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Body Systemic Response to Optimize Reparative Bone Regeneration

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Abstract Reparative regeneration of bone tissue and its optimization is a topical problem in both clinical and fundamental science. It can be performed with the help of local and systemic factors [A.S. Avrunin et al. 2018; A.S. Grioryan et al. 2015; Y.M. Iryanov, 2012; I.V. Mayborodin et al. 2015; G.A. Onoprienko, V.P. Voloshin, 2017; I.DuniS-Cule et al. 2014; W.Li et al. 2015]. They have a significant impact on angiogenesis, differentiation of osteoblasts and osteoclasts, osteogistogenesis [O.L. Grebneva et al., 2012; M.V. Stogov, 2013; A.M.Ali, 2015; Y.H.Kim et al., 2015; W.Li et al., 2015; N.Oxata et al., 2015; V.Shi et al., 2015, etc.].] The overwhelming number of works devoted to the improvement of treatment results is based on a mechanical approach based on the need for comparison of fragments and reliable fixation [G.V. Dyachkova et al., 2018, etc.]. However, according to researchers, the restoration of blood supply and metabolism, correction of neuroimmune endocrine relationships is no less critical K.V. Sudakov et al., 2010; L.B. Reznik et al., 2015; W.Li et al., 2015; V.Shi et al., 2015, etc.].

Keywords Succinate, The surface of a tibial bone, Diaphysis, Index of induction, Glycerophosphate dehydrogenase, Succinate dehydrogenase

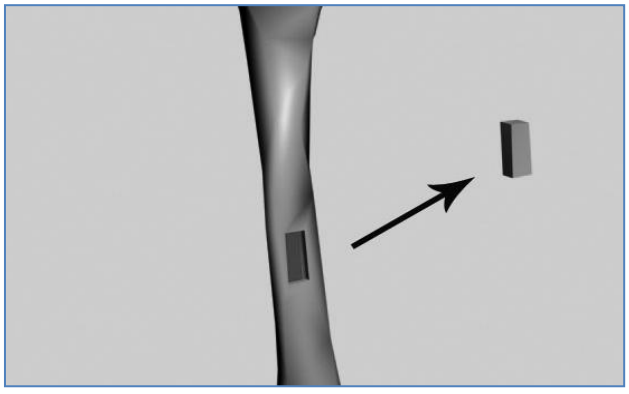
1. The Aim of the Research

To study the effect of infusion of physiological solution and succinate on the functionally metabolic activity of neutrophils in reparative regeneration of diaphysis bone tissue defect.

2. Research Material and Methods

Characteristics of the experiment.

The experiments were conducted on 42 rabbits of Shinshilla breed weighing 2.0-2.5 kg. After examination of them by the veterinarian, stating the absence of any diseases, under local anesthesia 0.5% solution of novocaine of the middle third of the tibia, a cut of skin and subcutaneous tissue was made. The muscles are bluntly separated from the tibia surface and removed from the middle third of the diaphysis. Then a sharp scalpel made two longitudinal 1.0 cm at a distance of 0.5 cm from each other and two transverse incisions of the periosteum (the scheme of defect formation is shown below), connecting them. The rectangular periosteum strip was removed after separation from the subject bone tissue. Then, per its width (0.5 cm) and length (1.0 cm), boron-machine created the defect of bone tissue to the endoscope. After removing small pieces of bone from the recess, the defect is covered with soft tissue from above. The skin and subcutaneous tissue are sutured with several transverse sutures. The wound is treated with iodinol. The sutures are removed on 8-9 days after the bone defect is created. No complications were observed after the operation.



Scheme of bone defect formation in the tibia diaphysis area

All animals were divided into 3 groups: I - control (n=18); 1 time per day during 5 days after the creation of the bone tissue defect, the animals were injected into/in the physiological solution; II. – experience (n=18); once a day, during 5 days after the defect creation, the animals were injected into/in the Succinasol solution(12 mg/kg); III (n=6)-intact animals; to study the normal bone structure in the diaphysis defect area. Withdrawal of animals from the experience was made on 7.15 and 30 days after the defect

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was created in accordance with the European Convention for the Protection of Animals used in Scientific Research [38; p.34-36]. The terms of study of reparative regeneration of bone tissue defect in I and II gr. have been established on the basis of works of G.I. Lavrischeva, G.A. Onoprienko [57; p.208]. In all series of experiments, 6 rabbits (42 in total) were used for each term.

3. Biochemical Research

The functional-metabolic activity of neutrophils (FMAN) is established in the NST-test by the B.N. Park method with co-authors in the modification of M.G. Shubich and V.G. Mednikova [92; p.515-518]. FMAN allows judging about the phagocytic-metabolic activity of granulocytes. The activity of neutrophils in NST-test was taken into account by the semi-quantitative method. The activity of SDG and α-GFDG was determined by N. Novikoff, B. Masek method [140; p.287-302]. The ratio between the activity of SDH and α-HFDG and the NCT-test in the dynamics of the experiment and intact animals is considered as an induction index (AI).

4. Statistical Methods of Reserach

Morphometric and biochemical results are processed using standard methods of variation statistics using the t-criterion of Student Excel 2010. The arithmetic mean, its average error and reliability (P) were calculated. The difference between the compared values is reliable at $P<0.05$.

5. Results of Research

Under physiological conditions circulating in the blood neutrophils are characterized by relatively low activity of SDH (0.71± 0.94 c.u.) and α- GFDG (0.75± 0.06 c.u.); NST-test index is 0.74± 0.6. (Table 1).

Table 1. Dynamics of cytochemical parameters of peripheral blood neutrophils and index of induction (AI) at the optimization of reparative regeneration after the creation of bone tissue defect of the tibial bone diaphysis of rabbits (M±m; n=6)

Cytochrome indicators	Intact	7 days		15 days		3- days	
	Activity / Intact	Activity	II	Activity	II	Activity	II
NCT - test	0,74±0,06	1,83±0,11 1,98±0,12	2,49 2,68	1,33±0,08 1,80±0,16	1,78 2,53	1,04±0,06 1,55±0,23	1,42 2,08
SDG	0,71±0,04	1.55±0,14 1,98±0,09	2,18 2,79	1,17±0,08 1,48±0,09	1,66 2,08	1,20±0,09 1,38±0,08	1,69 1,96
α-GFDG	0,75±0,06	1,80±012 2,18±0,15	2,41 2,91	1,30±0,11 1,67±0,11	1,75 2,23	1,25±0,07 1,60±0,11	1,67 2,13

Note: The AI index of induction reflects the multiplicity of increase in the activity of the enzyme; NCT test - the total activity of enzymes of the cell; SDG-succinatedehydrogenase α-GFDG - α-glycerophosphate dehydrogenase.

Numerator - values of indicators in the I group; denominator - values of indicators in the II group of investigated animals.

At animals IIgr. after the creation of defect of bone tissue in an average part of a diaphysis in 7 days activity SDG and α-GFDG and NST-test more essentially raises, than in Igr. (P<0,001). These FMAN transformations influence the processes occurring in the defect area and reflect the adaptation of the neutrophilic-macrophageal functional system of the body. AI NST test, SDG and α-GFDG activity in IIg. (Table) Consequently, after the experimental creation of bone tissue defect diaphysis already in the initial stage of inflammation, FMAN increases more than twice on average, but after the infusion of succinasol animals II gr. The degree of growth of AI, FMAN is more significant than in 1 gr.

In 15 days after the creation of bone tissue defect in I Gr. animals FMOAN in 1,7 on average (P<0,001) becomes more than intact animals. In animals of II Gr. the activity of SDH and α-HFDG, the index of NST-test is still higher than in I Gr. FMAN and AI, respectively, are twice as high on average than in intact animals (Table).

Thus, after 15 days of experience, FMAN is high; AI activity of enzymes and NST-test in IIgr. Animals are significantly higher than in Igr.

By 30 days of experience of AI SDG, α-GFDG and NST-test in I and II gr. on the average 1.6 and 2 times higher respectively than in intact animals. However, the activity of α-HFDG and NCT-test in animals of II gr. is reliably higher (P<0,05) than in I gr. (Table 1).

Thus, in reparative regeneration of the tibial bone defect, according to the activity of SDG, α-HFDG and HST test, the dynamics is always higher with succinasol than without the drug.

6. Discussion

Blood as the internal environment of the body, as a result of damage to the structure and function of bone tissue of the diaphysis, is adaptively involved in the process of inflammation, whose task is to restore the defect of bone tissue of the diaphysis. Inflammation as a protective and adaptive process limits the damage zone, mobilizes

hematopoiesis organs and immunological protection, multiple activates functional and metabolic properties of peripheral blood leukocytes, etc. Regulation of the interaction of cooperating cells (neutrophil-macrophage-lymphocyte, etc.) is carried out due to the feedback of the structures of functional systems. A small number of phagocytes, neutrophils and macrophages are customarily recorded. The impact on the body of a factor that violates the homeostasis of tissues of this or that organ dramatically changes the phagocytic-metabolic activity (PMA), primarily regulatory systems that mobilize all systems to the reactions that correlate homeostasis in the damage zone (inflammation trigger, neutrophil-macrophagal-connective tissue reaction). The stereotype of mobilization of functional-metabolic activity of phagocytic blood and connective tissue cells allows to consider the dynamics of activity of lysosomal and membrane enzymes, energy and oxidative metabolism, processes of synthesis and secretion, migration, proliferation and differentiation. Both in the clinic and the experiment, the most informative, reflecting the reactive shifts in the system of oxidative metabolism, is to determine the ability to spontaneous and induced recovery of nitro blue tetrazol [Nagoyev B.S., M.R. Ivanova Indicators of spontaneous and induced NST-test of leukocytes in patients with hepatitis V. // Epidemiol. and infectious diseases.] From a large number of intracellular enzymes informative, integrally characterizing cell metabolism, we, like other researchers, chose SDG, α-HFDG. They are as informative as possible, characterizing the functional completeness of the cell under study.

The SDH transfers hydrogen from succinic acid through ubiquon to cytochrome in the respiratory chain. It's like a mitochondrion marker that reflects the state of oxidation in the cell. α-GFDG is a component of the α-glycerophosphate shunt, which provides continuity and synchrony in the processes of glycolysis and respiration; it transfers hydrogen to the cytochrome C of the respiratory chain. In the study of the functional-metabolic activity of leukocytes attach great importance to HST-test, which detects the induction of peroxidase systems of leukocytes in response to various effects. It is a biochemical marker of cell peroxidase enzymes. Processes occurring in the damage zone after the injury, are presented as follows: when exposed to the polynuclear substrates of cell damage or intercellular substance occurs phagocytosis of the resulting complex of HST-heparin-fibrinogen and its transport to the phagosome. Here, under the influence of enzymes, the recovery of HST and transformation into dark blue formasan. In general, NST-test reflects the degree of activation of oxygen-dependent metabolism, i.e., the function of the hexozomonophosphate shunt and the associated production of free radicals. According to our data, the recovery of NST occurs in neutrophils and macrophages. In the dynamics of inflammation increases the proportion of NST-active neutrophils 4-6 times. Normalization of NST-test parameters occurs only at the stage of completion of inflammation and restoration of damaged tissue. Optimization of the course of inflammation causes both a reliable and significant increase in the rate of NST-test, and return to normal at the stage of stabilization of adaptation and restoration of homeostasis. Our experiments have shown that inflammation (injury) with a seemingly local character is systemic, and the study of enzyme systems of polymorphonuclear leukocytes reflects both its local flow and the body's systemic adaptive response aimed at restoring and preserving homeostasis of the internal environment.

The increase in NST-test indexes by 2-3 times indicates the maximum induction of FMAN in the dynamics of inflammation and regeneration, characterizes the rapid and pronounced degree of activation of the phagocytic stage of inflammation in traumatic damage to bone tissue and the formation of blood clots, necrobiotically altered cells and bone particles. A more significant increase in FMAN in the dynamics of reparative regeneration in animals after the administration of succinasol indicates the restoration of oxidative and other processes occurring in mitochondria, normalization of stages of the Kreps cycle.

1. Neutrophils are at rest while moving through the system of vessels from bone marrow to tissue or inflammation center. They are active in tissues when performing a specific function, in inflammation (respiratory explosion, phagocytosis, degranulation, extracellular secretion, extracellular lysis of damaged tissues, structures, the decaying neutrophils themselves and other cells) [Serov V.V. Inflammation. -M.: Medina, 1995.-640 p.

2. Naasag-Weber M., Hort W.H. Dysfunction of polymorphonuclear leucocytes in uremia.// Semin. Nephrol. -1996. -Vol 16, №3.-192-201.]. From a cell level to an organ violation of structural, functional, metabolic homeostasis, relations of functional systems at tibia fracture (defect) by the formed factors modulates reaction of neutrophils, inflammation. Under conditions of oxygen adequacy, neutrophils realize their functions in full. Ejected proteases open the basal capillary endothelial membrane, provide cleavage of complement, antibodies and other factors with the formation of some mediators that affect both neutrophils and other inflammatory cells. Hydrogen peroxide generated at the same time promotes the activation of collagenase, resulting in a full-fledged enzyme enters the intercellular medium. The splitting of plasma-lemm phospholipids by neutrophil enzymes causes the formation and secretion of some mediators: thromboxanes, prostaglandins, leukotrienes. Neutrophils interact with humoral factors and numerous cells of connective tissue [O.L. Grebneva, M.A. Kovinka, S.N. Luneva et al. The study of humoral components stimulating osteogenesis. // The genius of orthopedics.]. The peculiarity of neutrophils is the presence of phagosome and digestible cellular detritus in the cytoplasm.

Neutrophils and macrophages at early stages of inflammation, granulation tissue formation and reparative bone tissue regeneration influence migration, differentiation of fibroblastic and osteoblastic differons, bone tissue remodeling, development of growth factors, vascular formation and development.

7. Conclusions

The functional-metabolic activity of neutrophils of peripheral blood, according to the activity of SDG, α-GFDG and NST-test in the dynamics of reparative regeneration of bone tissue of diaphyse, infusion of succinasol is significantly more significant than without the administration of the drug.

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