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Research article

REPARATIVE OSTEOGENESIS DURING TREATMENT OF FRACTURE UNDER TRANSOSSEOUS OSTEOSYNTHESIS AND INTRAMEDULLARY INSERTION OF WIRES WITH HYDROXYAPATITE COATING

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ABSTRACT

Background: The problem of improving medical care for patients with the locomotor system injuries is very important especially last time. **Material and Methods:** Canine open comminuted tibial fractures modelled experimentally, wires with hydroxyapatite coating inserted intramedullary, osteosynthesis performed with the Ilizarov fixator. Regenerated bones investigated 14-360 days after surgery using the techniques of light microscopy, scanning and transmission electron microscopy, and X-ray electron probe microanalysis for histologic sections . **Results:** It has been found that a zone of active reparative osteo- and angiogenesis forms around the wires, as well as a bone sheath with the properties of osteogenesis conductor and inductor. Fracture consolidation occurs early according to the primary type without cartilaginous and connective tissue formation in bone adhesion. Presented morphological characteristics endovasal angiogenesis. **Conclusion:** The results of the study evidence of the positive effect of intramedullary wires with hydroxyapatite coating on the course and intensity of reparative osteogenesis during fracture healing

Key words: Transosseous osteosynthesis, Intramedullary wires, Hydroxyapatite coating, Fracture healing, Reparative osteogenesis, Angiogenesis.

INTRODUCTION

The problem of improving medical care for patients with the locomotor system injuries every year is becoming increasingly important due to the increase of injured persons in number, to that of disability and mortality from injuries not having downward tendency. However, osteosynthesis real terms remain to be significant. The technique of directed stimulation of bone tissue regeneration process is practiced by using intramedullary wires with calcium phosphate coating in order to optimize the conditions for regenerated bone formation, as well as for treatment period reduction, and complication prevention [1, 2]. At the same time, the process of reparative osteogenesis using those or other implants inserted into the regenerated bone is poorly understood, as well as both their effectiveness characterization and mechanism of action are absent. The purpose of the present work consists in studying the morphological features of osteogenesis process for consolidation of long tubular bone fractures under transosseous osteosynthesis and intramedullary insertion of wires with hydroxyapatite bioactive calcium phosphate coating.

MATERIAL AND METHODS

16 mongrel dogs, males and females, at the age from one to five years with the body weight of 20±2.9 kg were used for experiments. The keeping, surgical

interventions, and euthanasia of the animals were made in compliance with European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986); they were approved by the Ethics Committee of RISC RTO.

Procedures: The animals underwent intramedullary reinforcement of right tibia with two wires under general anesthesia. Wires of titanium alloy were used with bioactive coating of hydroxyapatite of 20-40-μm thickness and 2-8% porosity; the alloy was obtained by the technique of anodic oxidation in the arc mode. The coating presented a multilevel ultraporous system consisting of macro- and micropores of the diameter from 50-100 nm to 1-2 μm. Osteosynthesis was performed with the Ilizarov fixator, and an open comminuted fracture of leg bones was modeled in the shaft middle third. The Ilizarov fixator dismounting made after 28 or 35 days of fixation. Radiography performed in the course of the experiment. The animals were divided into four groups: 14, 21; 28, 35; 42, 90; 180, 360 days after surgery, four animals in each group and two ones – for each time point. Clinical observation of the animals carried out throughout the experiment. Radiography was made using «Premium Vet» X-ray machine (Sedecal, Spain) in direct and lateral views immediately after surgery and during the experiment. Tibias of three intact adult dogs were investigated for comparison. After euthanasia of the animals the shaft portions of the operated bones were sawed lengthwise, fixed in 2% solution of paraformaldehyde and glutaraldehyde, embedded in celloidin (after decalcification) and Araldite (without decalcification). Histotopographic sections were prepared using Reichert microtome (Germany) and stained with hematoxylin-eosin, and with picrofuchsin by Van Gieson. Research and photomicrography of histological sections were performed using «Stemi 2000-C» stereomicroscope and «AxioCam ERc 5s» digital camera completed with «Zen blue» software (Carl Zeiss MicroImaging GmbH, Germany). Araldite blocks were smoothed and investigated with INCA-200 Energy X-ray electron probe microanalyser (Oxford instruments, England). Facture zone image was obtained in characteristic X-ray radiati characterizing the degree bone tissue maturity was calculated. The index of compactness was calculated as well (bone tissue/non-mineralized structure content ratio).The blocks are then sawed ultra thin sections were prepared prepared on an ultra microtome LKB-8800 (Sweden), contrasted and examined using a transmission electron microscope JEM-2010 (Jeol, Japan). Then Araldite blocks were treated in sodium ethyolate 2% solution in order to remove the embedding medium, and they were investigated with scanning electron microscope JSM-840 (Jeol, Japan). The results of quantitative studies were processed using standard methods of variation statistics. The significance of differences between the values was estimated using nonparametric Mann-Whitney U-test. Differences were considered statistically significant for $p \leq 0.05$.

RESULTS

Transverse fractures have been produced in tibial shaft middle third of all the animals after surgery (Figure 1, a). The height of diastasis between the fragments is 0.5-1.0 mm. The signs of periosteal reaction as cloud-like shadows appear in close proximity to the fracture line by 8-10 days after surgery. The formation of new bone cortex is determined by X-rays 35 days after surgery (Figure 1, b).

Fig 1: X-rays of canine tibia after fracture modeling and intramedullary osteosynthesis: a – shaft middle third fracture, immediately after surgery; b – newly formed cortex at the fracture site 35 days after surgery

The regenerated bone is located all over the bone diameter. Numerous anastomosing trabeculae of reticulofibrous bone tissue in grow towards each other from the periosteal and endosteal surfaces in the intermediary zone, and they form strata on the ends of fragments. Primary endosteal-periosteal and intermediary union is formed. The content of calcium and phosphorus in the intermediary zone of regenerated bone is 19 % and 20 %, respectively, and that of bone tissue – 26 % of the values of the shaft cortex in normal intact dogs (Table1)

Table1: Content of calcium, phosphorus, and bone tissue in the intermediary zone of regenerated bone, and in the cortex of intact shaft (M±m,%)

Measures	Period of experiment, days				Cortical layer
	14, 21	28, 35	42, 90	180, 360	
Calcium	4.96±0.31	11.02±0.65	17.27±1.08	23.30±1.31	25.82±1.10
Phosphorus	2.95±0.18[1]	6.44±0.36	9.06±0.56	11.07±0.69	11.75±0.53
Bone tissue	5.74±1.53[1]	53.43±2.89	80.07±4.90	94.13±5.49	96.15±4.44
Ca/P	1.68±0.13[1]	1.72±0.14	1.91±0.18	2.10±0.14	2.20±0.13
Index of compactness	0.35±0.02	1.15±0.07	4.02±0.26	16.04±0.67	24.97±1.28

Significant changes comparing with the measures of intact animal shaft cortex.

By 28, 35 days after surgery the ends of fragments lose clear boundaries due to massive deposits in the intermediary space of mature lamellar bone tissue. Periosteal strata of 2.5-3-mm height become compacted, and they combine the ends of fragments in fracture zone by the flattened fusiform "sleeve". Ribbon-like spreads are formed in the periosteal area near fragmental ends as small-looped network of lamellar-structured bone trabeculae bridging fracture line.

The regenerated bone in the intermediary zone is represented by spongy and compact bone tissue closely adhered with cortex of bone fragments. The phase of organogenesis and remodeling is observed evidenced by reorganization of primary trabeculae into organotypical osteon structures forming cortex (Figure 2, a, b). Gradual reorganization of the trabecular structures of coarse-fibered bone tissue into more mineralized and mature lamellar ones is also observed in the bone sheath round the wires. The content of calcium and phosphorus in the intermediary zone of regenerated bone in this period increases up to 43-55 %, and that of bone tissue – up to 56-57 % of the measures of the shaft cortex in normal intact dogs (Table 1).

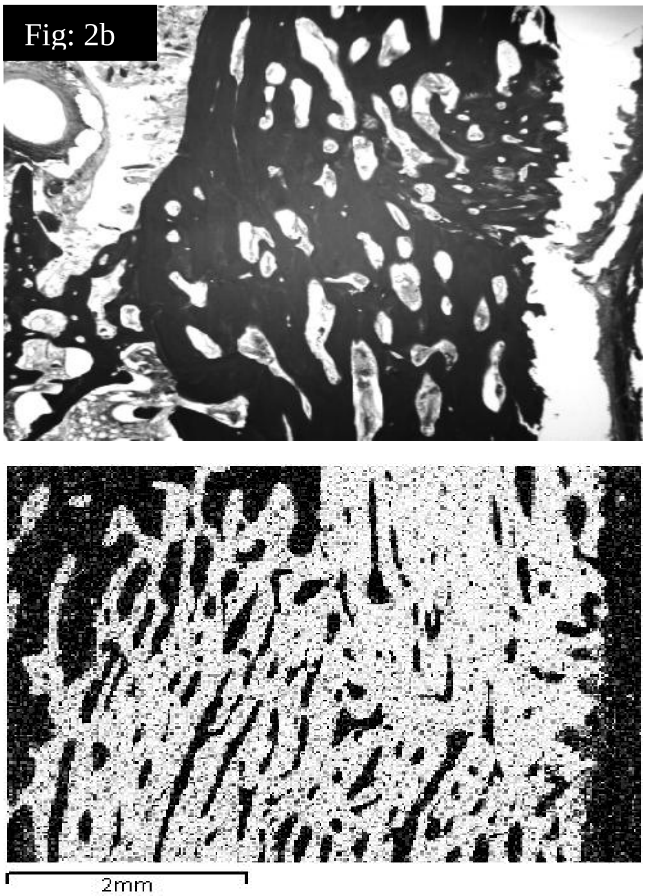

Fig 2: The newly formed cortex at fracture site 35 days after surgery: a – staining according to Van Gieson. Lens 2.5, eyepiece 10; b – the map of electron probe microanalysis, the image in characteristic X-ray radiation of calcium, magnification x15.

By 42, 90 days after surgery the ends of fragments in the intermediary space are connected by narrow-looped network of bone trabeculae, as well as by osteons of different maturity with compaction signs all over cortex width, and practically complete periosteal, intermediary, and endosteal bone union is revealed. The fracture healing occurs by the type of primary consolidation due to the fact that osteogenic cells of Haversian canals forming bone trabecular and osteons across the fracture line grow into the diastasis from the ends of fragments along blood vessels. The bone sheath around the wires is formed by compact bone of lamellar structure with forming osteons and spongy bone tissue which spreads not only into the diastasis but it also fills the medullary cavity of fragments thereby binding them like a pin. Both osteogenesis intense process and bone tissue remodeling is also seen in the periosteal parts of regenerated bone where numerous osteoblasts and functionally active osteoclasts are located, reorganization of the cortex of the fragmental ends is observed with vascular channel expansion and extensive stratification on the fragmental ends of the bone trabeculae surrounded by some layers of large osteoblasts. Secondary osteons of lamellar bone tissue are formed in the new cortex at fracture site, however, the intermediary zone of regenerated bone still differs significantly from the cortical layer of animals' intact shaft by mineralization degree and organospecificity (Table 1). The content of calcium in the intermediary zone of regenerated bone during this period is 66-67 %, that of phosphorus – 76-77 %, and that of bone tissue – 83-84 % of the values of intact shaft cortex.

By 180, 360 days after surgery the endosteal part of regenerated bone is rather small being represented by web-like network of thin lamellar-structured bone trabeculae in the expanded intertrabecular spaces of which vascular channels with wide lumens are located, as well as hematopoietic-and-fatty bone marrow. The bone sheath around the wires sharply becomes thinner, and it is fragmented, multiple functionally active hypertrophied osteoclasts and resorption lacunae are located on its outer surface. The content of calcium, phosphorus, and bone tissue in newly formed cortex at this stage of the experiment approximates to the measures of the shaft cortex in intact animals (Table 1).

The investigations of the content of bone tissue and of the main mineral components in the intermediary zone of regenerated bone evidence of the fact that as far as the experiment duration increases, calcium and phosphorus content in the newly formed bone increases steadily as well thereby reflecting gradual prolonged mineralization of the regenerated bone throughout the experiment. The rise of Ca/P coefficient with increasing the experiment duration indicates qualitative changes in the mineral phase of regenerated bone tissue which are characterized by gradual decreasing the proportion of soluble calcium phosphate, as well as by increasing the proportion of hydroxyapatite and bone tissue maturity degree thereby approximating for these measures to the shaft cortex of intact animals. The index of bone tissue compactness in the newly formed part of regenerated bone cortex increased gradually as well, reflecting the rise in its organospecificity degree. At the same time, the index of bone tissue compactness in the regenerated bone intermediary zone amounted to 64.24±3.73 % of the values of intact shaft cortex even by the end of the experiment thereby evidencing of incompleteness of remodeling processes.

DISCUSSION

Intramedullary osteosynthesis is known to provide little-damage fixation of fractures, to allow earlier weight-bearing of the operated limb, and to be one of the main standard techniques for treating femoral and tibial shaft fractures in most countries [3,4]. The main disadvantage of intramedullary osteosynthesis is considered the risk of damaging vessels and circulation system of medullary canal which weakens the osteogenic and osteoinductive potential of bone marrow stromal pluripotent cells [5]. Experimental studies have demonstrated that insertion of even thin implant into the medullary cavity results in significant blood supply disorders of the medullary canal and cortex inside [6]. The possible mechanism of the stimulating effect of intramedullary wire insertion is connected with prolonged formation of the local foci of granulation tissue in the medullary cavity. The characteristic feature of the granulation tissue is the expression of endotheliocyte migration phenotype, and as a consequence – angiogenesis activation evidenced by intense formation of numerous endothelial sprouts which generate capillary buds and endovasal spreads localizing in vascular lumens (endovasal angiogenesis).

The creation of such foci provides the increase of osteoproducing cell population in fracture zone both endocrinally and paracrinally, as well as it stimulate regeneration angiogenesis, and thereby contribute to osteoreparation process activation. The additional coating of titanium implant surface with hydroxyapatite nanostructured high-porosity layer provides high biocompatibility with the tissue structures of regenerated bone, increases osteointegration rate, decreases the output of metal ions, and prevents the formation of fibrillary connective tissue and cartilage in the regenerated bone [6,7]. The zone of active appositional osteogenesis and angiogenesis is formed around the wires, as well as the bone sheath with osteogenesis conductor and inductor properties, which provide directed growth of bone tissue, prolonged stimulation of angiogenesis and reparative osteogenesis. Fracture consolidation occurs by the primary type early without cartilaginous and connective tissue formation in bone adhesion.

CONCLUSION

Thus, the results of the study evidence of the positive effect of intramedullary wires with hydroxyapatite coating on the course and intensity of reparative osteogenesis during fracture healing. The data obtained allow recommending this relatively little-invasive method of osteoreparation optimization to be used in combination with other methods of conservative and surgical treatment of bone fractures, especially for sluggish reparative processes in children, elderly and senile persons, as well as in debilitated patients.

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Conflict of Interest: Nil

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