OSSEOINTEGRATION OF THE INTRAMEDULLARY IMPLANT IN FRACTURE OF THE DIAPHYSIS OF A LONG BONE

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Abstract: In the experiment on dogs, nails coated with hydroxyapatite were introduced intramedullary in the tibia. By using light microscopy of histological sections and scanning electron microscopy, bone marrow space around the implant was studied. It was found that an area of active apposition bone formation is formed around the nails, and bone case is formed with the properties of osteogenesis conductor and inductor, providing directional growth of bone tissue and reparative bone formation. The extent of osseointegration of intramedullary implant was determined by separation method when removing intramedullary nail a month after the surgery. Active implant osseointegration provides guaranteed diaphysis fracture healing in 2-3 weeks.

Keywords: Intramedullary osteosynthesis, Hydroxyapatite coating, Reparative bone formation, Osseointegration.

INTRODUCTION

In the treatment of patients with bone fractures, the implants are often used in the form of rods arranged in the medullary canal (intramedullary osteosynthesis). The purpose of such osteosynthesis is to ensure maximum immobility of reduced fragments, i.e. optimal conditions for reparative regeneration of bone in the fracture zone.

These conditions are achieved by purely mechanical means: reaming of the medullary canal for the diameter of the nail and fixing screws [1]. During recent years, in the treatment of diaphyseal fractures, it was proposed to use flexible intramedullary nails made of titanium alloys 2-4 mm thick, including those with a bioactive coating [2,3,4], which according to the authors, has stimulated reparative bone formation and shortened fracture healing period.

The purpose of this study is to investigate the process of osseointegration of the implant with a bioactive coating in the medullary canal.

MATERIALS AND METHODS

Preoperative Period

Prior to start the experimental research, the approval of Ethics Committee of The Federal State-Financed Institution “Russian Scientific Center Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics of the RF Ministry of Healthcare was received (Extract from the protocol No. 2(19) of the Ethics Committee Session of The Federal State-Financed Institution “Russian Scientific Center Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics of the RF Ministry of Healthcare, dated June 6, 2011). The experiments were performed in accordance with the requirements of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Strasbourg, 1986) and were approved by the Ethics Committee of the FSFI Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics of the RF Ministry of Healthcare.
The experiments were performed using the base of vivarium of the FSFI Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics of the RF Ministry of Healthcare. The animal subjects were kept in separate boxes (individually). The animals were provided with similar, standard, nutritive balanced fares and pure drinking water. Every animal subject was identified by individual four-digit number.

**Surgical Technique**

12 mongrels (males and females) were used in the experiments, the age ranged from 1 to 3 years old with a body mass of 20±2.9 kg. In each case, the osteosynthesis was performed by the same surgical team. All the animal subjects were dehaired on the right lower thigh. After that, in order to achieve maximum sedation, 0.05 mg/kg atropine sulfate dose was injected subdermally. Thiopental sodium was used for general anaesthesia which was injected intravenously in a dose of 12.45 mg/kg. During the surgery, cefazolin (1 g) was injected intravenously.

Intramedullary nailing of tibia was performed with two nails in 12 animals under general anesthesia (figure 1). Nails used were made of titanium alloy coated with a bioactive hydroxyapatite. The coating with a thickness of 20-40 microns and porosity of 2 to 8% was obtained by anodic oxidation in an arc mode (5). Multilevel ultraporous system consists of macro- and micropores of 50-100 nm to 1-2 microns in diameter. Nails with a slightly curved end were introduced from the medial and lateral sides on the level of the crest of the tibia in tuberositas tibia to the level of the distal metaphysis and then were cut at the level of the surface. Then, osteosynthesis was performed with Ilizarov fixator and an open fracture of the tibia bone was simulated in the middle third of the diaphysis by means of osteotomy.

**Post-operative Period**

During 7 days after the surgery, cefazolin (0.5 g b.i.d.). Wounds were observed daily.

The animal subjects were watched till bone consolidation between fracture fragments and 31 day after thereof. During that period, in 7, 10, 14, 21, 2 and 45 days after the surgery, radiography of the treated segment was performed in two standard projections.

The fracture consolidation was determined from radiographic findings and clinical tests results. For clinical test, the nails connecting Ilizarov fixator subsystems were removed, and rotational and flexional loads were applied to fracture fragments. The absence of pathologic motion in the area of osteotomy was the criterion of consolidation. The nails introduced into bone marrow were not removed during intravital observation of the animal subjects. The Ilizarov fixator was disassembled after fracture consolidation was reached.

At different stages of the fracture consolidation, the animals were subjected to euthanasia. For euthanasia, firstly, thiopental sodium in a dose of 12.5 mg was slowly injected intravenously. After achievement of general anesthesia, thiopental sodium was injected intravenously in a lethal dose (500 mg).

After the death of the animal subject was certified, the areas of treated bones diaphyses were freed from soft tissues. Intramedullary nails were removed. Then, lower thigh CT was performed. Upon execution the CT, areas of diaphysis of the bones which underwent surgery were sawed lengthwise and in transverse directions, fixed and embedded in paraffin to produce histologic sections. Histotopographic sections were stained with hematoxylin eosin and picro-fuchsin according to Van Gieson.

X-ray study in the dynamics of the experiment was carried out using Premium Vet (Sedecal, Spain) X-ray machine, and after dismantling of the Ilizarov fixator and removal of intramedullary nails – additionally with multihelical computer tomograph Light Speed VCT by GE. MSCT study was conducted in helical scan mode in tomography steps of 1 mm and a minimum slice thickness of 0.7 mm. Extremity (knee) study protocol.

**RESULTS**

After surgery, a transverse fracture in the middle third of the shin bone is determined
in all animals by radiographs (figure 1a). The edges of the pieces have an uneven surface, the height of diastasis between them is 0.5-1.0 mm. After 8-10 days from the operation, in the vicinity of the fracture line, there are signs of periosteal reaction in the form of cloud-like shadows at the edges of the bone, which gradually compacts after 2 weeks of the experiment (figure 1b). Throughout the experiment, the stable state of the bone fragments was determined. Intramedullary nails also remained almost motionless, no shifts were observed. Formation of a new cortical bone layer at the fracture site is radiographically determined after 28±5.0 days after the operation, a full contact and healing of bone fragments in the damage are noted; intermediate regenerate is clearly expressed (figure 1c). The damage zone of Thickening of the bones is observed in the damage area due to remaining periosteal layers.

A clinical sample for consolidation has confirmed the absence of any mobility of bone fragments in the fracture area after 14±1.0 days of osteosynthesis.

Computer tomography after removing the nails shows the presence of bone case that has formed around the implant (figure 2c).

The thickness of the mineralized wall of this “case” reaches up to 3 mm, and in the future, it is securely bond with endosteum, and the medullary canal along the entire length of the intramedullary nail is filled with cancellous bone (figure 3 a, b).
Histological studies showed that 14 days after the surgery, there occurs endosteal-intermediary fracture healing. Formation of primary bone regenerate in the fracture zone is observed within 14-21 days after surgery (figure 2a). In this period, the active bone formation process is noted with trabecular growth vector directed toward the diastasis, manifested in the mass proliferation of osteogenic periosteal cells, endosteum and bone case around the nails and formation of considerable volume of periosteal and endosteal bone-osteoid growth overlapping diastasis.

Bone case is formed around intramedullary nails throughout their length, which remains until the end of the experiment (figure 2b). It consists of a thin osteoid capsule 30-40 microns thick, circularly oriented plates of compact bone formed by apposition bone formation on the nail surface and adjacent trabecules of cancellous bone. After 14-21 days after surgery, coarse-fibered osteoid forms around the nails with a fibrous skeleton consisting of sleeve-like accumulations of bunches of collagen fibers of flat, ribbon-like shape, located in the longitudinal, transverse and circular directions. Bunches of osteoid collagen fibers are firmly fixed to the roughened nanostructured surface of the nail coating and the endosteal bone surface closest to the nail.

They are tied together with thin, branching elastic fibers that form the elastic frame of the capsule (figure 4). Areas of granulation tissue with numerous blood vessels and perivasculocites at various stages of the osteogenic differentiation are located in the inter-trabecular space of the bone case.

Medullary canal is gradually formed by newly formed cancellous bone (figure 3b). This kind of bone “nail” reinforced by intramedullary nail ensures immobility of the bone fragments and rapid fracture consolidation.

Figure 3: Wet mount of the dog’s tibia: a) a cross section shows that the medullary canal is completely filled with cancellous bone; b) a kind of bone “nail” has formed over the medullary canal (cortical bone layer and cancellous bone are partially removed-intramedullary nail is visible).

Figure 4: Bone trabeculae growing into the pores of hydroxyapatite on the implant surface, that were formed from the bundles of collagen fibers.
DISCUSSION

Integration (Latin integratio — «conjunction») — the process of combining parts into a whole. Depending on the context, it can mean: political integration, data integration, system integration, economic integration. In traumatology and orthopaedics, osteointegration, apparently, should be considered as a special case of the inclusion of a foreign body (implant) in the tissue environment of a living organism.

All metals and their alloys, which are used as implants for temporary fixation of a fractured bone (extramedullary plates, intramedullary rods) can be estimated by the activity of the effect on reparative ability of the latter: biotolerable materials (stainless steel and cobalt chrome alloys) — the surface of such implants separates from the adjacent bone by a layer of fibrous tissue; reparative regeneration of the damaged bone occurs in the usual time and at some distance from the implant (distant osteogenesis); bioinert materials (aluminum and titanium oxides) — do not cause formation of fibrous tissue, reparative osteogenesis occurs in direct contact with the implant surface, but consolidation occurs in the usual time. Modern medicine does not know bioactive metals, which would accelerate reparative osteogenesis.

The most bioactive materials include calcium phosphate ceramics (in particular, hydroxyapatite) and silicon-based bioglasses characterized by formation of a very tight chemical bond with the bone (binding osteogenesis). They improve bone formation reactions starting from the implant surface and induce formation of a continuous bond on the tissue to its surface. However, calcium-phosphate ceramics, having undoubted biological activity, is too fragile to use as a bulk material under loading conditions and cannot be used as independent or massive extramedullary and intraosseous implants. The practical way out of this situation is usually found in the possibility of applying CaP-ceramics on the surface of metallic implanted materials with the aim of combining mechanical strength of metals with its excellent biological properties.

Bioactivity phenomenon is mainly determined by chemical factors such as the crystalline phase and molecular structure of material, as well as physical factors — roughness and porosity of material surface, and surface design [6, 7, 8]. In our opinion, intramedullary osteosynthesis with bioactive coated nails has two main functions — it provides additional stability of bone fragments, as well as all osteoconductive and osteoinductive conditions for reparative bone tissue regeneration inherent in biomaterials based on hydroxypatite.

From the economic point of view, the technology for manufacturing the nails with a bioactive calcium phosphate coating is rather inexpensive process, and intramedullary position of the implant eliminates the need for additional cultivation of expensive growth and cell factors. The method of flexible intramedullary nailing (FIN) with the pins with a bioactive HA coating allows to influence the activity of the bone marrow, stimulating endosteal bone formation. This osteoinduction method is characterized by low traumatic rate, it does not damage a. nutricia, excludes the possibility of decelerated consolidation, i.e. it prevents formation of pseudarthrosis, secondary bone deformations, and ensures early functional activity of the patient.

When discussing a number of existing hypotheses of osteoinduction mechanism, we do adhere to the one that associates this process not only with the material, more precisely, with surface design of an intramedullary nail, but also with a biological phenomenon in the form of aseptic inflammatory reaction occurring after implantation of biomaterials [9] and even more so in case of bone trauma. A.I. Caplan and B.D. Boyan [10] hypothesized that the initial inflammatory response after implantation of biomaterial stimulates factors attracting mesenchymal stem cells. One of them may be prostaglandin E2 (PGE2), which is produced by macrophages present around biomaterials during the inflammatory phase [11, 12]. It was reported that microroughened surface, unique to osteoinductive materials, stimulates PGE2 production by macrophages and PGE2 has
chemoattractive properties against human mesenchymal stem cells and stimulates their osteogenic differentiation.

Hydroxyapatite coatings induce bone formation around the implant, which provides practical implant osseointegration and improves its fixation virtually without any gaps between the bone and the implant.

Among traumatologists, it is assumed that the main drawback of the standard intramedullary osteosynthesis is a danger of damage to the blood vessels and circulatory system of the medullary canal, which weakens osteogenic and osteoinductive potency of pluripotent stromal cells of bone marrow [13].

Relatively less traumatic FIN virtually eliminates damage to the intraosseous artery and destruction of endosteum. A possible mechanism of the stimulating effect the intramedullary pins introduction is associated with prolonged formation of the local pockets of granulation tissue in the medullary cavity; their formation in endocrine and paracrine ways of influence provides an increase in the population of osteo-producing cells in the fracture zone, stimulates regeneration angiogenesis and thereby contributes to activation of osteoreparation process [14].

Additional coating of the surface of titanium implants with nanostructured layer of highly porous hydroxyapatite provides high biocompatibility with tissue regenerate structures, increases the rate of osseointegration, reduces the yield of metal ions and prevents formation of fibrillar connective tissue and cartilage in the regenerate [5, 6, 15, 16] A zone of active apposition bone formation is formed around pins, and a bone case is formed with the properties of osteogenesis conductor and inductor, providing directional growth of bone tissue and reparative bone formation. Fracture consolidation is carried out in short terms according to the primary type without formation of cartilage and connective tissue in the bone junction. Thus, the results of research show the positive influence of intramedullary nail with a coating of hydroxyapatite on the course and intensity of reparative bone formation in fracture healing. The data obtained allow recommending this relatively noninvasive way to optimize osteoreparation for use in combination with other methods of conservative and surgical treatment of bone fracture, especially in case of low-intensity reparative processes of children, elderly, senile and debilitated patients.

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