Bone union and structural changes in the articular cartilage of the knee joint after immediate and delayed antegrade locked intramedullary nailing of femoral shaft fractures. Experimental findings

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INTRODUCTION

There is a lot of clinical research on the use of locked intramedullary nailing (IMN) for a number of long bone injuries [1–2]. Current literature that describes the management of diaphyseal fractures of the femur also provides much evidence regarding the use of antegrade and retrograde IMN that yields high union rates and optimal bone healing [2]. Antegrade locked IMN is considered to be a method of choice for repair of femoral shaft fractures. The range of motion (ROM) in the hip and knee returns to normal over time [5] but several clinical studies of femoral shaft fracture repair with the use of IMN fixation reported nonunion, mal-alignment, residual impairment such as hip abduction weakness, knee extensor weakness, pain in the lower limb, anterior knee pain, and gait abnormalities [2, 3–6].

However, the issues of functional limb recovery, including knee joint functions, are more challenging in case of high energy injuries [3-5]. Associated disorders of blood supply, hypokinesia and inflammation, incomplete or absent weight-bearing during fracture healing as well as initial osteoarthritis (OA) may add to deterioration of the articular cartilage [3–6].

It is impossible to obtain the information about the structural changes in the joint tissues due to injury or treatment method on the basis of clinical studies. Therefore, it seemed reasonable to set up an experimental research in order to provide knowledge on the condition of the articular cartilage during IMN fracture repair to reveal the phenomena that could be influenced by the method and the time the osteosynthesis is performed.

The purpose of our study was investigation of bone healing and structural changes in the articular cartilage of the knee joint in the conditions of antegrade locked IMN used for femoral shaft fractures on a canine model.

MATERIAL AND METHODS

The study was carried out on five adult intact and 12 experimental dogs of both sexes aged 1.1 ± 0.05 years, mean body weight of 15.95 ± 1.09 kg, and femur length of 18 ± 0.8 cm.

Transverse fractures in the middle third of the femur were modelled with a chisel under general narcosis using sodium triopenthal in the dose of seven to 10 mg/kg. Preliminary medication was a mixed solution of 0.1 ml/kg of 2 % rometar, 0.1 ml/kg of dimedrol, and 0.01 ml/kg of atropine. Antegrade locked IMN with the nails that were 6 mm thick and from 13.5 mm to 16.0 mm long (I-Loc system, BioMedtrix, USA) was used for fracture fixation immediately after the osteotomy in group 1 (n = 6) and four days after the fracture in group 2 (n = 6) following skeletal traction (Figs. 1a, 1b)
Antero-posterior and lateral radiographic views of the limbs were taken with the Premium Vet system (Sedecal, Spain) before operation, after fracture modeling, and on days 14, 28, 35, 42, 56, 70, 85 and 100 postoperatively.

The points of time for histological study were days 42 (n = 3) and 70 (n = 3) in group 1, and days 70 (n = 3) and 100 (n = 3) in group 2. Euthanasia was conducted with intravenous sodium thiopental in the dose of more than 40 mg/kg. The knee joint was opened, and the articular cartilage from the lateral femoral condyle was harvested for a histological study. The samples obtained were fixed in adeldig osmium and then contained in araldite. Semithin sections (0.5 to 1.0 mcm) of a large area [7] were excised using an ultratome (Nova, LKB, Sweden), stained with toloidin blue and methylene blue base fuxin. The preparations were studied on a photomicroscope (Opton, Germany).

Histomorphometric measurements in the articular cartilage samples were performed using the DiaMorph complex (Diamorph, Moscow, Russia) with the VT-Master-Morphology software (VideoTest, St. Petersburg, Russia). The following parameters were measured in the articular cartilage: thickness (h, mcm), volumetric density of chondrocytes (Vvch), chonrocyte numeric density (NAch), the portions (%) of empty lacunas (NNem. lac.) and isogenic groups (NNis. gr.) from the total of chondrocytes (Table 1). Intact articular cartilage samples from five intact control dogs were studied using morphometry.

Wilcoxon test was used for statistical analysis (AtteStat software version 1.0 for Microsoft Excel 97) with a significance value of p < 0.05

![Radiographs after fracture modeling](a), antegrade locked intramedullary nail placement (b); bone union condition on day 70 in group 1 (c) and group 2 (d)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vvch (%)</th>
<th>NAch (M ± m)</th>
<th>NNem. lac (%)</th>
<th>NNis. gr. (%)</th>
<th>h, mcm (M ± m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9.03 ± 1.04</td>
<td>6.1 ± 0.78</td>
<td>13.6</td>
<td>14.5</td>
<td>475.5 ± 1.3</td>
</tr>
<tr>
<td>Fixation time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 70 days</td>
<td>4.94 ± 0.41</td>
<td>8.21 ± 0.69</td>
<td>16.6</td>
<td>10.9</td>
<td>428.98 ± 1.41</td>
</tr>
<tr>
<td>Group 2 70 days</td>
<td>4.25 ± 0.52*</td>
<td>6.84 ± 0.71*</td>
<td>17.5</td>
<td>3.4</td>
<td>505.61 ± 7.65*</td>
</tr>
<tr>
<td>Group 2 100 days</td>
<td>4.95 ± 0.32</td>
<td>8.68 ± 0.65</td>
<td>22.3</td>
<td>4.4</td>
<td>601.68 ± 10.28</td>
</tr>
</tbody>
</table>

Note: bold type are significant differences with the control group; * – difference between group 1 and group 2 (p < 0.05)

RESULTS

All dogs had mild edema in the femoral area and an increase in the body temperature of 0.5 degrees after the first two or three days. Group 1 animals started weight-bearing within three to seven days after the operation but group 2 dogs recovered weight-bearing only after 10 to 14 days following the operation.

Knee joint range of motion was normal in group 1. It was within 100 to 110 degrees during two to three weeks after the operation in group 2. There was no evident hind limb muscle atrophy in both groups.

Perosteal response was seen from day 14 and endosteal osteogenesis was visualized from day 28 in the
radiographs of group 1 dogs. On day 42 after the IMN fixation, the periosteal layers were becoming compact and the cortex was seen on both sides that proved consolidation. The radiographs of group 2 dogs showed a weak periosteal response on the fragments ends from day 14 through 42.

On day 70, a uniform bone marrow cavity was revealed in the radiographs in group 1 (Fig. 1c). The unified cortical plates were approximating in density and diameter to the maternal bone plates. In group 2, bone union was observed by day 70 (Fig. 1d) but the cortical layer density was similar to the maternal bone cortex only by day 100 of fixation.

Histological study revealed that the articular cartilage of the femoral condyles maintained its zonal structure at both experimental points of time, days 42 and 70, in group 1 (Fig. 2a). The intercellular substance of the joint surface was homogenous. The chondrocytes were functionally active, had clear homogenous nuclei and a basophile cytoplasm. In the intermediate layer, mostly solitary chondrocytes were detected. Two-member isogenic chondrocyte groups were not frequent. In the deep layer, the chondrocytes were positioned in columns; non-cellular spaces were also detected. Empty lacunas were more frequent. The basophilic line had clear contours and was uninterrupted. The calcified cartilage was of uneven thickness. The subchondral plate was absent in several areas of the subchondral bone, and a calcified cartilage was in direct contact with the intertrabecular space of the cancellous bone. A significant reduction in the cartilage thickness and volumetric density of chondrocytes was revealed by morphometry in group 1 on day 70. Numeric density of chondrocytes grew due to an increased cellularity in the superficial zone. The portion of empty lacunas was higher than the norm by 3 %, but the portion of isogenic groups was lower by 3.6 % (Table 1).

On day 70, the homogeneity of the intercellular substance of the superficial layer was affected in group 2. Blood vessels penetrated into the cartilage from the subchondral zone (Fig. 2b). The study of the samples harvested on day 100 showed that the destructive changes continued to progress. Defibration foci on the joint surface (Fig. 2c) were more frequent and blood vessels were seen in the cartilage (Fig. 2g). The thickness of the cartilage increased. Its height was maximal due to an expressed disorganization of the intercellular substance and its swelling in the defibration foci (Table 1). Low volumetric density of chondrocytes was retained; the numeric density exceeded the control values due to the increase in the cells on the superficial layer. There were considerable changes in the number of empty lacunas and isogenic groups.

![Fig. 2 Semithin sections of the femoral condyle cartilage. Methylene blue base fuxin staining. Magnification: ×78.75 (a, b); ×500 (c, d). Group 1: 70 days of experiment. General view of the cartilage: non-cellular spaces in the deep zone (a). Group 2: 70 days of the experiment. General view of the cartilage: break in the basophilic line continuity, penetration of vessels into the cartilage (b). Group 2: 100 days of experiment. Defibration of the superficial zone; chondrocytes with pycnotic nuclei of abnormal shape; present non-cellular spaces (c). Group 2: 100 days of fixation. Penetration of vessels into the deep cartilage zone from the subchondral bone (d)]

**DISCUSSION**

Locked IMN used for femoral shaft fractures provides stable fixation that can be applied using indirect reduction techniques. This method yields high union rates and low complication rates in regard to bone and function [1–6]. Our experiment confirmed uneventful bone union in both groups. However, it occurred in different periods. We attribute the retardation of the union in group 2 to a later time of fracture fixation though the interval between the injury and fixation was only four days. It proves the fact that the osteosynthesis should be performed as early as possible to exclude prolongation of the treatment process. The reported time of femoral shaft fracture union with nails in clinical settings ranges from 10 to 28 weeks [8]. Therefore, our experiment lasted for 70 days in group 1 and 100 days in group 2.

It is known that antegrade locked IMN is less traumatic in regard to the knee joint as far as nail insertion is performed from the proximal femur [6]. Our experiment showed that the destructive changes in the knee joint were mild and the structure of the femoral condyle cartilage was...
not broken in group 1. Such changes could be explained by a disturbed limb trophics condition. Once limb functions are gained completely, those changes are reversible.

On the contrary, significant changes such as defibration of the articular surface accompanied by breakage of the basophilic line, penetration of vessels into the cartilage and decreased chondrocyte proliferation were revealed in group 2. Our findings also showed that the changes were more profound in the deeper cartilage zones in both groups. The disturbance in the limb trophic condition, hypokynesia due to injury and locked IMN result in the development of those dystrophic changes which were more marked in group 2 due to the delay in fixation.

Moreover, associated local factors such as fragment displacement, damage to intraosseous blood supply and soft-tissue injury encountered in real orthopaedic practice may aggravate the conditions of bone healing and joint disorders thus stimulating the destruction of the cartilage.

It is well known that the proportion of total joint replacements due to posttraumatic OA has been growing [9]. Difficulties in the diagnosis of early secondary degenerative articular changes derive from the fact that plain radiographs are limited in the detection of a pre-osteoarthritic situation [10–11]. They were not detected in the radiographs in our experimental series preoperatively and postoperatively. However, they were proven by the morphological study of the articular cartilage in group 2. Our histomorphometric findings indicate that IMN osteosynthesis increases the treatment period and the risk factors of developing knee OA.

Ethical standards statement
The study was approved by the ethics board of the institution.
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest statement
The authors declare that they have no conflicts of interests.

REFERENCES

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