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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

OPTIMIZING REPARATIVE OSTEOGENESIS WITH IMMUNOCORRECTION IN COMPLEX TREATMENT OF MANDİBULAR FRACTURES
(Experimental and Clinical research)

Specialty: 3226.01 – Dentistry

Field of science: Medicine

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GENERAL CHARACTERISTICS OF THE STUDY

Actuality of the problem. The variety of clinical implications of trauma due to the complexity of their mechanism and displacements and limited patients' knowledge of trauma and their treatment further complicate their diagnosis and treatment.[1] General traumatology lays special emphasis on facial trauma. Studies have shown that mandibular fractures make up 60-70% of craniofacial injuries.[2,3]

In recent years, in the overall structure of traumatism, the average share of maxillofacial injuries has increased. Herewith, an aggravation of major trauma is observed, especially in the maxillofacial and largely in the middle facial region.[4]

Statistics show that maxillofacial injuries are mainly caused by traffic (41%), home (2%), and sports accidents (18%).

Maxillofacial fractures caused by traffic accidents can be open or closed. Injuries in this anatomic area were found to make 23.68%.[5] Initial complications of mandibular fractures included pain, soft-tissue edema, and masticatory muscle trauma.

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However, complications may occur at any other rehabilitation stage. Drug treatment and physiotherapy applied by clinicians are sometimes unsuccessful. Therefore, the search for more effective treatments continues to prevent complications and hasten rehabilitation.[6]

Many complications occur after mandibular fracture surgery. E.g., fractured bone fragments in various localizations occur during mandibular osteotomy, damage to teeth and alveolar tissue comes along with segmental osteotomy, overheating of bone tissue and large incision of interdental bone (leading to circulatory disorders) occur during forage.[7]

A number of conservative and surgical treatment techniques have currently been developed in maxillofacial surgery. Each of them has its benefits and shortcomings. Herewith, the conservative treatment frequency currently tends to be reduced.[8]

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5. *Масляков, В.В. Анализ результатов лечения сочетанных и изолированных повреждений лицевого черепа, полученных в результате дорожно-транспортных происшествий, в условиях региональных травмоцентров / В.В. Масляков, Ю.Е. Барачевский, О.Н. Павлова [и др.] // Политравма, – Ленинск-Кузнецкий: – 2021. №4, – с. 6-17.*
6. *Гасымзаде, Д.К. Эффективность зубочелюстного тренинга в реабилитации пациентов с переломами нижней челюсти / Д.К. Гасымзаде, С.С. Ксембаев, Ф.В. Тахавиева [и др.] // Российская стоматология. – Москва: – 2021. №4, – с. 30-32.*
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Despite modern preventive measures, the frequency of post-surgery complications at mandibular fractures does not decrease and makes 5,5-14,1% of all complications. Mandibular osteomyelitis has been shown to occur due to the body's low immune response. In this regard, eliminating the infectious process in the mandibular fracture treatment and applying effective methods to stimulate local and general immunity is an urgent problem.[9]

The biologists' recent fundamental research at the molecular-cellular level has enhanced insight into reparative osteogenesis. Attention was paid to the multifunctionality of osteocytes formed in the bone tissue.[10]

In the first posttraumatic days, inflammation and resorption of the bone fragment end occur in the fracture region. At this stage, it is advisable to stimulate osteoclastic resorption and immunity.

One study has shown that in patients with mandibular fractures, general immune changes such as an increase in leukocytes and CIC and a decrease in lymphocytes occur in the first days and 7th and 14th days, compared to healthy subjects.[11]

When searching for modern immunocorrection techniques, the efficacy of the adaptogenic drug (Septilin) having non-specific immunomodulatory and anti-inflammatory effects in various secondary immunodeficiency conditions (in vitro, in vivo) attracts our interest.

Therefore, it was decided to study the impact of Septilin on reparative osteogenesis at mandibular fractures in experimental and clinical research.

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9. *Rakhimov, Z.K. Immuno correction of post-traumatic inflammatory complications in patients with fractures of the lower jaw // The Pharma Innovation Journal, – 2020, 9(6), – p. 592-594.*
10. *Оноприенко, Г.А. Современные концепции процессов физиологического и репаративного остеогенеза / Г.А. Оноприенко, В.П. Волошин // – Москва: Альманах клинической медицины, – 2017. №2, – с. 79-93.*
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The object of research. The experimental research was performed at the Scientific Research Center of the Azerbaijan Medical University on 24 completely healthy chinchilla rabbits of both genders, weighing 2,0-3,0 kg. 80 patients treated for maxillofacial fractures in the Maxillofacial Surgery Department of the Clinical Medical Center No. 1 in 2015-2017 were involved in the clinical trial.

The aim of the study. Improving the treatment outcome for patients with mandibular fractures by immunocorrection based on experimental and clinical research.

The study objectives included:

1. Determining changes in blood immunological parameters in the post-traumatic period at mandibular fractures in experimental research.
2. Studying the Septilin action mechanism in reparative osteogenesis in experimental research.
3. Identifying local and general immune disorders in patients diagnosed with mandibular fracture based on laboratory tests in clinical trials.
4. Estimating the efficiency of clinical application of immunological diagnostic and treatment algorithms proven themselves in experimental research.
5. Estimating the impact of immunodeficiency treatment with adaptive and immunomodulatory drugs on the reparative osteogenesis in patients with mandibular fractures in the post-traumatic period.

Methods of research:

-experimental
-morphological
-biochemical-immunological
-beam diagnostics
-clinical-laboratory
-thermometry
-mathematical-statistical.

The main statements submitted for dissertation defense:

1. Along with the conventional examination of patients with traumatic injuries accompanied by mandibular fractures, the study of the immune system conditions is advisable.

2. Inclusion of not only local but also total-body treatment in the mandibular fracture treatment procedure is effective.

3. Correction and modulation of the immune system at mandibular fractures have a positive impact on the treatment outcome.

4. Septilin is effective in mandibular fracture treatment.

Scientific novelty of the research. The specifics of reparative osteogenesis in mandibular fractures were identified, and the efficiency of new treatment algorithms was proved in clinical and experimental studies based on immunological, histomorphological, radiological, and mathematical-statistical indicators. SEPTILIN commonly used for immunodeficiency as an adjunct, was first used in the research to treat mandibular fractures.

Practical significance of the study. Along with the immunomodulatory action, the anti-inflammatory and analgesic effect of the herbal drug without any additional adverse effects facilitate its practical application. The new tactics added by Septilin in the complex treatment of mandibular fractures have advantages for practical use, minimizing possible complications.

Approbation of study results. The research results have been discussed at the following conferences:

24th International Congress of the European Association for Cranio Maxillo Facial Surgery, September 18-21, 2018. Munich, Germany;

1st International Congress of the Azerbaijan Society of Oral and Maxillofacial Surgeons, 2019, Baku, Azerbaijan;

The ARSSS (Advanced Research Society For Science And Sociology) International Conference, October 20-21, 2019. Istanbul, Turkey;

The dissertation materials were discussed at an extended meeting of the Department of Oral and Maxillofacial surgery with the participation of employees of other specialized departments of the Azerbaijan Medical University (25.01.2022, protocol No. 5), a scientific seminar operating under the Dissertation Council ED 2.05 (11.05.2022, protocol No. 15)

Applying the results scientific work. The scientific and practical research results are applied in the complex treatment of mandibular fractures in the Maxillofacial Surgery Departments of the

Clinical Medical Center No. 1 and the Teaching Surgery Clinic of the Azerbaijan Medical University.

The institution where the dissertation work is performed. The study was performed at the Department of Oral and Maxillofacial Surgery of the Azerbaijan Medical University, the Scientific Research Center of the Azerbaijan Medical University, and the Department of Oral and Maxillofacial Surgery of the Clinical Medical Center No. 1.

Published works. 12 scientific works have been published on the thesis topic. Of them, 9 papers and 5 theses, including 2 papers and 2 theses in the foreign journals.

The volume and structure of the dissertation. The dissertation work is written on 144 pages (210.851 symbols). The dissertation consists of an introduction (8.766 symbols), a literature review (63.785 symbols), a chapter on materials and methods of research (29.548 symbols), 2 chapters covering their own research (8.507 symbols), discussion of the obtained results, practical recommendations (366 symbols). The scientific work is illustrated with 16 tables, 5 graphs, 14 figures. The list of references included 160 sources. 17 of these in our own language.

THE MAIN CONTENT OF THE WORK

Experimental research. Experimental research was performed at the AMU Scientific Research Center on 24 completely healthy chinchilla rabbits of both genders, weighing 2,0-3,0 kg (Fig. 2.2.1). In animals, a traumatic mandibular fracture model was developed and a day later, osteosynthesis was performed with titanium mini-plates for all fractures. Biochemical-immunological analysis of blood was performed before the surgery and at different treatment stages, the fracture line was radiologically examined, and the fracture line biopsy samples were studied by light microscopy. The experimental animals were divided into two basic groups – the control and test ones. A model of mandibular fracture was created in all animals by the technique proposed by M.V. Morozova (2000).

Appropriate analgesia and anesthesia measures have been taken as follows. As premedication, 30 minutes before surgery, all animals received intramuscular injections of 50% Analgin (50-70 mg/kg), 1%

Diphenhydramine (1-1.5 mg/kg), 0.1% Atropine Sulfate (0,1-0,2 mg/kg), and 0,25% Droperidol solution (0,5 mg/kg). Rabbits' subcutaneous ear veins were then catheterized, and calypsol was introduced intravenously (3-4 mg/kg) for non-inhalation anesthesia. Intravenous anesthesia with calypsol was continued during the surgery.

Under general anesthesia, the skin in the mandibular angle region was removed, a linear incision was made in the submandibular area after treatment with an antiseptic solution, the mandibular angle bone was exposed by sharp and blunt dissection of surrounding tissues, and a fracture model was created with light trauma by linear decortication.

The fractures were then repositioned and rigidly fixed with titanium mini-plates and screws. Animals were conventionally treated for 7 days with 25 mg/kg Ampicillin, 0,3 mg/kg Dexamethasone, and 0,3 mg/kg Ketanol. After the development of the mandibular fracture model, 30 mg/kg Septilin immunomodulator had been given orally to 12 second-group rabbits twice a day for 30 days along with conventional treatment.

On the 1^{st}, 15^{th}, and 60^{th} days of the experiment, the animals' blood samples were sent to the Biochemistry Department of the AMU Scientific Research Center to determine CIC, complement, and lysozyme. 7, 21, and 60 days after the surgery, a monoblock sample was taken under anesthesia for histological examination from the mandibular angle of animals while completely saving the fracture line and sent to the Morphology and Histology Department of the AMU Scientific Research Center.

In the morphological experiment, biopsy samples, taken from the fracture line on the 7^{th}, 21^{st}, and 60^{th} days after the surgery, were examined with a light microscope at 400 magnifications.

Clinical research. During the peace period, mandibular fractures may occur for many reasons (injuries obtained in the home, sports, industrial, or traffic accidents). The mandibule has a horseshoe shape, a certain thickness in different places, and a large curvature. Mandibular fractures may occur as a result of bending or compression and are more common among craniofacial injuries.

Mandibular fractures may differ by location (unilateral, bilateral), number (single, double, multiple), and nature (complete, incomplete) and can be 1) non-displaced, 2) displaced, 3) linear, 4) fragmented, 5) open, and 6) closed.

In the second stage, in 2015-2017, 80 patients treated for maxillofacial fractures in the Department of Oral and Maxillofacial Surgery of Clinical Medical Center No. 1 were involved in the clinical trials (Table 1).

Table 1

Distribution of Patients by Gender, Etiology of Trauma, and Clinical Symptoms

Indicators	Main group (n=40)		Control group (n=40)		p
	Abs. n.	%	Abs. n.	%	
Male	31	77,5	36	90	> 0,05
Female	9	22,5	4	10	
Mean age	1-61	27,4±1,79	5–63	30,6±2,09	> 0,05
Etiology of trauma:					
Home	24	60,0	27	67,5	> 0,05
Conflict	11	27,5	10	25,0	> 0,05
Traffic accident	4	10,0	1	2,5	> 0,05
Sports	1	2,5	2	5,0	> 0,05
Edema, swelling	40	100	40	100	> 0,05
Soft tissue injury	40	100	40	100	> 0,05
Positive load symptom	40	100	40	100	> 0,05
Disturbed bite	34	85	36	90	> 0,05

Of the patients, 13 were women and 67 were men. Depending on the treatment regimen, the patients were divided into two groups: the main group of 40 patients and the control group of 40 patients. The mean age of 40 control group patients was 30,6 ± 2,9, the youngest was 5, and the oldest was 63. The mean age of 40 main group patients was 27,4±1,79, the youngest and the oldest were, respectively, 1 and 61 years old.

The number of patients who had applied for treatment immediately after the trauma in the control and main groups was, respectively, 23 and 19. In general, the average application period was 2,75 days after trauma. In the control group, 27 patients were injured at home, 10 in a conflict, 1 in a traffic accident, and 2 during sports. In the main group, 24 patients were injured at home, 11 in a conflict, 4 in a traffic accident and 1 during sports. After the trauma, all patients of both groups were visually diagnosed with edema, swelling, soft tissue injury, and positive load symptoms. The disturbed bite was reported in 36 and 34 patients, respectively, in the control and main groups. Patients applied within 1-7 days after the trauma (table 1).

In the control group, mandibular angle fractures made 52,5%, trochlea fractures – 17,5%, mental fractures – 45%, body fractures – 20,0%, ramus fractures – 1%; symphysis and alveolar fractures were not observed. In the main group of patients, mandibular angle fractures made 40.0%, trochlea fractures – 40,0%, mental fractures – 30,0%, body fractures – 20,0%, symphysis fractures – 22,5%, alveolar fractures – 2,5%, and ramus fractures – 2,5%. The mean bed days in the control and main groups were, respectively, 8,9±0,74 and 10,2±0,94.

Patients applied within 1-7 days after the trauma. According to the indication, mandibular osteosynthesis and bimaxillary mandibular surgery were performed, respectively, in 71 (88,75%) and 9 (11,25%) patients. Osteosynthesis surgery was performed with titanium mini-plates and screws and bimaxillary splintage with Vasiliev's splints according to the AO/ASIF (Association for Osteosynthesis/Association for the Study of Internal Fixation) concept.

In contrast to the control group of patients, the main group had received an adaptive immunomodulator Septilin for a month, 2 tablets per day, along with the conventional antibacterial, anti-inflammatory, symptomatic, and hygienic oral cavity treatment with antiseptics.

During the clinical examination, we have determined whether any of the basic diagnostic signs have a positive load symptom at mandibular fractures. In this case, applying pressure to the specific submandibular or reciprocal mandibular angle area is characterized by

pain in the appropriate place. It is usually possible to determine whether the load symptom is positive in another way. In this case, affecting the specific submandibular or reciprocal mandibular angle area is required. In both cases, pain indicates a positive load symptom clinically confirming the mandibular fracture. The role of bimanual palpation, confirming a complete mandibular fracture, is irreplaceable.

Along with the mandibular bone, other facial bones were also visually examined and palpated.

The facial skin color, the face structure, pathological elements in the face and mouth, the condition of the tongue, lips, cheeks, and palate, and whether they are exposed to other pathology have also been considered.

Patients were examined by radiology, orthopontography, computed tomography, and X-ray diagnostics. In the complex treatment of patients, we consulted with various specialists on concomitant pathologies, as well as planned surgeries. Before osteosynthesis, patients were mainly consulted by specialists such as neurotraumatologists, therapists, anesthesiologists, and cardiologists and were prepared for surgery. Immunological examinations were performed to determine local and general resistance in patients: phagocytic activity, functional activity of neutrophils migrating from the cheek, alkaline phosphatase activity, lysozyme activity, immunoglobulin G, A, M, complement system activity, and T-lymphocyte subpopulations have been measured. The obtained quantitative indicators were processed by mathematical-statistical techniques.

A pediatrician examined the trauma of children with mandibular fractures. In addition to the maxillofacial region, patients were consulted by neurotraumatologists for pathologies in other parts of the body, including brain damage. Some patients were examined by dental orthodontists due to anomalies and developmental defects in the dental system. Consultations of orthodontists have played an important role in the complex treatment of children treated with conservative and conservative surgical methods.

Examinations and consultations of specialists involved in the complex treatment process in the identification of concomitant

pathologies of the organism were important for the effective treatment of patients.

Prior to osteosynthesis, patients were mainly consulted by specialists such as neurotraumatologists, therapists, anesthesiologists, cardiologists and prepared for surgery.

Immunological examinations were performed to determine local and general resistance in patients: phagocytic activity, functional activity of neutrophils migrating from the cheek in patients, alkaline phosphatase activity, lysozyme activity, immunoglobulin G, A, M, complement system activity, subpopulations of T-lymphocytes. The obtained quantitative indicators were developed by mathematical-statistical methods.

It is now known that T and B lymphocytes carry certain receptors and markers. In humans, in particular, T cells can be identified by the formation of rosettes with erythrocytes in sheep blood. Human lymphocytes are involved in the formation of the rosette, which attracts at least 4 sheep erythrocytes. To assess the T-system of immunity, it was immediately determined by changing the formation of rosettes.

Leukocyte count is determined in heparinized blood (25 units of heparin, 1 ml of blood) and a smear is prepared to calculate the blood formula. The concentration of lymphocytes is brought to 2x10 ^ 6ml by adding the isolated phycol-verograph speda-199.

Dilute the washed sheep's blood with speda-199 to 0.56 to 0.1 ml. 0.1 ml of lymphocyte suspension is added to the mixture. The mixture remains in the thermostat at 37 C for 5 minutes. The mixture is then centrifuged for 5 minutes at 750 rpm. After that, it remains at a temperature of 12 C for 1 hour. One hour after incubation, the cells are fixed with glutaraldehyde. (0.5 ml of 0.6% solution) and ointments are prepared after washing. The ointments are fixed in memanol and painted with Romanevsky-gimza.

The activity of lysozyme in the blood is determined nephelometrically. (M.Q. Dorofeychuk 1968). Based on the degree of turbidity of the test microbe (Micrococcus hysodecticus) suspension in the mixed phosphate buffer (PH 7.2-7.4), it is determined in a tub of 3 ml in a standardized 540 wave.

Circulating immunocomplexes are determined by M. Digeon et al. The principle of this method is that in this amount of PEC 6000 solution there is a special precipitation of antigen-antibody complexes. The amount of circulating immunocomplexes is confirmed by the density of the precipitate obtained by photometry at 1x1 450 waves on a spectrophotometer. The result is measured in units of density.

In the blood, Ig is determined by the method of G, A, M (G. Mancini, 1965), which is based on the change of precipitation circles. The serum is transferred to the cut-out agar-containing wells, where the pre-monospecific serum is dispersed. The diameter of the precipitate circle is proportional to the concentration of the immunoglobulins studied. The amount of immunoglobulins was determined in comparison with the known concentration of immunoglobulins in standard human serum.

The activity of the complement system in the blood is determined by the photocoarimetric micro method. (F.Y.Qarib, L.N. Sharapov 1973). The degree of hemolysis is measured by FEK-56. The amount of complement is calculated by the following formula.

$$C = \frac{\text{FEK experience indicator x40}}{\text{50\% hemosystem index of hemolysis}} = \text{FEK unit of scale}$$

Subpopulations of T-lymphocytes have been identified by sensitivity to T-helper and T-suppressors (A. Shore et al., 1978) theophylline.

The obtained numerical data were processed by statistical methods, taking into account modern requirements. Average values (M) for group indicators, their standard error (m), minimum (thousand) and maximum (max) values of series, as well as the frequency of occurrence of quality indicators in groups were determined.For the initial assessment of the difference between the variance series, the Student's t-criterion parametric method was used, the average difference between the indicators selected for the pair-related variants, and the assessment of the difference between the parts. Non-parametric criteria - Wilcoxon's (Manna-Whitney) U-

criterion and Pearson's $\chi 2$ criterion for frequency analysis were then used to verify and clarify the results obtained. Correlation analysis was performed to determine the strength of the relationship between the studied indicators.

It should be noted that the statistical processing of the results obtained during the research was carried out using the computer program Statistica 7.0.

RESULTS AND THEIR DISCUSSION

In healthy rabbits involved in the experimental research, the level of blood immunological indicators such as CIC, lysozyme, and complement was measured. In all rabbits, a mandibular fracture model was created using the technique proposed, and surgery was performed to fix the fracture fragments with titanium mini-plates and screws. In the experimental research, the main group of animals received an adaptive immunomodulatory drug – Septilin along with conventional complex treatment. At different stages of treatment, in the experimental research, histological-morphological changes in blood immunological indicators and the fracture line healing have been compared for the control and main group of animals. After creating the mandibular fracture model and stable fixation of the fracture fragments, changes in blood immunological parameters at different stages of treatment were identified for both groups and compared. Based on the results, new algorithms have been proposed for the complex treatment of mandibular fractures (Table 2).

Table 2

Immunological Indications Before and 1 Day After Surgery (M±m, min-max)

Indicators	Figures	Before the operation	After the operation	P
CIC (c.u.)	10	28,8±0,36 (27-31)	22,5±0,48 (20-25)	<0,01
Complement (%)	10	57,8±2,10 (46-65)	53,0±2,10 (41-60)	<0,01
Lysozyme (%)	10	28,3±0,32 (26,8-29,8)	23,3±0,39 (21,5-25,5)	<0,01

Note: P is the statistical accuracy of the difference compared to the pre-surgery indicator (Wilcoxon test)

On the 15th and 60th days after treatment, immunological examinations of blood samples were performed, and faster recovery of immunological parameters was observed.

The mean CIC in the blood of healthy rabbits was 28,8 c.u. A day after the surgery, it decreased sharply to an average of 22,5 c.u. (Diagram 1). On the 15th day of treatment, the CIC index in the control group of rabbits was close to the average value in healthy rabbits and amounted to 28,2 c.u. while in the main group of rabbits, this indicator increased substantially to 31,6 c.u. On the 60th day of treatment, the indicators were within the norm in both the control and main groups and differed within the statistical accuracy amounting, respectively, to 29,2 and 30,0 c.u.

The mean complement value in the blood of healthy rabbits was 57,8%. A day after the surgery, it decreased sharply to an average of 53,0%. On the 15th day of treatment, the complement index in the control group was 56,9%, approaching the mean value in the healthy rabbits while in the main group of rabbits, it increased to 59,3% (Diagram 2). On the 60th day of the experiment, in the control and main groups, complement values averaged 60,8 and 62,5%, respectively, and the difference was not statistically significant.

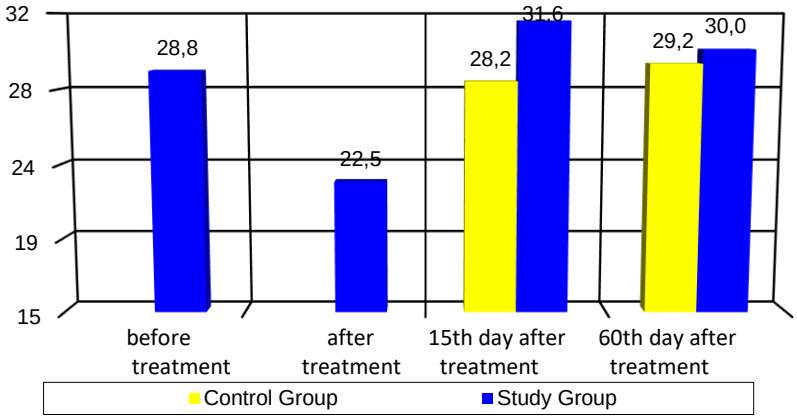

Diagram 1. The level CIC before and after the treatment

The mean lysozyme content in the blood of healthy rabbits was 28,8%. A day after the surgery, it dropped sharply to an average of 23,3% (Diagram 3). On the 15th day of treatment, in the control group, the lysozyme index was close to the mean value in healthy rabbits and amounted to 27,2% while in the maingroup, it was 28,4%. On the 60th day of treatment, lysozyme levels increased to 28,7 and 29,4% in the control and main groups, respectively.

Along with blood-forming elements (some leukocyte types), many immune system components are actively involved in the recovery of injured tissues at pathology. Experimental research has shown that immunomodulators have a positive effect on the regeneration of injured tissues in many pathological processes.

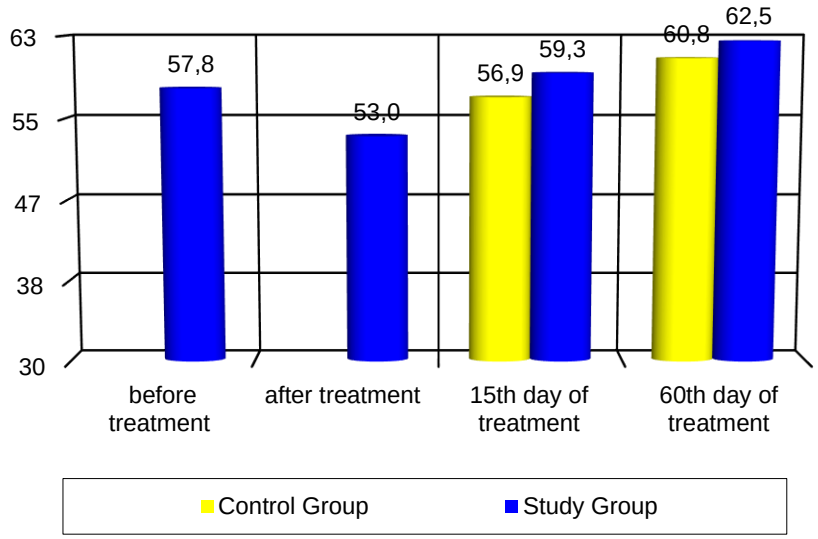

Diagram 2. The impact of treatment on complement

The histological examination results showed that within 7 days after the surgery, all the test animals had well-developed connective tissue elements in the mandibular fracture area. Irregularly arranged fibrin fibers were thin and soft. Weak blood capillaries were being

formed between them. Proliferative activity of regenerative cells was observed between irregularly arranged fibers.

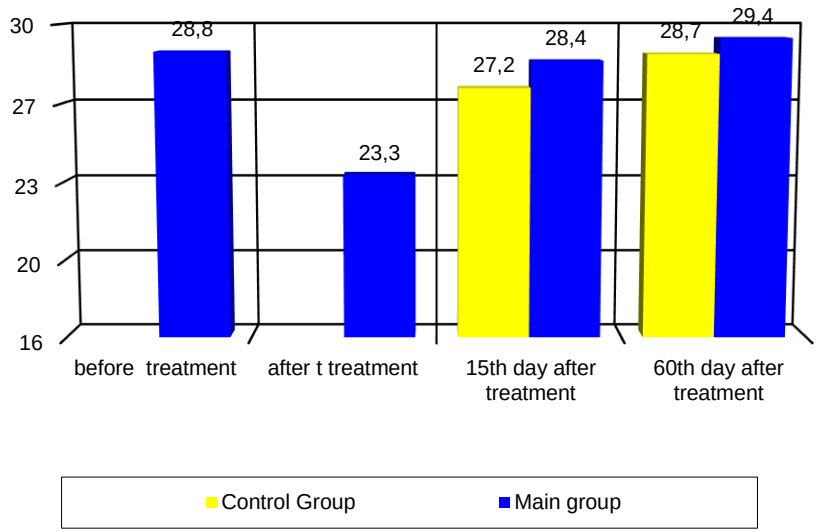

Diagram 3. The Impact of Treatment on Lysozyme

At the early stages of osteogenesis, bone samples taken from three conventionally treated rabbits had no obvious differences. In two of the 3 main group rabbits, the initial osteogenesis stage was successful. Connective tissue elements were intensively developed in the fracture area. Irregularly arranged fibrin fibers seemed to be slightly thicker and denser than in the control group (Fig. 1). The proliferative activity of osteoblasts between fibrin fibers was quite high. Their density was particularly noticeable along the fracture line. Individual osteocytes were observed in some areas. In main group animal, the first osteogenesis stage differed little from the control group. No acute edema or inflammatory reactions were reported in the mandibular bone area of all the control and main group animals.

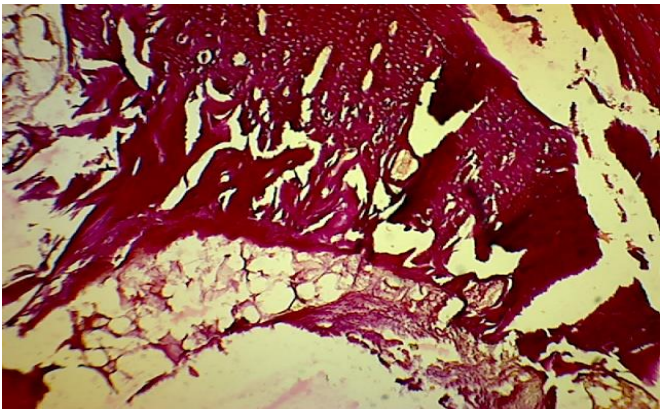

Figure 1. Light microscope view of a block taken from the fracture line in the main group rabbit 7 days after treatment at 400 magnification

On the 21st day of the experiment, part of the control and main group animals (3 rabbits each) were decapitated, and the mandibularbones were fixed in 10% formalin solution. Histological analysis showed that connective tissue elements were well-developed in the fracture area in all samples over the past 14 days. Sufficient osteocytes have been found between collagen fibers regularly arranged between fractured bones. Collagen fibers have become much thicker, and mineral salts have accumulated on them. Compared to the control group, bone plates were observed between sufficiently well-developed collagen fibers in the mandibular fracture area of the test animals receiving Septilin (Figure 2).

On the 60th day of the experiment, part of the control and main group animals (3 rabbits each) were decapitated, and the mandibule bones were fixed. Histological analysis showed that the initial bone elements had started recovering in the samples over the past 60 days. Connective tissue elements were well-developed in the fracture area. In animals additionally treated with Septilin, immunomodulatory drugs had a positive effect on the timely and uncomplicated completion of reparative osteogenesis compared to conventionally treated animals.

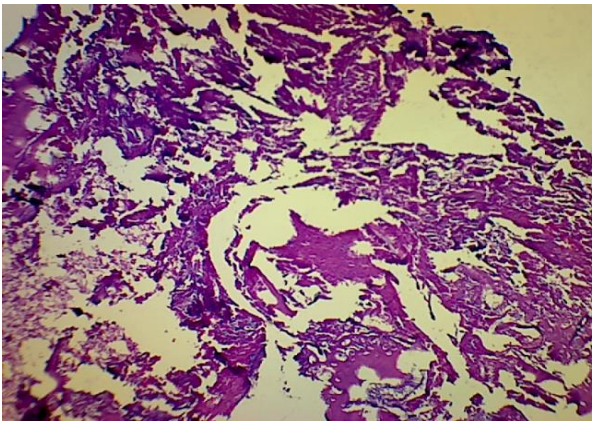

Figure 2. Light microscope view of a block taken from the fracture line in the main group rabbit 21 days after treatment at 400 magnification

The studied immunomodulator Septilin has a stronger effect at the initial osteogenesis stage, maintains the quantitative and qualitative balance of leukocytes, increases the activity of cytokines, and creates conditions to eliminate the risk of infection; thus, therapeutic-drug immunocorrection allows for improving the efficiency of pathogenetic treatment in reparative osteogenesis.

According to the clinical trial results, the average level of overall post-traumatic resistance decreased in both the control and main groups of patients.

Thus, at the time of hospitalization, in the control group of patients, T-lymphocyte, B-lymphocyte, and T-helper/T-suppressor indicators averaged 52,2±0,98, 13,9±0,92, and 2,4±0,09%, respectively. On the 7^{th} day of treatment, these indicators changed relatively and reached 58,6±1,02, 16,7±1,00, and 2,6±0,08%, respectively. On the 15^{th} day of treatment, the indicators increased to 65,1±0,91, 18,8±0,75 and 3,1±0,10%, respectively, and approached the norm. In the main group of patients, these values were, respectively, 55,4±0,66, 16,2±0,48, and 2,2±0,06% at the time of hospitalization, 61,3±0,72, 18,3±0,51 and 2,5±0,07% on the 7^{th} day of

treatment, and were within the norm on the 15th day of treatment, having reached the values 65,4±1,04, 20,7±0.50, and 2.8±0.09%.

In the control group of patients, at the time of hospitalization, the mean CIC, lysozyme, and complement values were 52,8±0,76 c.u., 34,1±0,58%, and 56,0±1,11%, respectively. On the 7th day of treatment, these indicators changed relatively and reached 58,5±0,78 c.u., 37,4±0,33%, and 60,1±1,02%, respectively. On the 15th day of treatment, the indicators increased to 63,9±1,10 c.u., 42,3±0,45%, and 65,1±0,98%, and approached the norm. In the main group of patients, these values were, respectively, 53,8±0,98 c.u., 30,4±0,58% and 48,2±0,98% at the time of hospitalization, 56,1±1,00 c.u., 34,6±0,72%, and 34,6±0,72% on the 7th day of treatment, and were within the norm on the 15th day of treatment, having reached the values 63,1±0,70 c.u., 39,2± 0,5%, and 39,2±0,85%.

On the 7th and 15th days of treatment, in the main group of patients, the indicators increased more intensively and approached the normative indicators faster than in the control group.

In the control group patients, at the time of hospitalization, immunoglobulins IgG, IgA, and IgM averaged 52,8±0,76, 34,1±0,58, and 56,0±1,11 g/l, respectively. On the 7th day of treatment, these values changed relatively and reached 58,5±0,78, 37,4±0,33, and 60,1±1,02 g/l, respectively. On the 15th day of treatment, the indicators increased to 63,9±1,10, 42,3±0,45 and 65,1±0,98 g/l, respectively, and approached the norm. In the main group of patients, these values were, respectively, 53,8±0,98, 30,4±0,58 and 48,2±0,98 g/l at the time of hospitalization, 56,1±1,00, 34,6±0,72 and 34,6±0,72 g/l on the 7th day of treatment, and were within the norm on the 15th day of treatment, having reached the values 63,1±0,70, 39,2±0,85 and 39,2±0,85 g/l. In the maingroup of patients, the indicators increased more intensively and approached the normative indicators faster than in the control group. Inflammatory complications were observed in 8 control group patients. In 5 of these patients, titanium mini-plates and screws placed in the fracture line have been removed. The other 3 patients refused the offered help.

In the main group, only one patient had an inflammatory complication in the surgery area. This patient also underwent surgery

to remove titanium mini-plates and screws, and the signs of inflammation were eliminated.

Thus, the results of our clinical and biochemical studies show that adaptive and immunomodulatory drugs used to eliminate the immune deficiency that may occur during the post-traumatic period at mandibular fractures has a positive effect on the timely and uncomplicated completion of reparative osteogenesis. The studied immunomodulatory drug Septilin has a stronger effect at the initial osteogenesis stage, maintains the quantitative and qualitative balance of leukocytes, increases the activity of cytokines, and creates conditions to eliminate the risk of infection. Thus, therapeutic-drug immunocorrection allows for improving the efficiency of pathogenetic treatment in reparative osteogenesis at bone fractures.

According to the examination results, the main group of patients had the following body resistance indicators during treatment. At the time of hospitalization, the number of neutrophils migrating from the mucous membrane of the cheek, increased moderately to 20,0±1,4 c.u., in contrast to healthy subjects ($p>0,05$). The alkaline phosphatase activity amounted to 46,8±2,0 c.u. ($p>0,005$).

10-14 days after hospitalization, the resistance indicators of migration from the mucous membrane of the cheek in patients increased significantly compared to healthy subjects: the number of neutrophils and the alkaline phosphatase activity were, respectively, 33,7±1,5 and 73,8±2,6 c.u. ($p<0,001$). At the time of discharge from the clinic, in the main group of patients (uncomplicated mandibular fractures), the number of neutrophils migrating from the mucous membrane of the cheek reduced to a value close to normal and amounted to 18,6±1,1 c.u. ($p> 0,05$). The alkaline phosphatase activity correspondingly approached the norm and was 40,7±1,4 c.u. ($p>0,05$).

Thus, according to the indicators, at the time of discharge from the clinic, local non-specific resistance in patients with uncomplicated mandibular fractures was normal.

In the control group, from the first days of hospitalization, the number of neutrophils migrating from the mucous membrane of the cheek and the alkaline phosphatase activity were being increased in patients with various post-traumatic complications and at the time of

discharge from the clinic, have reached, respectively, 24,7±1,2 and 47,5±1,6 c.u. ($p<0,001$). In patients examined 10-14 days after treatment, the number of neutrophils migrating from the mucous membrane of the cheek and the alkaline phosphatase activity significantly increased in contrast to healthy subjects and the start of treatment and reached, respectively, 47,5±1,6 and 86,3±2,5 c.u. ($p<0,001$). In the control group, at the time of discharge from the clinic, the number of neutrophils migrating from the mucous membrane of the cheek remained at a high level and amounted to 41,2±1,5 c.u. ($p<0,001$), and the alkaline phosphatase activity was 77,0±2,1 c.u. ($p<0,001$).

The aforementioned allows concluding that during treatment in the control group, local non-specific resistance has decreased, and on days 10-14 of treatment, an increase in the number of neutrophils migrating from the mucous membrane of the cheek and high alkaline phosphatase activity has observed. At the time of discharge from the clinic, these indicators did not approach the norm and were completely different. This confirms the low local immunity in the examined patients. Abnormal values in these patients were also accompanied by low local non-specific resistance factors and inflammation signs in the mucous membrane and around the bone.

Indicators of general nonspecific resistance in the main and control groups of patients were also studied. In the main group of patients, at the time of hospitalization, the phagocytic activity of leukocytes in the peripheral blood was almost unchanged compared to healthy subjects and amounted to 72,9±1,4% or 6,3±0,3 c.u. ($p>0,05$), and at the time of discharge, it was 73,7±1,2% or 6,5±0,2 c.u. ($p>0,05$). In the control group of patients, at the time of hospitalization, the phagocytic activity of leukocytes in the peripheral blood was almost unchanged: 73,6±1,3% or 6,2±0,3 c.u. ($p<0,001$). However, upon discharge from the clinic, the phagocytic activity of leukocytes significantly decreased: 61,9±1,2% or 5,0±0,4 c.u. ($p<0,02$).

The Schiller-Pisarev test was used to identify inflammation in the fracture line. In the main and control groups of patients, at the time of hospitalization, Svrakov's iodine number was, respectively, 6,4±0,7 (intensive inflammatory process) and 6,8±0,6 (intensive inflammatory

process) points. On the 3-4th day of drug treatment, the main and control groups of patients showed, respectively, 4,2±0,4 (moderate inflammatory process) and 5.5±0.3 (intensive inflammatory process) points. On the 6th-7th day of treatment, 3,3±0,7 (moderate inflammatory process) and 5,4±0,8 (intensive inflammatory process) points were recorded, respectively, in the main and control groups of patients. On the 9th-10th day of treatment, the Schiller-Pisarev test score in the main and control groups of patients was, respectively, 2,2±0,6 (slight inflammatory process) and 5,1±0,4 (intensive inflammatory process) points.

In both groups of patients, the alveolar ridge mucous membrane fracture line and the indicators of contact thermometry performed on the opposite side were determined. At the time of hospitalization, high thermoassimetry was observed in the main group of patients: 1,6±0,1°C ($p<0,01$), and approximately the same value was recorded in the control group: 1,7±0,2°C ($p<0,02$). On the 3-4th day of treatment, it was 1,2±0,2°C ($p<0,001$) and 1,5±0,1°C ($p<0,01$), respectively, in the main and control groups. On the 6th-7th day of treatment, it was 0.9±0.1 °C ($p<0,01$) and 1,3±0,2°C ($p<0,01$), respectively, in the main and control groups. On the 9th-10th day of treatment, thermoassimetry in the main group was close to normal: 0,6±0,2°C ($p<0,05$) while in the control group, relatively high values were recorded: 1,0±0,2°C ($p<0,01$).

In the main group, gingivitis was observed in 63,3% of cases as a result of the use of dental splints. This complication disappeared without intervention a few days after the removal of the splints. No other complications were reported in the main group.

In the control group, all patients had gingivitis; 26 patients had an inflammatory complication – post-traumatic osteomyelitis (2 patients in the mental and 24 patients in the mandibular angle regions). In 7 (26,9%) of the 26 patients with post-traumatic osteomyelitis, the fracture line teeth were removed at the time of hospitalization, and in another 17 (73,1%) – on days 11-23 of treatment. One patient underwent osteosynthesis surgery with titanium mini-plates 3 days after hospitalization. The patient left the clinic arbitrarily on the 14th day of treatment and was treated on an outpatient basis. On the 12th day after the surgery, dental splints were removed by himself, and the

treatment regimen was violated. The hygienic condition of the oral cavity was assessed as poor in all the control group patients.

Thus, developing inflammation in the mandibule bone can be forecasted using the technique of thermoassimetry by determining the number of neutrophils migrating from the mucous membrane of the cheek and the alkaline phosphatase activity. These indicators can be considered inflammation predictors.

CONCLUSIONS

1. The experimental study revealed pathological changes in the blood immunological indicators in the post-traumatic period: for the first day after the trauma, lysozyme decreased by 19,09% from 28,8 to 23,35%, CIC increased by 21,87% from 28,8 to 22,5 c.u., and complement decreased by 8,3% from 57,8% to 53%. 1 day after the surgery, the blood CIC decreased sharply by 6,3 c.u. compared to healthy rabbits. On the 15^{th} day of treatment, in the main group, it by 3,4 c.u. compared to the control one. On the 60^{th} day of treatment, it was within the norm in both control and main groups; statistical accuracy had not changed. A day after the surgery, the complement index decreased sharply by an average of 4,8%. There was no statistically significant difference between the groups on days 15 and 60 of treatment [5].

2. When studying reparative osteogenesis in experimental research groups, histological examination (decapitated mandibule bones) showed well-developed connective tissue elements and osteocytes formed between regularly-arranged collagen fibers between broken bones in all samples. Compared to the control group, in the main group (receiving Septilin), bone plates were formed between the sufficiently well-developed collagen fibers in the mandibular fracture area [8].

3. Clinical trials revealed pathological changes in the blood immunological parameters in the post-traumatic period: for the first day after the trauma, at the time of hospitalization, CIC, lysozyme, and complement, respectively, amounted to an average of 53,8±0,98 c.u., 30,4±0,58% and 48,2±0,98% in the main group and an average of 52,8±0,76 c.u., 34,1±0,58% and 56,0±1,11% in the control group. On

the 15th day of treatment, these indicators were 63,1±0,70 c.u., 39,2±0,85% (statistical accuracy $p_1<0,01$) and 39,2±0,85% ($p_1<0,01$) in the main group and 63,9±1,10 c.u., 42,3±0,45%, and 65,1±0,98% in the control group. In the main group, they increased more intensively and approached the norm faster than in the control group.

After trauma, T-lymphocyte, B-lymphocyte, and T-helper/T-suppressor values decreased compared to the norm and were, respectively, 55,4±0,66, 16,2±0,48 and 2,2±0,06% in the main group and 52,2±0,98, 13,9±0,92 and 2,4±0,09% in the control group. After the treatment, they increased to 65.4±1.04 ($p_1<0,05$), 20,7±0,50 and 2,8±0,09% ($p_1<0,05$), reaching the norm in the main group.

On the 15th day of treatment, the control group showed IgG, IgA, and IgM, respectively, 63,9±1,10, 42,3±0,45, and 65,1±0,98 g/l, and the main group, respectively, 63,1±0,70, 39,2±0,85 and 39,2±0,85 g/l ($p_1<0,001$). In the main group, the indicators increased more intensively and approached the norm faster than in the control group [7,9].

4. At the time of hospitalization, the number of neutrophils migrating from the mucous membrane of the cheek increased moderately (norm: 18,6±1,1 c.u.) compared to healthy subjects by an average of 20,0±1,4 c.u. ($p>0,05$) in the main group and 24,7±1,2 c.u. ($p<0,001$) in the control group; 10-14 days after treatment, it increased significantly by 33,7+1,5 c.u. ($p<0,001$) in the main group and 47,5±1,6 ($p<0,001$) in the control group with high statistical accuracy, and at the time of discharge from the clinic, it was within the norm 18,6±1,1 c.u. ($p>0,05$) in the main group and remained statistically high 41,2±1,5 c.u. ($p<0.001$) in the control group. Before treatment, alkaline phosphatase activity increased compared to healthy subjects (norm: 40,9±2,2 c.u.) by 46,8±2,0 c.u. ($p>0,005$) in the test group and 47,5±1,6 c.u. ($p<0,001$) in the control group. After treatment, alkaline phosphatase activity was 73,8±2,6 c.u. ($p<0,001$) in the main group and 86,3±2,5 c.u. in the control group. At the time of discharge from the clinic, it approached the norm 40,7±1,4 c.u. ($p>0,05$) in the main group and remained statistically high 77,0±2,1 c.u. ($p<0,001$) in the control group [3].

5. The use of adaptive and immunomodulatory drugs to eliminate the immune deficit in the post-traumatic period in patients with mandibular fractures allowed reducing inflammatory complications from 12,5% to 2,5% in the course of reparative osteogenesis in clinical trials. Introducing an adaptive immunomodulatory drug in the drug therapy in the complex treatment of mandibular fractures increases the efficiency of pathogenetic treatment in the course of reparative osteogenesis [1].

PRACTICAL RECOMMENDATIONS

1. The successful application of Septilin in mandibular fractures in our research allows concluding on the possibility of widely using it by practicing surgeons for this purpose.
2. It is more appropriate to apply a complex treatment of mandibular fractures with not only conventional methods but also adaptive immune drugs.

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Abbreviations

CIC – Circulating immune complexes
IgA – immunoglobulin A
IgG – immunoglobulin G
IgM– immunoglobulin M

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