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Basic Science

Does elevated osteopontin level play an important role in the development of scoliosis in bipedal mice?

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Abstract

BACKGROUND CONTEXT: Previous studies implied indirectly that an elevated osteopontin (OPN) level might play a key role in the pathomechanism of adolescent idiopathic scoliosis. Nonetheless, up to now, no direct evidence was proposed to determine this issue.

PURPOSE: The aim was to determine the role of OPN in the pathomechanism of scoliosis.

STUDY DESIGN: This was an experimental study to investigate the role of OPN in a bipedal mouse scoliosis model.

METHODS: All procedures were performed under the approval and supervision of the Institutional Animal Care and Use Committee of our university. A new bipedal mouse model with elevated OPN level was established in this study. Amputation of forelimbs and tail was performed on 80 male C3H/HeJ mice at the age of 3 weeks. Then, these mice were randomly divided into two groups: Group A consisted of 40 mice treated with OPN 40 mg/kg daily and Group B consisted of the remaining 40 mice treated with saline. Then, 40 quadruped mice with saline were included in Group C. Body length, X-rays, and computed tomographic scans were obtained at the twentieth week. Then, scoliosis incidence, curve magnitude, and circulating OPN level were compared among groups.

RESULTS: Osteopontin level was significantly higher in Group A compared with that in Groups B and C. Spine deformity was identified in 37 mice in Group A, 21 mice in Group B, and 5 mice in Group C. The average Cobb angle was 29.8° in Group A, 20.9° in Group B, and 17.5° in Group C. Although no significant difference of body length was found, significant statistical difference was noted in terms of scoliosis incidence and curve magnitude, among the three groups.

CONCLUSIONS: The results of the present study indicated that the elevated OPN level might play an important role in the etiopathogenesis of scoliosis, that is, it not only raises the risk for scoliosis in bipedal mice but also contributes to curve progression.

Keywords: Bipedal mouse; Scoliosis; Elevated osteopontin level; Erect position; Endochondral ossification; Enzyme linked immunosorbent assay

FDA device/drug status: Not applicable.

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Introduction

Adolescent idiopathic scoliosis (AIS) is a common three-dimensional spinal deformity mainly affecting otherwise healthy girls during the pubertal growth spurt [1]. Although considerable advances have been achieved in the past decades, there is no consensus in reference to the proposed theories for the etiopathogenesis of AIS. Based on the current findings, it is universally received that AIS is caused by multifactors, including genetic defects, melatonin signaling dysfunction, abnormality of nervous system, and systemic platelet calmodulin dysfunction [1–4].

Osteopontin (OPN), a downstream effector regulated by melatonin, was reported to be involved in the development of AIS. Moreau et al. [5] performed an ELISA study and found that plasma OPN levels were significantly higher in AIS patients and correlated with curve severity. And then, in his other work, knocking out the OPN gene prevented successfully C57BL/6j bipedal mice from developing scoliosis [6]. The aforementioned findings both implied indirectly that elevated OPN level might play a key role in the onset and development of AIS. Nonetheless, up to now, no direct evidence was proposed to further determine the relation between elevated OPN level and the development of AIS. On this background, bipedal mice with high OPN level were established in this study to testify directly whether elevated OPN level could result in the onset and aggravation of scoliosis and provide more evidence about OPN hypothesis.

Materials and methods

All procedures in this study were performed under the approval and supervision of the Institutional Animal Care and Use Committee of our university. Male C3H/Hej mice, aged 3 weeks, were used in this study. A total of 120 mice were bred in group cages under specific pathogen-free room conditions. During the experimental course, temperature, light, and humidity were controlled (22±2°C, lights on from 7 AM to 7 PM, 50±5%).

Bipedal amputation was performed for 80 of 120 mice at the age of 3 weeks under anesthesia with admixture (1:1) of ketamine (0.05 g/mL) and diazepam (5 mg/mL). After anesthesia taking effect, bilateral forelimbs were amputated at the humeroscapular junction, and the tail was excised at the basal levels. One day after the operation, these 80 mice were randomly divided into two groups: Group A consisted of 40 mice which were treated with OPN 40 mg/kg daily by intraperitoneal injections and Group B consisted of the remaining 40 mice treated with saline water via the same approach. Then, the 40 remaining mice with no treatment were included in Group C. Two days later, three groups of mice were housed in special cages (shown in Fig. 1), in which the positions of food and water were elevated to induce these mice to maintain a standing posture.

X-rays and computed tomographic scans were obtained at the twentieth week after operation under anesthesia to determine the development of spinal deformity. Before radiographic examination, the mouse was placed in a prone position with the head fixed on a support. Then, the support was set at a slant of 30° relative to the horizontal plane to avoid postural scoliosis. Curve types were classified according to the Scoliosis Research Society (SRS) guidelines, and curve magnitude was measured via the Cobb method. Two more experienced spine surgeons were invited to remeasure the Cobb angle of scoliosis. Then, the intraclass correlation coefficient was measured to be more than 95%, indicating a very high intertester reliability. Then, the distance between the nose and the anus was measured to evaluate the body length of mice on the ventral side. After that, blood samples were collected in serum gel tubes and centrifuged for 20 minutes at 3,000 rpm at 4°C; serum samples were then stored at −80°C. Then, serum OPN levels were determined for each mouse by an ELISA kit (Quantikine. Mouse Osteopontin; R&D Systems, Minneapolis, MN, USA).

The scoliosis incidence, curve magnitude, and serum OPN levels were recorded in each group. Differences between the two groups were compared in terms of the aforementioned indices with an analysis of independent-sample *t* test and chi-square test analysis. Statistical Package for Special Software (SPSS 13, Chicago, IL, USA) was used for all statistical analysis. Statistical significance was set at $p<.05$.

Results

Twenty weeks after operation, each mouse in this study was identified to have the capacity of maintaining standing position (shown in Fig. 1). The result of ELISA experiment (shown in the Figure 2) demonstrated that OPN level was significantly higher in Group A (207.3±85.1 ng/mL) than that in Group B (74.6±20.1 ng/mL) and Group C (83.7±32.2 ng/mL). Nonetheless, no significant difference of body length was found among the three groups.

According to SRS scoliosis criteria, results of radiographic examination (shown in Figure 3 and Table) showed that spine deformity was identified in 37 mice (92.5%) in Group A and 21 mice (52.5%) in Group B. The average Cobb angle of scoliosis mice was calculated to be 29.8° in bipedal mice with elevated OPN level, 20.9°in control bipedal mice, and 17.5° in quadruped mice. Significant statistical difference was noted, in terms of scoliosis incidence and curve magnitude, between Groups A and B. No significant difference of mice height was found among the three groups.

Discussion

The present study established a bipedal mouse model with elevated OPN level to research directly on the relationship between OPN and AIS. The results of our study showed that an obvious rise in scoliosis incidence and curve magnitude