THE FEATURES OF CANINE M. PSOAS MINOR HISTOGENESIS IN THE PERIOD OF ACTIVE GROWTH DURING MODELING THE LUMBAR SPINE SCOLIOSIS

G.N. Filimonova, A.E. Kobyzev, V.V. Krasnov
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russia

Objective. To identify the features of *m. psoas minor* histogenesis in growing dogs under conditions of the development of spinal scoliotic deformity.
Material and Methods. Experiments were performed on 16 four-month-old mongrel dogs, males and females. In animals of Series I, the deformity was created by gangliotomy at five lumbar motion segments (L2—L6), in animals of Series II — by fixation of adjacent L3—L6 vertebral bodies with nickel titanium staples possessing thermochemical shape memory effect, and in animals of Series III — by implantation of titanium plates into the subchondral zone of vertebral growth plates, together with the staples. The control series included intact age-matched dogs. The X-ray examination of animals of Series I—III was performed in dorsoventral and lateral views at days 14, 30, 60, 90, and 180 after surgery. Paraffin and semi-thin sections of *m. psoas minor* from concave and convex sides of the lumbar scoliotic deformity zone were studied using light microscopy in 3 and 6 months after surgery.
Results. Standard signs of degenerative-dystrophic changes and reparation by restitution/substitution type characterize the histogenesis of *m. psoas minor*. The most marked destructive changes in the muscles on both sides of the deformity retained in the long-term only in animals of Series III.
Conclusions. The study results can be used for evaluating the adaptation and plasticity potential of paravertebral muscles, as well as for developing models of the spine scoliotic deformity.
Key Words: induced deformity of the spine, *m. psoas minor*, histology.

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Scoliosis is a complex multi-level pathology occurring in 6–8 % of school-age children [1]. Its treatment remains one of the most difficult and topical issues in modern orthopedics. The molecular-genetic mechanisms of scoliosis development [5], the etiology and pathogenesis of idiopathic scoliosis [6] are under study.

Paravertebral muscles play a major role in the pathogenesis of scoliosis [14]. Studies of patients with scoliosis revealed the presence of dystrophic changes in deep and superficial muscles [11]. In addition, the functions of the muscles of the body and lower extremities have been found to change [2]. Deformity of the vertebral column may be caused by hereditary systemic diseases (Marfan syndrome, Ehlers-Danlos syndrome, dysplastic syndrome); in particular, congenital muscular dysplasias are one of the factors in the development of scoliosis [10]. Some studies describe histological and morphological changes in paravertebral muscles in patients with chronic spinal pain, including radicular one; however, such changes in patients with scoliosis of the lumbar spine remain poorly understood [18].

Papers dealing with the histological analysis of paravertebral muscles in experimental animals in various models of scoliotic deformity development have not been found.

This study aims to identify the features of *m. psoas minor* histogenesis in growing dogs in the setting of of spinal scoliotic deformity development.

Material and Methods

The experiments were performed using 16 mongrel four-month-old dogs, males and females, with a body weight of 5.7 ± 0.5 kg. The animals were kept under standard vivarium conditions. Surgery and euthanasia were performed in accordance with the requirements of the European Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and were approved by the Ethics Committee of the Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics.

Operations were performed under sterile conditions using thiopental intravenous anesthesia. Scoliotic deformity of the lumbar spine was modeled in three experimental series. In series I (n = 4), the deformity was created by one-sided gangliotomy at five lumbar motion segments L2–L6 (a modified method of G. I. Gayvoronskiy using the EasyGo minimally invasive technique) [3]. In series II (n = 4), the deformity was created by fixation of adjacent vertebral bodies L3–L6 with titanium nickelide staples possessing thermochemical shape memory effect. In series III (n = 4) — by implantation of 0.3 mm titanium plates into the subchondral zone of vertebral growth plates at the ipsilateral side, together with titanium nickelide staples [7, 8]. A control series (n = 4) consisted of intact age-matched dogs. Radiography was performed using the Premium Vet x-ray apparatus (Spain) in dorsoventral and lateral views before the operation, after producing a model of scoliotic deformity, and after euthanasia. To assess changes in the shape of the spine, morphometric radiography on 14, 30, 60, 90 and 180 days after surgery was performed. The animals were euthanized on 3 and 6 months.

For morphological studies, we excised fragments of the psoas minor muscle (*m. psoas minor*) from concave and convex sides of the lumbar scoliotic deformity zone and fixed in 10% neutral formalin solution, embedded in paraffin or in epoxy resin. The sections were made using a Historange Microtome LKB Bromma 2218 microtome (Sweden), stained with hematoxylin and eosin and using the Van Gieson's stain. Semi-thin sections were made from epoxy blocks using a Nova ultratome (Sweden) and stained according to M. Ontell. The sections were examined using a light microscope (Germany). The images were digitized using the hardware and software complex DiaMorph built-in camera (Russia) and processed using the Color software. Data were processed using methods of nonparametric statistics in the AtteStat 13.1 software built in Microsoft Excel [4]. The significance of differences was determined using the W-Wilcoxon test for independent samples. Statistically significant differences were considered at $p \leq 0.05$.

Results and Discussion

In the analysis of radiographs of the lumbosacral spine in series I animals, signs of scoliotic curvature (wedge-shaped discs, vertebral frontal plane angular displacement, projection displacement of spinous processes to the concave side, changes in ratios between transverse processes) appeared on the 30th day. In addition, rotations of the lumbar vertebrae were observedsince the inception of lateral deviation of the axis of the vertebral column. Spinal curvature was always directed towards the side of gangliotomy, localized at the gangliotomy level and also extended to one segment above the uppermost and below the lowermost injured ganglia (Fig. 1). The animals of series II and III demonstrated scoliotic changes in the shape of the intervertebral discs and the vertebrae as early as on the 14th day, with the lateral deviation of the axis of the spine. However, the pace of deformity development and its severity in all experimental series were different (Fig. 1, Table).

At the macroscopic dissection, the bundles of *m. psoas minor* fibers had strict longitudinal orientation. The muscle of intact animals had muscle fibers polygonal in shape and relatively uniform in diameter, two phenotypes of fibers, few own resting nuclei, and thin layers of endomysium and perimysium (Fig. 2). Arterioles with moderately marked *t. media*, which contained circularly oriented smooth muscle cells (SMCs), and *t. adventitia* normal connective tissue outer sheath were seen.

In all experimental series, the features of structural reorganization typical of striated muscle were observed on the 90th and 180th days of the experiment in *m. psoas minor* on both sides of scoliotic deformity, reflecting an adaptive response of muscles to experimental influences. The muscle fibers lost their polygonal shape and had an increased variability in their diameters; increased numbers of nuclei in muscle fibers and fibrosis in the interstitial space was observed (Figs. 3–5). Isolated adipocytes or vast fields that had replaced degenerated muscle fibers were found. The numbers of fibroblasts, macrophages, and labrocytes increased in the interstitial space.

In series I on 90th day after surgery, the structural features of the muscles on both sides of the induced scoliosis were the most marked. Muscle fibers round in profiles and with different diameters dominated, with most of the residual muscle fibers undergoing degeneration with light halos and having centrally localized nuclei (Fig. 3a). Vast fields showed fatty degeneration where bundles of fibers had been replaced by fibrous or adipose tissue. Adventitial fibrosis and massive *t. media* with loss of circularly oriented SMCs were seen in arterioles. Six months after surgery, the histological presentation of the muscles restored and profiles of the fibers acquired a polygonal shape (Fig. 3b). Single fibers in the initial stage of fatty degeneration, fibers with accumulations of macrophages were seen, but some fibrosis of the endomysial and perimysial spaces remained.

In series II on the 90th day of the experiment, profiles of *m. psoas minor* on two sides of the forming deformity were polygonal in shape with slightly smooth contours and an insignificantly increased interstitial tissue size. Spindle-shaped multinucleated fibroblasts were seen in the perimysium. Muscle fibers with changed contractures, muscle fibers with ridges, and fibers with light halos (Fig. 4) were seen. After 180 days, the histological structure of both muscles was maximally close to the intact normal structure.

In series III on the 90th day of the experiment, a variety of pathological signs of adaptive processes in muscle tissue were observed in *m. psoas minor* on both sides of the scoliosis. Vast fields of adipocytes, fibers with centrally localized nuclei, and paired myoblasts were identified on the convex side of the induced deformity (Fig. 5a). In 6 months, the whole range of the above-mentioned

features was observed in *m. psoas minor*, including fibers in the initial stage of degeneration with light halos, fibers with multiple internal nuclei reflecting invasion of macrophages – myophagy, and cellular infiltrates in the interstitial tissue (Fig. 5b).

A comparative analysis of the results of morphometric radiography of the vertebral column in animals of the experimental series indicates faster development and greater severity of scoliotic deformity in the case of segmental blocking of the subchondral vertebral zones. This fact confirms that disturbed nutrient supply via segmental arteries to the subchondral region of the vertebrae influences the shaping of the vertebrae and formation of scoliotic deformity of the spine [15].

An increase in the number of nuclei in the muscle fibers and in the interstices is the main symptom of the activation of reparation/physiological regeneration of muscle tissue, when the muscle tissue changes the state of functional activity into the state of plastic reorganization. It is known that the cambial elements of the muscle tissue (satellite cells) are activated, for example, under slight compression, in tensile, after training, when exposed to cold, and during denervation [13, 16]. By definition, satellite cells proliferate (undergo cell division), fuse and give rise to new fibers (hyperplasia) or are involved in hypertrophy of pre-existing fibers [12, 17, 19].

A histopathological examination of the psoas minor muscle has revealed that in the animals of series I three months after gangliotomy, destruction processes dominated in the muscle tissue characterized by an extensive loss of muscle fibers, severe interstitial fibrosis, and replacement of the contractile structures with connective tissue or fat conglomerates. However, the histological structure of the muscle almost completely recovered in 6 months. In series II, such adaptive structural responses were mostly observed in the muscles on both sides of the scoliosis on the 90th day, as reversible contractures of muscle fibers of I–II stages, helical fibers, a small number of fibers in the initial stage of degeneration, and slight fibrosis in the intercellular space. The histological presentation of the muscle restored in 6 months. Apparent destructive changes in the muscles on both sides of the deformity in the long-term period of the experiment remained only in series III.

Based on the results of a pathomorphological examination of the psoas minor muscle by 6 months of the experiment, the creation of the lumbar scoliosis with titanium nickelide staples and metal plates (series III) was the best model. Similar results were also observed in the study of intervertebral discs in the same experiment [9].

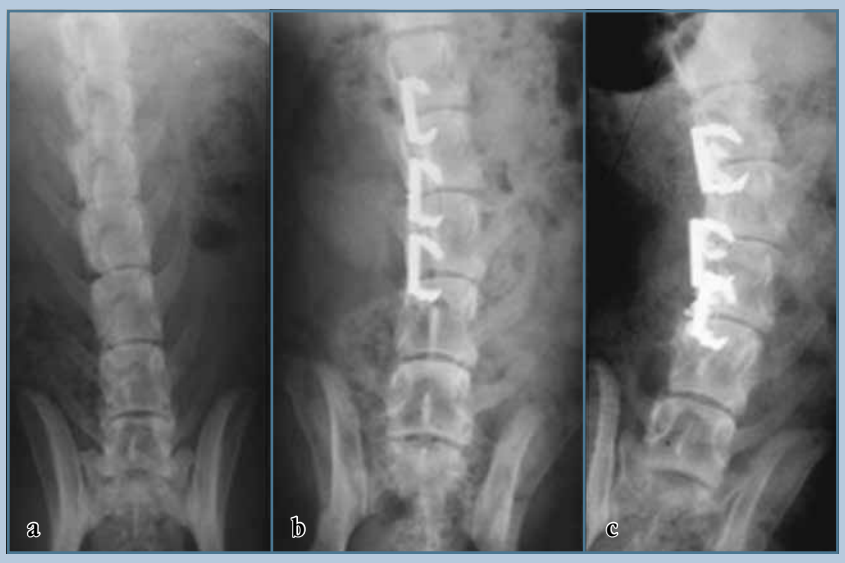

Fig. 1
Radiographs of the lumbosacral vertebral column of a dog in the frontal projection 180 days after surgery: **a** – the endoscopic coagulation of the spinal ganglia; **b** – spinal motion segments fixed with titanium nickelide staples; **c** – spinal motion segments fixed with titanium nickelide staples and plates inserted in the metaphyseal regions of adjacent vertebrae

Table
Magnitude of the spinal deformity in dogs, degrees ($M \pm m$)

Series of the experiment	Period of observation, days				
	14	30	60	90	180
I	–	3.80 ± 0.17	4.60 ± 0.18	7.30 ± 0.23	9.60 ± 0.24
II	3.20 ± 0.12	6.30 ± 0.27	7.30 ± 0.11	12.40 ± 0.28	17.30 ± 0.16
III	5.10 ± 0.18	8.50 ± 0.31	10.50 ± 0.39	27.60 ± 0.26	34.20 ± 0.18

Differences between the series are reliable ($p \leq 0.05$).

Conclusions

In this study, new data about the features of the canine *m. psoas minor* morphogenesis in the period of active growth with scoliotic deformity of the vertebral column induced in various ways have been obtained. The results can be used to assess adaptive and plastic capabilities of paravertebral muscles that play an important role in the

pathogenesis of scoliosis and in creation of scoliosis deformity models in order to develop, approve new procedures and improve existing techniques of its surgical correction.

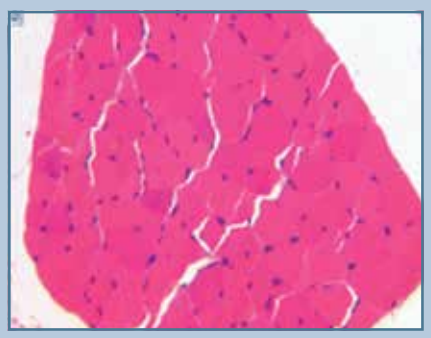

Fig. 2
The histological structure of *m. psoas minor* of intact animals: two phenotypes of muscle fibers, polygonal profiles, and thin layers of connective tissue are identified; paraffin section stained with hematoxylin and eosin, magnification: lens 6.3, ocular 12.5

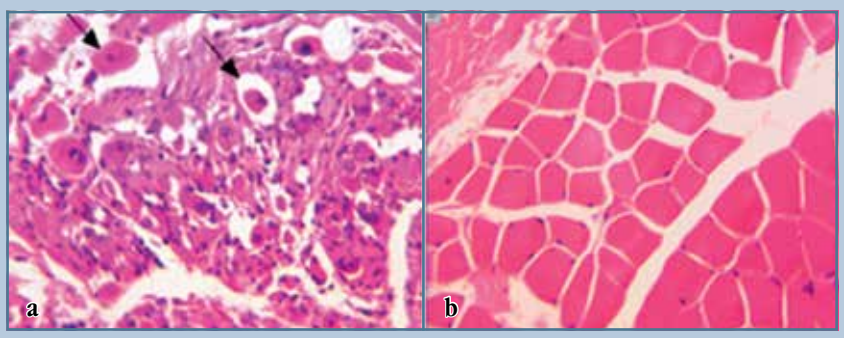

Fig. 3
Features of the histological structure of m. psoas minor in series I on the concave side of the deformity on the 90th **(a)** and 180th **(b)** day of the experiment: **a** – rounded profiles of muscle fibers of varying diameters, residual degenerating fibers with centrally localized nuclei (arrows), adipocytes; **b** – polygonal profiles of the fibers, insignificant fibrosis of the endomysium and perimysium; paraffin sections stained with hematoxylin and eosin, magnification: lens 16, ocular 12.5

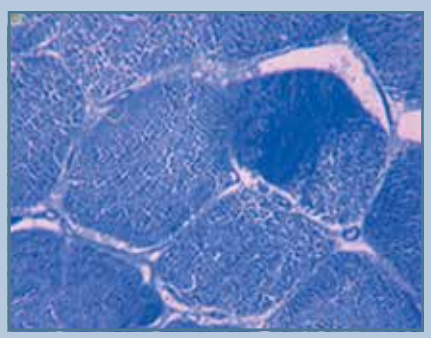

Fig. 4
The histological structure of the psoas minor muscle on the concave side of the deformity in series II after 90 days: onset of the fibers degeneration (light halos); cross semi-thin section stained by M. Ontell, magnification: lens 40, ocular 12.5

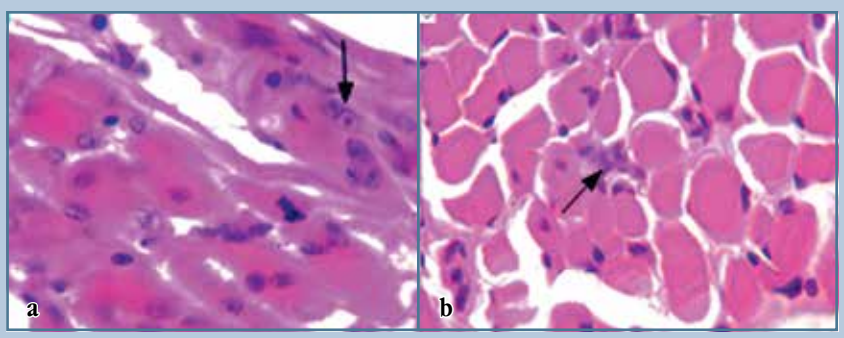

Fig. 5
Features of the histological structure of the psoas minor muscle on the convex side of the deformity in series III on the 90th **(a)** and 180th **(b)** day: **a** – activated nuclei in the fibers, centrally localized nuclei, paired myoblasts (arrow); **b** – initial stage of degeneration (light halos), cellular infiltration in the interstitial tissue (arrow); paraffin sections stained with hematoxylin and eosin, magnification: **a** – lens 40, ocular 12.5; **b** – lens 16, ocular 12.5

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Address correspondence to:
Filimonova Galina Nikolaevna
Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, M. Ulyanovoj str., 6, Kurgan, 640014, Russia,
galnik.kurgan@mail.ru

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Galina Nikolaevna Filimonova, PhD in Biology, senior researcher, Laboratory of Morphology in Clinical and Diagnostic Department, Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russia;
Andrey Evgenyevich Kobyzev, MD, PhD, deputy managing director, Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russia;
Vitaly Viktorovich Krasnov, DSc in Biology, leading researcher, Laboratory of pathology of the axial skeleton and neurosurgery, Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, Kurgan, Russia.