SCIENCE AND EDUCATION

ISSN 2181-0842 VOLUME 4, ISSUE 10

OCTOBER 2023

SCIENCE AND EDUCATION

SCIENTIFIC JOURNAL

ISSN 2181-0842

VOLUME 4, ISSUE 10

OCTOBER 2023

www.openscience.uz

SCIENCE AND EDUCATION

SCIENTIFIC JOURNAL VOLUME 4 ISSUE 10

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Post-traumatic immunosuppression and the possibility of their correction

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Abstract: Serum concentrations of soluble activation receptors (sCD25 and sCD 95), cytokines (IL-2, IL-6,and TNF-α) were studiedin the dynamics of traumatic disease treatment. Those submitted data points out the development of posttraumatic immunosuppression in the early stages of observation, such as neutrophilic leukocytosis, relative lymphopenia, increase of sCD25 and sCD95, cytokines IL-6 and TNF-α, reduction of IL-2, which are more pronounced in patients at risk of posttraumatic complications development. Basic therapy corrects only some parameters of immunity, but the usage of polyoxidonium entirely recover affected immunological indicators. It is recommended to include polyoxidonium into basic therapy in patients with traumatic disease at early stages.

Keywords: traumatic disease, therapy, immunocorrection, polyoxidonium, soluble receptors, cytokines

Introduction

Immunodepression in case of injury occurs, inspite of the systemic activation of the immune system. It can be represented by different pathogenetically heterogeneous processes [2, 5, 9]. Severe mechanical trauma is accompanied by long-lasting antigenemia, and due to extensive soft tissue hematomas, traumatic endotoxicosis gradually increases, which leads to the development of early purulent complications [1, 10]. At the first stage, the immune system reacts to such antigenic increase by activation of nonspecific reactions caused by secretion of proinflammatory cytokines,when maintenance of homeostasis is impossible withoutthe last one. According to the literature, post-traumatic immunosuppression can be considered as a useful component of post-aggressive reactions aimed at preserving the viability of damaged tissues, which is undergoing a critical state and thereupon partially take the autoantigen properties [5, 9]. This is confirmed by data indicating an increase of lymphocytes amount which is exposed to apoptosis after a thermal injury. It means that

lymphocyte apoptosis can be considered as one of the main pathogenetic mechanisms in the development of posttraumatic immunosuppression [2, 13, 14]. In this regard, the study of the functional state of the immune system in injuries is an important direction in the diagnostic prediction of the outcomes of traumatic disease treatment. The deepening of knowledge in this area will significantly improve the effectiveness of medical care for injured, providing an opportunity for earlier treatment correction at the pathogenetic level.

Objective: to study some mechanisms of immunosuppression in traumatic disease and the possibility of their correction by polyoxidonium.

Material and methods of research

The objects of clinical study were 48 people admitted to the Department of traumatology of the 2nd Tashkent Medical Academy clinic in a state of traumatic shock, aged 18 to 47 years, men were 34 (70.8%) and women-14 (29.2%). Distribution was carried out by type of damage according to the AO-ASIF classification as follows: A1-16; A2-8; A3-1; B1-3; B2-1; C1-7; C2-9; C3-3. Injured people were more often affected aftercar accidents and outdoors, less often in the workplace and at home (falling from a height). The combined injuries were observed as follows: a closed craniocerebral injurywith a cerebralconcussion was diagnosed in 24 cases, closed craniocerebral injurywith brain contusion in 5 cases, closed fracture of the humerus with brachial artery injury in 2 cases, fractures of the ribs complicated by hemothorax in 2 cases.

Multiple injury: in 3 cases there was a fracture of the shin bones in combination with a compression fracture of the lumbar and thoracic vertebrae, in 5 cases - a fracture of the femur and pelvic bones, in 5 cases - a fracture of the shin bones and humerus, in 3 cases - a fracture of the femur and shin bones, in 1 case - a fracture of the Shin bones of both limbs. We produced primary surgical debridement with the rod device osteosynthesis, compressive-distractive osteosynthesis by Ilizarov, supraosteal, intramedullary and blocking intramedullary osteosynthesis, in case of pelvis fractures setting pin-and-rod apparatus was perfomed, and if in the control X-ray investigation standing of long bone fractures standing was satisfactory- we continued conservative therapy.

All patients in the study underwent complex treatment: infusion-transfusion therapy; inotropic, vascular and respiratory support; enteral nutrition; antibacterial therapy, 27 patients of them continued basic therapy (control group - №1), and 21 patients additionally took immunocorrective therapy with the addition of polyoxidonium for 10 days at a dose of 6 mg, intramuscularly, on alternate days, №5 (main group - № 2).

We carried out clinical, radiological (X-ray study, computer tomography (CT), magnetic resonance imaging (MRI)), electrophysiological, laboratory (blood and urine

samples, biochemical blood analysis, etc.), pathomorphological studies toassess the general condition of the patient's organism, the characteristics of homeostatic functions abnormalities, as well as the effect level of the method of complex treatment on the course of the wound process. The analysis of the spectrum of interleukins (IL) changes: IL-2, IL-6,and TNF-α by solid-phase enzyme immunoassay (EIA) using sets of firm "Biomedica" (Austria)were conducted. SAPO-1/FAS (sCD95+) and Il-2 receptor (sCD25+) content were also determined by enzyme immunoassay on the ELYZA apparatus (Germany) with using Bender Medsystems equipment. The studies were performed dynamically on the 3rd, 7th and 14th day of treatment. The analysis of quantitative indicators is carried out on the personal computer Pentium IV under control of the operating system Microsoft Windows 2000 Server, with use of statistical programs Microsoft Excel and Microsoft Access, with the determination of average values (M), average error (m). The reliability of the indices differences was determined using the Student's t-test with the accuracy of $p \leq 0.001$ and $p \leq 0.005$.

Results and discussion

Interpretation of the results we started with the determination of the relative value of lymphocytes in the blood. Initially, both groups had leukocytosis, manifested by neutrophilia, which is associated with the activation of neutrophils and tissue macrophages in the early inducible period. Exactly neutrophils and tissue macrophages in combination with NK-cells produce pro-inflammatory cytokines during the early inducible response period. The relative content of lymphocytes in the peripheral blood of the examined patients was significantly lower than the normative values (Fig. 1), but an increase in absolute values of lymphocytes reflects the release of relatively immature T-lymphocytes into the bloodstream.

The decrease in the percentage of lymphocytes in the peripheral blood of injured was observed from from 21.95±1.04% and 20.46±1.72 % (34.45±1.65% in practically healthy individuals) on the 3rd and 7th day after injury ($p<0.001$) respectively (Fig.1). Lymphopenia depended on the severity of the injury and the patient's condition, as we observed wide variability in the level of lymphocytes. A more pronounced decrease in the relative content of lymphocytes in the peripheral blood was observed in patients with the risk of development of purulent-septic complications, which is associated with the migration of lymphocytes from the bloodstream to the tissues, especially to the foci of inflammation. According to the literature [8], during the first day after the injury, there arecellular devastation of the thymus and spleen, accompanied by dystrophic and metabolic (inhibition of DNA and protein synthesis) changes in stromal cells. In this case, the number of mature lymphocytes cannot be restored for a long period of time due to impaired maturation of blast forms of lymphocytes. The results obtained by us coincide with the data of other researchers and indicate the possibility of using this test in predicting complications since relative lymphopenia is characteristic of the initial

stage of the infectious-toxic process [2, 5]. One of the causes of lymphopenia in the development of purulent-septic processes in the post-traumatic period is apoptosis, which at an early stage performs an adaptive function aimed at limiting the systemic inflammatory reaction, and at a late stage, if unfavorable outcome occurs, it contributes to the lymphopenia development [2, 9]. It should be said that apoptosis is a regulator of adaptive immunity, therefore, apoptotic reactivity of lymphocytes, determined by the number of cells expressing membrane Fas-receptors (CD95), is considered as a marker of prognosis of posttraumatic sepsis [5].

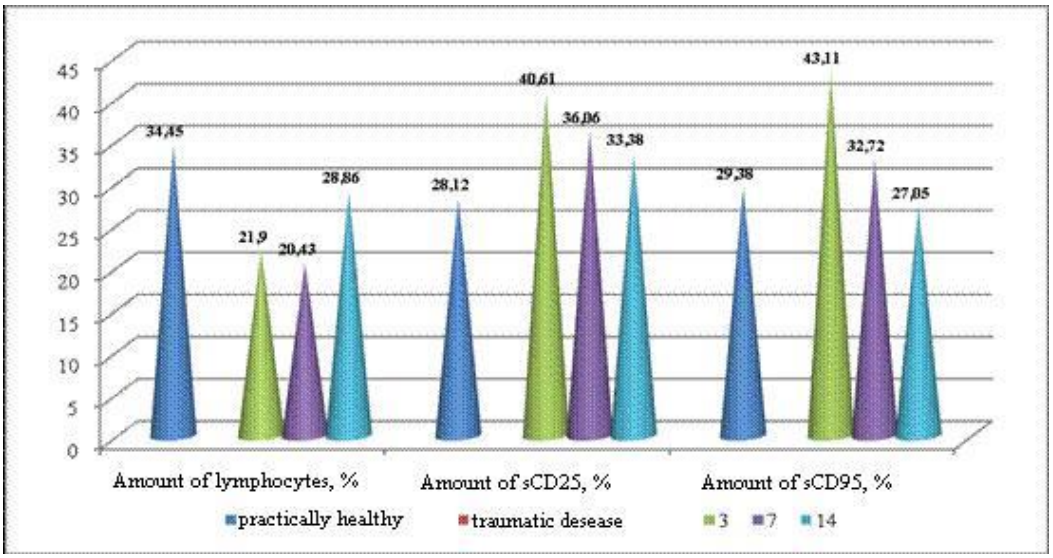

Figure. 1. Dynamics of changes in the relative content of lymphocytes, cd95,and cd25 in traumatic disease

Indeed, the determination of sCD95 content on the 3rd day of observation showed a significant increase to 43.06±2.18% with the value of this indicator in healthy individuals 29.38±1.09% (Fig. 1). Subsequently (after 7 days) it decreased to 32.70±2.12% and approximation to the values of healthy individuals on the 14th day of observation (decrease to 27.55±1.76%) was noted. More pronounced changes were typical for injured with combined trauma. Apparently, an increase in the content of sCD95 thatwe revealed on the 3rd day of observation is aimed at inhibiting the inflammatory process, and it can be considered as a marker of the prognosis of post-traumatic sepsis.

To diagnose immunodeficiency in patients with the traumatic disease, information about the functional activity of immunocompetent cells is needed also, in particular about their membrane receptor apparatus. CD25 is a single-chain glycoprotein, an early marker of cell activation and proliferation, identified as a low-affine receptor for IL-2 [12]. This receptor is expressed predominately on activated T and B lymphocytes. The studies carried out in this context showed an increase in sCD25 to 40.60±2.16% with the value of this indicator in practically healthy individuals 28.12±1.33% (Fig. 1). It should be noted that in the subsequent periods the level of this indicator gradually decreases, amounting to 36.06±1.98% and 33.38±2.16%, on the 7th and 14th day of

the study, respectively, but we did not bring out full recuperation. The greatest changes were typical for patients with combined trauma and with the risk of post-traumatic infectious and inflammatory processes.

Indeed, in the analysis of clinical data, the following complications were observed: infectious complications-in 6 (22.2%) and 2 (9.5%) patients, involvement in the pathological process of lungs and bronchi was observed in 10 (37%) and 4 (19%) patients, bedsores of the sacral, heel and scapula - in 7 (25.9%) and 1 (4.8%) patients, suppuration of the postoperative wound - in 4 (14.8%) and 2 (9.5%) patients, in the 1st and 2nd groups, respectively.

The results obtained by us coincide with the data of other authors, in which the increased expression of sCD95 and sCD25 receptors by peripheral blood lymphocytes of affected by thermal and mechanical trauma in the early stages of post-traumatic disease was established [2; 3]. According to the researchers, the activation processes in the immune system are the initial elements of the development of post-traumatic immune deficiency, i.e. there is a partial release of the membrane receptors to the liquid phase.

A possible task of apoptosis is the utilization of cells without ingress of the cell contents into the intercellular space, without the induction of inflammatory phenomena, in contrast to necrosis. Cell destruction by apoptosis provides minimal tissue damage compared to other mechanisms of cell death. In patients with a traumatic shock, from the first day of injury, occurs development of both adaptive and pathological reactions of the immune system.Cytokines play an important role in the reaction above [12]. These bioregulatory molecules determine the type and duration of the immune response, control cell proliferation, angiogenesis, haemopoiesis, inflammatory response, wound healing, etc.

The studies showed a statistically significant increase in the level of IL-6 in the injured people 1.85 times as many on the 3rd day of the study (table.1). In subsequent periods, its level gradually decreased, but still exceeded the normative values of 1.69 and 1.39 times on the 7th and 14th day of the study. The same dynamics were characteristic for the content of TNF-α in the blood serum of the injured. The level of cytokines in the blood serum depended on the severity and dynamics of shock, as well as the development of infectious and inflammatory processes. Apparently, expression of cytokines caused by the inflowof lysosomal enzymes, active metabolites of oxygen, tissue antigens into the bloodstream from damaged tissues leading to activation of cytokines synthesizing cells [2, 3, 4], as large-molecular components of damaged tissuesstimulates the formation of proinflammatory cytokines.

Table 1

Dynamics of changes serum cytokine level in patients with trauma, M±m

Groups	Practically healthy	Period of study, days		
		3	7	14
IL-6, пг/мл	23,22±1,65	43,02±2,33[a]	39,35±2,29[a]	32,28±1,83[a]
IL-2, пг/мл	29,38±1,32	17,51±1,29[a]	22,35±1,61[a]	28,34±1,62
TNF-α, пг/мл	15,33±0,98	26,80±1,55[a]	23,55±1,33[a]	21,54±1,18[a]

Note: a-differences between healthy individuals and patients were significant ($P<0.05$).

In contrast to the above cytokines, the level of IL-2 significantly decreased in the acute period of injury and gradually increased with the patient's condition improvement. The results we obtained correspond to the literature data, where the decrease in the expression of receptors to IL-2 CD4+ and CD8+ lymphocytes in response to bacterial superantigens [2, 3] is shown. According to the authors, the direct mechanisms of secondary immune insufficiency formation in case of injury may be: a decrease in the number of cells necessary for adequate manifestation of immune system reactions; functional failure of cellular components of immunoreactivity systems; quantitative or qualitative imbalance of factors and mechanisms of immunoreactivity; violation of relationships and imbalance of regulatory integrative systems: immune, nervous, endocrine.

In this regard, the additional inclusion of immunomodulator polyoxidonium in the complex of therapeutic measures was of interest. Studies have shown a gradual increase in the relative content of lymphocytes in the peripheral blood of injured, especially in 7 days, and the approximation of its values to the norm on day 14, while patients receiving basic treatment, maintained a reduced level of lymphocytes (table. 2).This was confirmed by a decrease in the high level of sCD95 in treatment with additional use of polyoxidonium and its approximation to the values of healthy individuals. The same dynamics were observed in the study of the level of sCD25 in the blood serum of patients. Consequently, polyoxidonium helped to restore the number of lymphocytes and increase the functional activity of the last one.

Table 2

Dynamics of changes in the immune system in patients with the traumatic disease during treatment, M±m

Groups	Practically healthy	Period of study, days		
		3	7	14
Relative lymphocyte value,%	34,45±1,65	21,95±1,04[a] 26,30±1,41[a]	20,46±1,72[a] 29,62±1,81[б]	28,97±1,45[a] 32,86±2,17
Absolute lymphocyte value, 10^9/l	2,75±0,14	1,76±0,07[a] 2,10±0,11	1,64±0,08[a,б] 2,37±0,12	2,32±0,16 2,63±0,18
sCD25, %	28,12±1,33	40,60±2,16[a] 36,85±2,14[a]	36,06±1,98[a] 35,06±0,76[a]	33,38±2,16 29,21±1,82
Amount ofsCD95, %	29,38±1,09	43,06±2,18[a] 31,32±2,15[б]	32,70±2,12 30,03±0,72	27,55±1,76 27,77±1,23

Cytokines				
IL-6, pg/ml	23,22±1,65	43,58±2,49[а] 39,87±2,12[а]	37,40±2,03[а] 29,62±1,81[а,б]	32,76±1,57[а] 28,65±1,78[а]
IL-2, pg/ml	29,38±1,32	18,12±1,67[а] 23,57±1,44[а]	22,84±1,98[а] 24,14±1,47[а]	27,79±1,62 29,25±1,28
TNF-α, pg/ml	15,33±0,98	26,24±1,84[а] 28,77±1,23[а]	23,69±1,29[а] 17,90±1,50	21,65±1,67[а] 17,20±1,19

Note: 1) the numerator presents the values of the control group of patients, the denominator - the main group; 2) a - the differences between the indicators of healthy individuals and patients are significant (P<0.05), b - the differences between the indicators of the main (2-group) and the control group (1-group) patients are significant (P<0.05).

High values of IL-6 and TNF-α were more distinctly reduced when polyoxidonium was included in the treatment complex, also low values of IL-2 reached normative values, while in the control group they retain increase tendency. Analysis of the positive activationmarker ratio index (sCD25) to negative activation marker (sCD95) showed a certain dynamics of this indicator. In healthy individuals this ratio was 0.95, in patients of the 1st group in 3 days - 0,93, on the 7th day - 1,11, and 14 day - of 1.23. That is, in patients who are on basic treatment, starting from the 7th day of admission to the hospital, activation of lymphocytes proliferation is observed. In the 2 groups, the ratio index was 1.17, 1.17 and 1.05 on day 3, 7 and 14, respectively. The inclusion of polyoxidonium contributed not only to a faster increase in the activation of proliferative processes but also to the normalization of this process. These data are confirmed by cytokine values in the dynamics of observation also. Secretion of IL-2 is detected after 3-4 hours after stimulation (trauma) and quickly stops. The growth of the cytokines IL-6 and TNF-α level increases the production of IL-2 in the following period, which protects the activated cells from apoptosis. Indeed, on the 7th day of observation, the indicator sCD95 was detected within the control values. Thus, the recovery processes in traumatic disease with polyoxidonium prescription realized more actively in the early stages after injury in comparison with the basic treatment.

Immunomodulator polyoxidonium is a high-molecular physiologically active compound with a pronounced immunotropic activity. It has an effect on all parts of the organism's protection from foreign agents of antigenic nature, increasing reduced and lowering increased immunity factors, i.e. it is a genuine immunomodulator [6,7,11]. The target cells for polyoxidonium are natural resistance factors: macrophages, monocytes, neutrophils, and NK-cells [6,7]. Apparently, the acceleration of differentiation and maturation of thymocytes under the influence of polyoxidonium leads to an increase in the number of T-lymphocytes, strengthening their functional activity in the conditions of the entire organism, which leads to the activation of both cellular and humoral immunity [11]. In addition to the immunomodulatory effect, polyoxidonium is characterized by the presence of detoxifying, antioxidant and

membrane-stabilizing activity. The combination of these properties makes it an indispensable drug in the treatment and prevention of immunodeficiency conditions. Thesefindings allow us to recommend the use of polyoxidonium in traumatic disease, especially with the risk of septic complications.

Conclusions

1. In the acute period of the traumaticdisease, we observed neutrophilic leukocytosis and relative lymphopenia, increased levels of sCD95 and sCD25 lymphocytes, more clearly defined in patients with combined trauma and the risk of post-traumatic infectious and inflammatory processes.

2. In the early stages of the traumatic disease, there is an increase of proinflammatory cytokines IL-6 and TNF-α and a decrease in IL-2 production, more pronounced in patients with extensive hematomas and severe injury.

3. The additional inclusion of polyoxidonium in the therapeutic complex increases the content of lymphocytes, reduces the high values of sCD95 and sCD25 in 7 days, and leads to the restoration of the balance of cytokines, especially when disposition to systemic inflammatory response syndrome exists.

4. The obtained results allow us to recommend the use of polyoxidonium in the early stages of the traumatic disease, especially in patients at risk of septic complications.

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