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Effect of neurocentral cartilage destruction on spinal growth in immature rabbits

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Abstract

Objective: This study was performed to observe the effect of neurocentral cartilage (NCC) destruction on spinal growth in immature rabbits.

Methods: The NCC of the lumbar vertebrae of 24 4-week-old female rabbits was destroyed through posterolateral and anterior approaches, and three-dimensional computed tomography examinations were performed 3 months after the procedure.

Results: Scoliosis was successfully induced in all rabbits of both the anterior and posterolateral approach groups. The scoliosis exceeded 10 degrees in three rabbits, which exhibited coronal scoliosis of the spine, unequal length and thickness of the bilateral pedicles, and rotation of the vertebrae. Scoliosis was not observed in the control group.

Conclusions: Destruction of the unilateral NCC in immature rabbits can induce structural scoliosis, similar to the pathological features of human scoliosis. The Cobb angles are similar after NCC destruction by a posterolateral approach and under direct vision via the anterior approach.

Keywords

Scoliosis, etiology, neurocentral cartilage, rabbits, computed tomography, Cobb angle

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Introduction

The etiology of idiopathic scoliosis involves heredity, metabolic, and neurogenic factors; the paravertebral muscles; bony development; and other factors. However, there is a lack of consensus.[1–5] As our in-depth understanding of the bony development of

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the spine has grown in recent years, bony factors have also been recognized as a cause of scoliosis. Studies in this field have been based on the following three points: 1) scoliosis is a three-dimensional structural malformation with multiple planar aberrations involving coronal scoliosis, sagittal kyphosis, and horizontal rotation; 2) growth and development of the spine, as part of the axial skeleton, also follow the general rules of skeletal development; and 3) load in the vertical direction plays a role in growth and follows the Hueter–Volkmann law. According to the general law of bone growth and development, spinal growth depends on several important surrounding ossification centers, including the vertebral bodies, bilateral articular processes, transverse processes, and spinous processes. The vertebral epiphysis mainly includes the upper and lower vertebral growth plates, circular epiphysis at the middle segment of the vertebrae, and the neurocentral cartilage (NCC) at the junction of the vertebrae and pedicles. Based on this anatomy, many scholars have successfully established experimental models of structural scoliosis in a variety of animals (rats, rabbits, pigs, and goats) using distinct etiological hypotheses in the scoliosis process.[6–9] Structural scoliosis models can be used to study human scoliosis only if there are structural rather than postural changes; i.e., the spinal structure shows bony changes similar to those in human scoliosis, including coronal scoliosis, vertebral rotation, and pedicle asymmetry. The primary cause of scoliosis remains controversial. Some scholars believe that growth imbalance in the coronal plane (longitudinal growth asymmetry of the vertebrae) leads to vertebral rotation and aggravated scoliosis.[10] Others have proposed that growth imbalance in the horizontal plane (front and rear growth asymmetry of the vertebrae) leads to vertebral rotation, subsequently inducing scoliosis.[11]

The NCC is a cartilage connection between the vertebrae and bilateral arches and is formed during the development of the spinal bony structure.[12] It is a cartilaginous area associated with the upper and lower growth plates of the vertebrae, upper and lower articular processes, left and right transverse processes, and spinous epiphysis; its two-way ossification feature enables simultaneous effects on the development of the vertebrae and arches.[13] In 1980, Beguiristain et al.[14]used screws to selectively destruct the NCC of the right thoracic vertebrae (4–5 segments) via the pedicle through the posterior approach in eight pigs (2–3 months old) and achieved structural scoliosis, with the nonsurgical side convex to the surgical side. Since then, several scholars have repeated such experiments but failed to construct scoliosis models.[15–17] A study by Schlösser et al.[18] in 2013 suggested that pre-existent rotation of the spine is related to asymmetrical closure of the neurocentral junctions. Therefore, whether NCC destruction can cause scoliosis remains greatly controversial.

In the present study, we destroyed the unilateral NCC in immature rabbits using two methods, (the use of screws through the posterolateral approach and under direct vision through the anterior approach) and comparatively assessed the two scoliosis models. This study was performed to elucidate the role of the NCC in spinal growth and thus facilitate treatment of scoliosis according to its etiology in the future.

Materials and methods

Animals

Twenty-four 4-week-old (immature) New Zealand female rabbits were included in the study. Their body weights ranged from 400 to 600 g, and they were free from diseases such as growth and developmental abnormalities, spinal deformities, and spinal tumors.

Grouping

The 24 rabbits were randomized into three equally numbered groups. In the first group (posterolateral group, n = 8), NCC destruction was performed through the posterolateral approach; a screw with a diameter of 1.0 mm (threaded portion removed) was inserted into the front vertebrae through the base of the transverse processes of the L3 to L5 vertebrae to vertically destroy the NCC (Figure 1). In the second group (anterior group, n = 8), NCC destruction was carried out through the anterior approach; the NCC was completely destroyed from the lower to upper part of the lower lumbar disc under direct vision using a needle tip (Figure 2). In the third group (sham-operation group, n = 8), the NCC was exposed until the anterolateral spine was reached using the same surgical approach as in the other two groups; however, no operation was performed on the spine before suturing.

Surgical and destruction methods

The animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (2 mL/kg). The rabbits were subsequently placed in the left lateral position, and a horizontal incision of about 1.5 to 2.0 cm in length was made at the lateral margin of the right erector spinae muscles along the level of the L4 vertebra and downward to the surfaces of the erector spinae and lumbar quadrate muscles. The erector spinae and lumbar quadrate muscles were bluntly dissected along the space between them to expose the right transverse process at the L3 to L5 vertebrae. To reduce intraoperative bleeding and trauma, exposure was terminated at this site in the posterolateral approach and sham-operation groups.

Figure 1. Diagram of neurocentral cartilage destruction through the posterolateral approach.

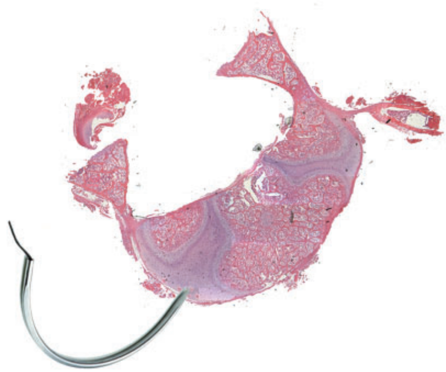

Figure 2. Diagram of neurocentral cartilage destruction through the anterior approach.

In the anterior approach group, a hook was used to gently pull the greater psoas muscle to expose the vertebra and disc in the front, where white longitudinal cartilage was visible at the lateral margin of the vertebra and near the transverse process; this structure was the NCC (Figure 3). In the posterolateral approach group, a 1.0-mm hole perpendicular to the surface of the transverse process was drilled in the base of the transverse process using an electric drill (Figure 4), through which a 1.0-mm screw was inserted into the front of the vertebra (Figure 5). In the anterior approach group, a fine needle tip was used to completely

Figure 3. Neurocentral cartilage structure under direct vision through the anterior approach. NCC, neurocentral cartilage.

Figure 4. Destruction of neurocentral cartilage through the posterolateral approach.

destroy the white cartilage under direct vision until a slightly blended sensation was felt, indicating that part of the cartilage had been removed from the inner wall of the spinal canal. Hemostasis was then applied, and stratified suturing was performed using 5-0 Coated Vicryl Plus Antibacterial Suture (Ethicon, Inc., Somerville, NJ, USA). Within 3 days of surgery, the animals were given an intramuscular injection of 40,000 U/kg of penicillin daily to prevent infection.

Imaging

Three months after the operation, the animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (2 mL/kg), and a three-dimensional computed tomography scan of the lumbar vertebrae was performed for three-dimensional reconstruction.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA). Multiple-group comparisons were conducted by the Kruskal–Wallis H test. Pairwise group comparisons were conducted by the Nemenyi test. A P value of <0.05 was considered statistically significant.

Ethics statement

This study protocol was approved by the ethics committee of the Children's Hospital of Fudan University.

Results

All 24 animals survived postoperatively with no signs of wound infection and with good wound healing. They showed no manifestations of nerve injury and exhibited good limb motion and normal urination and defecation. Scoliosis was successfully induced in all rabbits of both the anterior approach group (Figure 6) and posterolateral approach group (Figure 5). In three rabbits, the scoliosis exceeded 10 degrees; the scoliosis-related changes included coronal scoliosis of the spine, unequal length and thickness of the bilateral pedicles, and rotation of the vertebrae (Figure 8).

The anterior and posterolateral approach groups showed statistically significant differences compared with the sham-operation group ($P < 0.05$) (Figure 7). However, no significant difference was found between the anterior and posterolateral approach groups ($P > 0.05$) (Table 1).

Figure 5. Radiograph and three-dimensional computed tomography image (ventral view) of a rabbit in the posterolateral approach group. The Cobb angle was 18.96 degrees, and the scoliosis was convex to the nonsurgical side.

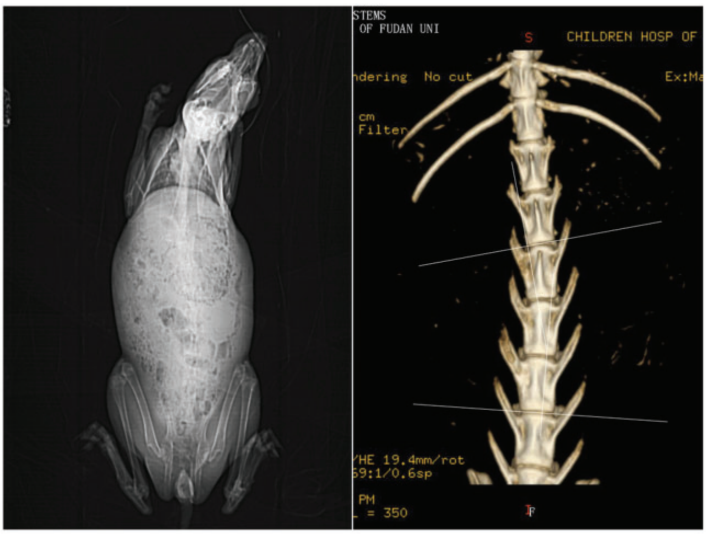

Figure 6. Radiograph and three-dimensional computed tomography image (ventral view) of a rabbit in the anterior approach group. The Cobb angle was 16.53 degrees, and the scoliosis was convex to the non-surgical side.

Discussion

To date, studies on scoliosis models for bony development have focused on two aspects: scoliosis generated through a tethered cord in the coronal plane and rotation of vertebrae in the horizontal plane. This also reflects the scholars' interpretation of

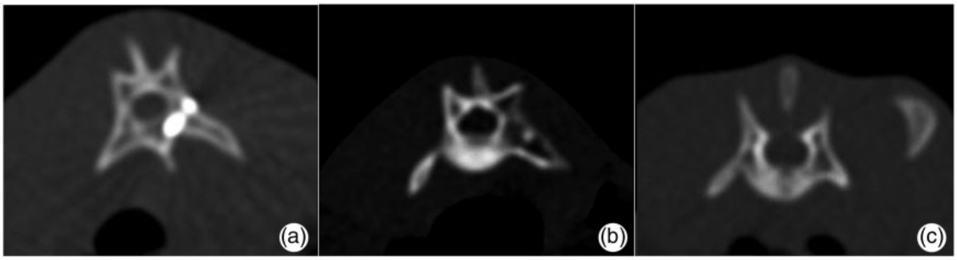

Figure 7. Vertebral changes in computed tomography images, including unequal length and thickness of the bilateral pedicles and rotation of the vertebrae. (a) Posterolateral approach group. (b) Anterior approach group. (c) Control group.

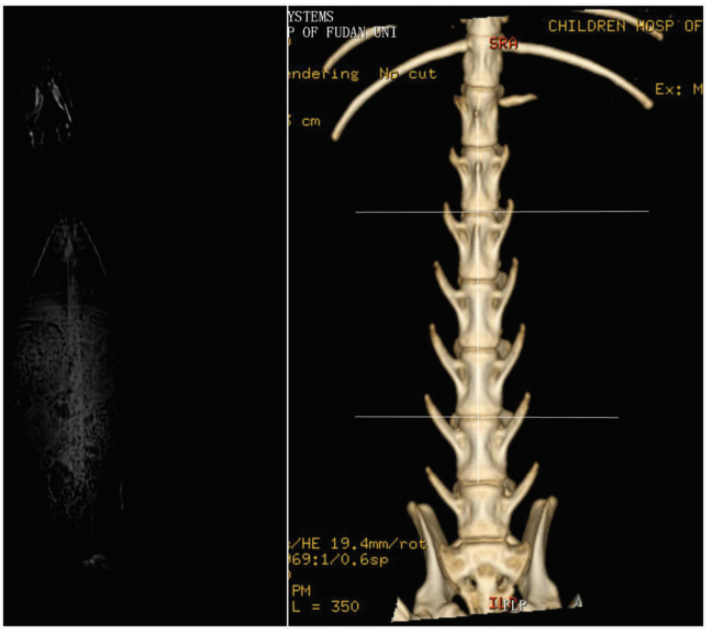

Figure 8. Radiograph and three-dimensional computed tomography image (ventral view) of a rabbit in the sham-operation group. The Cobb angle was 0 degrees.

the primary cause of scoliosis: whether i) scoliosis in the coronal plane causes secondary rotation in the horizontal plane or ii) asymmetry of the left and right vertebrae in the horizontal plane causes scoliosis in the coronal plane. Roaf[11] proposed that rotation of the vertebrae in the horizontal plane is the trigger of scoliosis. However, Dickson et al.[10] suggested that sagittal deformation of the apical vertebra is the starting point of scoliosis. Sarwark et al.[19] successfully established a scoliosis model by suturing the lower angle of the scapula and the ipsilateral pelvis in mice. Tsuang et al.[20] then repeated this experiment in rabbits and established a scoliosis model. Carpintero et al.[21] used silk to suture the transverse and upper spinous processes in rabbits to

Table 1. Cobb angles in the three groups.

Cobb angles in the posterior-lateral approach group	Cobb angles in the anterior approach group	Cobb angles in the sham-operation group
18.96	16.53	0.00
14.18	7.62	0.00
10.91	6.46	0.00
9.83	11.04	1.00
9.60	9.66	0.00
8.54	9.53	1.12
8.34	19.59	2.55
8.49	7.30	0.00

All values are given in degrees

induce scoliosis, which showed progression within the observation period and was characterized by vertebral rotation. Bundling and tethering have since been used to construct scoliosis models in large animals. For example, Braun et al.[22] successfully constructed a scoliosis model in goats by posterior tethering, costectomy, contralateral rib bundling, and other operations. Schwab et al.[9] generated a scoliosis model in swine by performing unilateral tethering via the posterolateral approach using pedicle screws. In 2008, Zhang and Sucato[23] constructed a scoliosis model in swine by destroying the unilateral NCC. In 2011, the Spanish scholars Caballero et al.[24] constructed a scoliosis model by anterior thoracoscopic-assisted galvanocautery of the NCC. In these reports, analysis of the pathological changes of scoliosis revealed that the vertebrae indeed showed changes in the horizontal plane in scoliosis constructed by means of tethering, but with no asymmetry of the bilateral pedicles. In addition, the vertebrae often act as a whole when they rotate with respect to the thorax and ribs, rather than forming a relative angle between the central axis of the anterior vertebra and that of the posterior vertebral lamina and process. Moreover, coronal wedging deformation of scoliosis caused by vertebral asymmetry in the horizontal plane is similar to that of human scoliosis. Therefore, horizontal asymmetry is more likely to be the earliest pathological change of scoliosis based on the findings of the above-mentioned experimental study.[24]

The animals currently used to study scoliosis mainly include chickens, mice, rabbits, swine, and goats. Of these, chickens and mice undergo operations in the upright position (bipedal mice) and show the most similarities to the human spine in terms of the vertical load; however, their vertebrae are small and difficult to operate. The vertebrae of swine and goats are larger and more similar to the shape of the human spine at the evolutionary level, but they lack stress in the vertical direction. The growth and development of the spine follows the Hueter–Volkmann law, and stress in the vertical direction is one of the main factors that cannot be ignored in the formation of scoliosis. Thus, scoliosis in swine and goats is not the most appropriate model to simulate the load of the human spine, and this has become an unavoidable drawback. Meanwhile, the lumbar vertebrae of rabbits are close to the upright state when they are in the sitting position with load in the vertical direction (Figure 9). According to our preliminary experiments, the NCC of the rabbit spine is similar to that of the human spine in terms of structure and location, and it is easier to be destroyed. Thus, we selected rabbits as the subjects in this study.

The ribs indisputably play a certain role in the development of scoliosis. Previous scholars established scoliosis models by incision of ribs alone, tethering of unilateral ribs, or extension of ribs.[25–27] Therefore, the effect of ribs on scoliosis should be excluded when selecting experimental segments; this will help to understand the relationship between the NCC structure and scoliosis

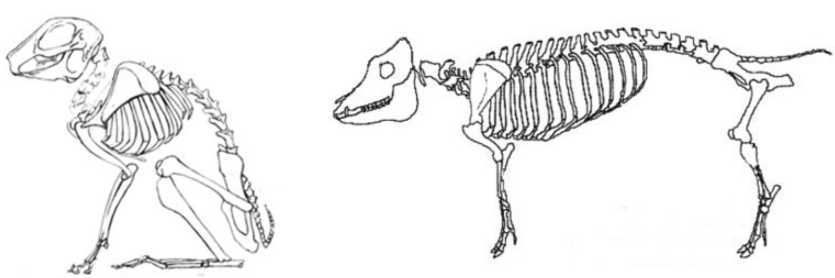

Figure 9. Lumbar vertebrae of rabbits are close to the upright position and more likely to simulate the vertical load of the human spine.

and is a major reason why we selected the lumbar vertebrae of rabbits. Because the ribs, rib complex, and transverse processes do not interfere with the stability of the lumbar vertebrae, the lumbar vertebrae can be used as independent experimental subjects to explore the relationship between the NCC structure and scoliosis and were selected as the experimental segments in this study.

NCC destruction through the posterolateral approach is a novel aspect of the present study. Beguiristain et al.[14] and Zhang and Sucato[23] used pedicle screws to destroy the NCC directly through the posterolateral approach. Because the pedicle screw is moved forward to destroy the NCC, the development of asymmetry of the bilateral pedicles after modeling is not entirely due to the destruction of the growth-promoting ability of the NCC. This is partly because the bone channel of the pedicle is occupied by the pedicle screw, which prevents the pedicle from achieving bony accumulation and thereby leads to bilateral asymmetry. In this study, the screw was inserted obliquely to the anterior medial side in the posterolateral approach group, near the transverse base at the posterolateral vertebrae (Figure 4); this prevented the screw from occupying the pedicle, which resulted in a more reliable effect of the NCC on pedicle development. The diameter of the screw was 1.0 mm (the threaded portion was removed) and increased to about 1.2 mm when including the threaded portion, and the length was 5.0 mm, which was sufficient to pass through the NCC. Postoperative computed tomography confirmed that the screw had passed through the NCC and induced satisfactory destruction.

At 3 months postoperatively, scoliosis was successfully induced in all rabbits, three of which developed scoliosis that exceed 10 degrees in the posterolateral and anterior approach groups; however, the scoliosis in the sham-operation group reached less than 3 degrees. The postoperative Cobb angle was significantly different between the posterolateral approach and sham-operation groups as well as between the anterior approach and sham-operation groups (all $p < 0.05$), indicating effective NCC destruction using both approaches. However, the postoperative Cobb angles showed no significant differences between the posterolateral and anterior approach groups. These findings indicate that both methods can be used for NCC destruction with no significant difference in the outcomes of NCC destruction. According to the principle of epiphyseal destruction in children, local damage to the epiphyseal plate does not necessarily lead to loss of function; the effect of the damage on function depends on the damaged area as well as the absence or presence of bony bridging. If bony bridging is absent and instead, a small area of the epiphyseal plate is damaged or objects (such as adipose tissue or screws) occupy the bone channel, the epiphyseal plate may only display declined function or remain unaffected instead of showing loss of function. However, if a large area of the epiphyseal plate is damaged or the entire plate is completely damaged, a large bone bridge forms and the epiphyseal plate loses growth ability.[28] Thus, the posterolateral approach group in the present study may have displayed reduced growth ability at the surgical site because the bone channel was occupied by the screw. Meanwhile, because the NCC was completely removed under direct vision in the anterior approach group, the destruction was even more thorough, and complete loss of growth ability may have occurred at the surgical side alongside excessive growth at the nonsurgical side, resulting in more severe scoliosis.

Caballero et al.[24] and Zhang and Sucato[23] developed scoliosis models by destruction of the NCC, in which they derived concave and convex NCCs at the surgical side, respectively. We believe these outcomes may have been caused by decreased vertebral height on the surgical side. In the present study, most cases of scoliosis were convex to the left side (i.e., concave on the surgical side), corroborating the findings reported by Caballero et al.[24] while differing from those obtained by Zhang and Sucato.[23] Three possible reasons for this discrepancy are as follows. First, Caballero et al.[24] applied anterior thoracoscopic galvanocautery to completely destroy the NCC, while Zhang and Sucato[23] used screws for destruction. According to the principle of epiphyseal destruction, the occupying screw does not lead to the formation of a bone bridge and the undamaged NCC still has growth ability, indicating that destruction is not as thorough as in the anterior approach. Second, in the study by Zhang and Sucato,[23] the pedicle screw occupied the space of the pedicle, leading to pedicle bone deficiency. This affected the pedicle growth, thereby causing a vertebral rotation direction different from that in the present study, in which the pedicle space was not occupied by the screw. Therefore, different directions of scoliosis were achieved. Third, Zhang and Sucato[23] adopted nonupright animals in their experiments. The lumbar vertebrae of rabbits are in a semi-upright state, and stress in the vertical direction may cause uneven load on both sides of the spine, resulting in inconsistent scoliosis.

Conclusions

This study demonstrated that destruction of the unilateral NCC in immature rabbits can induce structural scoliosis characterized by coronal curvature, vertebral rotation, and unequal length and thickness of the pedicle, similar to the pathological features of human scoliosis. In addition, no significant differences were found in the Cobb angles between scoliosis caused by NCC destruction using the posterolateral approach and scoliosis caused by NCC destruction under direct vision via the anterior approach.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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