Muscle study in experimental scoliosis in rabbits with costotransversectomy: evidence of ischemic process

Lineu C. Werneck · Vlademir A. Cousseau ·
Xavier S. Graells · Mauricio C. Werneck ·
Rosana H. Scola

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Abstract Scoliosis involves the central nervous system diseases, ligaments, articulations and skeletal muscles, but there is no consensus on its pathogeny and progression of muscle abnormalities. In this study, we investigate the morphologic changes in the muscle of rabbit submitted to experimental scoliosis, with special emphasis on abnormalities related to blood supply. We studied 26 rabbits subjected to costotransversectomy by pulling out six transverse processes at thoracic level and six rabbits were used as controls. All the animals operated upon developed scoliosis showing an average angle of 29.1° on the 60th day, with its apices located at T4 and T12 when they were subjected to paraspinal muscle biopsy on both sides. The muscle biopsies were subjected to histological stains and histochemical reactions, as well as to a morphometric study. On the concave side, the changes were not statistically significant regarding the control group. On the convex side conjunctive tissue proliferation, infiltration by adipose tissue, central nucleus excess, inflammatory reaction, segmental fibrosis, type 1 fiber hypertrophy, type 2 fiber hypertrophy and atrophic angular dark fibers in the unspecific esterase were statistically significant. The segmental fibrosis reached a circumscribed muscle segment, compatible with an ischemic phenomenon. The histological diagnoses on the concave side of the animals had unspecific alterations (atrophy and hypertrophy) in 13, myopathy

in 3, denervation in 3 and normal in 7. The convex side diagnoses were myopathy in 14, denervation in 8, mixed in 3 and normal in 1. The procedure determined morphologic changes on the convex side indicating possible denervation or myopathy of ischemic origin.

Keywords Scoliosis · Muscle biopsy · Experimental scoliosis · Ischemic scoliosis

Introduction

Scoliosis is a backbone three-dimensional deformity combining a lateral curvature with vertebral body rotation. This deformity is caused by force alterations, which permit abnormal backbone equilibrium, unusual movements and deformities. This process involves ligaments, articulations, paravertebral structures, skeleton muscles and central nervous system influence [1, 33, 35, 38, 41].

Diseases that affect the above-mentioned structures, such as spine dysraphia [24, 45], muscle dystrophies [29, 30, 31, 42], congenital myopathies [6, 16, 21, 32, 36], spine muscle atrophies [5, 11], cerebral palsy [34, 39], non-progressive cerebral lesions [13] and spine cerebellar degenerations [22], are well-known causes of scoliosis.

In most cases, it is not possible to determine an etiology, so the term established was idiopathic scoliosis. There are several hypotheses to explain why idiopathic scoliosis appears: a primary skeleton disturb, muscle unbalance due to different factors, metabolic hypothesis, genetic defect, hormonal defect, conjunctive tissue disease and vestibular dysfunction [2, 19, 26, 43]. Whatever the basic defect is, the paraspinal muscles show changes, which seem to be specifically located and the hypothesis of a systemic muscle disease was rejected [27]. The changes mentioned

L. C. Werneck (✉) · M. C. Werneck · R. H. Scola
Department of Internal Medicine/Neurology,
Universidade Federal do Paraná, Rua Gal. Carneiro,
181-3 andar, Curitiba, PR 80060-900, Brazil
e-mail: werneck@ufpr.br

V. A. Cousseau · X. S. Graells
Department of Surgery/Orthopedic,
Universidade Federal do Paraná, Curitiba, PR, Brazil

are the differences between convex and concave muscle fiber types regarding the histochemical pattern transformation. Some papers report that the concave side is more affected than the convex side [4, 28], and others report that the convex side is more affected than the concave [8, 18]. Most of these papers study the muscle by needle biopsy or small fragments, usually taken from the peripheral region.

To explain the muscle changes occurring in the idiopathic scoliosis, there are several experimental studies in dogs with experimentally induced syringomyelia [12], rabbit unilateral electrical stimulation [23, 47], rat electrical stimulation [20], rabbit spinous and transverse apophyses ligature [10], rabbit transverse processes and facet articulation removal [37] and costotransversolysis with transectomy to release the paraspinal muscles [17]. Most of the papers focus on the muscle fiber type distribution, comparing the changes within the convex and concave sides. Little or no mention is made regarding the abnormalities in muscle blood supply or vessels abnormalities.

The present work aims to study the paraspinal muscles of rabbits with scoliosis induced by unilateral costotransversectomy looking for all changes in the muscle regarding fiber type composition and those induced by ischemic processes.

Materials and methods

The study focused 32 male and female rabbits (*Oryctolagus cuniculus*) with an average weight of 750 g belonging to the same lineage. Twenty-six of them were used in the experiment and six of them compose the control group.

In the night before surgery, the animals were subjected to a trichotomy in the previously defined surgery region and to fasting for 12 h. After being weighed, the rabbits were given the pre-anesthetics xylazine hydrochloride (5 mg/kg) and intramuscular atropine sulfate (0.2 mg/kg). Total anesthetic was obtained with ketamine hydrochloride (50 mg/kg) and intramuscular acetilpromazine maleate (0.75 mg/kg).

With the animal lying prone, a cutaneous incision was made in the median posterior level of the thoracic spine and deepened toward the fascia; the skin edges were drawn back with the use of Farabeuf retractor; the interspinous ligament overlying the spinous processes was identified; the cartilaginous cover of the spinous process was crenated and its right half was separated to the side with the help of a Penfield detach; the spinous process was exposed and the subperiosteal was detached; after exposing six spinous processes, the retractor was introduced to prevent muscular rupture; the trapezius, rhomboid and serratus muscles were reflected laterally toward the median raphe exposing the

costotransversal processes, partial extraction of the semispinous and multifidos muscles; six transverse processes were withdrawn with a Luer bone pincer; the prevertebral musculature and fascia were sutured with a 3/0 absorbable thread and the skin with a 3/0 nylon thread. No dressing was used, just iodated alcohol was applied. As infection prophylaxis it was applied a dose of 200,000 u.i. procaine penicillin procaine and 250 mg/kg of dihydrostreptomycin sulfate. The animals of the control group were subjected to the same procedure in the right side, except for the costotransversal process withdrawal.

The operated animals and the control ones were maintained under room temperature in a rabbit cage during the whole experience period. All the animals that were operated on a developed progressive scoliosis with the convexity turned to the side where the transverse processes resection was made. The scoliosis average angle on the 7th day was 8.5°, on the 30th day 19.9° and on the 60th day 29.1°, ranging from 16° to 39°, with apex located at T4 to T12 (Fig. 1). These animals were subjected to biopsy of paraspinal muscles at the scoliotic curve apex on both concave and convex sides. The same technique aforementioned was used to access the paraspinal muscles. The six control group animals were subjected to biopsy of paraspinal muscles on just one side. The muscle fragments, generally measuring $0.6 \times 0.6 \times 1.5$ cm, were covered with gauze moistened with physiologic serum and sent to the laboratory. After the muscle withdrawal, the animals were killed.

In the laboratory, the muscle fragments were assembled on a metallic base with adraganth 7%. Then, they were covered with talcum powder, frozen in liquid nitrogen and stored at -120°C .

The muscle fragments were cut in cryostat at -20°C with 8 μm and subjected to staining by hematoxylin and eosin, modified Gomori trichrome, ATPase 9.4, 4.3 and 4.6 and unspecific esterase according to the techniques already described in this area of literature [14, 44]. When examining these biopsies, we always tried to compare the normal controls with the study animals, using the abnormality found in human beings as parameters. The biopsy alterations were classified according to intensity as 0 (normal or absent), 1 (discrete, light or rare), 2 (moderate or occasional) and 3 (important or frequent), regarding the items: conjunctive tissue proliferation, infiltration by adipose tissue, abnormal vessels, abnormal nerves, central nuclei, nuclear clumps, necrosis, phagocytosis, inflammatory reaction, segmentation, basophilic fibers, hyper-contractile fibers, perifacicular atrophy, segmental fibrosis (replacement of muscular tissues for fibrosis tissues in a certain biopsy area), predominance or deficiency of a type of muscular fiber, atrophy or hypertrophy of a specific fiber, grouping of the same fiber type and presence of dark

Fig. 1 Progressive spine angulations in rabbits with unilateral costotransversectomy. **a** Control, **b** after 60 days 35°

Fig. 2 Muscle from control group. **a** Slight variation in fiber size (Hematoxylin-Eosin). **b** Type 2 muscle fiber atrophy (ATPase 9.4, dark fibers are type 2). Bar 100 μ m

atrophic angular fibers in the unspecific esterase. To fiber variation diameters we used the following criteria: 0 (normal without important variation), 1 (atrophic polyhedral or hypertrophic rare fibers), 2 (polyhedral atrophic or

angular fibers with occasional hypertrophic), 3 (atrophic polyhedral, angular or frequent hypertrophic fibers). The fiber type distribution and morphometrical study done using digitalized pictures through the Image-Pro® Plus

Table 1 Histological findings comparing controls and scoliotic rabbits with to costotransversectomy

Type of alteration	Side	Absent	Present	<i>P</i> *
Connective tissue proliferation	C	6	0	
	X	25	1	1.000
	Y	7	19	0.005
Infiltration of adipose tissue	C	5	1	
	X	25	1	0.345
	Y	4	22	0.005
Vessels abnormalities	C	6	0	
	X	26	0	–
	Y	23	3	0.923
Nerves abnormalities	C	6	0	
	X	25	1	1.000
	Y	26	0	–
Change in fiber size	C	1	5	
	X	6	20	1.000
	Y	1	25	0.255
Central nuclei	C	6	0	
	X	25	1	1.000
	Y	6	20	0.002
Nuclear clumps	C	6	0	
	X	26	0	–
	Y	24	2	1.000
Necrosis	C	5	1	
	X	24	2	1.000
	Y	9	17	0.087
Phagocytosis	C	5	1	
	X	24	2	1.000
	Y	9	17	0.087
Inflammatory reaction	C	6	0	
	X	24	2	1.000
	Y	10	16	0.024
Fiber splitting	C	6	0	
	X	24	2	1.000
	Y	20	6	0.468
Basophilic fibers	C	6	0	
	X	26	0	–
	Y	21	5	0.585
Hypercontractile fibers	C	6	0	
	X	25	1	1.000
	Y	25	1	1.000
Perifascicular atrophy	C	6	0	
	X	26	0	–
	Y	22	4	0.732
Segmental fibrosis	C	6	0	
	X	26	0	–
	Y	12	14	0.050
Dark small angulated fibers—esterase	C	5	1	
	X	21	5	1.000
	Y	6	20	0.024

C control group, X concave side, Y convex side

– No calculation, because one of the variables is constant; values in bold are statistically significant

* Chi-square test in relation to control group

(Media Cybernetics, Silver Spring, MD, USA) program on convex and concave sides using the ATPase pH 4.6 (This reaction differentiates the type 1, 2A and 2B fibers). Was counted at least 100 fibers in middle of the biopsy and an average area on micron was calculated.

Results

The control group animals when compared to human muscles showed some alterations, such as: atrophy of type 2 fibers, discrete variation in fiber diameters and, to a lesser extent, infiltration by adipose tissue, necrosis, phagocytosis, type 1 fiber atrophy and dark angular atrophic fibers in unspecific esterase (Fig. 2; Table 1).

The histological alterations were more frequent and important on the convex side than on the concave, mainly represented by the adipose tissue infiltration, conjunctive tissue proliferation, central nuclei, necrosis and phagocytosis, inflammatory reaction, dark atrophic angular fibers in unspecific esterase, fiber splitting, basophilic fibers and segment fibrosis. These abnormalities were generally located in a circumscribed biopsy area, thus suggesting that there was a vascular component involved, like ischemia (Figs. 2, 3, 4, 5, 6; Table 1).

Concerning fiber qualitative variations, the convex side was also the one that showed a greater number of abnormalities such as: type 1 atrophy, type 1 fiber hypertrophy, type 2 fiber hypertrophy. Both sides showed a similar number of diameter variations and type 2 fiber atrophy. The other pathologic variables show a similar number of abnormalities (Fig. 7; Table 2).

In order to check the importance of the abnormalities concerning the control animals, we performed Chi-squared tests. To that end, the alterations were grouped as abnormal, when there were any abnormalities, and absent or normal (always taking into consideration the human pattern). Regarding the controls, we only found statistically significant alterations ($P < 0.05$) on the convex side for the following variables: conjunctive tissue proliferation, adipose tissue infiltration, central nuclei excess, inflammatory reaction, segmental fibrosis, type 1 fiber atrophy, types 1 and 2 fiber hypertrophy and dark atrophic angular fibers in the unspecific esterase (Figs. 7, 8; Table 2). The average area of type 1, 2A and 2B fibers was similar for the controls and concave side. On the convex side, the types 1 and 2A was smaller compared with the controls and concave side (Table 3).

The animals altogether were given several diagnoses, and abnormalities were found in all of them including the control ones. Type 2 fiber atrophies were found in five control animals and just one of them was normal. On the concave side the diagnosis that predominated was type 2

Fig. 3 **a** Fibers with necrosis and phagocytosis (*arrow*); **b** area of inflammatory infiltration in necrotic fiber (*arrow*) and atrophic fibers in the convex side (scoliotic group). Haematoxylin-eosin. Bar 50 μ m

Fig. 4 Connective tissue proliferation, segmental fibrosis in the perifascicular area (*arrows*), inflammatory reaction, excess of central nuclei, atrophic fibers in the perifascicular region (*star*) and hypertrophic fibers. Scoliotic group, convex side. Haematoxylin-eosin. Bar 100 μ m

Fig. 6 Large area of muscle infarct (*arrows*). Scoliotic group, convex side. Haematoxylin-eosin. Bar 100 μ m

Fig. 5 Vessel with the lumen occluded by thrombi. Scoliotic group, convex side. Haematoxylin-eosin, Bar 50 μ m

Fig. 7 Type 2 fiber atrophy (*arrows*) and type grouping (*star*). Scoliotic group, concave side. Type 2 fibers with strong reaction. ATPase 9.4, Bar 100 μ m

Table 2 Qualitative fiber type variations comparing controls and scoliotic rabbits with costotransversectomy

Type of alteration	Side	Absent	Present	<i>P</i> *
Type 1 fiber predominance	C	6	0	1.000
	X	25	1	
	Y	26	0	
Type 2 fiber predominance	C	6	0	0.136
	X	15	11	
	Y	18	8	
Type 1 fiber deficiency	C	6	0	0.373
	X	19	7	
	Y	20	6	
Type 2 fiber deficiency	C	6	0	1.000
	X	25	1	
	Y	26	0	
Type 1 fiber atrophy	C	5	1	0.592
	X	16	10	
	Y	4	22	
Type 2 fiber atrophy	C	0	6	1.000
	X	10	16	
	Y	2	24	
Type 1 fiber hypertrophy	C	6	0	0.923
	X	23	3	
	Y	7	19	
Type 2 fiber hypertrophy	C	6	0	0.732
	X	22	4	
	Y	7	19	
Type grouping	C	6	0	–
	X	26	0	
	Y	19	7	

C control group, X concave side, Y convex side

– No calculation, because one of the variables is constant; values in bold are statistically significant

* Chi-square test in relation to control group

fiber atrophy and on the convex side the predominating diagnosis was active and chronic myopathy. As some of these diagnoses are unspecific or not significant, we regrouped the diagnosis as: normal or minimum abnormalities, unspecific abnormalities, myopathy and denervation. We noticed that the convex side showed a greater number of abnormalities, myopathy being predominant and followed by denervation. Unspecific abnormalities, as atrophy and hypertrophy, predominated on the concave side. They were also present in the control animals (Table 4).

Discussion

The presence of histological alterations was more severe on the convex side and could be related to the experimental

Fig. 8 Dark small angulated fibers (arrows). Scoliotic group, convex side. Non-specific esterase, Bar 100 μ m**Table 3** Fiber type distribution in controls and scoliotic rabbits with costotransversectomy (mean area in μ m)

Fiber type	Controls	Concave	Convex
Type 1	32,36	32,38	27,50
Type 2A	32,31	31,89	27,54
Type 2B	32,44	33,86	32,27

technique used, since this side is the one that is stretched the most. Although, this procedure tries to reproduce just scoliosis, it could affect the vascular bundles that provide the corresponding space to the paraspinal muscles (multifidos). Such process could compress the arteries, which supply the muscles and determine a progressive muscle ischemia, which would become worse as the scoliosis angle increases, causing a more severe muscular lesion.

The convex side alteration found in our experiment was more affected and showed alterations that can be found singly in myopathies and denervations process. Nevertheless, the muscular fibrosis represents a circumscribed area of muscular tissue generally located peripherally, as described in experimental muscle infarct [15], and can remind us of perifascicular atrophy found in dermatomyositis, which is the result of muscle ischemia or infarct [3, 9, 14]. On the other hand, the presence of dark atrophic angular fibers in unspecific esterase is a strong evidence to recent denervation, although it can be seen in myopathies with a great degree of necrosis. In this case, the muscular fiber necrosis is partial and one of the segments remains without innervation and functions as a denervated fiber [7]. Therefore, the histological data is more likely to indicate an ischemic-origin myopathy.

The skeleton muscle has an important regenerating capacity when it is subjected to ischemia and this capacity

Table 4 Histological diagnosis in controls and scoliotic rabbits with costotransversectomy

	Controls	Concave side	Convex side
Normal and minimal abnormalities	1	7	1
Abnormal non-specific (Types 1 and 2 fiber atrophy, hypertrophies)	5	13	
Myopathy (active and chronic)		3	14
Denervation (recent and chronic)		3	8
Mixed			3
Total	6	26	26

is proportional to the process duration and severity. The need of restoring circulation is already known for a long time because if the lesion is not restored it can be permanent [3, 40]. In our experiment, probably the progressive compressions of the arteries, which supply the muscles lead to a definitive ischemia. The ischemic muscle become weak, leading to an abnormal equilibrium between the right and left sides and can be a factor of progressive scoliosis.

The denervation histological pattern we found has already been described in experimental scoliosis by costotransversectomy. In that experimental work, the authors stated that the histological alterations (myopathic) were a consequence of the postural deformity [4].

Statistically, we did not find a change in the fiber distribution pattern, which somehow is difficult to be analyzed regarding the human cases studied. Possibly, biopsies on humans are more superficial and scoliosis has a slower evolution which would allow muscle regeneration. During regeneration different fiber types react differently. So, in myopathies it is common to find greater atrophy and the predominance of type 1 fiber, and in denervations there is more atrophy of type 2 fibers [7]. However, in the ischemic processes determining myopathic and neuropathic alterations, there is not a unique directive for regeneration and a mixed pattern can happen, as it does in vasculitis. The smallness of fiber types 1 and 2A on convex side found in our experiment suggests a process of regeneration, which might happen during muscles ischemia [3, 40].

The concave side alterations are apparently a consequence of the lack of use and relative immobilization of these muscles, associated to the animal low mobility in the rabbit hutches. The alterations in the control animals are important and show that relative immobilization can also cause discrete alterations such as type 2 fiber atrophy. This happens in humans with prolonged immobilization [8, 9, 14].

We concluded that this experimental model presents histological alterations suggesting myopathy and denervation. Myopathy patterns are more common on the convex side. The concave side did not show statistically significant alterations concerning the control animals, and the few ones we found were likely to be secondary to disuse and

immobilization. The quantitative relation between fiber muscles types was not related to scoliosis production. The alterations noticed in this experimental model seemed to be due to muscular ischemia [15], either due to the progressive arteries stretching inside the convex side muscle or due to trauma during the costotransversectomy procedure. Further works are needed in human idiopathic scoliosis to see if the same pathological process happens in the paraspinal muscles using muscle blood flow measurement “in site” with magnetic resonance techniques [25, 46].

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