ANNOTATION
of the dissertation work by Kairanbayeva Gulgul Kairanbayevna on the topic "Metal-induced disorders of inflammatory process regulation in experiment and ways of their pathogenetic correction" submitted for the degree of Doctor of Philosophy (PhD) in specialty 6D110100 – «Medicine»

Urgency of the research

The accumulation of cadmium and lead in the organism induces adverse effects leading to various pathological conditions (Ebrahimi M., Khalili N., Razi S., Keshavarz-Fathi M., Khalili N., Rezaei N., 2020). The elevated disease risk is hypothesized to be attributable to the immunotoxic effects of lead and cadmium and their capacity to induce oxidative stress (Ahamed M., Siddiqui M.K., 2007). It is established that Tregs play a crucial role in suppressing inflammatory responses during the resolution phase of inflammation (Gootjes, C.; Zwaginga, J.J.; Roep, B.O. 2024). However, the potential impact of cadmium and lead salts on their early activation remains unexplored. Consequently, this project will investigate the functional activity of immunosuppressive subpopulations (Treg) during (aseptic) inflammation under heavy metal salt exposure.

The research findings will significantly enhance our understanding of inflammatory process pathogenesis mechanisms by providing data on effector T-lymphocyte involvement. Furthermore, based on evidence of Treg's prognostic role in inflammatory process regulation, the necessity of discovering novel approaches to modulate their expression activity becomes apparent. These new insights will enable recommendations for a differentiated approach to anti-inflammatory therapy, considering the phase, stage, and severity of the process, including the activation of specific inducers during early inflammation development.

Treg cells suppress immune responses by downregulating proliferation, differentiation, activation, pro-inflammatory cytokine production, and functional activity of a broad spectrum of effector cells in both adaptive and innate immunity, thereby maintaining immune homeostasis (Shevach E.M., 2019). Natural Treg cells undergo maturation in the thymus during normal T-lymphocyte biogenesis and, upon reaching the periphery, participate in maintaining peripheral immunological tolerance. Natural Treg cells are identified in peripheral blood and secondary lymphoid organs by their constitutive expression of CD4, CD25, and Foxp3.

One of the most significant molecules mediating Treg immunosuppression is the checkpoint class protein receptor CTLA-4 (Cytotoxic T-Lymphocyte Associated Protein 4) (Turley A.E., Zagorski J.W., Kennedy R.C., Freeborn R.A., Bursley J.K., Edwards J.R., Rockwell C.E., 2019). Under physiological conditions, Treg cells regulate the quality and magnitude of antimicrobial immune responses to protect against pathogenic microbes while preventing the development of adverse immunopathologies or inappropriate responses to commensal pathogens (Sakaguchi S., Yamaguchi T., Nomura T., Ono M., 2015). Conversely, their accumulation in the tumor microenvironment significantly contributes to establishing a tolerogenic microenvironment and promotes tumor progression (Zou W., 2019). Depletion

of natural Treg cells not only triggers autoimmune conditions but also enhances immune responses to alloantigens. For instance, Treg cell depletion in mice results in inflammatory bowel disease, likely due to an excessive immune response to commensal intestinal bacteria (Singh B., Read S., Asseman C., Malmstrom V., Mottet C., Stephens L.A., Stepankova R., Tlaskalova H., Powrie F., 2021).

Despite significant advances in understanding xenobiotic toxicity mechanisms, the influence of cadmium and lead on inflammatory processes and immune response regulatory mechanisms remains inadequately studied, particularly regarding the relationship between cadmium and lead exposure and Treg cell activity. Understanding inflammation regulation mechanisms is essential for appropriate selection of pathogenetic therapy, which largely prevents adverse outcomes of inflammatory processes.

Research Objective: to study the role of immune cells with regulatory and suppressor functions in the mechanisms of development of the inflammatory process in metal-induced immunodepression in order to find possible ways of pathogenetic correction using piperazine derivatives.

Research Tasks:

1. Conduct a microscopic examination of the thymus, mesenteric lymph nodes, and inflammatory focus on the skin of experimental rats with aseptic inflammation, pre-infected with cadmium and lead.
2. Study the dynamics of changes in the main hematological and immunological parameters associated with possible key mechanisms of inflammation regulation caused by two-week intoxication with cadmium and lead salts.
3. Conduct an experimental assessment of the regenerative potential of the Complex based on the nature of changes in immunomorphological reactions in the immunogenesis organs of experimental rats.
4. Assess the hematological parameters and phenotypic profile of splenic subpopulations in experimental rats with aseptic inflammation after correction with the Complex.
5. Establish key changes in the studied parameters under the influence of the Complex and polyoxidonium using discriminant analysis.

Research Methods

All animal experiments comply with recommendations outlined in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" and were approved by the Ethics Committee of Kazakh National Medical University named after S.D. Asfendiyarov (protocol № 4(95) dated 29.04.2020).

Laboratory Methods

Hematological Studies. To determine the number of formed elements of the blood, the content of hemoglobin, erythrocytes, reticulocytes, leukocytes, and platelets, the following hematological equipment and consumables were used: automatic hematological analyzer HumaCount 60TS, portable hematological analyzer DH 26 (manufactured by DYMIND).

***Immunological Studies*.**

Flow cytometry. The studies were performed using an Attune™ NxT acoustic focusing flow cytometer (Thermo Fisher Scientific, Waltham, MA, USA).

Enzyme immunoassay.

Rat-ELISA-Kit kits containing cytokine-specific capturing antibodies and a Thermo Scientific Multiskan FC microplate immunoassay spectrophotometer were used to determine pro- and anti-inflammatory cytokines.

Microscopic Examination

Microscopic examination was conducted to assess morphological changes in thymus, mesenteric lymph nodes, and inflammation focus in laboratory rats' skin tissues. Special attention was paid to studying the histomorphological picture of skin fragment in the injection area, as inflammation focus developed with purulent-necrotic changes, as well as thymus and mesenteric lymph nodes tissue. Tissue fragments 0.5 cm thick and 1-1.5 cm long were placed in buffered 10% formalin. Subsequently, tissue pieces were fixed in 10% neutral formalin solution, dehydrated in ascending strength alcohols, embedded in paraffin, then serial sections 5 μm thick were prepared on a microtome. For histological examination, sections were stained with hematoxylin and eosin and Van Gieson's picrofuchsin.

A blind semi-quantitative method was used with scoring from 0 to 3 points (0 - no pathology, 1 - mild, 2 - moderate, 3 - severe pathology). Ongoing aseptic inflammation was assessed by necrotic zone size, soft tissue swelling, leukocyte infiltration, blood vessel and fibroblast proliferation.

Statistical Data Processing

Using Excel application, arithmetic mean (M) and standard deviation (SD) were calculated. Graphs and tables contain information as arithmetic means (M)±standard deviation (SD). The significance of mean differences between two experiments was calculated using TTEST program. For comparison of more than two groups, one-way ANOVA with subsequent Tukey test was conducted. Differences were considered insignificant if the null hypothesis probability did not exceed 5% ($p>0.05$). GraphPad Prism computer statistical software was used for graphical representations, released by GraphPad Software, Inc.

Research Object

– The objects of the research work were peripheral blood, one of the central organs of the immune system – the thymus, spleen cells – splenocytes, skin with simulated aseptic inflammation and mesenteric lymph nodes of laboratory animals (rats).

For the experiment, 150 sexually mature non-linear male rats with a body weight of 170–250 grams (± 15 grams) were used.

Series 1 - Control
Series 2 - Rats with aseptic inflammation (AI)
Series 3 - AI/Polyoxidonium (PO)
Series 4 - AI/Complex
Series 5 - Rats receiving lead acetate and cadmium chloride (Me)
Series 6 - Me/AI
Series 7 - Me/AI/PO
Series 8 - Me/AI/Complex

Subject of the study:

– experimentally developed new model of metal-induced immunosuppression of experimental animals with aseptic inflammation.

Main Provisions for Defense

1. The course of aseptic inflammation in rats subjected to two-week oral inoculation with cadmium chloride and lead acetate is accompanied by destructive changes in the thymus, mesenteric lymph nodes and the site of inflammation at both study periods.
2. Preliminary intoxication with lead and cadmium compounds in experimental rats with aseptic inflammation causes pronounced degenerative-destructive changes in the lymph organs and their structural components throughout the experiment.
3. Lead acetate and cadmium chloride induce a suppressive background at the early stages of inflammation due to the activation of T-regulatory lymphocytes and their phenotypes CD4+CTLA4+, CD4+FoxP3+, inhibition of the proliferative activity of granulocytic and monocytic myeloid cells of the spleen, as well as a decrease in the cellular components of the peripheral blood, which contributes to an unfavorable outcome of the inflammatory process.
4. The complex improves structural and regenerative processes in the thymus and lymph organs of experimental rats
5. The complex, along with polyoxidonium, in the studied parameters of the peripheral blood of experimental rats with aseptic inflammation. The complex is able to be effective in eliminating the immunotoxic manifestations of heavy metals.
6. The corrective effectiveness of the Complex is comparable to polyoxidonium.

Research Results:

In this section, using a model of aseptic inflammation induced by subcutaneous turpentine injection, macroscopic and microscopic assessment of structural changes in the inflammation focus was conducted in experimental rats under conditions of preliminary lead acetate and cadmium chloride intoxication.

During microscopic examination of the material, special attention was paid to the study of histomorphological picture of a fragment of skin tissue in the area of injections with a focus of inflammation, represented by purulent-necrotic changes, as well as thymus and mesenteric lymph nodes.

It was established that metal exposure induced pronounced pathological changes in the immune system, skin, and tissues of thymus and lymph nodes in experimental rats. In the thymus at 7 and 14 days after exposure, a marked decrease in lymphocyte numbers and structural changes were documented, such as homogeneity of lymphocytic parenchyma, stromal edema, and reduction in Hassall's corpuscles number. These signs indicate degenerative processes and suppression of thymic immune function.

In mesenteric lymph nodes, dystrophic and necrotic cell changes were observed, along with decreased number of lymphoid nodules with germinal centers, diapedetic hemorrhages, and vascular congestion, indicating progressive deterioration of immune activity. Connective tissue proliferation and thickening of lymphoid nodule capsules and trabeculae additionally suggests chronification of the inflammatory process.

Examination of skin revealed extensive purulent-necrotic inflammation and leukocyte infiltration around the injection zone, indicating strong local reaction. At 7 days, the necrosis zone persisted, and granulation tissue formation began, indicating partial reparative activity in the damage zone.

Thus, the observed changes in thymus, lymph nodes, and skin indicate profound immune system and tissue damage under metal exposure, necessitating development of pathogenetically justified correction methods to prevent chronic immunodepression and structural disorders. Analysis of quantitative hematological parameters in laboratory animals revealed that inflammation progression in the Me+AI group was characterized by weak leukocyte response to turpentine-induced damage (Table 3.1).

In the first week of the study, blood analysis of rats previously exposed to cadmium and lead showed a 2.2-fold decrease from control in total leukocyte count (M=4.3, SD=0.7; pK=0.009), primarily due to lymphocytes, whose values were 3-fold lower than control (M=2.6, SD=0.3; pK=0.003). Meanwhile, by this study period, the AI group's leukocyte, lymphocyte, monocyte, and neutrophil indicators were significantly higher than Me+AI group by 3.5-fold (Mleukocytes=15.2, SD=2.2; pMe/AI=0.009), 4.3-fold (Mlymphocytes=9.4, SD=0.8; pMe/AI<0.0001), 3-fold (Mmonocytes=1.2, SD=0.5; pMe/AI=0.0038), and 2.7-fold (Mneutrophils=4.3, SD=1.3; pMe/AI=0.004) respectively. Thus, the acute inflammatory period in Me+AI group rats was characterized by sharp decrease in leukocyte levels, particularly lymphocytes. Notably, in the Me+AI group, under immunosuppressive effects of lead and cadmium salts, total leukocyte content and absolute lymphocyte content did not reach control levels even after 14 days.

Therefore, based on the studied parameters of nonspecific resistance, it can be concluded that throughout the experimental period, the inflammatory process in experimental animals was accompanied by low neutrophil activity, which was characteristic of immunological reactivity depression induced by lead and cadmium salts. The inflammatory course was complicated by anemia development.

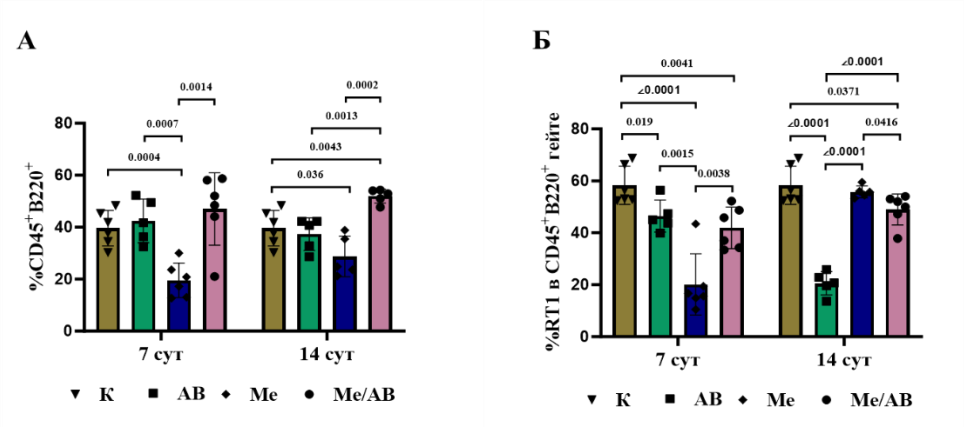
In the next stage of work, we evaluated heavy metals' effects on pro-inflammatory cytokine levels IL-6 and IL-1β. Special attention was paid to IL-1β and IL-6 cytokines' role in inflammatory process development and regulation. IL-1β and IL-6 are key pro-inflammatory cytokines that enhance inflammatory response by activating effector immune cells and stimulating their proliferation and differentiation. These molecules also play significant roles in acute inflammation induction and systemic organism response. IL-1β and IL-6 levels in animal serum were evaluated at 7 and 14 days after subcutaneous turpentine or metal administration by ELISA. After 7 days in the AI group, aseptic inflammation development was accompanied by more than two-fold statistically significant increase in IL-1β serum levels compared to control (*M=208.5, SD=58.0; pK=0.0052 versus control M=82.2, SD=11.2*). Under metal influence in Me group and after inflammation modeling in Me+AI group, IL-1β levels were significantly lower than AI by 3.6-fold (*M=57.1, SD=27.0; pAI=0.0091*) and 6.1-fold (*M=34.4, SD=17.0; pAI=0.0003*) respectively. After 14 days in AI group, serum IL-1β levels returned to control values, while Me and Me+AI groups remained at previous study period levels.

Based on serum cytokine studies, it was concluded that decreased IL-1β and IL-6 levels in heavy metal intoxication groups, compared to AI group, may indicate regulatory process disruption, likely due to immune cell proliferation suppression and enhanced apoptosis under toxic substance influence. By day 14, IL-6 levels in Me+AI group approached AI group values, suggesting partial restoration of inflammatory response

activity under metal influence. However, IL-1β levels in this group never reached near-control values, indicating prolonged but insufficient recovery process during continued toxic exposure. Overall data indicates that cadmium and lead not only suppress IL-1β and IL-6 synthesis but likely prevent full inflammatory response development, potentially creating prerequisites for chronic inflammation and deeper immune function disorders. IL-1β and IL-6 suppression during heavy metal intoxication indicates natural defense mechanism disruption and altered inflammatory process course, potentially having long-term consequences for immune system and general organism resistance to inflammatory stimuli. Anti-inflammatory cytokine TGFβ levels showed no significant changes, precluding assessment of its role in heavy metal exposure and inflammation response in this experiment.

Thus, obtained data indicates that cadmium and lead exert pronounced suppressive effects on key inflammation mechanisms, potentially leading to natural defense mechanism disruption and long-term negative consequences for immune system.

B-cell numbers were studied by flow cytometry, analyzing CD45+B220(CD45R)+-splenocyte proportion in lymphocyte gate (Table 3.2). The study found that CC and LA administration led to statistically significant reduction in relative B-cell content in Me group by 50.6% at 7 days (*M=19.6, SD=6.7; pK=0.0004*) and by 27.4% at 14 days (*M=28.8, SD=7.8; pK=0.0036*) compared to intact animals (*M=39.6, SD=6.8*).

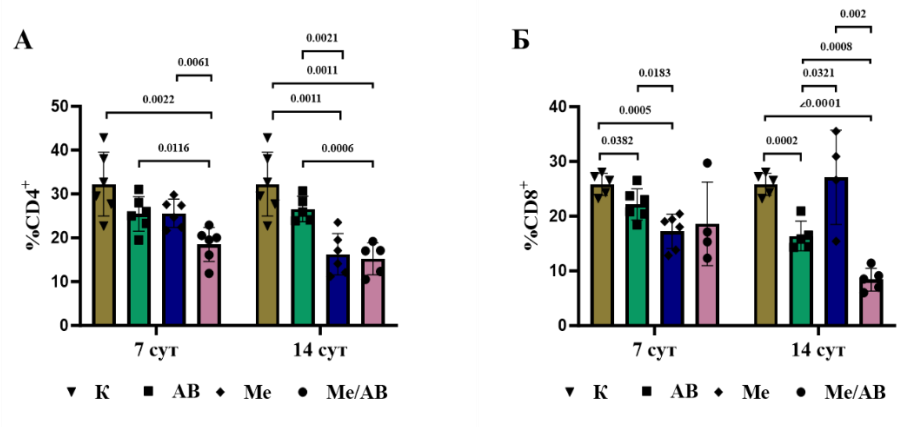


Aggregated data presented as M±SD.

Figure 3.2. - Proportion of CD45+B220+, RT1+ in CD45+B220+ gate in observation dynamics

While cadmium and lead salts induced increased proliferative activity of B-lymphocytes (CD45R(B220)) at 7 days, the capacity for MHC-II molecule expression (RT1+ in CD45+CD45R(B220)+gate) in the early stages of inflammation in Me+AI group was 28% lower than mean control values (*M=58.3, SD=7.3; pK=0.0041*).

Similar to the effect on B-cells, the immunotoxic action of CC and LA was also manifested in the assessment of main effector T-cell populations (Figure 3.3).



Aggregated data presented as M±SD.

Figure 3.3. - Proportion of CD4+, CD8+ cells in observation dynamics

Establishing balance between effector and regulatory T-lymphocytes was crucial during inflammation in rats subjected to cadmium chloride and lead acetate intoxication. These metals in Me+AI group reduced CD4+ lymphocyte percentage (*M=18.5, SD=3.9*) in the first week, lagging by 42.5% from control values (*M=32.2, SD=7.3; pMe/SI=0.0022*) and by 47.8% from AI (*M=25.5, SD=3.9; pMe/SI=0.0116*) (Figure 3.3 A). By day 14, continued statistically significant reduction in CD4+ lymphocytes was observed both relative to control by 52.8% (*M=32.2, SD=7.3; pMe/SI=0.0011*) and to SI by 42.7% (*M=26.6, SD=2.9; pMe/SI=0.0006*). CD4+ lymphocyte reduction at both time points is presumably associated with heavy metals disrupting proliferation and inducing lymphocyte apoptosis, confirmed by earlier data on increased Th-cell sensitivity to metal toxicity.

Further analysis of T-cell populations showed that aseptic inflammation was accompanied by gradual reduction in CD8+ T-cell content by 14.2% at 7 days (*M=22.1, SD=2.9; pK=0.0382*) and by 36.5% at 14 days (*M=16.4, SD=22.7; pK=0.0002*) compared to control (*M=25.8, SD=2.0*) respectively. Even greater reduction in CD8+ T-cell percentage was observed in Me+AI group at 14 days, with values lower relative to control (*M=25.8, SD=2.0*), AI (*M=16.4, SD=2.7*), and Me (*M=17.1, SD=8.6*) by 67.4% (*pK<0.0001*), 48.8% (*pAI=0.0008*), and 69% (*pMe=0.002*) respectively.

In this study, Treg cells were identified by CD4+CD25+ phenotype. One key aspect was monitoring the Treg population, which plays an important role in inflammation suppression. Classical course of aseptic inflammation, represented by AI group (*M=2.1, SD=0.9*), was accompanied by statistically significant two-fold reduction in total Treg population compared to control (*M=4.5, SD=0.7; pAI=0.0004*). At 14 days, Treg percentage still did not reach control level despite showing accumulation tendency.

Normal inflammatory process in AI group was accompanied by gradual reduction in CD4+CTLA-4+ cell content, reaching statistical significance compared to control animals by day 14 (p=0.01). Preliminary CC and LA administration to animals with inflammation led to significantly higher Treg cell content at days 7 and 14 compared to AI group. The

obtained data indicate that lead and cadmium compounds disrupted normal inflammatory process progression by inducing elevated immunosuppressive background, preventing adequate effector immunocyte response to antigen.

More pronounced inflammatory burden was associated with increased FoxP3+ expression and progression of its suppressive activity in Me/SI group, typically associated with inflammatory process inhibition.

During analysis of granulocyte cell population dynamics, we noted slight reduction in AI group at 7 days. Preliminary heavy metal intoxication in Me group at the same time points caused almost two-fold reduction compared to control (*M=7.5, SD=3.4; pK=0.0091 versus control M=13.5, SD=2.3*), while in Me+AI group (*M=2.2, SD=1.6; pK<0.0001*) they were 6-fold lower than control. At 14 days, in AI group, granulocyte population reduction continued and was statistically significantly 2-fold lower than control level (*M=7.8, SD=2.5; pK=0.0006 versus control M=13.5, SD=2.3*). Under metal influence in Me group, granulocyte population continued declining with more than two-fold reduction compared to control level (*p=0.0067*). Inflammatory reaction in Me+AI group at this study period showed two-fold increase compared to previous results but did not reach AI and control levels by 2-fold (*M=4.1, SD=1.2; pAI=0.0118 versus AI M=7.8, SD=2.5*) and 3.3-fold (*pK=0.0118 versus control M=13.5, SD=2.3*) respectively.

Thus, daily administration of cadmium chloride and lead acetate exhibited significant immunotoxic effects on animal organisms, manifested in reduced levels of key immune populations such as B-cells, CD4+ Th-cells, monocytes, and granulocytes in spleen. This reduction confirms heavy metals' suppressive effect on immune system and disruption of normal immune regulation. Dynamics of turpentine-induced aseptic inflammation revealed reduction in levels of MHCII-expressing B-cells, CD8+, CD4+CD25+ cells, Treg cells, monocytes, and granulocytes by day 14, potentially indicating these cells' migration to inflammation site. Simultaneously, this reduction creates favorable conditions for inflammatory reaction by reducing immunosuppressive background.

Cadmium chloride and lead acetate enhance inflammatory process dysregulation, manifested in reduced CD8+ and granulocyte populations, and increased B-cell and Treg cell proportions. This effect indicates immunosuppressive background dominance and Th2-directed immune response, potentially impeding full anti-inflammatory organism response.

The obtained data confirm that cadmium and lead intoxication disrupt balance between various immune system cell types, increasing immunodepressive effects and promoting immunological dysregulation. This may lead to increased tendency toward inflammatory process chronification and reduced overall immune response. Observations show that heavy metal exposure causes pronounced inflammatory process dysregulation, requiring further study to develop prevention strategies and correction of such immunotoxic disorders.

Complex application accelerates healing through rapid granulation tissue formation, favorably distinguishing it from Polyoxidonium, which does not provide equally effective restoration and leaves inflammation foci with edema and minimal fibrous tissue at day 14.

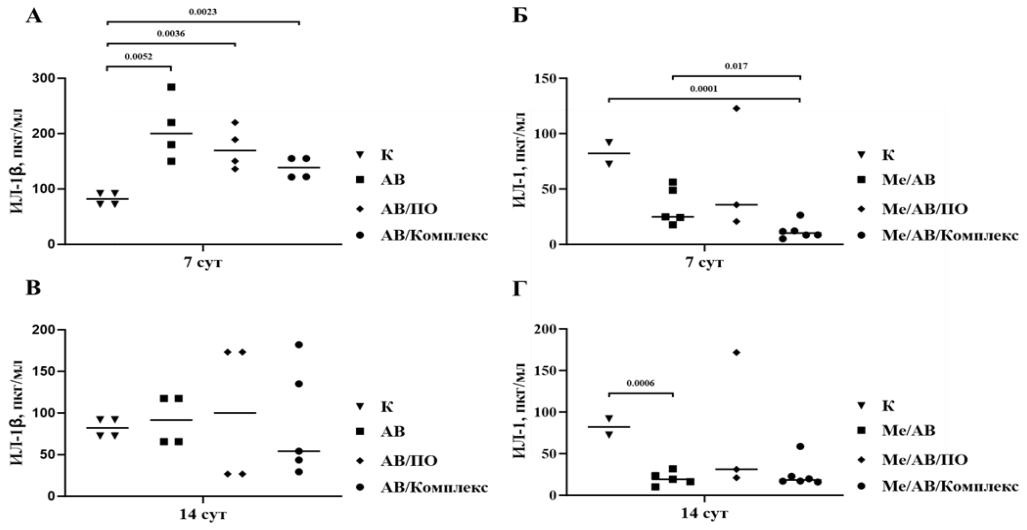
Experimental study of peripheral blood parameters in rats with experimental inflammation, previously exposed to cadmium and lead salts, after Complex correction

showed that Complex, like Polyoxidonium, promotes more than three-fold increase in leukocytes and lymphocytes compared to Me+AI, more than 2-fold increases neutrophil content, without reducing accumulation in subsequent study period.

Figure 3.4 presents IL-1β research results after Complex correction compared to Polyoxidonium. Complex correction during one week in AI group led to statistically significant 2.5-fold increase in IL-1β (*M=208.5, SD=58.0; pK=0.0023*) compared to control (*M=82.2, SD=11.2*), comparable to Polyoxidonium (Fig.3.4 A). At 14 days, no statistically significant changes in IL-1β concentration were observed in compared groups (Fig.3.4 B). Complex did not correct aseptic inflammation course induced against background of cadmium and lead salt exposure, and IL-1β values (*M=12.1, SD=7.5; pK=0.0001*) at 7 days were statistically significantly lower than not only control (*M=82.2, SD=13.8*) but also Me/AI (*M=34.4, SD=17.0, pMe/AI/Complex=0.017*) (Fig.3.4 B). At 14 days, similar pattern was observed (Fig.3.4 D).

Further, we studied serum IL-6 content. Results showed that Complex in Me/AI/Complex group at 7 days promoted inflammatory response activation. Significant reduction in this level at 14 days indicated partial normalization of inflammatory response, although it remained elevated compared to control. Similarity with Polyoxidonium effect suggests potential immunostimulating effect of Complex. In AI group, correction with Polyoxidonium and Complex led to lower IL-6 levels than in AI group, potentially indicating their role in inflammatory activity modulation and excessive response reduction.

Polyoxidonium and Complex administration led to B-lymphocyte content normalization in AI/PO and AI/Complex groups by observation day 14. Meanwhile, Complex correction of Me/AI group did not stimulate MHC-II molecule expression by B-lymphocytes either at 7 days exposure, with values 1.5-fold lower than control level (p=0.0004), or at 14 days, not reaching untreated rat levels (p<0.0001) and indicating sustained suppression of B-cell antigen-presenting function under heavy metal intoxication conditions.



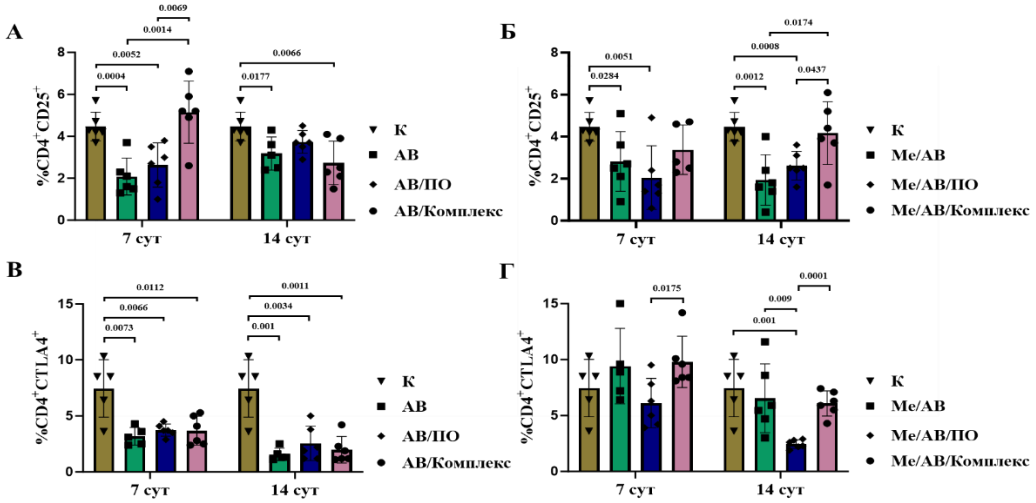
Aggregated data presented as M±SD. Statistical significance indicated as *p<0.05.

Figure 3.4. - IL-1 content in peripheral blood serum of experimental rats with aseptic inflammation after Complex correction.

This likely indicates that Complex is less effective in restoring antigen-presenting activity in the presence of cadmium and lead toxic effects.

Complex administration demonstrated significant restoration of cytotoxic T-lymphocyte (CTL, CD8+) levels by observation day 14, bringing their content to control level. This effect was statistically significant (p=0.00191) and proved more pronounced than with Polyoxidonium, indicating Complex's superior ability to maintain CTL numbers and activity.

Complex demonstrated significant immunostimulating effect on regulatory T-cells (CD4+CD25+), maintaining their levels at control values at 7 days and promoting their restoration by day 14. Under cadmium and lead intoxication conditions, Complex provided stabilization and increase in CD4+CD25+ cell proportion, exceeding Me/AI group parameters two-fold by study end. Polyoxidonium, conversely, did not exert similar effect on CD4+CD25+ cell proliferation, remaining below control values at both study timepoints. These data indicate Complex's advantage in supporting regulatory T-cell proliferative activity and ability to effectively modulate immune response, particularly under cadmium and lead intoxication conditions.



Aggregated data presented as M±SD. Statistical significance indicated as *p<0.05.

Figure 3.5. - Proportion of CD4+CD25+, CD4+CTLA4+ cells in experimental rats receiving Complex, in observation dynamics

Complex effect in Me/AI group restored CTLA4+ proportion to control level, however statistically significantly exceeded Polyoxidonium efficacy by 1.7-fold (p=0.0175) at 7 days and 2-fold (p=0.0001) at 14 days (Fig. 3.5 D). This possibly indicates Complex's ability to effectively normalize CTLA4+ levels under intoxication conditions, which may be important for immune function restoration after heavy metal exposure.

More than three-fold increase in CD4+FoxP3+ proportion compared to both control (p=0.0002) and Me/AI/PO was observed in Me/AI/Complex group (p=0.0001) (Fig. 3.5 B).

At 14 days, under both Complex and Polyoxidonium influence, CD4+FoxP3+ proportion statistically significantly decreased two-fold both relative to previous study period and to Me/AI, but remained equally significantly above control values.

Summarizing research results, it should be noted that Complex application had ambiguous effects on FoxP3+ cell levels under conditions of aseptic inflammation and cadmium and lead salt intoxication. In aseptic inflammation model, Complex promoted positive dynamics of immune response regulatory component, ensuring significant increase in FoxP3+ cell proportion by 14 days, potentially facilitating effective inflammatory process control. However, under conditions of preliminary cadmium and lead intoxication, Complex induced excessive FoxP3+ cell proportion increase by 7 days, potentially promoting excessive regulatory component activation and, consequently, possible immune response weakening. Despite partial FoxP3+ level reduction by day 14, it remained above control values, potentially maintaining immunodepression state.

Thus, despite initial CD4+FoxP3+ proportion increase in response to Complex application, results show its effect is not stable and T-regulator level decreases by day 14. This indicates Complex's inability to maintain high FoxP3+ levels long-term, potentially limiting its effectiveness in immune response modulation and inflammatory process control.

Further studies focused on examining granulocyte and monocyte population proportions in experimental rat spleens receiving Complex and Polyoxidonium. In Me/AI group, one-week Complex correction proved ineffective, while at 14 days Complex restored His48+hCD11b/c+ proportion to control values. We can hypothesize that observed spleen cellularity increase in Me/AI/Complex group at 14 days compared to 7 days was likely associated specifically with Complex's immunostimulating effect on granulocytes. Polyoxidonium proved ineffective in His48+hCD11b/c+ correction.

Thus, Complex application demonstrated significant immunostimulating effect under aseptic inflammation and intoxication conditions, especially regarding granulocytes and monocytes. Complex promoted immune parameter restoration, returning His48+CD11b/c- and His48+CD11b/c+ cell levels to control values by 14 days, indicating possible ability to strengthen immune response with prolonged use. Compared to Polyoxidonium, Complex proved more effective in immune cell stimulation.

Mathematical modeling of data array using discriminant analysis showed that variables CD45+CD45R(B220), CD4+, CD4+CTLA4+, and IL-6 allow discrimination of Me/AI, Me/AI/PO, and Me/AI/Complex groups. Data analysis shows that by 14 days, both Complex and Polyoxidonium reduce IL-6 activity but do not promote restoration of B-lymphocytes with CD45+CD45R(B220) phenotype and CD4+ lymphocyte proportion in rats with aseptic inflammation. However, Complex shows higher efficiency in CTLA4+ modulation, potentially significantly affecting immune response regulation and inflammatory process control.

Thus, drug effectiveness at 14 days distributed as follows: Complex → Polyoxidonium.

Scientific Novelty

The conducted comprehensive assessment, which included microscopic studies of the central and peripheral organs of immunogenesis and the inflammation focus, the study of

cellular and humoral components of immunity in the spleen and peripheral blood of experimental animals subjected to two-week intoxication with cadmium and lead, expanded the modern understanding that the disturbance of the inflammatory process is formed by a diverse complex of interdependent pathological mechanisms. It was established that among the studied indicators, for identifying the features of the inflammatory process after 7 and 14 days, the most interesting is the quantitative assessment of T regulatory lymphocytes involved in the mechanisms of inflammation regulation. An increase in the expression activity of CD4+, CD25+, Foxp3+ and CTLA-4+ after 7 days increases the likelihood of inflammation regulation disturbance. Decreased activity of CD4+, CD8+, His48+CD11b/c+, His48+CD11b/c- brought clarity to the understanding of the mechanism of development of aseptic inflammation induced against the background of preliminary intoxication with cadmium and lead salts. In rats with aseptic inflammation, pathogenetic correction of disorders caused by cadmium and lead was carried out. For the first time, a new piperazine derivative was used - Complex, synthesized in the laboratory of JSC "Institute of Chemical Sciences named after A.B. Bekturov", in comparison with polyoxidonium. For the first time, it was established that the Complex contributes to the improvement of regenerative processes in the thymus and inflammation tissue. The Complex restores % CD8+, increases IL-6 activity and shows higher efficiency in modulating CD4+CD25+ and CTLA4+ than polyoxidonium.

Theoretical and Practical Significance

The achieved results of the scientific research contribute to the development of the ecological approach to the study of human health and pathology, to identify the correlation between the level of air pollution with heavy metals and a decrease in cellular immunity in the population in ecologically unfavorable areas of the country. The ecological principle of studying health indicators serves to expand the understanding of the pathogenesis of the corresponding diseases. The practical significance of the dissertation research, as a fundamental work, is that the results obtained in the future will allow developing a more accurate approach to assessing the immune status of people living in conditions of increased air pollution, enriching scientific knowledge in the field of general patterns of functioning of the immune system in the process of adaptation to adverse environmental conditions. The materials and generalizations contained in the dissertation can be useful in developing programs for the prevention of respiratory diseases in the population in similar regions, contributing to the formation of the concept of immune system tension in a systemic approach to assessing the immune status.

Personal Contribution of the Doctoral Candidate

The dissertation candidate directly participated in research idea development, determining goals and objectives, and in designing and conducting experiments as a performer of scientific projects "Role of CD4+CD25+FOXP3+Tregs in inflammatory process regulation: metal-induced mechanisms of immunosuppression and search for new methods of pathogenetic correction in experiment" (HHG, 2019-2021) and as scientific supervisor of "Mechanisms of inflammation regulation disorder under influence of chemical ecotoxicants and new methods of their pathogenetic correction", GF Ministry of Science and Higher Education of the Republic of Kazakhstan "Zhas Galym" (2022-2024). The

candidate made substantial contributions to research method selection, data collection and analysis, and results interpretation. The candidate actively participated in all research stages, including sample preparation, biological material collection, data analysis, and statistical processing. The candidate took active part in scientific hypothesis proposal, conclusion formulation, and research result publication in specialized scientific journals and at conferences.

Conclusions

1. Microscopic studies of immunogenesis organs in rats with aseptic inflammation induced against preliminary cadmium and lead salt intoxication revealed pronounced structural changes in lymphoid organs throughout the experiment. In thymus, edema and pericapillary space expansion, stromal edema, vascular congestion, and microhemorrhages with lymphocyte apoptosis were observed (7 days). Capsule edema, decreased lymphocytes in cortical layer, reduced thymic corpuscles, congested vessels, and hemorrhages with leukocytes were observed at 14 days. In mesenteric lymph nodes at 7 days, increased numbers of large and medium lymphocytes were found, with presence of plasma cells, macrophages, neutrophils, and eosinophils. At 14 days, total lymphocyte count decreased while neutrophils remained elevated. In skin at 7 days, reactive inflammation zone around necrosis showed edema, leukocyte infiltration, fibroblasts, and capillaries. By 14 days, necrosis zone decreased, monocytes and leukocytes accumulated, granulation tissue formed, and capsule thickened.

2. Key indicators of inflammation regulation disorder in Me/AI group were established as quantitative reduction in leukocytes, lymphocytes, and neutrophils, with pronounced anemia. Heavy metal salts at early inflammation stages reduced MHC-II molecule expression (RT1+ in CD45+CD45R(B220)+gate) in Me+AI group by 28%. Inflammation course was aggravated by CD4+ lymphocyte reduction by 42.5% and 52.8% compared to control at both study timepoints, increased FoxP3+ and CTLA4+ proportion in early inflammation stage, creating suppressive background for inflammatory reaction development. Late-stage inflammation (after 2 weeks) was characterized by reduced differentiation of granulocytic leukocytes in spleen and decreased lymphocytic leukocyte pool. Cadmium and lead suppressed IL-1β and IL-6 synthesis at both study timepoints, impeding full inflammatory response development.

3. Polyoxidonium administration promotes structural changes in thymus and lymph nodes, reflected in increased lymphocytic activity and tissue regeneration. Restoration processes in thymus and lymph nodes indicate reactive immune system adaptation, however more pronounced fibrous skin healing does not occur. Piperazine derivative application in experimental animal group leads to notable cortical substance thickening and granulation tissue formation, improving regenerative processes. Significant reduction in necrosis areas and granulation tissue development indicates its higher efficacy compared to Polyoxidonium. Unlike Complex, Polyoxidonium application does not lead to notable healing acceleration. At day 14, inflammation zone retains edema and insufficiently developed fibrous tissue, confirming lower Polyoxidonium efficacy under metal intoxication-induced inflammation conditions.

4. Inflammation correction induced by cadmium and lead intoxication showed that Complex at 7 days increased leukocyte and lymphocyte counts more than 3-fold, and neutrophil content more than 2-fold, maintaining these levels in subsequent periods. Complex did not alter IL-1β levels but elevated IL-6. It did not stimulate MHC-II expression, remaining below control level. Complex restored CD8+ to control level (p=0.00191), more pronounced than with Polyoxidonium. Complex maintained CD4+CD25+ proportion at control level, exceeding Me/AI group values 2-fold by day 14. It restored CTLA4+ proportion, statistically significantly exceeding Polyoxidonium efficacy by 1.7-fold (p=0.0175) at 7 days and 2-fold (p=0.0001) at 14 days. However, Complex caused excessive FoxP3+ cell increase at 7 days. At 14 days, Complex restored His48+hCD11b/c+ proportion to control values. Polyoxidonium promoted more than three-fold increase in leukocytes and lymphocytes at 7 days but did not cause significant changes in neutrophil levels, IL-1β, and did not stimulate CD4+CD25+ cell proliferation. Under cadmium and lead intoxication, Polyoxidonium remained below control values, and its efficacy in restoring CTLA4+ proportion and CTL levels was significantly lower than Complex, indicating less pronounced ability to maintain immune response.

5. Discriminant analysis results showed that variables CD45+CD45R(B220), CD4+, CD4+CTLA4+, and IL-6 allow discrimination of Me/AI, Me/AI/PO, and Me/AI/Complex groups. Data analysis showed that by 14 days both Complex and Polyoxidonium decreased IL-6 activity but did not promote restoration of B-lymphocytes with CD45+CD45R(B220) phenotype and CD4+ lymphocyte proportion in rats with aseptic inflammation. However, Complex showed higher efficacy in CTLA4+ modulation, potentially significantly affecting immune response regulation and inflammatory process control. Drug efficacy at 14 days distributed as follows: Complex → Polyoxidonium.

Dissertation Results Approbation

Main provisions of the dissertation work were presented and discussed at:

1. 1st International Forum "Asfen.Forum, new generation-2023" with oral presentation "Study of aseptic inflammation dysregulation mechanisms under conditions of metal-induced immunodepression and search for new methods of pathogenetic correction" June 5-6, 2023, Republic of Kazakhstan, Almaty.
2. International Scientific-Practical Conference «Physiology in Focus 2023» (Tartu, Lithuania).
3. VII International Scientific-Practical Conference "Allergology and Immunology: Achievements and Prospects" with oral presentation "Determination of T regulatory cell activity during inflammation against background of heavy metal salt action using flow cytometry" Republic of Kazakhstan, Almaty, September 21-23, 20.
4. International Scientific-Practical Conference of Young Scientists and Students "KSMA Science Days-2024", dedicated to 85th anniversary of Kyrgyz State Medical Academy, Symposium "Issues of Fundamental and Forensic Medicine", with oral presentation "Cadmium and lead compounds disrupt T-regulatory cell activity in experimental inflammation" Kyrgyz Republic, Bishkek, April 11, 2024.

Published works on the results of the thesis research:

1 article - in a publication indexed in Scopus (Q2 (Molecular medicine) CiteScore 6.7.), *4 articles - in publications recommended by the Committee for Control in the Sphere of Education and Science of the Republic of Kazakhstan, 4 abstracts – in collections of international scientific conferences, 1 utility model patent, 1 copyright certificate.*

Dissertation Structure and Volume.

The work consists of introduction, literature review, description of research materials and methods, chapter of original research, conclusions, and practical recommendations. The bibliography contains 210 sources. The dissertation is presented on 117 pages, contains 20 tables, 31 figures, and 2 appendix.