Original Article
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Morphological Changes in the Tibial Nerve During the Treatment of Large Tibia Defects Using Ilizarov Apparatus Combining with the Masquelet Technique: Experimental Study

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Abstract

Background. The use of Masquelet technique in combination with non-free osteoplasty according to Ilizarov in order to compensate large defects of the lower leg bones provides proper bone union and recurrence-free course of the disease, but the problem of patient rehabilitation remains relevant. The course and duration of the recovery period depend on the morphofunctional state of the tibial nerves. ***The purpose*** of this study was to determine morphological changes in the tibia nerve of dogs during the experimental treatment of large tibial defects using Ilizarov apparatus combining with the Masquelet technique. ***Materials and Methods.*** A defect of the upper third of the tibia in the form of false joint was created in 10 mongrel dogs. Then this defect was replaced with 25 mm diastasis, into which a cement spacer was placed. After 30 days, the latter was removed. At the level of the lower third, transverse osteotomy was performed, as well as distraction of 1 mm rate for 4 times per day, until complete contact of the fragments in the defect zone. The tibial nerves were studied within the periods of 60 days of fixation (F60) and 30 days after the fixator removal (FR30). ***Results.*** There were no mechanical damages of the nerves. During the experiment a part of epineural veins and arteries had obliterated lumens, two-fold decrease in the numerical density of endoneural arterioles, venules and capillaries was observed in FR30 — 97.5±2.5 in 1 mm^2 (normally — 182.0±22.0), that evidenced of microcirculatory disorders progressing towards the end of the experiment. Patterns of Wallerian degeneration were found along with typical damages to fibers during distraction osteosynthesis — demyelination and axonal degeneration. In F60 the proportion of modified fibers was 7.7±1.5%, which was 4.8 times higher ($p = 0.52 \times 10^{-5}$) above normal, the numerical densities and dimensional characteristics of fibers decreased. At the end of the experiment, the proportion of modified conductors 2.3-fold exceeded the norm ($p = 0.33 \times 10^{-4}$) — 3.7±0.4%, the numerical density of fibers remained 10.2% below the norm ($p = 0.0362$), making up 17436±865, but the average axon diameter and thickness of myelin sheaths were restored. ***Conclusion.*** Microcirculatory dysfunction, axonal atrophy, demyelination, Wallerian degeneration of a part of myelin nerve fibers and a decrease in their number revealed in the tibial nerves during the treatment of large tibia defects using the Masquelet technique combining with Ilizarov non-free bone grafting indicated the need for the use of adequate neurometabolic pharmacotherapy and effective rehabilitation.

Keywords: tibia defect, Masquelet technique, tibial nerve, histomorphometry.

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Introduction

Replacement of large bone defects caused by trauma, osteomyelitis, tumor resection, or congenital false joint is a fundamental problem for orthopaedic and plastic surgeons [1, 2, 3, 4, 5, 6, 7, 8]. The femur and lower leg bones are most often affected by this pathology [5, 6, 9, 10]. The critical size of bone defects, exceeding the internal regenerative potentials of the bone, leads to disruption and delay in its restoration [8].

To solve this problem, various surgical technologies have been developed, the most commonly used of which are methods using a free vascularized graft, the Ilizarov method and the Masquelet method [11, 12, 13, 14, 15, 16, 17, 18].

The Ilizarov method in replacing large diaphyseal defects has no alternative, since the dosed dislocation of a fragment together with soft tissues and the available blood supply is very biological, but its only drawback is long-term treatment (the period of distraction lasts 3-4 months) [19].

The Masquelet method has number of advantages: shorter treatment times, high healing rates; it is positioned as the most technically simple, reliable and reproducible [4, 18, 20, 21, 22]. It is based on a two-stage surgical procedure. The first stage includes a total excision of the altered bone and placing a cement spacer to aid in the formation of an "induced membrane". At the second stage, the spacer is removed, and the resulting cavity is filled with bone autograft [3, 6, 23, 24, 25, 26, 27].

Despite the indisputable success in reconstructive surgery of large diaphyseal bone defects (up to 20 cm long) using the Masquelet method [6, 28], orthopaedic surgeons state high risks of the process recurrence and a rather high frequency of complications, averaging about 18% [23], fluctuating , according to different authors, from 9% to 59% [7, 23, 29, 30]. The main complications when using the Masquelet method are non-union of bone fragments, lysis and fractures of the bone graft, the development of wound infection, mismatch in the limbs length, joint stiffness, infection recurrency and amputations [5, 7, 28, 29, 30, 31].

The presence of complications and the recurrent nature of the process prompts clinicians to search for new technological solutions based on a combination of surgical techniques and approaches, the use of combined options for external and internal osteosynthesis [32, 33]. The use of a combined osteoplastic technique, including the formation of the "induced membrane" according to the Masquelet technique and vascularized bone grafting according to Ilizarov, made it possible to achieve complete bone fusion of the lower leg congenital pseudarthrosis and recurrence-free course of the disease [33]. Nevertheless, after large defects replacing in the lower leg bones by various techniques and walking resumption, patients need 2-3 years to achieve stable functional result [9]. Complaints of pain after reconstructive surgery of the tibia were reported in 21% of patients, and lack of sensitivity – in 12% [34]. The pathogenesis of emerging neurological disorders in bone injuries due to the multifactorial nature of the peripheral nerves lesions is considered rather complex and poorly studied [35]. There are no experimental studies with morphological changes analysis in the tibial nerves during the replacement of the lower leg bones large defects in the available literature, which determined the aim of this study.

The aim of this study was to evaluate morphological changes in the tibial nerve of dogs during the replacement of large defects of the tibia with the Ilizarov apparatus in combination with the Masquelet method in the experiment.

Materials and Methods

Surgical technique

Three surgical procedures were performed on 10 mongrel dogs (weight 16.8 ± 0.4 kg; age

3.9 ± 0.3 years; tibia length 17.43 ± 0.68 cm). The first stage, a defect-false joint (RF patent No. 2539627) was performed at the level of the proximal third of the tibia using the Ilizarov method. At the second stage, a 25 mm of the false joint zone was resected, which was 15% of the initial length of the tibia. In the formed defect-diastasis, a cement spacer was placed, which was removed after 30 days of fixation. At the third stage, a transverse osteotomy of the tibia was performed in the lower third of the leg, and 7 days later, a longitudinal transfer of the intermediate bone fragment in the proximal direction was performed for 25–28 days at a daily rate of 1 mm in 4 steps until contact of bone fragments at the level of diastasis was achieved. After 60 days, the apparatus was removed. The animals were sacrificed after 60 days of fixation (n = 5; F60) and 30 days after removing the apparatus (n = 5; BA30).

Technique of morphological research

Fragments of the tibial nerves excised at the level of the lower leg middle third were fixed for 7 days in a mixture of glutaraldehyde and paraformaldehyde with the addition of picric acid, then additionally fixed in a 1% solution of osmium (IV) oxide and embedded in epoxy resins. One-micron semithin sections were stained with toluidine blue and polychrome methods: methylene blue, azure II, and basic fuchsin. Full-color images of nerves histological specimens were obtained using an AxioCam digital camera and AxioScope.A1 stereomicroscope (Carl Zeiss MicroImaging GmbH, Germany). The proportion (%) of reactive-destructively altered myelinated nerve fibers and their numerical density in 1 mm^2 was calculated, the numerical density of endoneural microvessels (arterioles, venules and capillaries) in 1 mm^2 of the bundle area. In the program "VideoTesT Master-Morphology 4.0", the average diameters of myelin fibers (Dmf), their axons (Dax), and the thickness of the myelin sheath (Lm) were measured. The coefficient G (Dax / Dmf) was calculated. Histograms of fiber diameter distribution (step -1 μm) was formed. Control – tibial nerves of 5 intact dogs.

Research ethics

The maintenance and care of animals was regulated by: SP 2.2.1.3218-14 "Sanitary and Epidemiological Requirements for the Design, Equipment and Maintenance of Experimental Biological Clinics (Vivariums)"; GOST 33215-2014 "Guidelines for the maintenance and care of laboratory animals. Rules for laboratory equipping and organizing procedures"; GOST 33217-2014 "Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory predatory mammals. The experiment complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and Directive 2010/63 / EU of the European Parliament and the European Union Council on the protection of animals used for scientific purposes. The permission of the ethical committee of the institution was obtained (Protocol No. 2/57, 17.05.2018).

Statistical analysis

The arithmetic mean of the indicators (M) and the standard error of the mean (m) were calculated. The statistical significance of the differences was determined using the nonparametric U-test using the AtteStat software, version 9.3.1 (Russia). At $p < 0.05$, the differences were considered statistically significant.

Results

Clinical observation

At the stage of defect-false joint modeling in the first two days, swelling of the soft tissues of the limb from the tarsal joint to the mid-thigh with moderate pain developed, there was a supporting type lameness - the animals spared the limb, stepping on the fingertips. On the 5-7th day after the surgery, the edema disappeared, the support ability of the limb improved, but there was a gradual atrophy development of the thigh muscles.

At the second stage of the experiment, after the fragments edges resection and placement of the spacer, the postoperative tissue edema was located on the medial surface around the surgical site. There were no significant changes in limb function.

At the stage of removing the spacer and performing vascularized bone grafting, the support function was low; while walking, the animal kept the limb suspended.

At the stage of fixation, the support ability of the limb improved - the animals used the limb more confidently, and after the apparatus removing, it gradually recovered. By the end of the experiment, a slight disturbance in gait was observed.

Morphological study

When dissecting the tibial nerves in the studied time frame, their adhesion to the surrounding tissues was not found, hematomas, mechanical damage, and discontinuity were absent. The nerve trunks retained an even thickness, a natural pearl-white color, but had a more pronounced superficial vascular pattern.

Microscopic examination of the nerves connective tissue sheaths showed that in F60 and BA30 the epineurium retained its integrity, the number of fibroblastic, mast and perivascular cells increased in comparison with the intact nerve, and plasmacytic cells and macrophages appeared. The walls of the arteries and veins of the epineurium are hypertrophied, the lumens of the majority are dilated, filled with blood cells, and in some of the vessels they are narrowed or obliterated. Perineurium in F60 and BA30 in all fascicules retained a fine lamellar structure, signs of damage, perineural and subperineural edema were absent (Fig. 1).

Numerical density of endoneural arterioles, venules and capillaries in 1 mm2 of the bundle area in F60 increased ($p = 0.0425$) to 211.0 ± 11.0 (in the intact nerve 182.0 ± 22.0), in BA30 decreased 2.2 times compared to the previous period and was below normal ($p = 0,0061$) 1.9 times – 97.5 ± 2.5.

Most of the myelin fibers retained their normal structure. Some of the conductors showed signs of axonal, Wallerian degeneration and demyelination. In F30, axonal atrophy prevailed, and in BA30, a significant part of the large-diameter conductors had thickened myelin sheaths with signs of stratification and uneven coloration. There were isolated small diameter myelinating axons and single regeneration clusters.

The proportion of altered conductors in F60 was 7.7 ± 1.5%, which is 4.8 times higher

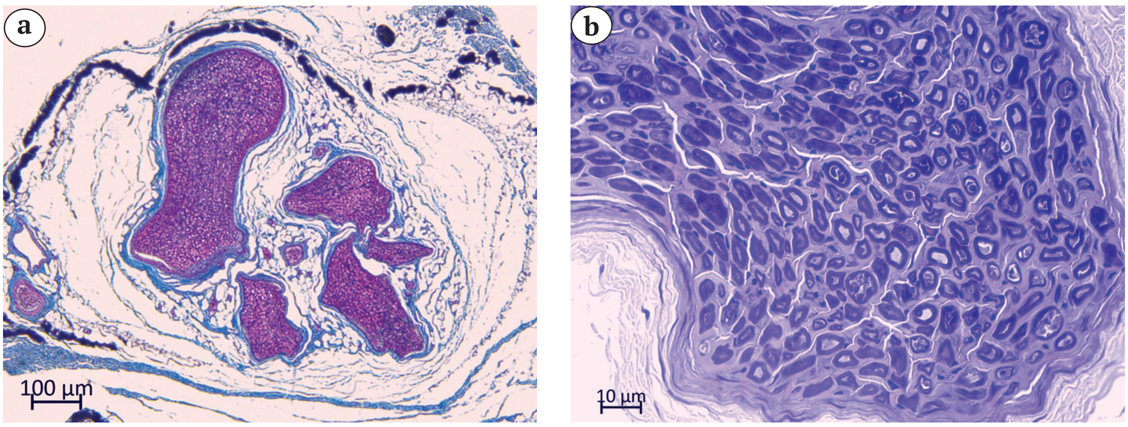

Figure 1. The dog's tibial nerve after 60 days of fixation. Semi-thin slides:
a — total cross section, polychrome staining with methylene blue, azure II and basic fuchsin, ×40;
b — fragment of a nerve fiber bundle, ×400

(p = 0.52 × 10-5) than the values of the intact nerve (1.6 ± 0.2%), in BA30 it decreased up to 3.7 ± 0.4%, but exceeded the norm (p = 0.33 × 10-4) 2.3 times. At the same time, the number density of myelin fibers in F60 was reduced by 6.4% (p = 0.0470) and amounted to 18189 ± 89 (in the intact nerve 19426 ± 649), in BA30 it decreased to 17436 ± 865, remaining below the norm by 10, 2% (p = 0.0362).

The study of the dimensional characteristics of myelin fibers showed a decrease in F60 of all indicators: Dmf by 19% (p = 0.0241), Dax by 22% (p = 0.0213) and Lm by 14% (p <0.0364), which indicates the predominance of axonal atrophy and the presence of demyelination processes. By the end of the experiment, all the dimensional characteristics of myelin fibers were restored, slightly exceeding the norm (Table 1).

In F60 and BA30, the histograms of the myelin fibers distribution by diameter retained a bimodal character (Fig. 2).

Their bases exceeded the base of the intact nerve by two discharges due to the appearance of fibers with diameter less than 2 μm, indicating the presence of regenerative processes at the given time of the experiment, as well as the appearance of 1% hypertrophied fibers with a diameter of 13.1-14.0 μm. In both F60 and BA30, the left peak of the histogram exceeded the right one due to a change in the population composition of nerve conductors: the proportion of small (D≤4.0 μm) increased to 28% and 24% (normal 22%), the proportion of medium fibers (D 4.1-7.0 μm) was 29% and 30% (normal 27%), the proportion of large fibers (D> 7.0 μm) decreased to 43% and 46 % (normal 51%), respectively.

Table 1

Dimensional characteristics of the myelin fibers of the tibial nerves (m±σ) after 60 days of fixation (F60) and 30 days without the apparatus (BA30)

Duration of the experiment	Dmf, μm	Dax, μm	Lm, μm	G (Dax/Dmf)
F60	5,46±0,76*	3,63±0,51*	0,91±0,12*	0,672±0,020
BA30	6,96±0,28	4,75±0,29	1,10±0,01	0,692±0,014
Intact nerve	6,75±0,28	4,63±0,33	1,06±0,05	0,686±0,013

* — the differences are statistically significant according to the U-test for independent samples at p<0.05.

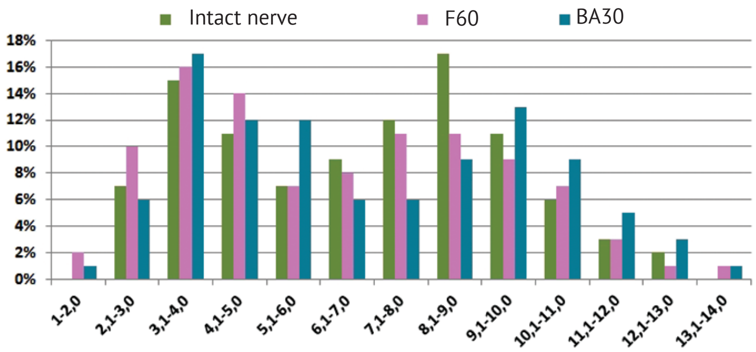

Figure 2. Tibial nerves myelin fibers distribution by diameter in intact and experimental animals after 60 days of fixation and 30 days after removing the apparatus. The abscissa is the size classes of fibers (μm), the ordinate is the proportion of fibers of each class (%)

Discussion

The problem of patients rehabilitation after the large defects of the lower leg bones replacement remains relevant, since in order to achieve a good functional result after resuming walking, according to A.C. Masquelet et al, takes several years [9]. The course and duration of the recovery period depends on the morphofunctional state of the tibial nerves. The use of a combined osteoplastic technique, including the formation of the "induced membrane" according to the Masquelet technique and vascularized bone grafting according to Ilizarov, showed good results – complete bone fusion and recurrence-free course of the disease [33].

An experimental study has shown that the replacement of large defects in the lower leg bones of dogs with this combined technique is accompanied by a closed injury of the tibial nerves with damage to the myelin sheaths, nerve fiber axons and subsequent Wallerian degeneration without affecting the nerve sheaths, which, according to the pathohistological classification of nerve injuries [35, 36], corresponds to neuropraxia and axonotmesis.

Throughout the experiment, the trunks of the tibial nerves and their connective tissue membranes remained intact, there were no signs of mechanical damage. In all animals, at all periods of the experiment, some of the epineural veins and arteries had closed or obliterated lumens, which, along with a twofold decrease in the number density of endoneural microvessels 30 days after the removal of the apparatus (175-178 days of the experiment) indicates the presence of microcirculatory disorders progressing towards the end of the experiment.

Most of myelinated nerve fibers (over 90%) retained their normal structure. Along with the typical damage to myelinated nerve fibers - demyelination and axonal degeneration, previously identified in distraction osteosynthesis of the limb [37], patterns of Wallerian degeneration of the conductors were found. After 60 days of fixation of the lower leg in the apparatus, reactive-destructive changes covered 8% of nerve conductors (4 times higher), a decrease in the number and dimensional characteristics of myelin fibers was revealed due to axonal atrophy and demyelination of some of them. At the end of the experiment, due to endoneural hypovascularization, the proportion of altered conductors exceeded the norm by 2 times, but the average diameter of axons and the thickness of myelin sheaths were restored and even slightly exceeded the control. It should be noted that in none of the animals in the tibial nerves the classical signs of traumatic neuropathies described in the literature were found – concentric structures called "onion-bulb" [38, 39].

Conclusion

Microcirculatory disorders, axonal atrophy, demyelination, Wallerian degeneration of a part of myelinated nerve fibers and a decrease in their number during the large defects of the lower leg bones replacement using the Masquelet method revealed in the tibial nerves in combination with vascularized bone grafting according to Ilizarov indicate the need for adequate neurometabolic pharmacotherapy and effective methods of rehabilitation for this category of patients.

Ethics approval

The permission of the ethical committee of the institution was obtained (Protocol No. 2/57, 17.05.2018).

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