ORIGINAL ARTICLE

Scoliosis induced by costotransversectomy in minipigs model

Javier Cervera-Irimia[1], Álvaro González-Miranda[2], Óscar Riquelme-García [3], Jesus Burgos-Flores[4], Carlos Barrios-Pitarque[5], Pedro García-Barreno[6], Azucena García-Martín[3], Eduardo Hevia-Sierra[7], Giuseppe Rollo[8], Luigi Meccariello[8], Luigi Caruso[9], Michele Bisaccia[9]

[1]Plastic, Reconstructive and Burns Surgery Department, University Hospital La Paz, Madrid, [2]Orthopaedic and Trauma Department, General Hospital of Villalba, Collado Villalba, [3]Orthopaedic and Trauma Department, Hospital Universitario Gregorio Marañón, Madrid, [4]Orthopaedic and Trauma Department, Ramón y Cajal University Hospital, Madrid, [5]Orthopaedic and Trauma Department, University Research Institute in Musculoskeletal Diseases, School of Medicine, Catholic University of Valencia, Valencia, Spain, [6]Surgery Department, School of Medicine, Complutense University of Madrid, Madrid, [7]Orthopedic and Trauma Department, La Fraternidad Hospital, Madrid; Spain, [8]Department of Orthopedics and Traumatology, Vito Fazzi Hospital, Lecce, [9]Division of Orthopedics and Trauma Surgery, University of Perugia, S. Maria della Misericordia Hospital, Perugia; Italy

ABSTRACT

Aim To validate surgical costotransversectomy as a technique for creating a scoliosis model in minipigs and to assess whether differences in approach (posterior medial approach, posterior paramedial approach and anterior approach by video-assisted thoracoscopy) lead to differences in the production of spinal deformity. Creation of disease models in experimental animals, specifically in minipigs, is controversial, as no appropriate technique has been reported.

Methods Surgical costotransversectomy was performed in 11 minipigs using 3 different approaches: posterior medial approach (4 animals, group I), posterior paramedial approach (3 animals, group II) and anterior approach by videothoracoscopy (4 animals, group III). A conventional x-ray study was performed in the immediate postoperative period. Follow-up lasted for 4 months. Specimens were humanely killed according to current protocols, and a second x-ray study was performed. A deformation was measured using the Cobb angle and direct observation of the rotational component.

Results Data from group I revealed a scoliosis deformation of 27º-41º (mean 34.5º) with a macroscopic rotational component. No deformity (<10º) or rotational component was observed in groups II and III. Only a posterior medial costotransversectomy produced a significant deformity in minipigs and established a valid model for studying scoliosis in these animals.

Conclusion Only a posterior medial costotransversectomy produces a significant deformity in minipigs and establish a valid model for studying scoliosis in these animals. A tensegrity model would elucidate such results and harmonize disparate conclusions. Further investigation is needed to demonstrate the reliability of tensegrity principles for spinal biomechanics.

Key words: experimental animal, model, spinal curvatures

Corresponding author:
Luigi Meccariello
Department of Orthopaedics and
Traumatology, Vito Fazzi Hospital
Piazzetta Filippo Muratore,
Block: A- Floor:V, Lecce, Italy
Phone: +39 329 941 9574;
Fax:+39 082 371 3864
E-mail: drlordmec@gmail.com
ORCID ID: http://www.orcid.org/0000-0002-3669-189X

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INTRODUCTION

Idiopathic scoliosis (IS) is defined as a lateral curvature or deviation (left or right) of the spine >10º that is associated with vertebral rotation (1). Its etiology is unknown. Although the most obvious abnormality is manifested in the frontal plane, vertebral rotation means that scoliosis is a three-dimensional deformity (2). The prevalence of IS ranges from 0.5% to 3%, depending on age group and sex. It is more common in adolescents and females (3.6:1 female-male ratio) (3). The prevalence of severe impairment (curves >30º) decreases to 0.15% to 0.3% (4). Three age groups have been established for this condition, as follows: infantile (<3 years), juvenile (3 to 10 years) and adolescent (>10 years) (2).

The treatment of IS varies according to severity and progression of the deformity and may be conservative (observation and orthopaedic measures) in the early stages or aggressive (surgery) in advanced or progressive forms. The use of braces to halt progression of scoliosis has limited usefulness in many cases and significantly impacts a child´s daily life (5). On the other hand, surgery is an aggressive intervention that limits the patient´s functional capacity and often needs to be repeated, with a complication rate of around 5.7%. Surgery has proven to be fatal in 0.03% of cases (6).

In this context, scoliosis models in experimental animals provide a reasonable approach to the pathophysiology of this disease and to the design of possible therapeutic options. More than 50 years of experience have been accumulated in this field and different procedures have been developed to establish the pathogenesis of the model. MacEwen (7) studied three possible models: natural scoliosis, scoliosis caused by systemic agents and scoliosis induced by surgical procedures. Natural scoliosis has been observed in several species, although few experimental studies have been performed, owing to the low caseload and the complexity in obtaining and working with these animals. Scoliosis induced by systemic agents is based on various publications about the "scoliogenic" potential of some substances (e.g. insulin injected into a chicken embryo or 6-aminonicotinamide injected into a mouse embryo) or some conditions affecting the organism (e.g. hypovitaminosis E or pregnant rat hypoxia). However, the appearance of multiple concomitant major malformations in other organs makes isolated study of the spinal deformation difficult. Scoliosis induced by surgical procedures is the most comprehensive and promising approach, because of its ability to generate animal models designed exclusively for IS studies and the lack of associated abnormalities that could distort results.

Numerous techniques have been described to create scoliosis models in the experimental animal. In the 1980s, Dubousset started the pinealectomy line of research, which was based on the ability of this technique to produce deformities in chickens that were similar to those of humans IS. Later investigations by Machida and Dubousset (8) and O'Kelly and Wang (9) support this model. Piggot (10) used unilateral costotransversectomy in experimental rabbits to generate scoliotic curvatures, which led to convex deformity on the operated side. The author carried out a clinical trial with 25 patients aged between 2 and 14 years, who suffered from rapidly progressive scoliosis (with an unknown etiology in most cases). Costotransversectomy was performed on the concave side of the curvature, resulting in process deceleration and, in some cases, a slight regression. The author highlighted the need for caution and further investigation before results could be considered conclusive. Subsequently, the validity of this principle has been confirmed when applied to pinealectomized chickens (11). Braun et al. (12-14) analysed the results obtained in costotransversectomized goats undergoing flexible posterior asymmetric tethering. The authors used a synthetic ligament analogue (made of polyethylene and polyester) to tether a row of ribs on the concave side (opposite to costotransversectomy), thus enhancing the "scoliogenic" process. Coillard et al. (15) studied the deformation produced by unilateral epiphysiodesis of the neurocentral cartilage on five consecutive vertebrae of the minipig, using a screwing method. The results suggest that the technique affects mainly the horizontal curvature, with little rotational involvement.

Despite the above-mentioned evidence, costotransversectomy has failed to produce scoliosis. For example, Robin and Stein (16) showed negative results in primates, as did Cañadell et al. (17) in bipedal rats.

Therefore, no evidence of a valid procedure to create scoliosis models in experimental minipigs has been reported. Moreover, no studies confirm the ability of costotransversectomy to generate spinal deformity in these animals and current interspecies data are contradictory (18). We performed this study to validate an experimental scoliosis model in minipigs. We have resolved the confusion surrounding the potential of costotransversectomy to produce spinal deformation and explained the mechanisms that lead to this condition.

The primary objective of this work was to validate costotransversectomy as a technique for creating a scoliosis model in the experimental minipig. A secondary objective was to assess whether differences in approach (posterior medial approach, posterior paramedial, and anterior approach by video-assisted thoracoscopy, VAT) lead to differences in the production of spinal deformity. Both the posterior paramedial approach and the VAT anterior approach leave the vertebral column intact, an interesting property when studying treatment of IS.

MATERIALS AND METHODS

Material and study design

We used 11 male minipigs, with a mean age of 36 days (range 30-54 days). Costotransversectomy (T6-T10) was performed using different approaches: the posterior medial approach (4 animals, group I), the posterior paramedial approach (3 animals, group II), and the VAT anterior approach (4 animals, group III).

An approval of the Institutional Animal Care and Use Committee was obtained for this study.

Methods

Surgical approaches. The posterior medial approach (mean operative duration 44±8.4 min) consisted of a longitudinal incision of about 12 cm over the midline, with detachment of the paravertebral musculature following the contour of the spine. The costovertebral joints were exposed medially to dissected muscular components. Costotransversectomy involved removal of about 3 cm of the rib head, including both the costovertebral and the costotransversal joints with their ligament complexes.

A similar incision was performed for the posterior paramedial approach (mean operative duration 51±7.2 min), although at about 2 cm from the midline of the column. In this case, dissection to expose the costotransversal area followed the paravertebral musculature laterally, although joint resection was similar to that of the previous group (Figure 1). The VAT anterior approach (mean operative duration 69 ± 9.8 min) was performed using three portals (incisions of 2 cm) over the anterior axillary line. The initial portal for the endoscopic camera was placed in the sixth or seventh interspace, while trying to prevent injury to the diaphragm, which is normally more caudal. The other 2 portals were placed two and three interspaces further up, directly over the ribs in order to allow placement above and below the rib at each level with a single skin incision. The consecutive T6-T10 levels were identified and only removal of about 3 cm of the rib head was performed, because reach of transverse apophysis was not possible by VAT anterior approach. Direct closure of pleura and skin was always possible and a chest tube was not necessary in any case.

Figure 1. Animal in prone position, head on the right side of the image. Posterior paramedial approach, 2cm from the midline of the column. It is observed that paravertebral musculature is undamaged. Dissection to expose the costotransversal area followed the paravertebral musculature laterally (Experimental Medicine and Surgery Department of Gregorio Marañón, 2015)

Radiography and macroscopic examination. Plain X-ray of the column was taken immediately after the surgery. Follow-up lasted for 4 months. The animals were humanely killed according to existing protocols (19-20), and the spine was extracted for macroscopic examination and radiography. Frontal plane deformity was measured in all cases using the Cobb method (21). In contrast, vertebral rotation was assessed in situ, that is, directly on the anatomical specimen extracted. There were no postoperative deaths or complications, so it was possible to include data from all 11 animals.

Statistical analysis

Independent samples were compared using the Mann-Whitney test. It made paired comparisons on data that are ordinal, or continuous but non-normally distributed, the Mann-Whitney test was use. In analysing the data, we considered the continued merits of these simple yet equally valid unadjusted bivariate statistical tests. However, the appropriate use of an unadjusted bivariate test still required a solid understanding of its utility, assumptions (requirements), and limitations. This understanding mitigated the risk of misleading findings, interpretations and conclusions.

RESULTS

The Cobb angle was 34.5º (27º to 41º) in group I, with significant macroscopic vertebral rotation. In group II, all cases had curvatures with angles <10º and negligible vertebral rotation, as did group III (Table 1).

Table 1. Cobb angle and vertebral rotation*

	Animal	Postoperative Cobb angle	4th month Cobb angle	Vertebral rotation
Group I (posterior medial approach)	1	<10	31	Yes
	2	<10	27	Yes
	3	<10	41	Yes
	4	<10	39	Yes
Group II (posterior paramedial approach)	5	<10	<10	No
	6	<10	<10	No
	7	<10	<10	No
Group III (VAT anterior approach)	8	<10	<10	No
	9	<10	<10	No
	10	<10	<10	No
	11	<10	<10	No

*Cobb Angle measured using frontal plain X-ray study. Vertebral rotation measured by direct observation of the extracted spine; VAT, Video-Assisted Thoracoscopy

The macroscopic postmortem study of the spine after dissection and detachment of the paravertebral musculature supported the radiological findings (Figure 2, 3). Bone disease other than iatrogenic disease was not found in any animal.

A statistically significant difference was found in the Cobb angle between the posterior medial approach and the posterior paramedial approaches (p<0.01). In the same way, a statistically significant difference was obtained in terms of vertebral rotation between the posterior medial approach and the posterior paramedial approaches (p<0.01). In addition, a statistically significant difference in Cobb angle and vertebral rotation was found between the open approaches and the VAT anterior approaches (p<0.01).

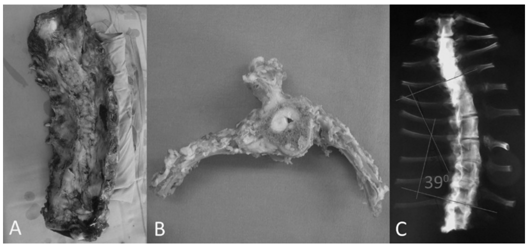

Figure 2. The macroscopic postmortem study. Posterior medial approach specimen (group I): A) extracted spine for macroscopical examination, B) evident rotational component, C) radiograph demonstrating a Cobb angle of 39º (Experimental Medicine and Surgery Department of Gregorio Marañón, 2015)

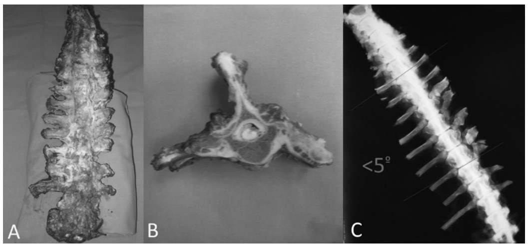

Figure 3. The macroscopic postmortem study. Posteriorparamedial approach specimen (group II): A) extracted spine for macroscopical examination, B) negligible rotational component, C) radiograph demonstrating a Cobb angle <10º (Experimental Medicine and Surgery Department of Gregorio Marañón, 2015)

DISCUSSION

The data obtained were homogeneous for each group, although they are controversial in that they confirm the original hypothesis, namely, that surgical costotransversectomy is a valid technique for creating a scoliosis model in the experimental minipig. Several authors have observed disparate results using costotransversectomy in other species. Piggot (10) suggested that retardation of posterior rib growth, removal of mechanical support from one side of the spine, and disturbance of proprioceptive impulses are the factors which could initiate the deformity. Sevastikoglou et al. (22) also showed unilateral loss of mechanical support provided by the ribs to the spine as the cause of deformation. Langenskiöld and Michelsson (23) pointed to muscle-ligament imbalance as responsible for scoliosis, as did Werneck et al. (18).

Further analysis of our data shows posterior medial costotransversectomy to be the only approach that leads to spinal deformity, whereas the alternative approaches (posterior paramedial and VAT anterior) leave the column almost unaltered. An alternative approach is found in Robin and Stein study in primates, with unsuccessful results. Posterior paramedial (2 cm from midline) approach is described. In the case of bipedal rats, Cañadell et al. (17) did not state the type of incision used for removal of the costotransversal joints. The results for the VAT anterior approach are similar, with no significant curvatures. Therefore, it is interesting to ask why the same osteoarticular injury leads to different structural results depending on the surgical approach used.

Tensegrity is a building principle that was first described by the architect R. Buckminster Fuller (U.S Patent Office, nº 3.063.521, "Tensile-Integrity Structures", patented Nov. 13, 1961) and first visualized by the sculptor Kenneth D. Snelson (U.S Patent Office, nº 3.169.611, "Continuous Tension, Discontinuous Compression Structures", patented Feb. 16, 1965). Ingber defined tensegrity systems as structures that stabilize their shape by continuous tension or "tensional integrity" rather than by continuous compression (24). Other authors describe tensegrity as a structural principle based on the use of isolated compressed components into a continuous network that is stretched in such a way that compressed elements (usually bars) do not touch each other and are united only through tensile elements (usually wires) (25). The most important feature of these structures is that their balance depends solely on tension and compression forces along the axis of each component. In other words, no torsional forces exist in such structures (or these forces are decomposed into force vectors axial to the elements of the structure). Therefore, a tensegrity structure shows the following characteristics: a set of discontinuous compression components interacting with a continuum of tensile components, a pre-stretched structure; dynamically linked components, so that any force applied to one of them is transferred instantly to the entire structure.

In 1998, Ingber used tensegrity successfully to explain some of the properties of the cytoskeleton and cellular signal transduction. A few years before, Levin applied the same concepts in biomechanics to further understand joint function (26) and he disagreed with the traditional model in which the spine is considered a pillar: the weight exerted by the head with anterior and lateral flexion movements should break the structure due to the shear forces generated. Moreover, the forces required to balance a spine whose centre of gravity is constantly changing would be incalculable. The author considers that the purpose of a pillar is stability, but the aim of the spine is flexibility and movement. Biological structures are mobile, flexible, low-energy, and functionally independent of the force of gravity. A pillar, on the other hand, needs a fixed base and a rigid conformation to remain stable and cannot be considered a useful model to explain the biomechanical properties of the spine. Therefore, the vertebral column could be considered a tensegrity structure (Figure 4) in which a set of discontinuous compression components (vertebral bones) interacts with a continuum of stretched components (ligaments and muscles). That is, soft tissues are not only motor appendages, but also part of the structure (27-30).

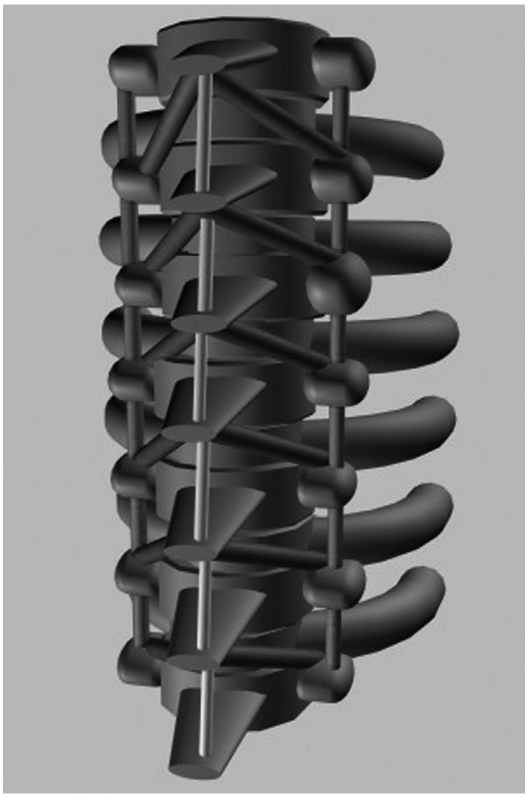

Figure 4. Tensegrity model of the spine: a set of discontinuous compression components (vertebral bones) interacts with a continuum of stretched components (ligaments and muscles). The vertebral column could be considered a tensegrity structure (Experimental Medicine and Surgery Department of Gregorio Marañón, 2015)

According to this new perspective, the experimental data obtained in the present study seem justifiable. The only surgical costotransversectomy procedure that produces frank damage to the muscle-ligament structures of the spine (tension elements) is posterior medial incision, as it requires detachment of the paravertebral musculature following the contour of the spine (see Animation, Supplemental Digital Content 1, which simulates the alteration of tensegrity forces produced by posterior medial approach costotransversectomy. Tensile component imbalance is observed; see Animation, Supplemental Digital Content 2, which simulates the deformation process resulting after tensegrity alteration. Both frontal and rotational deformity is observed). However, with a posterior paramedial or VAT anterior approach, this musculature remains intact, with the only tensional component injury occurring at the level of costotransversal joint ligaments. Similar results are observed in other studies, when a midline posterior approach was performed (22, 31) or a paraspinal muscular imbalance was produced (18,32).

We believe that our results, while not conclusive, are significant and may help to improve our knowledge of motor diseases, particularly IS. Further investigation is needed to validate the spine as a biotensegrity structure and to support any potential clinical applications arising from such an approach.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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SUPPLEMENTAL DIGITAL CONTENT (SDC)

Supplemental Digital Content 1. Animation that simulates the alteration of tensegrity forces produced by posterior medial approach costotransversectomy. Tensile component imbalance is observed.

http://ljkzedo.ba/supplemental-digital-content-sdc/

Supplemental Digital Content 2. Animation that simulates the deformation process resulting after tensegrity alteration. Both frontal and rotational deformities are observed.

http://ljkzedo.ba/supplemental-digital-content-sdc/