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Peculiarities of bone regenerate formation, structural changes in joint cartridge and tibial nerve in the conditions of 3-mm automatic distraction of the tibia with the Ilizarov method and application of achillotomy (experimental study)

E.N. Gorbach, T.A. Stupina, T.N. Varsegova, M.A. Stepanov, E.S. Gorbach

Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation

Introduction Reduction of the period of limb lengthening with the Ilizarov method and preservation of limb functions in large elongations are the tasks to be solved by modern traumatology and orthopedics. The aim was to study the functional state of the limb, dynamics of the tibial regenerate formation, histostructural changes in the articular cartilage and the tibial nerve under the conditions of automatic high-frequency elongation of the tibia with the method of transosseous distraction osteosynthesis at a rate of 3 mm using a preliminary Z-shaped achillotomy. **Material and methods** The 24-hour high-fractional mode provided with automatic distractor was used to lengthen tibiae of 12 adult mongrel dogs with the method of transosseous distraction osteosynthesis. Distraction rate was 3.0 mm per day in 120 steps. To prevent formation of foot equinus, a Z-shaped achillotomy was performed. Methods of light microscopy, morphometry and X-ray electron probe microanalysis were used to study the distraction regeneration in the tibia, the articular cartilage of the medial condyle of the femur and the tibial nerve. **Results** During the periods of distraction and fixation, the regenerate was characterized by a normotrophic structure with a large proportion of bone component which provided the limb support function after 45 days of the experiment. Thirty days after the removal of the apparatus, a newly formed bone of a typical structure was seen in the distraction gap. Achillotomy helped prevent equinus deformity of the foot and flexion contractures of the knee joint and the metatarsal joint. However, histostructural changes in the articular cartilage were detected at the stages of osteosynthesis. Despite the restoration of the thickness of the cartilage, there was a decrease in the number of isogenic groups and the presence of cells with chondoptosis by the end of the experiment. Not a single case of neuropathy of the tibial nerve was revealed histologically due to prevention of overstretching of the anterior surface of the tibia by an increase in the length of the calcaneal tendon with tenotomy. The proportion of destructively altered nerve fibers in all animals did not exceed 5 %. Necrobiotic changes in the epineural vessels were compensated by hypervascularization of the epineurium and endonevria, as a result of which the majority of nerve conductors retained their normal structure, numeric density, and restored dimensional characteristics at the end of the experiment. **Conclusions** The conditions of the experiment provide for functional restoration of the limb, promote active reparative osteogenesis and structural adaptation of the tibial nerve, do not cause any gross destructive changes in the articular cartilage and reduce the period with the Ilizarov frame on by 30 % as compared with the classical variant.

Keywords: achillotomy, transosseous distraction osteosynthesis, automated distractor, reparative osteogenesis, articular cartilage, tibial nerve

INTRODUCTION

The method of transosseous distraction osteosynthesis has been widely used in the management of bone defects, correction of bone deformities, and limb lengthening [1–7]. However, a long hospital stay is uncomfortable for patients and is costly for the health care systems. Therefore, many researches devote their developments to creating conditions that might reduce the period with the Ilizarov fixator on limbs. A more active process of bone formation, adaptation of soft and joint tissues to elongation were achieved with the use of fractional lengthening modes [8–10] with a daily rate of not less than 1 mm [11]. However, there are risks of foot equinus and development of contractures in adjacent joints in lengthening of more than 15 % of the initial leg length with a daily rate of more than 2 mm, despite active osteogenesis and rapid restoration of limb weight-bearing ability [12], [13–18]. It is associated first of all with a different capacity of the antagonist muscles and their tendons to stretching [19, 20].

Another possible complication of lengthening is neuropathy [21–23].

Achillotomy and osteotomy of the calcaneus are used as options for surgical correction of foot equinus [24].

In connection with the above, it seems of great value to study the tissues of adjacent joints and nerves in parallel with the study of conditions for osteogenesis to assess the clinical applicability of new technologies.

The **purpose** of the study was to investigate the functional state of the limb, histostructural features of the tibial regenerate, changes in the tissues of the

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articular cartilage and tibial nerve under the conditions of tibial lengthening using the method of transosseous distraction osteosynthesis with a daily distraction rate of 3 mm and a preliminary Z-shaped achillotomy.

MATERIAL AND METHODS

The experiment was conducted on 12 adult mongrel dogs with a tibia length of 19.5 cm, whose age ranged from one to 3 years. Exclusion criteria were age older than 3 years or less than 1 year old, presence of pathology, pregnancy, i.e. criteria that could affect the result of the experiment and the histological picture of the objects under study.

External fixation device (Ilizarov apparatus) was mounted on dogs' tibiae in the operating room. Flexion osteoclasia was used to break the integrity of the tibia. In order to prevent foot equinus, the calcaneal tendon tenotomy was performed according to the original technology [25]. After 5 days of the latent period, lengthening of the tibia continued 10 days to grow 15 % of its original length in a round-the-clock high-frequency mode, provided by automated power drives and a control unit. The rate of elongation was 3.0 mm per day in 120 steps. One step measured 0.025 mm.

Euthanasia of animals was carried out by an overdose of sodium thiopental after 10 days of distraction, after 30 days of fixation of bone fragments in the device and 1 month after dismantling the device. The experiment was performed in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes.

Sawn sections of the tibial regenerates were fixed in a 10 % solution of neutral formalin, decalcified in a mixture of solutions of hydrochloric and formic acids, dehydrated in ethyl alcohol at alternate concentrations from 70° to 100°. The decalcified material was poured into celloidin and paraffin.

Histological sections were prepared on a sledge microtome (Riechard, Germany) and stained with hematoxylin and eosin according to Van Gieson. Also, staining was performed using an immunohistochemical reaction of polyclonal antibodies to osteopontin (protocol and reagents of Abcam, Germany).

Light-optical histological study of the tibial regenerate was performed using an AxioScope.A1 stereomicroscope and an AxioCam ICc 5 digital camera (Carl Zeiss MicroImaging GmbH, Germany).

Content of Ca and P was determined in various zones of distraction regenerates using an X-ray electron probe microanalyzer INKA Energy 200 (Oxford Instruments Analytical) based on a scanning electron microscope JSM-840 (Japan).

Histomorphometric study of the AC of the femoral condyles was performed on epoxy sections, 1–2 μm thick, stained with methylene blue and basic fuchsin, using an Opton microscope (Germany) and Diamorph hardware-software complex (Russia). The morphometry of digital images of the preparations was studied with software VideoTesT-Master-Morphology. The following parameters were determined: 1) N_{Ach} – numerical density of chondrocytes; 2) Sch, μm^2 – area of cartilage cells; 3) V_{Vch} – volumetric density in the tissue substrate; 4) h, μm – cartilage thickness; 5) $N_{Nis.gr.}$, % – proportion of isogenic groups; $N_{Nem.lac.}$, % – proportion of empty lacunas.

Histological sample preparation of the sites of the tibial nerve excised at the level of distraction bone regenerate was performed according to the previously described method [12]. In the VideoTesT-Master-Morphology, 4.0 image analyzer in the images digitized using an AxioScope.A1 microscope and an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany) we determined: 1) D_{mf} – average diameters of myelinated nerve fibers; 2) D_{ax} – axon diameters; 3) D_{ax} / D_{mf} – coefficient G; 4) L_m is the thickness of the myelin sheath; 4) N_{Amv} – numerical density of endoneural microvessels in 1 mm^2 of the beam area; 5) N_{Amf} – numerical density of myelin nerve fibers; 6) N_{Aamf} – numerical density of non-myelinated nerve fibers; 7) Deg – proportion of destructively altered myelin nerve fibers.

The tibial diaphysis, the articular cartilage of the femoral condyles and the tibial nerve of seven intact dogs were also studied and were a control group. Statistical data analysis was performed with AtteStat software, version 1.0 [26], using nonparametric statistics methods. The Wilcoxon test was used to identify the significance of differences.

RESULTS

Five days after the operation as well as in the fixation period, the animals showed dynamic functioning of the operated limb with intermittent claudication. During the entire period of lengthening, slight swelling of the lower leg tissues was noted. Angles of passive extension in the knee joint were to 140–150° with full flexion of the joint. In the tarsus joint, the range of passive movements was from 40 to 50° (norm, 150°). Mixed

contracture was present. After 30 days of fixation, the weight-bearing function of the operated limb was restored in all dogs. The extension of the knee joint was 150–160° and 120° of the tarsus. There was no case of foot equinus. After a month of non-apparatus period, the functional activity of the limbs was close to that in the controls. Knee extension was 165° and 130° in the tarsus joint. Limb was physiologically aligned. No clinical signs of neurogenic disorders were observed.

Histological methods revealed that bands of bone tissue (bony sections), 9.5–12 mm long, represented by a reticulofibroous tissue of the trabecular bone extended in the longitudinal direction from the bone fragments deep into the gap after 10 days of the distraction (**Fig. 1, a**) .

Loose fibrous connective tissue with foci of hematopoiesis and numerous thin-walled capillaries with enlarged lumens was seen between the newly formed bone trabeculae by that period.

Between the bony portions, vascularized and longitudinally oriented fibrous connective tissue was visualized in the structures of which there were numerous bands of newly formed bone trabeculae.

In the zone of the connective tissue layer and the bony portions, cells with osteopontin expression were revealed that indicated an active replenishment of osteogenic cells in these areas. Such cells were located predominantly perivascular, but were also found on the surface and in the trabeculae of reticulofibrous structure (**Fig. 1, d**).

After 30 days of fixation of the limbs in the apparatus, the regenerate in the interfragmental area was represented by trabecular bone of medium-sized reticular cells, which was undergoing compaction from the side of the periosteum (**Fig. 1, b**). In the endosteal region, active osteoclastic resorption of bone trabeculae was observed. Formation of hematopoietic and fatty bone marrow was noted in the intertrabecularspaces of the proximal and distal parts of tibial regenerate while it was predominantly hematopoietic in the middle part.

After a month of non-apparatus period, the newly formed part of the tibia was similar in its structure to the intact bone (**Fig. 1, c**).

A continuous compact layer consisting of lamellar bone tissue and a continuous bone marrow cavity containing hematopoietic-adipose bone marrow with single bone trabeculae were formed by that period.

In the post-distraction period, cells with osteopontin expression were detected in the cambial layer of the periosteum, on the surface of bone trabeculae (**Fig. 1, e**), as well as on the surface of osteon canals and in the bonding lines.

According to X-ray electron probe microanalysis, the content of Ca after 10 days of distraction (end of the lengthening period) was generally low in the zones of the regenerate in the distraction gap – from 2.2 to 7 % by weight (Table 1). The lowest values were detected in the area of the connective tissue layer. The studied parameters were lower than in the intact dogs: in the zone of the proximal bone part – 3.5 times lower ($p = 0.0089$); in the area of the connective tissue layer – 10.5 times lower ($p = 0.0026$), in the distal bone part – 3 times ($p = 0.0017$) lower.

By the end of the fixation period, zonal changes were minor. Compared to the distraction period, the content of Ca in the proximal and distal bone parts increased by 2.63 and 2.32 times, respectively ($p = 0.0082$ and $p = 0.0069$), and in the area of the connective tissue layer became bigger by 5.95 times ($p = 0.0038$).

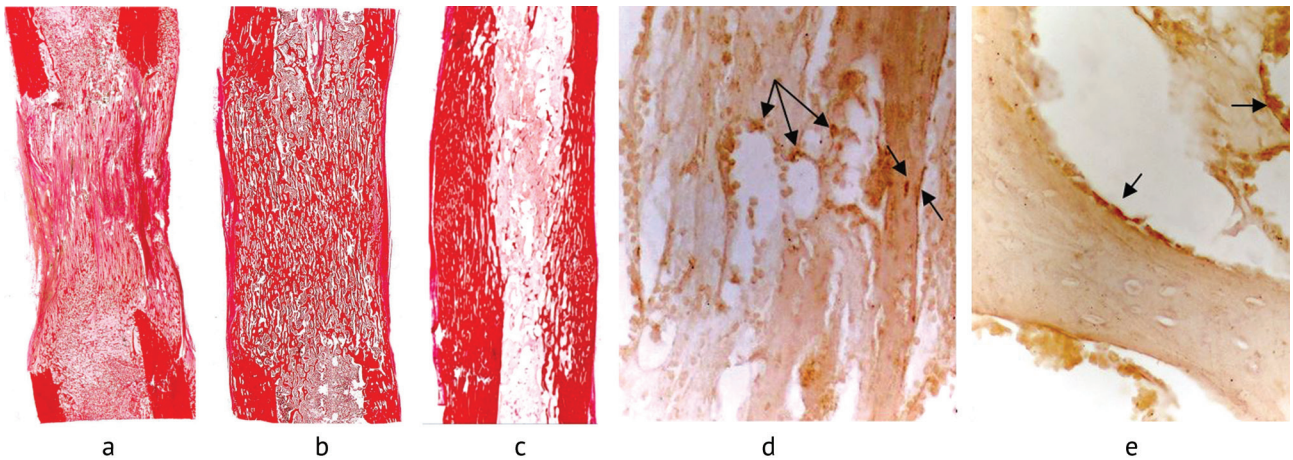


Fig. 1 Histotopograms of longitudinal sawn section of the tibial diaphysis: **a** – after 10 days of distraction; **b** – after 30 days of fixation; **c** – 30 days after apparatus removal. Van Gieson staining; magnification 1.5 ×; **d** – expression of osteopontin after 10 days of distraction (arrows); **e** – expression of osteopontin after 30 days of fixation (arrows). Immunohistochemical staining; magnification 600 ×

Table 1

Ca content in tibial regenerate zones at stages of lengthening

Experiment stage	ω_{Ca}, % (M ± m) in regenerate zones		
	1	2	3
Distraction, 10 days	6.0 1 ± 0.12*	2.2 ± 0.78*	7 ± 0.27*
Fixation, 30 days	15.8 ± 0.59*	13.1 ± 0.54*	16.3 ± 0.79*
After frame removal, 30 days	21.2 ± 0.93**	18.9 ± 0.81**	20.6 ± 0.96**
Intact dogs	21 ± 0.95		

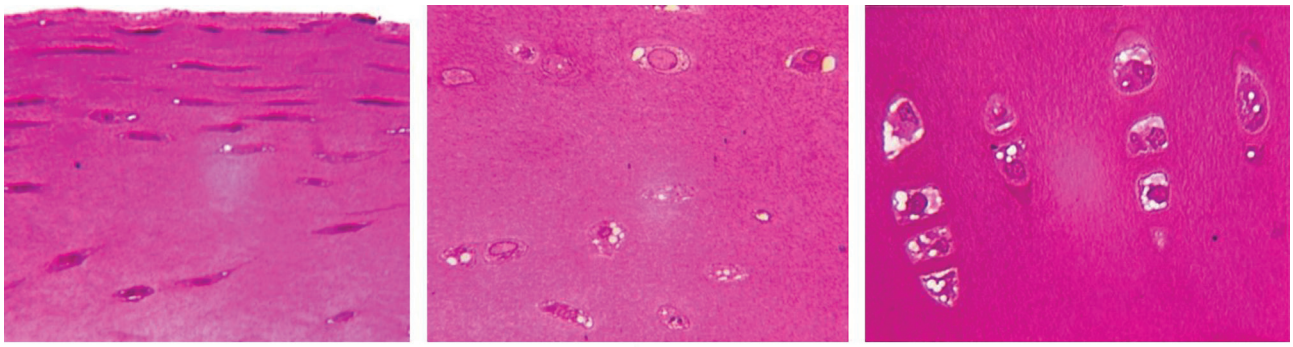
* – at $p < 0.01$ compared with intact animals; ** – at $p \geqslant 0.05$ compared with intact animals. Regenerate zones: 1 – proximal bone part; 2 – connective tissue layer; 3 – distal bone part

One month after the removal of the device, no reliable zonal changes in the newly formed part of the diaphysis were detected. In the compact plate, in the projection of the formation (at the previous stages of the experiment) of the proximal bone part, the connective tissue layer and the distal bone part, the content of Ca increased on average by 1.3–1.4 times ($p = 0.035$, $p = 0.021$, $p = 0.039$). Compared with the intact dogs, no significant difference was found in all the studied areas of the newly formed part of the diaphysis ($p = 0.092$, $p = 0.059$, $p = 0.064$).

Histological study of the articular cartilage showed that its zonal structure was preserved at all stages of the experiment. However, some changes in the qualitative and quantitative characteristics of its structural components were identified. So, by the end of the elongation period (10 days), the structure of the superficial zone was disordered, and there was defibration of the intercellular substance in the superficial part (**Fig. 2, a**). In all zones of the cartilage, chondrocytes with vacuolated cytoplasm were present, empty lacunae were found, few isogenic groups were noted (**Fig. 2, b, c**). In the deep zone of the cartilage, the portion of cell-free fields increased. The histomorphometric method detected a significant decrease in the cartilage thickness ($p = 2.34E^{-06}$) relative to the intact animals, a decrease in the volumetric density of chondrocytes ($p = 1.15E^{-06}$) due to a decrease in their area ($p = 1.23E^{-07}$), and also a decrease in the portion of isogenic groups (Table 2). Due to an increase in the number of cells on the surface zone, the total numeric density of chondrocytes exceeded the control values ($p = 0.00011$), while the portion of empty lacunae was comparable to those in intact animals (Table 2).

After 30 days of fixation in the apparatus, chondrocytes with signs of "chondroptosis" were detected in all zones of the cartilage. Such cells were hypertrophic and had vacuolated cytoplasm. Relative to the previous period of the experiment and to intact animals, the portion of empty lacunae significantly increased (Table 2); the isogenic groups were solitary, and the chondrocytes in them showed signs of destruction. However, compared with the end of the distraction period, there was a tendency to an increase in the thickness of the cartilage and the area of chondrocytes; a decrease in the numeric density of cells was noted which remained significantly increased as compared with the control values ($p = 0.024$). The indices of volumetric density of chondrocytes ($p = 5.62E^{-07}$) remained significantly reduced.

A month after the removal of the apparatus, defibration remained in the upper superficial zone (**Fig. 3, a**). Compared with the previous period, the fraction of empty lacunae significantly decreased. A tendency to an increase in the thickness of cartilage was revealed (Table 2). The volumetric density of chondrocytes ($p = 1.93E^{-07}$), the cell area ($p = 6.14E^{-07}$), and the portion of isogenic groups remained low. At the same time, the values of numeric density remained significantly higher than in the control ($p = 0.037$).



a b c

Fig. 2 Articular cartilage of the femoral condyles 10 after the start of distraction. Semi-thin sections; staining with methylene blue-basic fuchsin. Lens – 40; eyepiece – 12.5×. Cartilage zones: **a** – superficial; **b** – intermediate; **c** – deep

Table 2

Morphometric characteristics of the articular cartilage of the lateral condyle of the femur at the organ and tissue levels at the stages of the experiment

Parameters	VV_{ch} (%, M ± m)	NA_{ch} (M ± m)	S_{ch} (mcm^2, M ± m)	h (mcm, M ± m)	$NN_{em.\ lac.}$ (%)	$NN_{is.gr}$ (%)
Intact dogs	9.03 ± 1.55	6.12 ± 0.64	87.51 ± 3.79	475.55 ± 1.31	13.6	14.5
Distraction, 10 days	4.01 ± 0.33*	9.49 ± 0.66*	45.25 ± 2.69*	375.99 ± 3.81**	14.55	3.9
Fixation, 30 days	3.89 ± 0.25*	7.67 ± 0.75*	62.98 ± 3.45*	420.99 ± 3.26**	32.8	3.88
Without apparatus, 30 days	3.13 ± 0.26*	6.67 ± 0.45*	48.06 ± 3.38*	431.94 ± 7.26**	18.1	3.85

Note: the Wilcoxon test was used for reliability of differences with the controls for VV_{ch}, NA_{ch}; for h – Student's test. The differences are significant: ** – at $p < 0.001$, * – at $p < 0.05$.

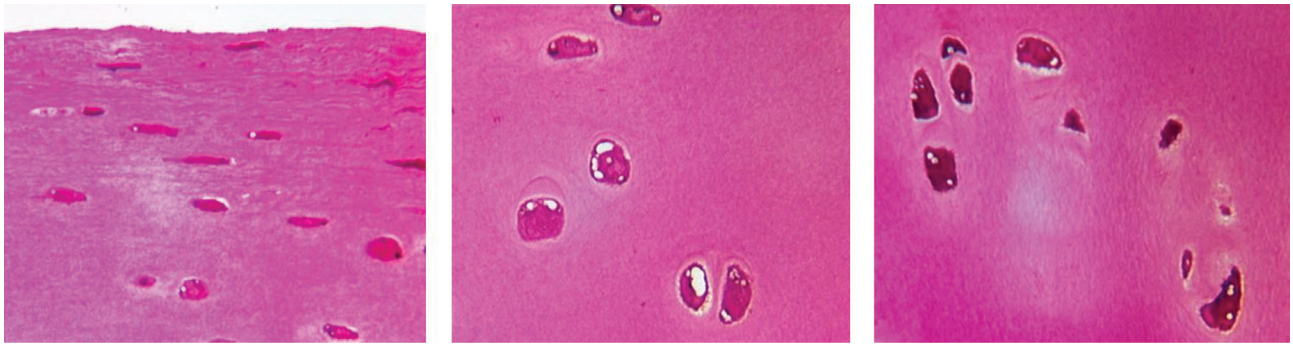


Fig. 3 Articular cartilage of the femoral condyles 30 days after removal of the apparatus. Semi-thin sections; staining with methylene blue-basic fuchsin. Lens – 40; eyepiece – 12.5×. Cartilage zones: **a** – superficial; **b** – intermediate zone; **c** – deep

Functionally active chondrocytes, which had bright homogeneous nuclei and basophilic cytoplasm, prevailed in the superficial zone. In the intermediate and deep zones, the cytoarchitecture of the deep zone was disturbed – there was no column arrangement of cells (**Fig. 3, b, c**).

Integrity of the basophilic line was maintained in all periods of the experiment. In some areas, its separation, thinning, partial fragmentation and/or absence of calcified cartilage in some areas were seen.

The tibial nerves retained the continuity, integrity of the membranes and normal relationships with surrounding organs.

The morphological study showed that the epineurium of the tibial nerve under lengthening contained an increased number of microvessels during all periods of this experiment. Fuchsinophilic collagen fibers, fibroblasts and fibrocytes, mast and perivascular, plasma and macrophage cells were detected in it. There was some vasodilatation of a number of vessels.

Perineurium was continuous and had a thin lamellar structure. The layers of loose fibrous connective tissue between the cell layers of the perineurium were thickened; the number of cell nuclei increased. Throughout the experiment, extensive subperineral and some endoneural edemas were detected in single large and small nerve bundles. At the same time, most of the endoneurium vessels did not differ in structure from the norm. Hypervascularization of endoneurium was found at all periods of the experiment. The numeric density of arterioles, venules and capillaries was increased (Table 3) relative to the intact animals after completion of the distraction, fixation stages and at the end of the experiment by 33 % ($p = 0.0057$), 71 % ($p = 0.0012$) and 46 % ($p = 0.0024$), respectively.

Histological examination of the conductive portion of the tibial nerve showed no signs of neuropathy in any animal, although some of the myelinated nerve fibers showed signs of demyelination and axonal degeneration.

The portion of destructively changed conductors was maximum (Table 3) after the end of distraction (exceeded the norm by 3 times, $p = 1.22E^{-08}$); at the end of fixation and a month after removing the device it decreased gradually, but exceeded ($p = 3.01E^{-08}$) the norm two times. NA_{mf} increased at the end of the distraction stage (Table 3) by 9 % ($p = 0.0300$) relative to the norm, and NA_{amf} increased by 42 % ($p = 0.0024$) at the end of the fixation, which was accompanied by an increase in the NA_{amf} / NA_{mf} ratio. In the remaining periods of the experiment, the numerical density of the

fibers did not differ from the values of the intact nerve.

Study of the dynamics of the dimensional characteristics of myelin nerve fibers showed (Table 3) that after 10 days of distraction with an increased daily rate, the average fiber diameter decreased by 6.4 % (p = 0.0421) due to pronounced axonal atrophy (axon diameter decreased by 12, 3 %, G coefficient by 5.8 % (p = 0.0410) with an insignificant increase in the average thickness of the myelin layer by 3.8 % due to the stratification of myelin lamellae. After 30 days of fixation, the mean diameter of myelin fibers did not change, myelin thickness and G were normal; average diameter of axons increased relative to the previous period, but remained lower than the norm by 8.2 % (p = 0.0420). All the dimensional characteristics of myelinated fibers recovered at the end of experiment.

Table 3

Values of quantative features of tibial nerve fibers

Parameter	Intact nerve	Distraction, 10 days	Fixation, 30 days	30 days after frame removal
NA_{mf} per 1 mm^2	19426 ± 1717	21099 ± 1054*	20141 ± 505	18787 ± 1060
NA_{amf} per 1mm^2	10347 ± 1921	10569 ± 1674	14697 ± 799*	9893 ± 376
NA_{amf} / NA_{mf}	0.5 ± 0.04	0.5 ± 0.09	0.8 ± 0.09*	0.5 ± 0.03
Deg (%)	1.64 ± 0.54 %	4.57 ± 0.47 %*	3.81 ± 0.47 %*	3.04 ± 0.18 %*
NA_{mv} per 1 mm^2	182.29 ± 6.36	243.33 ± 46.31*	312.33 ± 37.26*	266.67 ± 48.07*
D_{mf} (mcm)	6.75 ± 0.01	6.32 ± 0.06*	6.35 ± 0.23*	6.82 ± 0.09
D_{ax} (mcm)	4.63 ± 0.13	4.06 ± 0.15*	4.25 ± 0.19*	4.63 ± 0.04
L_m (mcm)	1.06 ± 0.02	1.13 ± 0.06	1.05 ± 0.05	1.10 ± 0.05
G	0.69 ± 0.01	0.65 ± 0.02*	0.68 ± 0.01	0.69 ± 0.01

* – differences between the values of the dimensional characteristics of the experimental and intact nerves are significant according to Wilcoxon test for independent samples at $p < 0.05$.

DISCUSSION

The results of this study demonstrated the possibility of the formation of a new part of the diaphysis able to weight bearing after 45 days of the experiment, as evidenced by the absence of refractures after the removal of the apparatus in 100 % of cases. The same results were obtained by us in lengthening of the tibia with a rate of 3 mm for 180 steps and 3 mm for 120 steps without the use of achillotomy [12, 27]. However, in this study, active osteogenesis was observed at all stages of osteosynthesis. Compared with the previously obtained results [27], during the period of distraction and during the fixation period, the regenerate differed in a normotrophic structure with a greater portion of bone component, which contributed to an earlier completion of organotypic restructuring and the formation of the new bone of a more typical structure 30 days after the apparatus was removed. It was confirmed by greater mineralization of the compact plate and the filling of the medullary canal mainly with fatty bone marrow. We associate the obtained effect with an improvement in the blood supply of the bone formed, both from the periosteum and soft tissue components, and from the intraosseous vascular bed, as a result of the prevention of overstretching of the tissues of the anterior surface of the tibia by increasing the length of the calcaneal tendon with Z-shaped tenotomy.

In this experiment with the elongation of the tibia with a rate of 3 mm in a highly fractional mode, the use of achillotomy significantly improved the functional state of the limb when compared to the elongation in the same mode without it. Z-shaped achillotomy was effective in preventing the formation of foot equinus and flexion contractures of the knee and tarsal joints. However, at the stages of osteosynthesis, we identified histostructural changes in the articular cartilage, which, according to the histological classification of the International Society for the Study of Osteoarthrosis OARSI (2006), can be referred to early signs of osteoarthritis [28].

Under the conditions of the present experiment, the integrity of the basophilic line was preserved; defibration of the surperficial zone was less pronounced as compared with the elongation in the conditions we had studied earlier with a 2-mm rate in manual mode and an automated distraction of 3 mm in 180 steps [12].

In the conditions of a similar automated distraction with a rate of 3 mm for 120 steps but without the use of achillotomy, the thickness of the cartilage was

more reduced at the stages of fixation and one month after removal of the apparatus, while the values of volumetric density and of area of chondrocytes, on the contrary, increased when compared with the results of this experiment [29].

Despite better preservation of the superficial zone by the end of the period of distraction, viability of the intermediate and deep zone chondrocytes was distorted and there was a decrease in the number of isogenic groups during the period of fixation. At the same time, the total numeric density of chondrocytes remained high relative to the intact value, and the number of empty lacunae had a decreased dynamics.

In general, the function of the joints (angles of flexion and extension) in this experiment was comparable to that when the mode of automatic lengthening of the tibia with a daily rate of 1 mm in 60 steps had been used [30]. However, the apparatus period in this experiment was reduced by 20 days due to high lengthening rate, what may be the cause of incomplete restoration of the articular cartilage histostructure.

With the use of this lengthening technique, not a single case of tibial nerve neuropathy was recorded histologically: the portion of destructively altered nerve fibers did not exceed 5 % in all animals as was the case with elongation in the same mode without achillotomy performance. Necrobiotic changes in epineural vessels were compensated by hypervascularization of the epineurium and endoneurium, as a result of which the majority of nerve conductors retained their normal structure, numerical density, and restored their dimensional characteristics by the end of the experiment. Thus, the automated distraction mode of "3 mm per day in 120 steps" with the use of achillotomy turned out to be relatively good for the tibial nerve.

CONCLUSION

Thus, automated lengthening of the tibia with a high daily rate of 3 mm for 120 steps after Z-shaped achillotomy reduces the period with the frame on by 30–31 % compared with the classical variant, prevents foot equinus, minimizes flexion contracture of the knee and tarsal joints. The conditions created are more optimal for functional limb recovery, reparative osteogenesis and structural adaptation of the tibial nerve. Gross destructive changes in the articular cartilage did not develop during the experimental period (75 days). However, despite the restoration of cartilage thickness, the reduction in the number of isogenic groups and the presence of cells with signs of chondroptosis by the end of the experiment should be taken into account, and therefore it is advisable to apply therapeutic measures to prevent osteoarthritis.

Conflict of interest: not declared

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Information about the authors:

1. Elena N. Gorbach, Ph.D. of Biological Sciences,
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation,
Email: gorbach.e@mail.ru
2. Tatyana A. Stupina, Ph.D. of Biological Sciences,
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation,
Email: StupinaSTA@mail.ru
3. Tatyana N. Varsegova, Ph.D. of Biological Sciences,
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation,
Email: varstn@mail.ru
4. Mikhail A. Stepanov, Ph.D. of Veterinary Sciences,
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation,
Email: m-stepanov@mail.ru
5. Evgenii S. Gorbach,
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russian Federation,
Email: gorbach.evg@mail.ru