micro organism (antibiotics, immunoglobulin medicines and other). For this consideration it is important to use the methods of blood extracorporeal perfusion (hemi sorption, plasma sorption, lymph sorption and other), by means of which it is possible to eliminate microbes and their toxins out of organism. Unfortunately use of these appeared to be of less effective while sepsis treatment; as, while conducting, erythrocytes, lymphocytes and other components necessary for life are deposited and damaged together with pathological components.

Hence this, it gets actual to create and conduct such means, which are able to perform selective deposition of pathological components out of blood and lymph, having been circulating in organism (Z. S. Kheladze and other, 1986). Immunosorbtion is one of such means – blood extra corporation perfusion on immunosorbment. Immunosorbtion was first used experimentally and clinically in 1982, when they eliminated staphylococcus microbes and toxins out of blood of a patient sick with staphylococcus disease (Z. S. Kheladze and other, 1984).

Material and Methods

The work includes stand, experimental and clinical studies. 5 stand studies were carried out. For the stand study they took blood of 4 mongrel dogs of 15-18 kg of mass, 10-45 minutes before.

Before the study the animals have been infused with 10 ml of golden staphylococcus of 100 billiards culture in thigh vein, which contained more than 30, including plasma coagulants and laycocidin synthesized pathogenic stems. At the same time, they intensively were infused with 2 ml of staphylococcus α-toxins, tirer of which was 24 LH. In the result, all the animals were lost and for the stand study the blood was taken after heart stoppage from thigh artery by means of roller type pump. The stand was created between perfusion glass vessel and the vessel with immunosorbment connected to it by perfusion tubes. 0.5 liters of animal blood was in the perfusion vessel and immunosorbment was taken in the volume of 250 ml. Blood perfusion was done with the speed of 0.05-0.1 l/min by means of roller shaped pump placed between vessel pot and immunosorbment in the duration of 15-30 minutes.

Experimental study was done in mongrel dogs of 18-19 kg of mass. In 5 of them after heparanization (80-100 per kg) immunosorbtion was conducted between thigh artery and vein. Blood flow speed was regulated by means of bow-typed pump with 0.05-0.1 l/min speed, and perfusion was continuing during 30-60 minutes.

In 5 animals of the third group they were infusing 10 ml of golden staphylococcus of 100 billions culture and 2 ml staphylococcus α-toxin, tirer of which was 24 LH. After 10-45 minutes from infusing these components they were making blood extra-corporate perfusion between thigh artery and vein with the speed of 0.05-0.1 L/Min during 30-60 minutes.

Clinical material contains treatment by means of immunosobption of 9 aged patients sick with sepsis. Existence of sepsis of staphylococcus genesis in each of them was proved by means of taking out of golden staphylococcus culture from blood and counting containment of staphylococcus α-toxin in blood.

These patients were symphonized with poly-trauma, cranium-brain severe trauma, and acute failure of blood flow in head brain, peritonitis, bronchi pneumonia and flagmony of legs, as parallel pathologies. Their treatment was done by means of lungs artificial ventilation, parentheral feeding and other traditional means of intensive therapy. Antibacterial therapy was conducted with carba- and meropens, III-IV age cephalosporin, amino-glycosides and vancomicine group medicines. Immune sorption in the process of treatment of these patients was used because of effective traditional methods with the background of poly-organic failure. Immunosorbtion was done by 250 ml of immunosorbment placed in perfusion pot; immunosorbment was placed between thigh vein and under collar-bone vein, blood flow speed was 0.05-0.1 L/Min. Perfusion duration – 60-150 minutes.

Immunosorbment for immunosorbtion was prepared in advance using original method (Z. S. Kheladze and other, 1984). Activated bio-gel granules were used as matrix (“Bio-Rad”, USA). “catcher” components were presented by anti-staphylococcus globulin, which were obtained by means of grounding of golden staphylococcus culture of aged horses (I. Giorgadze, P. K. Soloviev, 1973); anti-toxic tirer of staphylococcus in the animals’ hyper-immunized serum constituted 1:2048.0. Anti-staphylococcus globulin molecules were attached to bio-gel granules by means of 25% of glutar aldehyde (“Sigma”, USA). Either in stand as well in experimental and clinical studies before immunosorbtion and after it they studied concentration of staphylococcus α-toxin in blood and immunosorbment, herewith materials taken out from blood and immunsorbtion were sowed in order to discover (observe) staphylococcus culture.

Here with they were studying blood cellular and humor components. Namely they were studying by standard methods containing of erythrocytes, eozinofile. Herewith, they studied containing of immune competitive T and B lymphocytes (helpers, suppressions). In parallel they studied containing of albumen, globulin, urea, creatinine, bolesterine, triglycerides, lipids, bilirubynes, phybrinogen, alaninaminotransferaza, asparatataminotransferanza, creatinfosfokanaza, amilaza, alkali phosphataza, electrolytes (K, Na, Ca, Mg) gas partial pressure (PCO2, PO2) and immunoglobulins (A, M, G). Before contributing immunosorbtion, immunosorbment was studied for pyrogeny, safety and allergy. Study of pyrogenic characteristics was done on 5 rabbits of Shishila breed of mass of 1.5-2.5 Kg. The animals were placed at the temperature of 15-18 three days earlier.

They made them starve during the last night, on the other day they infused them in ear vein with 1 ml/kg doze of immunosorbment and after 1, 2, 3 hours they measured temperature in rectum during 5 minutes (before starting the test temperature of the animals was 28.5-39.5 C). Safe nature of immunosorbment was studied in 5 guinea-pigs of mass of 0.25-0.35 kg; the animals were infused in both sides under skin with 5 ml of immunosorbment and during 7 days they observed development of inflammatory infiltrate and behavior wrongness. Study of allergic nature was done in 10 guinea-pigs of the mass of 0.25-0.35 kg. On the 1^{st}, 3^{rd} and 6^{th} day they infused 0.2 ml of immunosorbment and on the 21^{st} day they were infusing 10 times more doze (2.0 ml) and observed development of anaphylaxis or other reaction of allergic nature. In order to study immunosorbtion canserogenic effect, the group of intact animals (dogs) which were treated with immunosobtion, were treated without infusion of staphylococcus culture and α-toxin, they after one year of immunosorbtion, were treated with autopsy.

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For the same purpose, after a year autopsy was done in the animals (rabbits, guinea-pigs), which previously were used for the study of immunosorbments phylogenic, safety and allergic nature.

Results and Review

The study results, given in the Schedules #1 and #2, shows that before stand studies, abruptly positive (+ + +) culture of golden staphylococcus was taken out from blood, and concentration of staphylococcus α-toxin in blood constituted 921.6 109.8. Regarding immunosorbment, it was sterile before perfusion and there was not observed even staphylococcus α-toxin. After immunosorbtion, slightly positive (+) culture of golden staphylococcus was taken out in three cases, in one case blood was sterile. On this background volume of staphylococcus α-toxin was decreased 2.8+0.2($P<0.001$); Herewith, after perfusion abruptly positive culture (+ + +) of staphylococcus was taken out and staphylococcus α-toxin tirer was 2457.6+439.6 ($P<0.001$).

Same changes were observed while experimental studies in the group of animals, which after infusion of staphylococcus culture and α-toxin, were treated with immunosorbtion. The same Schedules show that before treating immunosorbtion, abruptly positive culture of golden staphylococcus (+ + +) was taken out from the animals' blood and staphylococcus tirer was 819.2 109.2; while after finishing immunosorbtion, abruptly positive (+ + +) culture of staphylococcus was taken out from immunosorbtion and staphylococcus α-toxin tirer constituted 2638.6 659.2 ($P<0.001$), while before perfusion the immunosorbment was sterile. It is to be mentioned that after immunosobtion, all the animals of this group stayed alive. As well all the individuals of those groups which were treated with immunosorbtion without infusion of staphylococcus culture and α-toxin were alive.

Herewith, in this group of animals, immunosorbtion have not caused more or less important changes ($P>0.05$) of blood cellular and humoral components (erythrocytes, thrombocytes, leucocytes, immunoglobulin, ferments and other). With the difference from those groups, all the individuals, without immunosorbtion died in 10-45 minutes after infusion of staphylococcus culture and α-toxin and in future the blood of these animals was used for stand studies.

Results of sowing of staphylococcus culture

	Study group	Blood		Immunosorbent	
		Before perfusion	After perfusion	Before perfusion	After perfusion
d1	Stand studies	Abruptly positive +++	Slightly positive +	Negative -	Abruptly positive +++
12	Intact animals	Negative -	Negative -	Negative -	Negative -
33	Animals after infusion of staphylococcus culture and α-toxin	Abruptly positive +++	Slightly positive +	Negative -	Abruptly positive +++
34	Patient	positive +++	Negative -	Negative -	Abruptly positive +++

As well it is important to mention the results of study of the immunnosorbments, which are connected to establishment of its progeny, safety and allergic nature. It appeared that the immunnosorbment prepared by such rule, is inpirogenic, and causes more or less change of temperature of animals during the first hours after its infusion. (Difference in the temperature of rabbits did not exceed 0.5 C); also its safe nature was established, as there was not observed inflammatory changes in animals (guinea-pig) during the first 7 days after infusion and their behavior was not importantly changed. As well it was not confirmed allergic nature of this immunnosorbments, as on every stage of "immunization" by immunosorbment, including the 21st stage, anafilaxial or allergic reaction of any other type was not developed in guinea-pigs. Results of immunosorbments carcinogenic nature were negative.

“treated” animals (dogs, rabbits, guinea-pigs). Regarding clinical study, while immunosorbtion, there were not developed important changes and effects in the patients and immunosorbtion was adopted by the patients without any difficulties. #1 and #2 Schedules show that before immunosorbtion, golden staphylococcus culture with positive result (+ +) was taken out from the patients blood, as well staphylococcus α-toxin contaminant was high in the blood 91.4 7.1, while after immunosorbtion staphylococcus culture was not taken out from blood in 7 patients, in two patients it was slightly positive (+) and α-toxin tirer constituted 11.6 3.3 ($P<0.001$). After perfusion staphylococcus abruptly positive culture (+ + +) was taken out from immunosorbtions which before were sterile

Schedule №2

Changes of staphylococcus α-toxin tirer

3#	Study group	Statistics data	Blood		Immunosorbent	
			Before perfusion	After perfusion	Before perfusion	After perfusion
1	Stand studies	X±m n	921.6+109.8 4	2.8+0.2 4 <0.001	- 4	2457.6+4396 4 <0.001
2	Intact animals	X±m n	4.4+1.3 5	3.2+1.3 5 >0.05	- 5	- 5
	Animals after infusion of staphylococcus culture and α-toxin	X±m n	819.2+109.9 5	6.4+0.009 5 <0.001	- 4	2638.6+659.2 5 <0.001
	Patient	X±m n	91.4+7.1 9	11.6+3.3 9 <0.001	- 9	1992.9+365.7 9 <0.001

and staphylococcus α-toxin was abruptly increased (1992.9 365.7 P<0.001). It is to be mentioned that in 3-19 hours after immunosorbtion there was observed tendency of increase of staphylococcus α-toxin. This was more abruptly (P<0.005) observed in the patients infection base of which was pumped not completely. This was accompanied by worsening the condition of patients – cold, shiver, increase of temperature and other. This last one indicates less effectiveness of immunosorbtion in the patients whose infection base is not pumped completely. Herewith there appears issue of necessity of repeated immunosorbtion sessions. For example, treatment history of 21 years old man. The patient B.V.G. was moved to clinic from Gardabani regional hospital on 1982/08/05, where he was placed on 20.04 due to working trauma; diagnose – combine trauma, body general licking, burning of second stage of 10% the body surface, cranium-brain severe

This was proved after one year from autopsy immunosorbtion of trauma, break of right collar-bone, meningitis, sepsis. The patient was operated in order of evocation of head brain subdural hematoma. The patient was transported from Gardabani regional hospital on the background of lungs artificial ventilation and it was continuing during the following days. While examination, abruptly positive (+ + +) culture of staphylococcus was taken out from the patient blood, α-toxin tirer was 1:136.0 due to worsening of results of effectiveness of treatment (antibiotics, parentheral and enthrall feeding and other) and condition with septic shock on 15.05.82. Immunosorbtion was done. Perfusion duration was 120 minutes. Perfusion speed – 0.1 l/min. Blood flow direction – from right thigh vein towards the right collar-bone vein. After the immunosorbtion the condition of the patient was gradually getting better. From the second day septic shock was liquidated, after a month consciousness and spontaneous breath was restored.

For further treatment the patient was moved to neuro-surgery clinic. He was released from the clinic in healthy condition. Generally 3 patients from 9, treated with immunosorbtion were died. This equals 33% of lethality.

This data indicates perspectives of usage of immunosorbtion for treatment of critical care condition due to staphylococcus sepsis. Though, in case of appropriate anti-bodies, this method may be successfully used for treatment of anti staphylococcus genesis sepsis. Moreover, in case of appropriate “catcher” components (anti-bodies and anti-gens) many components of pathological nature may be deposited from blood or other biological fluids. This may become certain pre-condition for treatment of many diseases and pathological conditions.

იმუნოსორბციის გამოყენება სეფსისით გამოწვეულ კრიტიკულ მდგომარეობათა სამკურნალოდ

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მოწოდებულია კრიტიკულ მდგომარეობაში მყოფ ავადმყოფთა მკურნალობის ახალი მეთოდი ექსტრაკორპორული იმუნოსორბციის სახით. იმუნოსორბენტის მომზადებისას გააქტივებული ბიოგელის გრანულებზე ანტისტაფილოკოკური იმუნოგლობულინის მოლეკულები გლუტარის ალდეჰიდის მეშვეობით იყო მიკერებული. იმუნოსორბცია წარმატებით იყო წარმოებული კრიტიკულ ავადმყოფთა სამკურნალოდ, რომელთა კლინიკური სურათი გართულდა სტაფილოკოკური სეფსისით. გამოთქმულია მოსაზრება ამგვარი წესით მომზადებული სელექტიური სორბენტების სარგებლიანობის შესახებ.