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Impact of Transphyseal Elastic Nailing On the Histostructure of the Tibia in Growing Animals (Non-Randomized Controlled Experimental Study)

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Keywords: Transphyseal nailing; Elastic intramedullary nail; Growing animals; Tibia; Histology

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Abstract

BACKGROUND: The use of intramedullary elastic nailing is a method of choice for prevention of complications in children with osteogenesis imperfecta. However, the morphology of the growing long bones in the conditions created was not investigated.

AIM: The purpose of our experiment was to study the impact of elastic intramedullary nailing on the histostructure of long bones in their physiological growth.

METHODS: Six mongrel dogs underwent intramedullary elastic transphyseal nailing of the intact tibia with two titanium wires. Six months after nailing, a light-optical microscopic and histomorphometric study of the operated and contralateral tibiae was performed.

RESULTS: It was found that asymmetric lesion of the distal physis induces a decrease in the height of the distal epimetaphysis. Adaptive changes in the hyaline cartilage of both articular ends were revealed corresponding to the initial stage of chondropathy. Intramedullary nailing promotes an increase in the thickness of the compact bone and the volume of the trabecular bone.

CONCLUSIONS: Elastic transphyseal nailing of the intact tibia has a shaping effect which is expressed by an increase in the volume of spongy and compact bone, adaptive changes in the hyaline cartilage. Asymmetric damage to growth zones should be avoided to prevent deformities.

Introduction

Osteogenesis imperfecta (OI) is a systemic bone disease that is accompanied by bone fragility that results in fractures and deformities [1] [2]. In 1987, J.P. Metaizeau proposed the use of counter directed intramedullary transphyseal elastic nailing to prevent deformities and treat fractures of the tubular bones. Currently, the use of transphyseal intramedullary nailing is a method of choice for prevention and treatment of complications in OI children [1] [2] [3] [4] [5] [6]. The advantage of elastic rods is the possibility to use them in a small bone canal, which is characteristic for OI children [3] [7]. Specific features of the method include possible elongation of the intramedullary fixator to stabilise bone fragments and to prevent bone deformities in the period of segment growth [3] [6] [7] [8] [9].

Earlier, we performed an X-ray diffraction study of the effect of the central retrograde and eccentric antegrade transphyseal placement of intramedullary elastic rods on the growth of the tibia without compromising its integrity. It was found that the segment lost the length and there was a change in the plane of distal articular surface inclination [10]. However, the morphology of the tibia in the conditions created by this previous study was not investigated.

The purpose of the present research was to study the shaping effect of bipolar transphyseal elastic intramedullary nailing on the histological structure of an intact long bone that continued physiological growth.

Material and Methods

This non-randomized controlled study was performed on six littermate healthy dogs of both sexes in the period of their active growth in 2014-2017 by the experimental department of the Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopedics. The age of the animals at the time of surgery was six months and the weight range from 10 to 12 kg. The choice of the species was due to the similarity of the bone reparative process in dogs and humans in orthopaedic situations [11]. Approval was obtained from the institutional ethics committee before the experiment. All manipulations were carried out by “On the Approval of the Rules of Laboratory Practice”, those of the “European Convention for the Protection of Animals used for Experimental and other Scientific Purposes” and approved by national ethics regulations.

Counter bipolar transphyseal elastic intramedullary nailing of the intact tibia on the right side was produced in dogs in the conditions of the operating room under intravenous anaesthesia with 20-25 mg of thiopental sodium per 1,000 g of body weight after premedication (0.1% atropine sulfate 0.1 ml, 1% dimedrol 1 ml, 2% xyiazinum 0.2 ml intramuscularly).

Two pre-curved titanium wires with a diameter of 1.8 mm and a length of 10 cm were used. The bending radius of the wires was 40°. One antegrade wire was inserted through the proximal paratendinous approach and the centre of the proximal tibial epiphysis. The second wire was introduced in the retrograde direction eccentrically at the level of the medial malleolus. The free ends of the wires were bent U-shaped and impacted into the epiphyses to prevent migration of the nails.

The experimental continued 180 days after the surgical intervention. At the time of its completion, the biological age of the animals corresponded to 12 months. Left tibia served as a control segment in all the animals.

The proximal and distal epiphyses, as well as the tibial diaphysis, were separated and fixed in 10% neutral formalin. Bone units were decalcified in a Richmann-Gelfand-Hill mixture, dehydrated in ethanol of increasing concentrations, and poured with celloidin. Histotopographic sections were cut with a sledge microtome (Reichard, Germany); sections 10-15 μm thick were stained with hematoxylin and eosin, Van Gieson and Masson methods.

Light-optical microscopic and histomorphometric study of epiphyseal preparations was carried out using AxioScope.A1 stereomicroscope and AxioCam ICc 5 digital camera supplied with Zen blue software (Carl Zeiss MicroImaging GmbH, Germany). The results of the microscopic investigation of the articular surfaces were objectified using the ICRS score system to assess the histological structure of the articular cartilage after mechanical or osteoarthritic damage [12].

Histomorphometric evaluation included the total thickness (h_{tot}, мм), height of non-calcified (h_{ncalc}, мм) and calcified (h_{calc}, мм) zones, cell density of chondrocytes in the articular cartilage (NA_{chc}, mm^{-2}); height of the epiphyseal parts of the tibia (h_{ep}, мм); thickness of the diaphysis compact plate (H_{cp}, мм); thickness of trabecular bone coupling around of the intramedullary wires (mm); trabecular bone area S_{tb} ($мм^2$) and its volume (S_{tb},%) in the bone marrow canal.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation of the mean (SD) were calculated. The Student’s test was used to compare the differences between the two independent groups. A probability value of $P < 0.05$ was considered statistically significant.

Results

After 180 days of the experiment, the proximal epiphysis of the tibia of the experimental and control extremities had an anatomically correct orientation and a typical histological structure (Figure 1 a-f) as in dogs of the similar age [13].

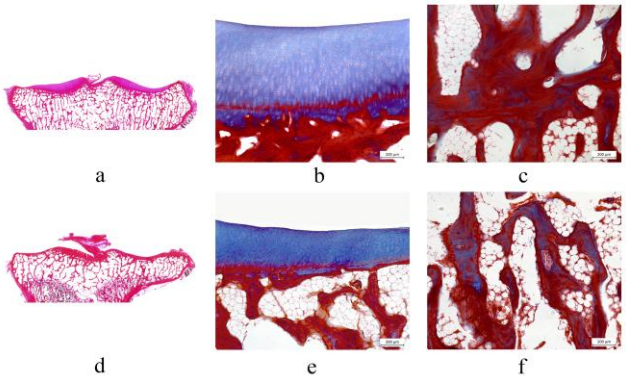

Figure 1: Histostructural organisation of the proximal epiphysis of the contralateral (a) and operated (d) canine tibiae of the dog after 6 months of transphyseal elastic intramedullary nailing. Changes in histoarchitectonics in the articular surface (e) and spongy bone substance (f) of the operated bone were noted in comparison with the corresponding sites (b, c) of the contralateral limb. Celloidine sections. Staining with hematoxylin and eosin (a, d), magnification × 6.5. Masson staining (b, c, e, f), magnification × 100

The articular surface of the tibial plateau in the control segment was covered with typical hyaline cartilage (Figure 1b). The ICRS score was I-VI: 3, which corresponded to the intact cartilage condition (Table 1).

Table 1: Semiquantitative histological evaluation of the articular surface of the tibial epiphyses of the control (C) and experimental (E) segments according to the ICRS scale [12]

Parameter and its histological evaluation (score)	Articular surface			
	Proximal epiphysis		Distal epiphysis	
	C	E	C	E
I. Surface Smooth/continuous (3) Discontinuities/irregularities (0)	3	0.3	3	0.3
II. Matrix Hyaline (3) Mixture: hyaline.fibrocartilage (2) Fibrocartilage (1) Fibrous tissue (0)	3	3	3	3
III. Cell distribution Columnar (3) Mixed.columnar-clusters (2) Clusters (1) Individual cells.disorganized (0)	3	2.3	3	1.3
IV. Cell population viability Predominantly viable (3) Partially viable (1) <10% viable (0)	3	3	3	3
V. Subchondral bone Normal (3) Increased remodeling (2) Bone necrosis.granulation tissue (1) Detached.fracture.callus at base(0)	3	3	3	2.3
VI. Cartilage mineralization (calcified cartilage) Normal (3) Abnormal.inappropriate location(0)	3	0.3	3	0.3

In the experimental segment, the total thickness of the hyaline cartilage of the articular surface was reduced, but the tidemark and the cement line retained a similar contour and continuity (Figure 1d). In the superficial zone, there was single surface undulation, but its smooth structure was retained. In the subchondral bone, there were alternating dense and rarefied areas; however, active restructuring or necrosis was not detected. The ICRS scores were I-VI: 3, very rarely-I:0.II: 3.III: 2.IV: 3.V:3.VI: 0 (Table 1).

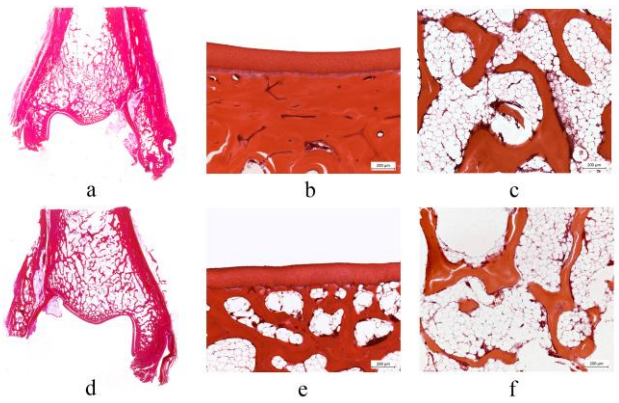

Figure 2: Histostructural organisation of the distal epiphysis of the control (a) and experimental (d) tibia of the dog after 6 months of transphyseal elastic intramedullary nailing. Changes in the histoarchitectonics of the articular surface (e) and spongy bone substance (f) of the operated bone were noted in comparison with the corresponding contralateral sites (b, c). Celloidin sections. Staining with hematoxylin and eosin, magnification × 6.5 (a, d); magnification × 100 (b, c, e, f)

The distal epiphysis of the control and experimental segments also had a typical histological structure (Figure 2 a-f). The control segment featured a smooth surface of the hyaline articular cartilage, its zonal cell organisation, homogeneity of the extracellular matrix, continuity of the basophilic line and the osteochondral junction. Numerous areas of the articular surface had signs of chondropathy in the experimental segment: strengthening of the fibrous matrix component in the upper third of the hyaline cartilage, the formation of rounded cell clusters in the zone of the columns, irregularity of the basophilic division lines and the osteochondral junction. On the ICRS scale, the score in the control segment was I-VI: 3, in the experimental one it was I-VI: 3 or II: 0. II: 3. III: 1. IV: 3. V: 2. VI: 0 (Table 1).

The tendency to decreased thickness of the zones and the overall height of the hyaline cartilage of the articular surface of the tibial epiphyses was statistically confirmed in its proximal part. The decrease in the thickness of the calcified zone was most pronounced, amounting to 78% and 25% for the proximal and distal epiphyses, respectively. The increase in the numeric density of chondrocytes in the articular cartilage of the proximal and distal epiphyses was 72% and 18%, respectively (Table 2).

Table 2: Histomorphometric evaluation of the parameters of the hyaline articular cartilage of the tibia of the control (C) and experimental (E) segments (M ± SD, μm)

Parameter	Proximal epiphysis		Distal epiphysis	
	C	E	C	E
h_{tot}	311±32.8	227±14.2[1]	223±39.1	216.8±42.1
h_{ncalc}	275±44.7	209±12.8[1]	198±32.6	185.9±31.9[1]
h_{calc}	87±40.6	19.3±9.6[1]	43.5±11.2	32.2±11.0[1]
NA_{xu}	2586±833.9	4439±709.2[1]	2739±422.8	3258±318.9[1]

[1]–statistical difference with control side, $p < 0.05$.

In the region of growth zone closure in the proximal tibial epimetaphysis of the control segment, a network of massive lamellar trabeculae with red or yellow bone marrow in the space between them was identified (Figure 1c). The height of the epiphyseal part at the level of the medial and lateral condyles of the articular surface did not differ significantly (Table 3).

Table 3: Histomorphometric evaluation of the height of the medial (M) and lateral (L) part of the tibial epiphyses, (M ± SD, μm)

Control segment				
Parameter	Proximal epiphysis		Distal epiphysis	
	M	L	M	L
h_{ep}	476 ± 8.1	473 ± 11.3	262 ± 8.92[1]	274 ± 10.7
Experimental segment				
Parameter	Proximal epiphysis		Distal epiphysis	
	M	L	M	L
h_{ep}	477 ± 14.0	460 ± 14.9[1,2]	190 ± 10.7[1,2]	271 ± 11.9

[1]–differences with the opposite part of the epiphysis are significant at $p < 0.05$; [2]–differences with the same parameter of the control segment are significant at $p < 0.05$.

In the experimental segment, a similar region was formed by a discontinuous network of lamellar and reticulofibrous bone trabeculae, including small cartilage foci (Figure 1f), while red or yellow bone marrow was slightly oedematous. The objective decrease in the height of the epiphysis at the level of the medial condyle of the articular surface was about 3% (Table 3). The growth plate of the distal epimetaphysis of the tibia in the control segment was replaced by a developed thick network of massive

lamellar trabeculae containing red or yellow bone marrow (Figure 2c). The height of the medial part of the epiphysis was reduced by 3% in comparison with its lateral part (Table 3). In the experimental segment, the spongy bone replacing the growth plate included atrophic trabeculae and red-to-yellow bone marrow and blood stagnation in the microcirculatory vessels (Figure 2f). The height of the distal epiphyseal part of the bone on the lateral side did not differ from the same value in the control segment but was reduced by 27% on the medial side (Table 3).

Significant differences in the structure of the experimental and control limbs were found on the anatomic and histological preparations of the tibial diaphysis. In the experimental limb, the tibial bone marrow canal was filled in with a trabecular bone substance forming a bone coupling around the wire which was 0.9-1.5 mm thick and closely adhered to the surface of the intramedullary implants. If the titanium implant contacted with the endosteal surface of the compact plate, its integration into the bone matrix was noted. The volume of the trabecular bone in the medullary canal of the experimental tibia was 6.7 ± 0.31 %, which significantly exceeded the value in the control limb equal to 1.9 ± 0.08 %. It meant a 2.8-fold increase in its absolute area. The thickness of the compact plate in the experimental diaphysis increased significantly, by 7.6 % as compared to the control tibia (Table 4).

Table 4: Morphometric parameters of the tibial diaphysis of the control (C) and experimental (E) segments after 180 days (M ± SD)

Thickness of compact plate. H_{cp} (μm)		The trabecular bone area in the bone marrow canal. S_{tb} (μm^2)	
C	E	C	E
2341 ± 8.42	2515 ± 9.11[1]	1669755 ± 8202	473512 ± 30.11[1]

[1]– the Significant difference with control segment, $p < 0.05$.

Discussion

Transphyseal insertion of implants implies some degree of damage to the articular cartilage and the bone growth zone. Previously, we reported that transphyseal intramedullary implants injure less than 6% of the physics area for 25 weeks. They did not lead to irreversible epiphysiodesis but caused a delay in bone growth with a loss of 15 to 18% of its length. We also found that the eccentric introduction of intramedullary nails through the medial malleolus resulted in ankle joint varus by the time of limb growth completion [10]. Radiographic study results correlated with histologically confirmed abnormalities of growth plates closure and a decreased rate of spongy bone formation in the distal tibial epiphysis on the side of the intervention. The findings obtained were confirmed by the studies of other authors who established the fact of angular deviation in the eccentric damage to the growth zone.

However, the central transphyseal introduction of elastic rods does not result in angulation [14] [15]. Seil R. et al. did not reveal any angular deviation during the subsequent growth of the segment in lambs after drilling the central canal [16]. Similar results were obtained in our study with the antegrade intramedullary insertion of the nails through the centre of the proximal tibial epiphysis. Our study also showed that elastic rods that passed outside the support areas of the articular surface and remained for 6 months in situ could cause initial signs of a dystrophic process in the hyaline cartilage of the articular surface. The decrease in the height of the articular cartilage without marginal osteophytes in the absence of fissuring and cracking of the cartilaginous matrix that was revealed using ultrasound [17] indicates the development of osteoarthritis grade I according to Outerbridge classification [18]. A similar to our data decrease in the height of the hyaline cartilage of the tibial plateau was observed in adolescents with arthropathies of non-inflammatory genesis. It is also one of the initial signs of osteoarthritis development in adults [19]. Reduction in the total height of the joint hyaline cartilage, and of the calcified zone thickness in the proximal femoral epimetaphysis in particular, was observed in the treatment of diaphyseal fractures in the conditions of locked intramedullary osteosynthesis [20]. Thinning of the calcified zone of the cartilage of the articular surfaces was also noted during its age involution [21].

The formation of both periosteal and endosteal bone callus during the healing of experimental fractures under the conditions of intramedullary elastic stress reinforcement was shown by us earlier [22]. Gradual traction of intramedullary elastic rods promotes the formation of the surrounding bone coupling, which was noted by limb bone elongation in experimental and clinical conditions [23].

In our experiment, traction of the implants was carried out due to spontaneous growth of the limb segment and was accompanied by thickening of the compact plate of the diaphysis and the formation of an endosteal formed spongy bone substance that was seen as a tight coupling around the intramedullary wires. Such an increase in bone mass along with augmentation in the mechanical strength of the bone due to its reinforcement with intramedullary wires could be useful for prevention of deformities and fractures and could be assisted with medication therapy in metabolic osteopathy [24].

Thus, bipolar transphyseal elastic intramedullary reinforcement has a form-building effect on the cost structure of the articular cartilage and subchondral bone of the epimetaphyses, as well as the on the diaphysis of the intact tibia in growing animals. Location of the rods (central or eccentric) affects the growth zone and the structural organisation of the articular cartilage by inducing initial changes of a dystrophic nature. These changes are less pronounced with the central positioning of the wires,

when the angular orientation of the epiphysis remains preserved, in comparison with their eccentric position inducing the formation of angular deformity.

The changes are adaptive, since quantitative rather than qualitative differences were observed in the histological structure of the control and experimental segments. Nevertheless, they might cause the development of osteoarthritis and deformity of the limb. Therefore, preventive measures are required. Transphyseal insertion of wires increases the opposition growth of the compact plate and stimulates the formation of additional trabecular bone in the medullary canal. The complex of intramedullary structures (wire + bone coupling round it) promotes an increase in the strength of the bone, which is important for the treatment of pediatric patients with systemic skeletal diseases.

We acknowledge the limitation of our study since the bone strength of the canine tibia was not affected as happens in osteogenesis imperfecta. Therefore, direct extrapolation of the results for osteogenesis imperfecta would need clinical trials.

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