DOI 10.1007/s10517-016-3401-7

MORPHOLOGY AND PATHOLORPHOLOGY

Morphofunctional Changes in the Tibial and Peroneal Nerves in Shin Lengthening Using High Speed Daily Distraction Technique

T. N. Varsegova, N. A. Shchudlo, M. M. Shchudlo, M. S. Saifutdinov, and M. A. Stepanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 161, No. 2, pp. 270-273, February, 2016
Original article submitted October 8, 2014

A sustained decrease in the M-response amplitudes of *m. gastrocnemius* and *m. tibialis anterior* was revealed during experimental orthopedic shin lengthening in dogs using Ilizarov external fixator with an automated drive (distraction 3 mm/day in 120 steps). Transverse contraction of intrafascicular content (by 13.2%), endoneural hypervascularization (by 28-95%), axonal degeneration and myelin decompactization, and destructive changes of no more than 5% fibers were detected in the tibial nerve. In the peroneal nerve, the contraction reached 17.3%. Endoneurium hypovascularization (by 12%), axonal degeneration and demyelinization, and destructive changes of more than 20% fibers were detected in 6 of 9 experiments.

Key Words: *shin lengthening; tibial, peroneal nerves*

Distraction osteosynthesis is widely used in orthopedics for repair of bone defects and for correction of shortened and deformed limbs. The daily rate of distraction optimal for bone regenerate formation is 1 mm [6]. The flaw of the method is long duration of treatment determining patient's discomfort and high expenditures of the public health. The use of fractionated distraction protocols [4,6,8] lead to more rapid osteogenesis and improve soft tissue adaptation to lengthening. The search for the optimal rate of highly fractionated distraction is a promising trend [1,3]. Neuropathies are among frequent complications of distraction [9], and hence, studies of limb nerve reactions are essential for evaluation of clinical use of new technologies.

We analyze morphofunctional changes in the tibial and peroneal nerves during shin distraction in dogs with the use of an autodistractor at a high daily rate of 3 mm in 120 steps.

MATERIALS AND METHODS

Five days after closed flexion osteoclasia of the tibial bones at the level of the middle third, the shin was distracted in adult dogs (n=9) by means of automated Ilizarov external fixator at a daily rate of 3 mm over 120 steps. Distraction was carried out over 10 days, after which the limb was fixed in the device over 30 days to attain consolidation of the bone regenerate, and the device was then removed. The animals were sacrificed by barbiturate overdosage (3 animals per period) at the end of distraction (D10), fixation (F30), and 30 days after the device removal (ND30). Evoked bioelectric activity (M-responses) of *m. gastrocnemius* and *m. tibialis anterior* was induced by stimulation electromyography [5] and recorded by DISA-1500 digital system (DANTEC). The biopotentials were re-

G. A. Ilizarov Center Repair Traumatology and Orthopedics, Ministry of Health of the Russian Federation, Kurgan, Russia. ***Address for correspondence:*** varstn@mail.ru. T. N. Varsegova

corded with monopolar electrodes with modified lead surfaces (needles). The active pole was injected in the test muscle belly, the indifferent one subcutaneously in the tendon area. The M-responses were evoked by stimulation of the sciatic nerve through a needle electrode with rectangular pulses (1 msec long) at *a priori* supramaximal intensity. The study was carried out with due consideration for the European Convention for protection of vertebrates used for experiments or other research purposes (Strasbourg, 1986).

Specimens of the tibial and peroneal nerves were routinely embedded in araldite. The images of total transverse semithin sections of the nerves stained with methylene blue and basic fuchsine were digitally processed on a Diamorph complex, and the following parameters were evaluated using VideoTestT-Master 4.0 software: summary area of fascicles and the mean parameters of myelin fibers (diameters of fibers (D_{mf}) and their axons (D_{ax}, thickness of myelin membranes (L_m)); the percentage of destroyed myelin guides in a sample of 500 fibers from each nerve was calculated. The numerical density of endoneural microvessels — number of arteriolar and capillary profiles per 1 mm^2 section area was evaluated as described previously [10]. Vascularization changes were verified by immunohistochemical reaction with von Willebrandt's factor on paraffin sections. Specimens of the tibial and peroneal nerves from 5 intact dogs served as the control.

The significance of differences was evaluated by paired two-sample *t* test and Wilcoxon's test using AtteStat 1.0 software [2].

RESULTS

Fibrillation and solitary positive acute waves were recorded in the *m. gastrocnemius* and *m. tibialis anterior* at the end of distraction and fixation. Spontaneous activity decreased 30 days after removal of the device, while the amplitude of M-responses increased significantly (Table 1).

Morphological studies detected no signs of nerve injury by elements of transosseous fixation or impairment of the epineurium integrity; however, many epineural vessels were partially or completely obliterated (Fig. 1).

Shrinkage of the summary fascicular area in the distracted nerve in comparison with the contralateral one at the end of distraction and fixation was more pronounced in the peroneal nerve (Table 2). Signs of destructive changes in the inner layer of perineural cells, endoneurium arterioles and capillaries at the end of distraction were manifest in the peroneal, but not in the tibial nerve. In the tibial nerve, the numerical density of the endoneural microvessels increased to 258±14 (end of distraction), 321±38 (end of fixation), and 211±24 (30 days after the device removal) and surpassed the control values by 56.4, 94.5, and 28% ($p<0.05$). In the peroneal nerve, this parameter decreased by 12% at the end of distraction ($p<0.05$) and was 161.0±14.6, after which it increased to 174.7±31.7 (end of fixation) and 179.3±41.2 (after removal of device), remaining slightly lower than in control. Similar proportions were found in immunohistochemical preparations.

Demyelination, axonal and Wallerian degeneration (Fig. 2), and regenerating nerve fibers (indicating previous destruction of the guides) were detected in both nerves. Destroyed fibers in the tibial nerve constitute no more than 5% of all fibers in all experiments, while in the peroneal nerve, this parameter surpassed 20% in 6 of 9 experiments.

Changes in the morphometric characteristics of myelin fibers (Table 3) indicated gradual reduction in the number of fibers with signs of axonal degeneration and emergence of fibers with myelin compactization in the tibial nerve. In the peroneal nerve, the axonal degeneration augmented at the end of fixation, while myelin decompactization, manifest at the end of distraction, was replaced by hypomyelinization of an appreciable number of fibers. The following correlations of M-response amplitudes were detected: with axon diameters ($r=0.985$ for tibial nerve and $r=0.810$ for peroneal nerve), medium correlations with myelin fiber diameter ($r=0.598$ for tibial and $r=0.618$ for peroneal nerve) and numerical density of myelin-free guides ($r=0.539$ for tibial and $r=0.610$ for peroneal

TABLE 1. Dynamics of M-Response Amplitude in *m. gastrocnemius* and *m. tibialis anterior* (*M*±*m*)

Muscle	Ai	Distraction (10 days)		Fixation (30 days)		After device removal (after 30 days)	
		A_e	$\Delta A_{ei}/A_i$	A_e	$\Delta A_{ei}/A_i$	A_e	$\Delta A_{ei}/A_i$
m. gastrocnemius	26.13±1.66	5.00±0.82*	-81	6.63±2.10*	-75	13.50±1.50*	-48
m. tibialis anterior	22.00±1.15	3.56±0.62*	-84	3.83±0.71*	-83	9.00±1.00*	-59

Note. A_i – initial amplitude; A_e – experiment amplitude; ΔA_{ei} – difference between experiment amplitude and initial amplitude values; $\Delta A_{ei}/A_i$ – difference between experiment and initial amplitude values in %. *$p<0.05$ *vs.* A_i (Wilcoxon's test).

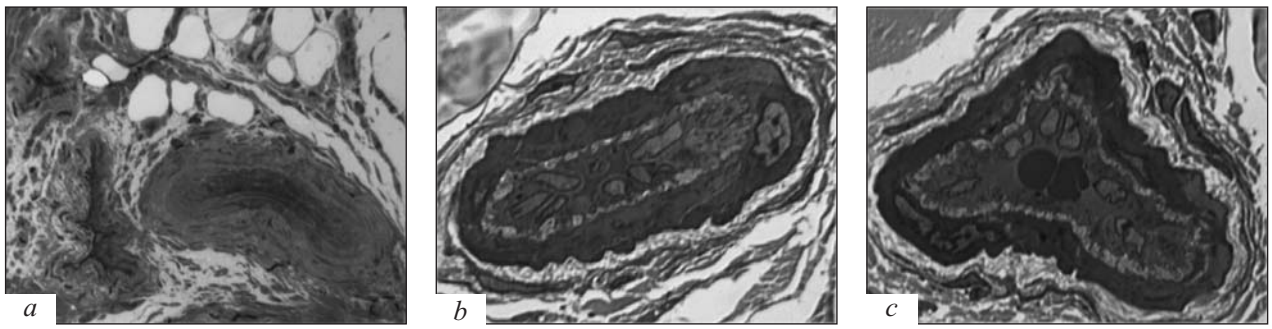

Fig. 1. Epineural vessels of the dog tibial and peroneal nerves. Vessels with obliterated lumens in tibial (*a*) and peroneal (*b*, *c*) epineurium. Fragments of semithin sections after 10-day distraction. Staining by methylene blue and basic fuchsine, ob. 16, oc. 12.5 (*a*), ob. 40, oc. 12.5 (*b*, *c*).

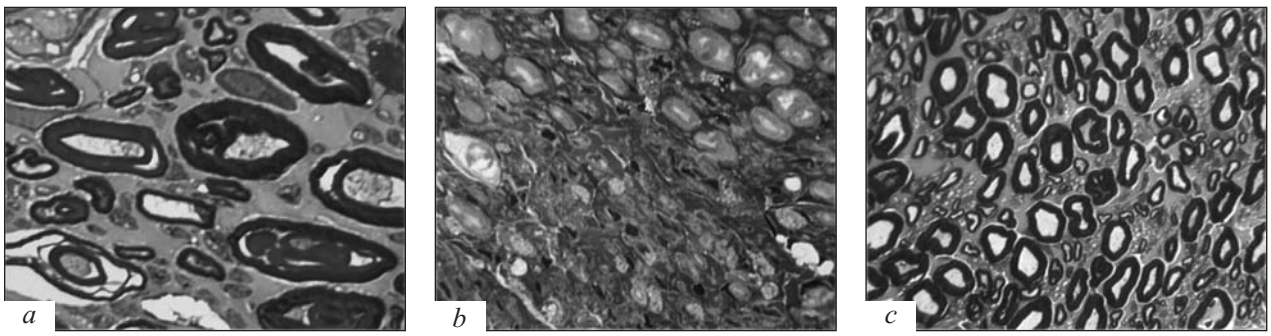

Fig. 2. Dog peroneal nerves. Fragments of semithin sections after 10-day distraction (*a*), after 30-day fixation (*b*), and 30 days after device removal (*c*). Methylene blue and basic fuchsine staining, ob. 100, oc. 12.5 (*a*), ob. 40, oc. 12.5 (*b*, *c*).

nerve), and a strong correlation with the percentage of degenerating myelin fibers (r=-0.873 for tibial and r=-0.993 for peroneal nerve).

The results indicate that in comparison with the manual protocol (1 mm/day in 4 steps) [4], a similar recovery of M-response amplitude after automated distraction (3 mm/day in 120 steps) was attained sooner (distraction period 18 days shorter, fixation period 30 days shorter). The severity of disorders in the muscle bioelectrical activity during shin lengthening is determined by disintegration of myofibrillar system, ischemization and denervation of intramuscular nerves [7], and by the severity of tibial and peroneal nerve injuries. Obliteration of the epineurial vessels and thinning of fascicles, more manifest in the peroneal nerves, indicates their hyperstretching. A similar phenomenon, called transverse contraction, is described *ex vivo* [11]. The most probable cause of more pronounced degeneration of myelin guides in the peroneal nerve in this experiment is maladaptation of the endoneural vascularization. Changes in the size of the myelin fibers in peroneal nerve reflect more intense degeneration of axons and neurolemmocyte dysfunction than in the tibial nerve. Hypervascularization of the endoneurium

TABLE 2. Summary Area of Nerve Fiber Bundles with Perineurium ($M \pm m$)

Day of study	TN			PN		
	experimental	contralateral	Δ%	experimental	contralateral	Δ%
Distraction (10 days)	64.37±2.56*	74.18±0.93	-13.2	22.18±3.78*	26.07±3.13	-17.3
Fixation (30 days)	87.56±7.16	87.73±7.01	-0.2	26.80±2.32*	28.23±2.74	-5.1
After device removal (after 30 days)	82.30±3.69	81.33 ±5.11	-1.2	29.84±3.27*	27.10±0.96	+10.1

Note. Δ% – difference between experimental and control limb values. *p<0.05 in comparison with the contralateral limb (paired two-sample t test).

TABLE 3. Parameters of Myelin Fibers at Various Stages of Experiment (*M*±*m*)

Parameter		Control	Distraction (10 days)	Fixation (30 days)	After device removal (after 30 days)
TN	fiber diameter, μ	6.75±0.28	5.82±0.24*	6.63±0.23	6.84±0.05
	axon diameter, μ	4.63±0.33	3.65±0.09*	3.78±0.14*	3.92±0.06*
	myelin membrane thickness, μ	1.06±0.05	1.08±0.08	1.43±0.19*	1.46±0.02*
PN	fiber diameter, μ	6.46±0.07	5.95±0.20*	4.45±1.02*	4.64±0.58*
	axon diameter, μ	4.39±0.08	3.56±0.10*	2.82±0.59*	2.98±0.29*
	myelin membrane thickness, μ	1.04±0.04	1.20±0.05*	0.81±0.22*	0.84±0.15*

Note. *p<0.01 in comparison with the control (Wilcoxon's test).

develops in the tibial nerve, and the structure of the majority of myelin fibers is retained. The decrease of M-response amplitude is more manifest in the *m. tibialis anterior*, the difference in comparison with *m. gastrocnemius* increasing by the end of experiment.

Hence, automated distraction by the protocol "3 mm/day in 120 steps" is rather sparing for the tibial nerve, but is fraught with a risk of the peroneal nerve neuropathy, and hence, this protocol cannot be considered as optimal.

The study was partially supported by the Russian Foundation for Basic Research (grant No. 14-44-00010).

REFERENCES

1. T. N. Varsegova, Reactive-and-destructive changes in tibial nerve at the lengthening of shin with use of fractional and high-fractional distraction in experiment. *Byull. Vostoch.-Sib. Nauch. Tsentra Sib. Otdel. Ross. Akad. Med. Nauk*, No. 4-1, 234-237 (2011).
2. I. P. Gaidyshev, *Analysis and Data Processing: Special Guidelines* [in Russian], St. Petersburg (2001).
3. E. N. Gorbach, T. A. Stupina, T. N. Varsegova, and A. A. Emanov, Study of osteogenesis dynamics, articular cartilage and tibial nerve state for leg lengthening of increased rate using an autodistractor experimentally. *Uspekhi Sovremn. Estesvoznan.*, No. 7, 42-47 (2013).
4. G. A. Ilizarov, M. M. Schudlo, N. R. Karymov, and M. S. Saifutdinov, The role of distraction rate in the realization of Ilizarov's effects in nerves of the lengthened limb. *Genii Ortopedii*, No. 1, 12-18 (1995).
5. V. N. Komantsev and V. A. Zabolotnykh, *Methodological Basics of Clinical Electroneuromyography: Handbook for Physicians* [in Russian], St. Petersburg (2001).
6. V. I. Shevtsov and A. V. Popkov, Surgical Lengthening of the lower extremities [in Russian], Moscow (1998).
7. M. M. Schudlo, N. A. Schudlo, T. N. Varsegova, and I. V. Borisova, Reaction of nerves to stretching and their structural adaptation to limb lengthening. *Genii Ortopedii*, No. 4, 48-55 (2009).
8. N. A. Shchudlo, T. N. Varsegova, and M. M. Shchudlo, The dynamics of morphological changes in the deep peroneal nerve and the anterior tibial muscle for experimental leg lengthening using distraction of high division with increased daily rate. *Uspekhi Sovremn. Estesvoznan.*, No. 11, 9-14 (2014).
9. M. P. Nogueira and D. Paley, Prophylactic and Therapeutic Peroneal Nerve Decompression for Deformity Correction and Lengthening. *Oper. Tech. Orthop.*, **21**, 180-183, doi: 10.1053/j.oto.2011.01.001 (2011).
10. S. Thrainsdottir, R. A. Malik, L. B. Dahlin, P. Wiksell, K. F. Eriksson, I. Rosén, J., Petersson D. A. Greene, and G. Sundkvist, Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. *Diabetes*, **52**, No. 10, 2615-2622 (2003).
11. K. S. Topp and B. S. Boyd, Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Phys. Ther.*, **86**, No. 1, 92-109 (2006).