DOI 10.26724 / 2079-8334-2017-4-62-132-136
UDC 616-003(616.314-089.23:616.71-007.234):599.323.4

A. M. Goltsev, O. O. Lykhytskyi
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, National Pirogov Memorial Medical University, Vinnitsa

THE ROLE OF IMMUNE-INFLAMMATORY PROCESSES AND OXIDATIVE STRESS IN THE MECHANISMS OF REPARATIVE OSTEOGENESIS IN RATS WITH AN OPEN FRACTURE OF THE MANDIBLE ON THE BACKGROUND OF OSTEOPOROSIS

e-mail: likhitskyi@yahoo.com

In the experimental study, the parameters of immune-inflammatory processes and oxidative stress in different terms of reparative osteogenesis in rats with an open fracture of the mandible on the background of osteoporosis were studied. It was proved that under these conditions the course of reparative osteogenesis was characterized by a number of metabolic changes: at 14 days after injury, the highest activity of immune-inflammatory reactions and oxidative stress was characteristic, and at 30 days - the maximum intensity of processes of angiogenesis and osteogenesis. Most of the biochemical parameters of blood serum of rats normalized at 45 days after fracture of the mandible.

Key words: immune-inflammatory reactions, oxidative stress, reparative osteogenesis, fracture of the mandible.

Reparative osteogenesis is a genetically programmed process, the course of which depends on the action of numerical exo- and endogenous factors [7, 9]. Among them, the group of factors that determines the osteoinductive potential of the organism and the activity of bone resorption / biosynthesis processes at the time of the injury, namely age, gender, the presence of metabolic disorders (diabetes mellitus, atherosclerosis), immunological status, etc., are important. [9, 10]. One of the important factors that modifies the course of reparative osteogenesis is osteoporosis [7]. However, molecular mechanisms integrated in the processes of osteoporosis under these conditions remain largely unknown. All this delayed the development of highly sensitive methods for controlling the course of reparative osteogenesis in fractures that occur in the background of osteoporosis.

Research purpose - to evaluate the role of immune-inflammatory reactions and oxidative stress in the regulation 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 traumatic damage to the lower jaw was restored: the rat was fixed on the back of the machine; under light hexanal (0.1 ml of 10% solution per 100 g of body weight) anesthesia in the right submandibular zone was performed damage on the 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 the external cortical plate with a separating disk, and then a full bone fracture with a bit on the line was applied, connecting the site of the fusion of the body and the branches of the jaw in the retro-molar region with a location 0.9 cm from the medial angle of the mandible. The surgical wound was connected with 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 Vinnitsa National Medical University named after Pirogov (Minutes No. 14 of 25.11.2010).

Animals were divided into two groups: 1 group (control) - pseudo-operated rats (PO); group 2 (experiment) - rats with a simulated open fracture of the mandible on the background of osteoporosis (MF + OP). The research was carried out at 7, 14, 21, 30 and 45 days after fracture simulation. Biochemical and immune-enzyme studies were carried out in blood serum, which was isolated according to the standard method [8]. The serum was obtained by centrifugation of blood at 600 g for 30 min. at 18-22oC. Blood serum aliquots were taken in Erpendorf microtubules and stored at -20°C until analysis. The content of TBC-reactive products (TBC-RP, secondary lipid peroxidation products) in serum was 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 activity of superoxide dismutase in blood serum (SOD, KF 1.15.1.1) was determined by inhibition of oxidation of quercetin [6], activity of NADPH-oxidase (KF 1.6.3.1) - by the decrease in absorption of NADPH at 340

nm [3]. The content of protein in serum was determined by the microbiouretic method [5]. 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 (TNFα) using the commercial kit "TNFα ELISA" ("Diaclone", France), and the content of the transforming growth factor beta (TGF-β1) - using the standard set of" "TGF-β1 ELISA kit" from DRG (France) in accordance with the instructions of the manufacturers.

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). The Student's t-criterion was used to estimate the intergroup difference. The difference was considered to be significant at $p < 0,05$.

Results and its discusssion. In rats with an open fracture of the mandible on the background of osteoporosis, the development of immune-inflammatory reactions is evidenced, with evidence of changes in the level of proinflammatory cytokine of TNF-α in different periods of reparative osteogenesis (Table 1). On the 7th and 14th day of the experiment, the content of TNF-α in serum increases significantly, respectively, by 58.5 and 76.5%, relative to the parameters of pseudo-operated animals. The maximum level of this cytokine is registered at 21 days after the injury (by 86.1% higher than the control rate). In this period of reparative osteogenesis, the content of TNF-α significantly exceeds by 23.5 and 12.0% the corresponding rates that were recorded at 7 and 14 days after the fracture. In the subsequent terms of the study there was a rapid drop in its blood level. On the 45th day of the experiment, the TNF-α returned to the level of control group of animals. Along with the activation of immune-inflammatory reactions under experimental pathology angiogenesis induction is recorded, evidenced by an increase in the content of VEGF in the blood (Table 1). The analysis of the serum VEGF index showed that during the first two weeks after the fracture, its level was not statistically significantly different from the control values and only 21 days after injury of the mandible the content of this cytokine was significantly higher than the rate of pseudo-operated animals by 12.8%. The most pronounced induction of angiogenesis is noted for 30 days of the experiment: serum VEGF levels increased by 24.7%, relative to control. On the 30 days, the level of this cytokine probably did not differ from the indicator of the control group of animals.

Under experimental pathology we have shown that on the 21st day after injury, expressive induction of osteoblasts is noted, as evidenced by the corresponding change in the growth factor of TGF-β1 (Table 1).

Table 1

Changes in blood serum concentrations of TNF-α, VEGF and TGF-β1 in rats with open fractures of the lower jaw against the background of osteoporosis in different terms of reparative osteogenesis (M ± m)

Terms of study	1 group (n=7): PO	2 group (n=7): MF + OP
TNF-α, pg/ml		
7 day	21,2±0,52	33,6±1,04*
14 day	21,0±0,63	37,1±1,68*
21 day	22,3±0,57	41,5±1,68*#
30 day	22,1±0,59	29,8±1,09*#&°
45 day	22,5±0,65	24,1±1,25#&°
VEGF, pg/ml		
7 day	67,5±2,18	69,8±2,44
14 day	64,4±2,29	70,6±3,18
21 day	65,8±1,96	74,3±2,91*
30 day	66,2±2,16	82,5±2,72*#&°
45 day	65,7±2,22	23,2±1,27°
TGF-β1, pg/ml		
7 day	130±2,64	134±4,37
14 day	128±2,54	135±5,22
21 day	126±2,50	185±2,99*#&
30 day	127±2,61	210±3,03*#&°
45 day	131±2,56	136±2,33°

Notes: 1. * - probable differences ($p < 0,05$) between the indicators of 1 and 2 groups of animals of the corresponding study period; 2. # - probable differences ($p < 0,05$) with respect to indicators for the 7th day of the study; 3. & - probable differences ($p < 0,05$) with respect to indicators for the 14th day of the study; 4. - probable differences ($p < 0,05$) with respect to indicators on 21 days.

It turned out that at 7 and 14 days of the experiment, the content of this cytokine probably did not differ from the control group. Instead, at 21 days after the fracture, TGF-β1 levels significantly exceeded the control value by 46.5%. Subsequently, its level first increased (as of 30 days, the TGF-β1 content

exceeded the control level by 65.3%), and then on 45 days after the fracture, it returned to the level of pseudo-operated animals.

In rats with an open fracture of the mandible, activation of free radical oxidation of lipids and proteins is recorded (Table 2). The TBC-RP and CGP blood levels have undergone changes already in the first week after the fracture: their level is likely to exceed the corresponding control by 54-60%. On the 14 day, the level of TBC-RP and CGP in serum was significantly higher by 84-100% relative to the group of pseudo-operated animals, and it was not statistically significantly different from that of the 7th day of the experiment. The maximum TBC-RP and CGP levels reached 21 days of the experiment: its value was 92-104% higher than in the control. In this term, the research of the content of oxide-modified proteins and secondary lipoperoxidation products were at the highest but statistically significantly not higher than the corresponding figure recorded at the 14th day of the experiment. In the subsequent terms of reparative osteogenesis, the content of TBC-RP and oxide-modified proteins significantly decreased by 22-37% (as of 30 days) and 22-28% (as of 45 days), compared to the 21st day of the experiment. In animals with a fracture of the mandible on the background of osteoporosis, an imbalance in the system of anti-oxidant enzymes is observed (Table 3).

Table 2

Changes in blood serum contents of products of peroxidation of lipids and proteins in open mouth fractures of the lower jaw against the background of osteoporosis in different periods of reparative osteogenesis (M ± m)

Terms of study	1 group (n=7): PO	2 group (n=7): MF + PO
TBC-RP, μmol/l		
7 day	3,25±0,14	5,20±0,28*
14 day	3,18±0,17	5,85±0,36*
21 day	3,20±0,19	6,14±0,41*#
30 day	3,22±0,26	4,80±0,44*&°
45 day	3,19±0,30	3,45±0,42&°
CGP, units of optical density / mg of protein		
7 day	45,2±1,06	69,6±1,73*
14 day	44,7±1,15	89,4±1,81*#
21 day	45,4±1,23	92,8±2,20*#
30 day	45,0±1,17	62,4±1,56*&°
45 day	45,8±1,44	48,9±1,57#&°

Notes: 1. * - probable differences (p <0,05) between the indicators of 1 and 2 groups of animals of the corresponding study period; 2. # - probable differences (p <0,05) with respect to indicators for the 7th day of the study; 3. & - probable differences (p <0,05) with respect to indicators for the 14th day of the study; 4. - probable differences (p <0,05) with respect to indicators for 21 days.

Table 3

Changes in the activity of pro- and antioxidant enzymes in serum of rats with open fractures of the mandible on the background of osteoporosis in different periods of reparative osteogenesis (M ± m)

Terms of study	1 group (n=7): PO	2 group (n=7): MF + PO
NADPH-oxidase, nmol/min ml		
7 day	2,40±0,06	3,25±0,07*
14 day	2,37±0,08	3,60±0,06*#
21 day	2,41±0,04	3,75±0,07*#
30 day	2,39±0,05	3,30±0,05*&°
45 day	2,42±0,07	2,55±0,07#&°
SOD, conventional units / mg protein		
7 day	45,2±1,06	69,6±1,73*
14 day	44,7±1,15	89,4±1,81*#
21 day	45,4±1,23	92,8±2,20*#
30 day	45,0±1,17	62,4±1,56*&°
45 day	45,8±1,44	48,9±1,57#&°

Notes: 1. * - probable differences (p <0,05) between the indicators of 1 and 2 groups of animals of the corresponding study period; 2. # - probable differences (p <0,05) with respect to indicators for the 7th day of the study; 3. & - probable differences (p <0,05) with respect to indicators for the 14th day of the study; 4. - probable differences (p <0,05) with respect to indicators for 21 days.

As of 7-14 days of the experiment, the activity of NADPH-oxidase significantly increases by 35.4-51.9%, while the activity of SOD is likely to decrease by 18.7-38.6%, compared with the control group. Maximum changes in enzymatic activity are noted as of the 21st day of the experiment: the activity of NADPH-oxidase is 55.6% higher, while SOD is 45.7% less than that of pseudo-operated rats. At a later date, the activity of investigated enzymes was registered and at 45 days it was not statistically significantly different from control. We have shown that in rats with an open fracture of the mandible on the background of osteoporosis in various terms of reparative osteogenesis a number of metabolic and

immunological changes are noted. Thus, as of 7 days, an increase in the activity of immune-inflammatory reactions is recorded, as evidenced by the probable increase in the serum content of proinflammatory cytokine of TNF-α by 58.5%, relative to control. It is known that this cytokine activates chemotaxis of neutrophils and monocytes / macrophages to the site of damage, increases their adhesion to endothelial cells, creates conditions for humoral and cellular cooperation of blood cells and bone tissue and the development of inflammatory response [14]. Along with this, activation of free radical lipid oxidation is recorded (TBC-RP content in serum increases by 60.0% compared to the control indicator, $p < 0.05$), oxidative degradation of proteins (serum CGP levels increase by 54.0%, relative to control; $p < 0,05$). The increase in the activity of lipid and protein peroxidation processes develops against the backdrop of imbalances in the system of anti-oxidant enzymes. Under these conditions, the growth of superoxide anion radicals is recorded (the activity of NADPH oxidase increases by 35.4% relative to the control $p < 0.05$) and the activity of its inactivation with the participation of SOD increases by 18.7%, as compared with the control group. As the 14th day of the experiment, an increase in the activity of immune-inflammatory reactions was observed (the level of TNF-α was significantly higher by 76.7%, relative to the control index, $p < 0.05$). An increase in the activity of free radical oxidation of lipids and proteins is also recorded (TBC-RP and CGP content exceeds by 12.5 and 28.5% for 7 days, $p < 0.05$). Along with this, an imbalance in the system of anti-oxidant enzymes increases (NADPH-oxidase activity is significantly higher by 10.8% and SOD is less by 23.0%, relative to 7 days). At this time of the experiment, the activity of the processes of angiogenesis and osteoblastic transformation coincides with that in the control group of animals. As of the 21st day of the experiment, the activity of immune-inflammatory reactions is high and is comparable to that for 14 days (the level of TNF-α in serum does not significantly differ from this state by 14 days). The processes of lipoperoxidation and oxidative destruction of proteins are roughly the same intensity as in the previous period of reparative osteogenesis (levels of TBC-RP and CGP probably do not differ from the indicator for 14 days). In addition, the intensity of angiogenesis increases, as evidenced by the likely increase in the serum VEGF content by 12.8% relative to the control index [2]. Cell osteoblastic differon is also activated, as evidenced by a statistically significant increase in TGF-β1 in serum by 46.5% relative to control. TGFβ1 enhances the proliferation of osteoblasts and the synthesis of collagen, induces the differentiation of mesenchymal cells in osteoblasts and chondrocytes, is a stimulator for the regeneration of fractures in the skeleton bones [11]. This cytokine is also involved in the process of angiogenesis, so the growth of its level may also indicate an increase in vascular growth processes [4]. As of 30 days, the manifestations of immune-inflammatory reactions and oxidative stress are minimal. Instead, the activity of osteoblasts and the intensity of the course of angiogenesis is maximal (TGF-β1 and VEGF levels in serum are likely to exceed 25-65% of the control group, $p < 0.05$). As of day 45, most biochemical and immunological parameters return to normal. Further research in this direction will reveal deep molecular mechanisms integrated into reparative osteogenesis in conditions of an open fracture of the mandible on the background of osteoporosis, which is an important prospect for the development of effective means of correction of this pathology.

Conclusions

1. As the 14th day of reparative osteogenesis in rats with an open fracture of the mandible on the background of osteoporosis, the maximum activity of immune-inflammatory reactions were detected (the level of TNF-α in serum increased by 76.5% relative to pseudo-operated animals, $p < 0.05$), and also oxidative stress (the content of carbonyl groups of proteins, nitrites and nitrates increased by 100 and 44.7% respectively, $p < 0.05$).
2. As the 30th day of reparative osteogenesis in rats with simulated pathology, the highest activity of the angiogenesis processes was recorded (VEGF content increased by 24.7%; $p < 0.05$) and osteogenesis (TGF-β1 content increased by 65.3%, respectively); $p < 0.05$).

Список літератури

1. Batura I. A. Osteonnyie konstruktsii bedrennoy kosti kryis pri dlitelnom vvedenii gidrokortizona / I. A. Batura // Visnyk Bilotserkivs'koho derzhavnoho ahrarnoho universytetu – 2005, Vol. 33, P.300-304.
2. Deev R. V. Effektivnost gen-aktivirovannogo osteoplasticheskogo materiala s plazmidnyimi DNK, soderzhaschimi gen VEGF, v zameschenii kostnyih defektov / R. V. Deev, I. Ya. Bozo, A. Yu. Drobyishev [et al.] // Probl. kriobiologii i kriomeditsinyi, - 2013, Vol. 23(3), P. 355-358.
3. Fukui T. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats / T. Fukui, N. Ishizaka, Rajagopalan [et al.] // - Circ. Res., - 1997, Vol. 80(1), P. 45-51.
4. Janssens K. Transforming growth factor-beta1 to the bone / K. Janssens, P. ten Dijke, S. Janssens [et al.] // - Endocr. Rev., - 2005, Vol. 26(6), P. 743-774.
5. Kochetov G. A. Prakticheskoe rukovodstvo po enzimologii / G. A. Kochetov // - M.: Vyissh. – 1980 shk.

6. Kostyuk V. A. Prostoy i chuvstvitelnyiy metod opredeleniya aktivnosti superoksiddismutazyi, osnovannyiy na reaktsii okisleniya kvertsitina / V. A. Kostyuk, A. I. Potapovich, Zh. V. Kovaleva // - Vopr. med. Himii, 2, - 1990, P.88-91.
7. Korzh N. A. Reparativnaya regeneratsiya kosti: sovremennyiy vzglyad na problemu. Sistemnyie faktoryi, vliyayuschie na zazhivlenie pereloma / N. A. Korzh, N. V. Deduh, O. A. Nikolchenko // - (Soobschenie 3). Ortopediya, travmatologiya i protezirovanie, - 2006, Vol. 2, P. 93-99.
8. Menshikov V. V. Laboratornyie metodyi issledovaniya v klinike: Spravochnik / V. V. Menshikov // - M.: Meditsina – 1987.
9. Rolik, O. V., & Zasadnyuk, I. A. Nezroshchennya dovhykh kistok (analiz, faktory ryzyku, likuval'na taktyka) / O. V. Rolik, I. A. Zasadnyuk // Ortopedyya, travmatolohyya y protezyrovanye, - 2005, Vol. 2, P. 61-64.
10. Saito M. Poor bone quality in diabetes and arteriosclerosis / M. Saito // - Clin Calcium, - 2009, Vol. 19(9), P.1257-1268.
11. Sikora V. Z. Nekollagenovyie belki kostnogo matriksa kak markeryi remodelirovaniya kosti / V. Z. Sikora, M. V. Pogorelov, Tkach // - Ukrayinskiy morfologichniy almanah, - 2011Vol. 9(3-D),P. 28-35.
12. Vladimirov Yu. V. Perekisnoe okislenie lipidov v biologicheskih membranah / Yu. V. Vladimirov, A. I. Archakov // - M.: Nauka – 1972.
13. Zaichko N. V. Okyslyuval'na modyfikatsiya bilkiv syrovatky krovi yak marker aktyvnosti revmatoyidnoho artrytu ta yiyi zminy pid vplyvom farmakoterapiyi amizonom, indometatsynom, nimesulidom / N. V. Zaichko // Visnyk Vinnyts'koho derzhavnoho medychnoho universytetu, - 2003, Vol. 7(2/2), P.664 - 666.
14. Zubov D. A. Tsitokinovaya immunoregulyatsiya reparativnoy regeneratsii kostnoy tkani kultivirovannyimi mezenhimalnyimi stvolovyimi kletkami / Zubov D. A., Oksimets, V. M. // - Travma,- 2008, Vol. 9(2), P.145-153.

Реферати

РОЛЬ ІМУНОЗАПАЛЬНИХ ПРОЦЕСІВ ТА ОКСИДАТИВНОГО СТРЕСУ В МЕХАНІЗМАХ РЕПАРАТИВНОГО ОСТЕОГЕНЕЗУ У ЩУРІВ З ВІДКРИТИМ ПЕРЕЛОМОМ НИЖНЬОЇ ЩЕЛЕПИ НА ТЛІ ОСТЕОПОРОЗУ

Гольцев А. М., Ліхіцький О. О.

В експериментальному дослідженні вивчені особливості імунозапальних процесів і оксидативного стресу в різні терміни репаративного остеогенезу у щурів з відкритим переломом нижньої щелепи на тлі остеопорозу. Було доведено, що в цих умовах перебіг репаративного остеогенезу характеризувалося низкою метаболічних змін: через 14 днів після травми була виявлена найвища активність імунозапальних реакцій і оксидативного стресу, а через 30 днів - максимальна інтенсивність процесів ангіогенезу і остеогенезу. Більшість біохімічних параметрів сироватки крові щурів нормалізувалися через 45 днів після перелому нижньої щелепи.

Ключові слова: імунозапальні реакції, оксидативний стрес, репаративний остеогенез, перелом нижньої щелепи.

Стаття надійшла 6.09.2017 р.

РОЛЬ ИММУНОВОСПАЛИТЕЛЬНЫХ ПРОЦЕССОВ И ОКСИДАТИВНОГО СТРЕССА В МЕХАНИЗМАХ РЕПАРАТИВНОГО ОСТЕОГЕНЕЗА У КРЫС С ОТКРЫТЫМ ПЕРЕЛОМОМ НИЖНЕЙ ЧЕЛЮСТИ НА ФОНЕ ОСТЕОПОРОЗА

Гольцев А. М., ЛИХИЦКАЯ А. А.

В экспериментальном исследовании изучены особенности иммуновоспалительных процессов и окислительного стресса в разные сроки репаративного остеогенеза у крыс с открытым переломом нижней челюсти на фоне остеопороза. Было доказано, что в этих условиях течение репаративного остеогенеза характеризовалось рядом метаболических изменений: через 14 дней после травмы была выявлена наивысшая активность иммуновос-палительных реакций и окислительного стресса, а через 30 дней - максимальная интенсивность процессов ангиогенеза и остеогенеза. Большинство биохимических параметров сыворотки крови крыс нормализовалось через 45 дней после перелома нижней челюсти.

Ключевые слова: иммуновоспалительные реакции, оксидативный стресс, репаративный остеогенез, перелом нижней челюсти.

Рецензент Шепітько В.І.

DOI 10.26724 / 2079-8334-2017-4-62-136-139
УДК [616.316:547.96]:615.24-056.5

Л. П. Гордієнко
ВДНЗ України «Українська медична стоматологічна академія», м. Полтава

ЕКСПЕРИМЕНТАЛЬНА КОРЕКЦІЯ МУЛЬТИПРОБІОТИКОМ ПРОТЕЇНАЗНО-ІНГІБІТОРНОГО ДИСБАЛАНСУ У СЛИННИХ ЗАЛОЗАХ ЗА УМОВ ОЖИРІННЯ

e-mail: hordiienko_lp@ukr.net

У статті наведено результати дослідження використання мультипробіотика для корекції патологічних змін у тканинах слинних залоз 29 щурів обох статей за умов ожиріння. Експериментальна корекція ожиріння із застосуванням мультипробіотика «Симбітер ацидофільний» нормалізує протеїназно-інгібіторний дисбаланс у тканинах слинних залоз. Отримані результати свідчить про ефективність пробіотикотерапії для попередження розвитку патологічних змін у слинних залозах за умов ожиріння.

Ключові слова: слинні залози, ожиріння, протеоліз, мультипробіотик.

Робота є фрагментом НДР «Механізми розвитку патологічних змін в органах порожнини рота за різних умов та їх корекція», № держреєстрації 0113U005913.

Надмірна вага та ожиріння збільшують ризик розвитку ряду захворювань, зокрема, органів порожнини рота. За даними наукових праць, ожиріння та асоційовані з ним патологічні стани характеризуються розвитком гіпосалівації, карієсу, патологічних процесів слизової оболонки