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# PROGNOSIS OF REPARATIVE OSTEOGENESIS IN RATS WITH OPEN MANDIBULAR FRACTURE ON THE BACKGROUND OF OSTEOPOROSIS

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In this work, mathematical prediction of the course of reparative osteogenesis in rats with an open fracture of the mandible on the background of osteoporosis has been carried out. It is shown that important biochemical markers for prognosis of osteo-reparation processes under conditions of simulated pathology are the content of VEGF, TGF-β1, carbonyl groups of proteins, nitrites and nitrates in serum. Among these biochemical indicators, the greatest value for controlling the processes of reparative osteoregeneration is the determination of the content of TGF-β1 and VEGF in serum, but less important is the study of the level of carbonyl groups of proteins and nitrites and nitrates in serum.

**Key words:** metabolic markers, reparative osteogenesis, fracture of the mandible, mathematical prediction.

Reparative osteogenesis is a complex multi-stage biological process, which is normally accompanied by a complete restoration of the structure of damaged bone tissue. Despite the fact that during this process, exist all the biological preconditions for normal healing of the fracture, the percentage of delayed fusion and various complications after traumatic bone damage to date remains rather high - 10-15% [6]. There are many factors that can modify the course of reparative osteogenesis, among which an important place belongs to osteoporosis [10]. Therefore, a very important issue of modern medicine is the definition of osteo-reparative potential in patients with osteoporosis before performing surgical interventions, which would allow, if necessary, to apply methods of stimulation of osteo-reparation in the early postoperative period to create optimal conditions for the course of reparative osteogenesis. In this regard, the prognosis of the course of osteo-reparation processes in fractures of the mandible, especially against the background of osteoporosis, is a very topical issue in dentistry to develop an effective treatment strategy for patients.

**The purpose** of the study is to develop an effective mathematical model for predicting the course of reparative osteogenesis in rats with an open fracture of the mandible on the background of osteoporosis.

**Material and methods.** Experimental osteoporosis in rats was induced by administration of 2.5% hydrocortisone acetate solution over a period of 60 days in a dose of 5 mg/kg body weight [1]. Subsequently, the drug was discontinued and made traumatic damage to the lower jaw: the rat was fixed on the machine on the back; under light hexanal (0.1 ml of 10% solution per 100 g of body weight) anesthesia in the right submandibular zone was performed damage of skin parallel to the lower edge of the mandible in the medial direction of 10-12 mm in length; the muscles dissected and skeletoned the lower jaw; separating cortical plate was cut through the separating disk, and then a full bone fracture was applied along the line connecting the site of the fusion of the body and the branches of the jaw in the retro-molar region with a position located 0.9 cm in the medial angle of the mandible. The surgical wound was connected to the oral cavity, the muscles and the skin were sutured with a catgut. All stages of experimental research have been performed in accordance with the International Humane Animal Health Practices Directive in accordance with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsya (Minutes № 14 of 25.11.2010). Animals were divided into two groups: 1 group (control) - pseudo-operated rats (PO); group 2 (trial) - rats with a simulated open fracture of the mandible on the background of osteoporosis (FM + OP). The research was carried out at 7, 14, 21, 30 and 45 days after fracture simulation. Biochemical and immunoassay studies were carried out in blood serum, which was isolated according to the standard method [9]. The serum was obtained by centrifugation of blood at 600 g for 30 min. at 18-22°C. Blood serum aliquots were taken in Erpendorf microtubules and stored at -20°C until analysis. The content of TBA-reactive products (TBA-RP, secondary lipid peroxidation products) in serum were determined by reaction with 2-thiobarbituric acid (TBA) [12], the level of carbonyl groups of proteins (CGP) - by the formation of phenylhydrazones having a characteristic spectrum absorption, with the interaction of carboxyl groups of aliphatic amino acids with 2,4-dinitrophenylhydrazine [13]. The content of nitrates and nitrites in serum was determined by reaction with the Gris reagent after precipitation of proteins with acetonitrile. Nitrates were pre-reduced to nitrite with a mixture of zinc powder and ammonia solution [5]. The level of medium weight molecules was determined after protein precipitation with trichloroacetic acid (TCA) [3]. The activity of superoxide dismutase in blood serum (SOD, KF 1.15.1.1) was determined by

inhibition of oxidation of quercetin [7], activity of NADPH-oxidase (CF 1.6.3.1) - at the decrease in absorption of NADPH at 340 nm [2]. The content of protein in serum was determined by the microbiouretic method [4]. The serum content of the human vascular endothelial growth factor (VEGF) was determined by the immune enzyme method using the standard set of "VEGF ELISA" (Invitrogen, Canada), the level of tumor necrosis factor alpha (TNPα) using the commercial kit "TNFα ELISA" ("Diaclone", France), interleukin-8 (IL-8) - using standard sets of the company" Diaclone"(France), and the content of the transforming growth factor beta (ТПА-β1) - using the standard set of" TGF-β1 ELISA kit "firm "DRG" (France) according to the manufacturer's instructions. The content of free and peptide-linked oxyproline in serum was determined by reaction with para-dimethylaminobenzaldehyde [11]. The content of total calcium in serum was determined by a standardized spectrophotometric method by reaction with o-krezolftalein complexon on the set of "Calcium" (Filisit-Diagnostika, Ukraine). The content of phosphates in serum was determined by a standardized spectrophotometric method by reaction with ammonium molybdate in an acid medium, using the “Phosphorus VIS” kit (Filisit-Diagnostika, Ukraine). Determination of the activity of acid (KF 3.1.3.2) and alkaline phosphatase (KF 3.1.3.1) in serum was performed by spectrophotometric method by reaction with p-nitrophenyl phosphate at pH 4.8 and 10.5, respectively [8]. Statistical processing of the results of the study was carried out in MS Excel XP and SPSS-10.0.5 for Window (license number 305147890). In determining links between the indicators used correlation analysis of Pierson and Spearman. The difference was considered to be significant at $p < 0,05$. One-factor dispersion and multiple linear regression analyzes were used to predict the course of reparative osteogenesis.

**Results and its discussion.** We first conducted a correlation analysis between collagen metabolism markers and biochemical parameters in rats with an open fracture of the mandible on the background of osteoporosis (Table 1). It was found that the content of free and peptide-linked oxyproline in blood serum most strongly correlated with the level of TGF-β1. Under these conditions, the growth of TGF-β1 is accompanied by a decrease in collagen degradation processes and an increase in collagen formation. Less than the correlation force appeared between the markers of collagen metabolism and the level of proinflammatory cytokine of the TNF-α. It turns out that the activation of immune-inflammatory reactions is accompanied by an increase in collagen degradation and a decrease in biosynthetic processes. The least strong interrelationships were between the level of oxyproline and the serum content of carbonyl groups of proteins and metabolites of nitrogen monoxide. Thus, the enhancement of oxidative modification of proteins and nitrosatitative stress activates catabolism and reduces the synthesis of collagen. Subsequently, we evaluated the correlation between markers of bone remodeling (activity in the serum of acid and alkaline phosphatase) and the activity of immune-inflammatory reactions, endotoxemia, nitrosatitative and oxidative stress (Table 2). Bone remodeling indicators revealed the strongest linkages with the level of growth factors VEGF and TGF-β1. Growth in the blood of the contents of these factors is associated with induction of osteogenesis and a decrease in the activity of destructive processes in the bone tissue. Less than the strength of the linkage occurred between the activity of phosphatase and serum levels of proinflammatory cytokines, TNF-α and IL-8. Under these conditions, the strengthening of immune-inflammatory reactions is accompanied by activation of osteolytic processes. The smallest modulus correlations arose between markers of bone remodeling and activity of superoxide anion (NADPH-oxidase), oxidative degradation of proteins (CGP) and nitrosated stress (nitrites and nitrates). Correlation analysis makes it possible to characterize only the force and direction of the relationship between the two variables, but does not reveal a causal relationship. In order to identify the independent biochemical parameters that allow us to predict the course of reparative osteogenesis in conditions of fracture of the mandible on the background of osteoporosis, we applied the method of multiple linear regression analysis. As possible regressors, we selected biochemical indicators that showed reliable links with markers of collagen exchange and bone remodeling. To these regressors, we took the content of serum contents of the TNF-α (X1), IL-8 (X2), VEGF (X3), TGF-β1 (X4), CGP (X5), nitrites and nitrates (X6) and NADFN- oxidase (X7). As a criterion, the dependent variable Y, we introduced a conditional indicator that characterizes the degree of mineralization of bone tissue, which we call the predicted index of mineralization (PIM). Since biological models have high intercollearnarity of regressors, we used the model of their turn-by-turn (Forward) in the regression equation, that is, at each step, the most informative parameters that had a high modulus partial correlation coefficient were included at each step and increased the determination coefficient of the linear regression equation. During the step-by-step inclusion of the selected regressors in the linear regression equation, four models were created (Table 3). The statistical analysis of these regressive osteogenesis regression models showed that mathematical model №4 is most suited to this type of forecasting, since it better describes the variance of the criterion variable Y ($R^2$ for Model №4 is significantly higher than that of other models) and has a lower standard error assessments. Therefore, in the future we used exactly this model of prediction of osteoporosis treatment. According to the mathematical

model №4 it was established that the most significant and independent biochemical parameters that play a significant role in predicting the course of reparative osteogenesis are the following: $X_3$ - content in the blood serum VEGF; $X_4$ - blood level of TGF-β1; $X_5$ - level in blood CGP; $X_6$ - blood level of nitrites and nitrates (Table 4).

Table 1

**Relationship between markers of collagen exchange and indicators of immune-inflammatory processes, oxidative and nitrosatitative stress in rats with an open fracture of the mandible on the background of osteoporosis (n=35)**

| Indexes | Free oxiprolin, μmol/l | Peptide-linked oxyproline, μmol/l |
|---|---|---|
| TNF-α, pg / ml | 0,38* | -0,40* |
| IL-8, pg / ml | 0,20 | -0,25 |
| VEGF, pg / ml | -0,15 | 0,21 |
| TGF -β1, pg / ml | -0,45* | 0,52* |
| TBA-RP, μmol / l | 0,10 | -0,14 |
| CGP, unit of protein / mg protein | 0,39* | -0,35* |
| NADPH-oxidase, nmol / min · ml | 0,20 | -0,24 |
| SOD, conditional units / mg of protein | -0,15 | 0,10 |
| MSM, units of protein. | 0,13 | -0,21 |
| Nitrites and nitrates, μmol / l | 0,35* | -0,38* |

Notes: * – reliability of the correlation coefficient at r>0,33 (p<0,05).

Table 2

**Relationship between biochemical markers of bone mineralization and immune-inflammatory processes, oxidative and nitrosatitistic stress in rats with an open fracture of the mandible on the background of osteoporosis (n=35)**

| Indexes | Alkaline phosphatase, U / l | Acid phosphatase, U / l |
|---|---|---|
| TNF -α, pg / ml | -0,45* | 0,48* |
| IL-8, pg / ml | -0,42* | 0,40* |
| VEGF, pg / ml | 0,54* | -0,62* |
| TGF -β1, pg / ml | 0,52* | -0,59* |
| TBA-RP, μmol / l | -0,14 | 0,22 |
| CGP, unit of protein / mg protein | -0,34* | 0,35* |
| NADPH-oxidase, nmol / min · ml | -0,33* | 0,36* |
| SOD, conditional units / mg of protein | 0,13 | -0,12 |
| MSM, units of protein. | -0,11 | 0,14 |
| Nitrites and nitrates, μmol / l | -0,34* | 0,37* |

Notes: * – reliability of the correlation coefficient at r>0,33 (p<0,05).

Table 3

**Statistical characteristic of models of prediction of bone tissue mineralization index in rats with open fracture of the mandible on the background of osteoporosis.**

| Model | R | $R^2$ | Adjusted $R^2$ | Standard error of estimation |
|---|---|---|---|---|
| № 1 | 0,766[a] | 0,587 | 0,584 | 0,059 |
| № 2 | 0,831[b] | 0,690 | 0,688 | 0,058 |
| № 3 | 0,899[c] | 0,808 | 0,803 | 0,073 |
| № 4 | 0,929[d] | 0,863 | 0,856 | 0,030 |
| a. Predictors: (constant), $X_4$ | | | | |
| b. Predictors: (constant), $X_4$ та $X_3$ | | | | |
| c. Predictors: (constant), $X_4$, $X_3$ та $X_5$ | | | | |
| d. Predictors: (constant), $X_4$, $X_3$, $X_5$ та $X_6$ | | | | |

Table 4

**Characteristics of regressors needed to predict bone mineralization index in rats with an open fracture of the mandible on the background of osteoporosis**

| Indexes | β | B | Standard error B | t | p |
|---|---|---|---|---|---|
| $X_4$ (TGF -β1) | 0,456 | 0,065 | 0,007 | 12,25 | 0,000 |
| $X_3$ (VEGF) | 0,327 | 0,058 | 0,006 | 11,54 | 0,000 |
| $X_5$(CGP) | -0,210 | -0,041 | 0,005 | -12,35 | 0,000 |
| $X_6$(Nitrites and nitrates) | -0,156 | -0,035 | 0,004 | -10,85 | 0,000 |

The statistical characteristic of these predictors is given in Table 4. It is established that the non-standardized regression coefficients of the model (B) are significant and reliable (t> 2, p <0.05), while the coefficients of the correlation are at an acceptable level, indicating an acceptable level of intercolinearity variables with each other. Comparison of regression coefficients β allows asserting a different contribution of selected predictors in predicting the course of reparative osteogenesis. It turned out that under these conditions, the most important role in osteoporosis is played by the level of TGF-β1 in blood, the VEGF content is slightly less important, and the least significant contribution belongs to the level of CGP, nitrites and nitrates. The obtained data indicate that a comprehensive study of the content of these biochemical markers in the

blood is necessary for monitoring the course of reparative osteogenesis in the fracture of the mandible on the background of osteoporosis.

## Conclusion

1. In animals with an open fracture of the mandible on the background of osteoporosis, the metabolism of collagen (the content of free and peptide-linked oxyproline in serum) most closely correlated with the level of TGF-β1. The increase in this serum cytokine level is accompanied by a decrease in collagen degradation processes ($r = -0.45$; $p < 0.05$) and a growth of collagen formation ($r = 0.52$; $p < 0.05$).
2. For modeling pathology, markers of bone remodeling show the strongest linkages with the level of growth factors TGF-β1 and VEGF. Increasing the level of these growth factors in the blood serum is associated with induction of osteogenesis ($r = 0.52$; 0.54; $p < 0.05$) and decrease in the activity of destructive processes in the bone tissue ($r = -0.59$; -0.62; $p < 0.05$).
3. The greatest diagnostic value for the control of osteoporosis in animals with an open fracture of the mandible on the background of osteoporosis is the determination of the content of TGF-β1 and VEGF in serum ($\beta = 0{,}456$; 0,327), less important is the study of the level of carbonyl groups of proteins, as well as nitrites and nitrates in serum ($\beta = -0.210$; -0.156).

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## Реферати

### ПРОГНОЗУВАННЯ ПЕРЕБІГУ РЕПАРАТИВНОГО ОСТЕОГЕНЕЗУ У ЩУРІВ З ВІДКРИТИМ ПЕРЕЛОМОМ НИЖНЬОЇ ЩЕЛЕПИ НА ТЛІ ОСТЕОПОРОЗУ

**Гольцев А. М., Ліхіцький О. О.**

В роботі проведено математичне прогнозування перебігу репаративного остеогенезу у щурів з відкритим переломом нижньої щелепи на тлі остеопорозу. Показано, що важливими біохімічними маркерами прогнозування процесів остеорепарації за умов модельованої патології є вміст VEGF, ТФР-β1, карбонільних груп протеїнів, нітритів та нітратів в сироватці крові. Серед цих біохімічних показників найбільшу цінність для контролю за процесами репаративної остеорегенерації має визначення вмісту ТФР-β1 та VEGF в сироватці крові, а менше значення належить дослідженню рівня карбонільних груп протеїнів та нітритів й нітратів в сироватці крові.

**Ключові слова:** метаболічні маркери, репаративний остеогенез, перелом нижньої щелепи, математичне прогнозування.

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### ПРОГНОЗИРОВАНИЕ ТЕЧЕНИЯ РЕПАРАТИВНОГО ОСТЕОГЕНЕЗА У КРЫС С ОТКРЫТЫМ ПЕРЕЛОМОМ НИЖНЕЙ ЧЕЛЮСТИ НА ФОНЕ ОСТЕОПОРОЗА

**Гольцев А. Н., Лихицкий А. А.**

В работе проведено математическое прогнозирование течения репаративного остеогенеза у крыс с открытым переломом нижней челюсти на фоне остеопороза. Показано, что важными биохимическими маркерами прогнозирования процессов остеорепарации в условиях моделируемой патологии является содержание VEGF, ТФР-β1, карбонильных групп протеинов, нитритов и нитратов в сыворотке крови. Среди этих биохимических показателей наибольшую ценность для контроля за процессами репаративной остеорегенерации имеет определение содержания ТФР-β1 и VEGF в сыворотке крови, а меньшее значение принадлежит исследованию уровня карбонильных групп протеинов и нитритов и нитратов в сыворотке крови.

**Ключевые слова:** метаболические маркеры, репаративный остеогенез, перелом нижней челюсти, математическое прогнозирование.

Рецензент Шепітько В.І.