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Original Article

Bone marrow-derived mesenchymal stem cells (BM-MSCs) inhibit apoptosis of spinal cord cells in a kaolin-induced syringomyelia-associated scoliosis rabbit model

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Abstract: The mechanisms and causes of scoliosis are believed to be multifactorial. Syringomyelia can often be found in scoliosis patients but the relationship between the two remains obscure. In this study, based on a rabbit model of syringomyelia-associated scoliosis, the involved pathological mechanism was explored in an attempt to further understand the relationship. This will also be helpful in determining how scoliosis occurred. In this study, a syringomyelia-associated scoliosis rabbit model was established by kaolin-injection technique. Spinal cell apoptosis following scoliosis and syringomyelia induction were analyzed. Furthermore, the effect of bone marrow-mesenchymal stem cell (BM-MSCs) transplantation on spinal cell apoptosis and on incidence of scoliosis and syringomyelia were assessed. Most of the experimental animals injected with kaolin developed progressive scoliotic curves and syringomyelia. Syrinx and scoliosis were found in 64.7% and 58.8% of the experimental animals. Syringomyelia-associated scoliosis appeared in 41.2% of the animals. Syrinx size and scoliotic curves increased with time. Apoptosis was found on postoperative day 3 both in surgical segments and adjacent segments in the spinal cord, peaking at week 6. The number of apoptotic cells was significantly lower in BM-MSCs transplantation group compared with the saline-injection group. Fewer rabbits in the BM-MSCs injection group developed scoliosis or syringomyelia by the end of the experiment. Our findings indicate the potential value of kaolin-induced scoliotic animal models. For the first time, we studied features of apoptosis of spinal cells in a syringomyelia-associated scoliosis rabbit model. Our results demonstrate that BM-MSCs transplanted into the spinal cord decrease both apoptosis of spinal cells and incidence of scoliosis and syringomyelia.

Keywords: Animal model, scoliosis, syringomyelia, apoptosis, mesenchymal stem cell

Introduction

Scoliosis is a common spine disease. The prevalence of scoliosis is approximately 1.02% among primary and middle school students [1]. The mechanisms and causes of scoliosis have not been identified, however, especially for idiopathic patients. The cause of scoliosis is believed to be multifactorial because of known associations between development of scoliosis and growth, hormonal secretion, gravity, and other factors. The association between apoptosis and scoliosis has been reported recently [2]. The mechanism of apoptosis in the pathogenesis of scoliosis and syringomyelia remains unclear, however, despite the number of studies performed.

To study spinal deformity and test novel treatments for scoliosis, many scoliosis models have been developed in recent years [3, 4]. Most of them apply mechanical tethering techniques. These tethering techniques are considered reliable approaches for generating animal models but not so optimal for scoliotic research. Currently, the surgical correction, fixation, and fusion process is a common choice for scoliosis patients. This provides a satisfactory deformity corrective effect but at the cost of spinal movement [5]. Previous studies have suggested that the decreased osteogenic differentiation ability of marrow-mesenchymal stem cells (MSCs) might be one of the possible mechanisms leading to low bone mass in adolescent idiopathic scoliosis [6]. Recently, use of stem cells for

Table 1. Summary of experimental groups

Group	n	Injection	Transplant	Survival time	Assay
A (Ctrl)	12	Saline	/	/	Apoptosis
B1	5*	Kaolin	/	3 days	Apoptosis
B2	5	Kaolin	/	2 weeks	Apoptosis
B3	5	Kaolin	/	4 weeks	Apoptosis
B4	5*	Kaolin	/	6 weeks	Apoptosis
B5	18*	Kaolin	/	12 weeks	SM/Scoliosis
C1 (TM)	5*	Kaolin	Saline	6 weeks	Apoptosis
C2 (TM)	5	Kaolin	BM-MSC	6 weeks	Apoptosis
C3 (TM)	18	Kaolin	BM-MSC	12 weeks	SM/Scoliosis

n, number of animals per group; SM, syringomyelia; *one animal died and failed to complete the assessment.

human disease has been widely investigated as a therapeutic strategy. Neural stem cells have been used for treatment of neurological diseases such as spinal cord injury, stroke, etc.

In this study, we first developed and validated a rabbit model of syringomyelia-associated scoliosis. We then examined time-dependent changes of spinal cord cell apoptosis to preliminarily explore the relationship between apoptosis and scoliosis occurrence. We further investigated the effect of bone marrow-mesenchymal stem cells (BM-MSCs) treatment on apoptosis and on incidence of scoliosis and syringomyelia. Our study sheds more light into the pathogenic mechanism of syringomyelia-associated scoliosis and may provide a new possible method for treating the disease.

Materials and methods

Experimental animals

All animal experiments were performed according to guidelines approved by the Ethics Committee of Kunming Medical University (Approval No. SYDW20080125001). A total of 78 Japanese white rabbits (purchased from Hunan SJA Laboratory Animal Co., Ltd, Hunan, China) weighing 1.8-2.5 kg were used for this investigation. Each animal was kept in an individual cage and had free access to food and water. The room temperature range was 20-28°C, with relative humidity of 35-60% and a 12 hour light-dark cycle.

BM-MSCs isolation and culture

Primary BM-MSCs were isolated from the fetus of Japanese white rabbits following published protocol [7]. Briefly, rabbits were anesthetized with pentobarbital sodium, then tibias and femurs from both left and right legs were dissected. 4 mL of BM-MSC culture medium consisting of DMEM/F12 (Gibco) supplemented with 15% fetal bovine serum (FBS, Hyclone) and antibiotics were injected into the bone to extrude marrow. Marrow cells were cultured at a density of 5×10^5 cells/cm^2 in 25 cm^2 tissue culture flasks (BD Biosciences, USA). Nonadherent cells were removed after 24 hours with the medium. Isolated BM-MSCs were defined as P0 and confluent cells were split 1:3 and passaged two times. Passage 2 BM-MSCs were used for transplantation after 7 days of cultivation. Before transplantation, cell surface markers CD34, CD45, CD29 and CD9 were checked by flow cytometry using specific antibodies (SC-7324PE-rcp, Santa Cruz; 561867, 562154, 561409, BD Biosciences) to be sure of their stem cell status [8]. Mesenchymal cells should be negative for CD34 and CD45 but should express CD29 and CD90. CD90 expression was used to estimate the purity of BM-MSCs. Purity of the BM-MSC preparations was > 90%.

Scoliosis/syringomyelia induction

Ten minutes before surgery, general anesthesia was induced by intravenous administration of 3% pentobarbital sodium (Sigma, America) through ear marginal vein at a dose of 30 mg/kg. The skin was shaved and prepared with povidone iodine. Animals were then placed prone on a self-made frame to raise the cervicothoracic junction and avoid interruption from the scapula to facilitate next steps. A midline incision was made over the cervicothoracic junction from C7 to T1. At the base of C7 spinous process, a hole was drilled through the bone using a high-speed drill (diameter of 2.0 mm) but never injuring the dura. The flaval ligament was thin and easily cut off for the exposure of dura. A 100 microliter glass microinjector (Qing Niu medical apparatus and instruments factory, Chengdu, China) was held in a stereotaxic apparatus (SR-6N, Narishige Group, Japan) and a 28-gauge needle was connected to puncture the dura for spinal cord injection. Site of puncture was along the drilled

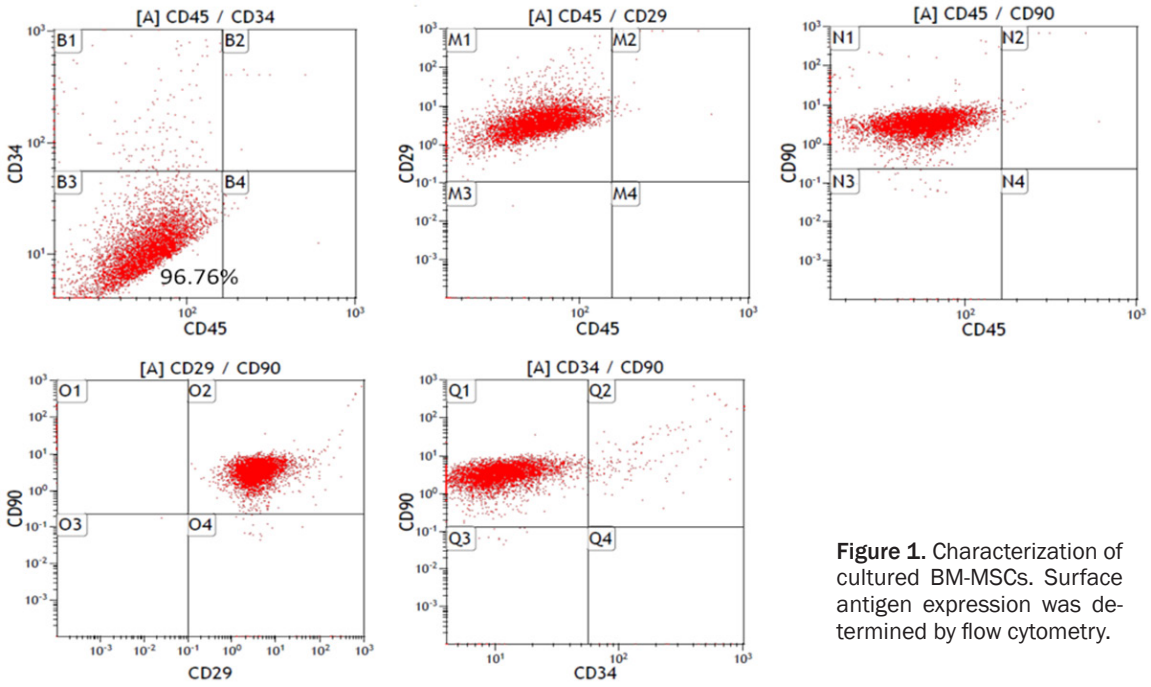

Figure 1. Characterization of cultured BM-MSCs. Surface antigen expression was determined by flow cytometry.

Table 2. Summary of incidence of syringomyelia and scoliosis in rabbit models after kaolin injection

	Postop time				
	Week 4	Week 6	Week 8	Week 10	Week 12
SM	0	23.5% (4/17*)	47.1% (8/17)	64.7% (11/17)	64.7% (11/17)
Scoliosis	0	29.4% (5/17)	58.8% (10/17)	58.8% (10/17)	58.8% (10/17)
SM & scoliosis	0	11.8% (2/17)	29.4% (5/17)	41.2% (8/17)	41.2% (8/17)

SM, syringomyelia; *one animal died and failed to complete the assessment.

bony hole as well as to avoid dorsal vessels and the depth was 2.0 mm under the dura. Then, sixty microliters of 25% kaolin (Sigma-Aldrich) were injected into the center of the spinal cord. Wounds were closed with a single layer silk suture. After surgery, all rabbits were under close observation.

Animal grouping

The 78 experimental rabbits were randomly divided into group A (n = 12), group B (n = 38), and group C (n = 28) (**Table 1**). In group A, sterile saline was injected as these animals were used as control. Group B was further randomly subdivided into 5 subgroups (n = 5 for B1, B2, B3, and B4 subgroup, n = 18 for B5 subgroup). Rabbits were sacrificed and tissues were taken at postoperative day 3 (group B1), week 2 (group B2), week 4 (group B3), and week 6 (group B4) to investigate progression of apoptosis of spinal cord cells. Group C was further subdivided into 3 subgroups (n = 5 for C1 and C2 subgroup, n = 18 for C3 subgroup). Rabbits received injections of 10 ul of BM-MSCs cell (C1) or sterile saline (C2) into the epicenter of lesion site at postoperative week 2 and week 4. Radiographs and MRIs were taken at different time points after surgical induction.

Radiological observation

In a prone position, coronal full-length posterior-anterior radiographs of the spine were taken with heads and bodies straightened at postoperative 4, 6, 8, 12 weeks under general anesthesia. The Cobb method was used to measure and observe curve on the coronal plane. Progression was then recorded.

MRI observation

MRI examinations were performed with a 1.5-T MR scanner (Sonata, Siemens) using cervical

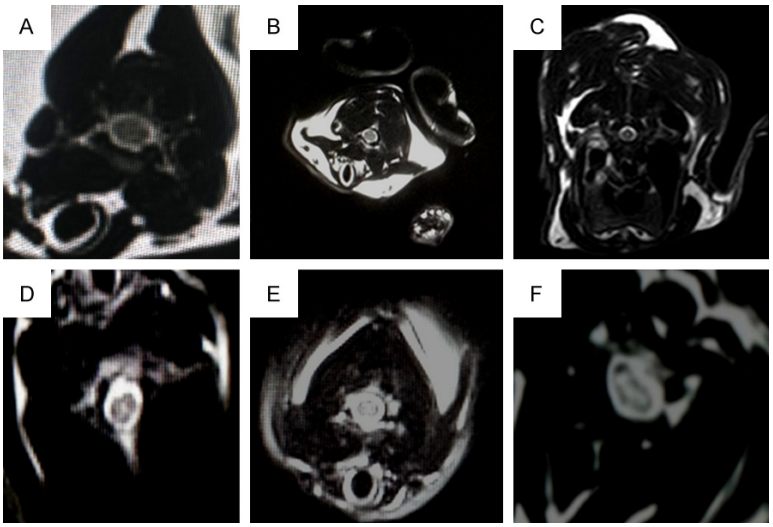

Figure 2. MRI (transverse) scan shows syrinx occurrence and progression. A. Preoperative MRI Scan; B. 4 weeks Postoperative MRI scan; C. 6 weeks Postoperative MRI scan; D. 8 weeks Postoperative MRI scan; E. 10 weeks Postoperative MRI scan; F. 12 weeks Postoperative MRI scan.

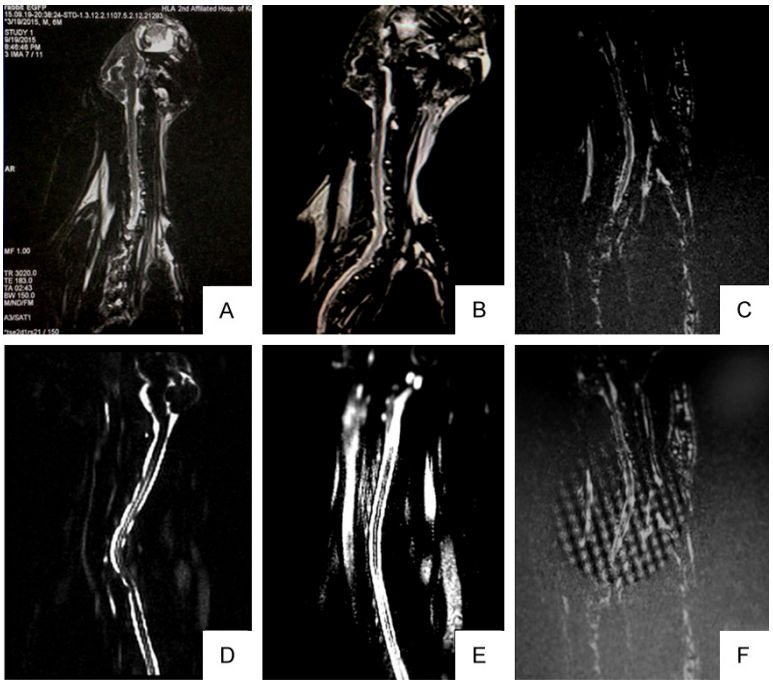

Figure 3. MRI (sagittal) scan shows syrinx occurrence and progression. A. Preoperative MRI Scan; B. Postoperative 4-weeks MRI scan; C. Postoperative 6-week MRI scan; D. Postoperative 8-week MRI scan; E. Postoperative 10-week MRI scan; F. Postoperative 12-week MRI scan.

and spinal coils. MRI scans were performed preoperatively and at 4, 6, 8, 10 and 12 weeks postoperatively. Ten minutes before MRI scanning, general anesthesia was induced by intramuscular injection of 3% pentobarbital sodium (Siagma, America) through the gluteus maximus at a dose of 30 mg/kg.

BM-MSC transplantation

Two weeks after surgery, the rabbits were prepared for transplantation. Animals were assigned randomly into three major groups: one group of rabbits was injected with 5 μL saline as control group (n = 5). The second group of rabbits were transplanted with BM-MSCs (n = 23). All animals were anaesthetized with pentobarbitone sodium (40 mg/kg body weight) and their spinal cords were exposed at the C7-T1 area, as described above in the primary operational section. About 1×10^5 cells/5 μL BM-MSCs were transplanted into the paracentral area of the spinal cord at a depth of 1 mm below the dorsal surface, at a rate of 1 μl/min. Subsequently, the muscles and skin were sutured. In experiment 1, treated rabbits were designed to test apoptosis of spinal cells after transplantation (n = 5). In experiment 2, the treated animals were used to evaluate clinical therapeutic effects (n = 18).

Tissue preparation

At day 3, week 2, 4, and 6 after surgery, animals were sacrificed under anesthesia and spines cord were harvested and post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight. Tissues surrounding spines were removed. Two spinal cord segments (C6-C7, C7-T1) and two adjacent segments (C4-C5, T1-T2) were isolated and investigated for each animal. TUNEL assay is described as follows.

TUNEL staining

TUNEL staining was performed using a commercial kit (Roche, Mannheim, Germany), acc-

ording to supplier's instructions. Briefly, 5 um spinal tissue sections were de-waxed in xylene, rehydrated, and pretreated with proteinase K for 15 minutes at 37°C. After rinsing in 2% hydrogen peroxide for 5 minutes, they were washed with PBS. Sections were incubated with TUNEL reaction mixture for 60 minutes at 37°C in a humidified chamber. After washing with PBS, sections were incubated in a converter-POD solution at 37°C for an additional 30 minutes. Slides were washed again three times in PBS and then reacted with DAB substrate at room temperature for 10 minutes. Eventually, slides were counterstained with hematoxylin and dehydrated in a series of alcohols, mounted under coverslips, and analyzed under a light microscope. Five random visual fields (× 200) were examined on each side in one sample at a magnification of 200. Results are expressed as an apoptotic index (the average number of positive cells per hundred spinal cord cells) to quantify apoptosis.

Statistical analysis

All data were analyzed by GraphPad prism (Graphpad 5.0). Two-tailed unpaired t-test was used to compare data within and between groups. For all tests, data are indicated by mean ± standard deviation and two-sided $P < 0.05$ was considered to be significant.

Results

Phenotype of rabbit BM-MSCs

After isolation, BM-MSCs adhered to the bottom of the flask and formed colonies. In culture, they displayed a fibroblast-like spindle-shaped morphology. No obvious morphological changes were observed during culture period. Cells from the second passage were analyzed and used for transplantation. Expression of cell surface antigens was examined using flow cytometry analyses (FACSCanto II, BD Biosciences, USA). The pattern of staining of the BM-MSC surface markers is shown in **Figure 1**. All cultured cells were CD45 and CD34 negative but positive for CD29 and CD90. The purity of BM-MSCs in culture was examined by CD90 expression. Purity of BM-MSCs was > 90%.

Characteristics of syringomyelia-associated scoliosis rabbit model

Postoperatively, most rabbits undergoing kaolin injection exhibited signs of lethargy and anorexia. Four animals died after the operation and failed to complete the assessment (**Table 2**). Two of them died of intraoperative anesthesia. The other two were excluded due to postoperative infections resulting in death. We assessed incidence of syringomyelia and scoliosis in animals at postoperative week 12.

Syrinx began to appear at postoperative week 6. Transverse and sagittal MRI scans showed syrinx in cervical-thoracic segments. Syrinx was found in 64.7% (11/17) of the experimental animals by the end of the experiment (week 12). Cavity size and amount increased with time during the course of the experiment (**Table 2** and **Figures 2**, **3**).

Scoliosis also began to appear at postoperative week 6 and the apexes of curves were at cervical-thoracic or upper thoracic segments. Scoliosis was found in 58.8% (10/17) of the experimental animals by the end of the experiment (week 12). Follow up spinal radiographs showed a gradual increase of coronal curve during the continuous phase after surgical induction (**Table 2** and **Figure 4**). Moreover, syringomelia-associated scoliosis appeared in 41.2% (8/17) of the experimental animals.

These findings indicate that our constructed model has typical syrinx and scoliosis representation. Therefore, this constructed animal model was close to clinical cervical syringomyelia accompanied with scoliosis.

Spinal cord cells apoptosis

To address the impact of apoptosis on incidence of scoliosis and syringomyelia in rabbits, we measured spinal cord cell apoptosis by TUNEL method. Animals were sacrificed under anesthesia at postoperative day 3 and weeks 2, 4, and 6 to test apoptosis. TUNEL-positive cells were found in all specimens. At postoperative day 3, compared to control group, a few more TUNEL-positive cells were observed in spinal cords of both subadjacent segments and surgical segments in the kaolin-induced group (**Figure 5**). At a 2-week time point, the percentage of TUNEL-positive cells within subadjacent segments was increased by 24 ± 3.2% ($P < 0.0001$), compared with surgical segments 17.8 ± 6.0% ($P = 0.014$). At a 6-week time point, surgical sections demonstrated the highest number of TUNEL-positive cells (24.3 ±

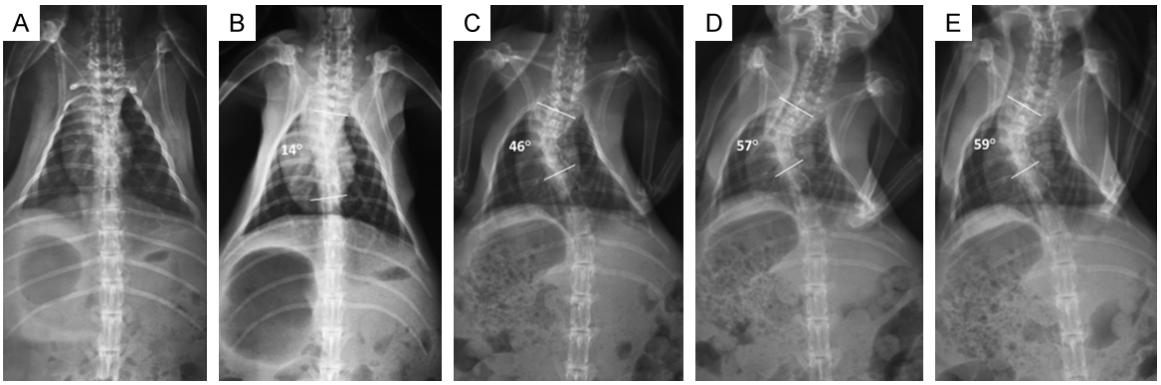

Figure 4. Posterior-anterior radiographs of the spine and scoliosis development (rabbit #21). A. Postoperative 4-week; B. Postoperative 6-week, scoliosis appeared and Cobb angle was 14°; C. Postoperative 8-week, scoliosis developed to 46°; D. Postoperative 10-week, scoliosis developed to 57°; E. Postoperative 12-week, scoliosis developed to 59°.

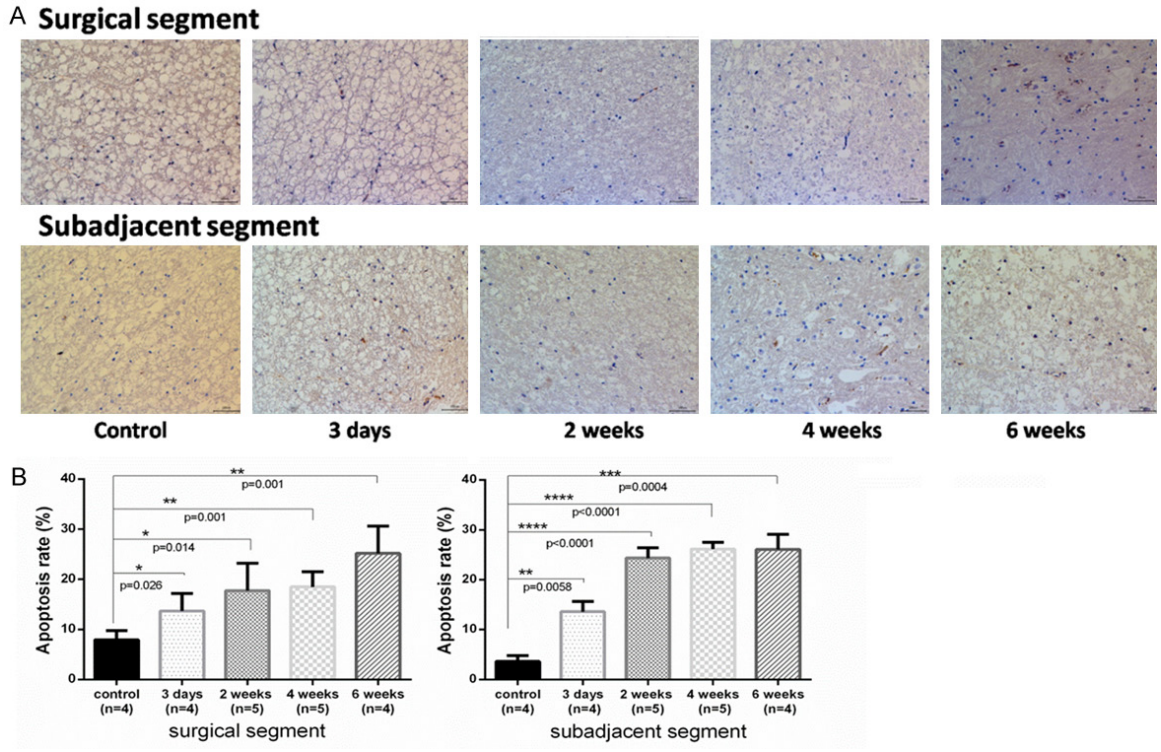

Figure 5. TUNEL staining of (x200) of apoptotic cells in spinal cord surrounding the surgical site at postoperative day 3, week 2, 4, 6. A. TUNEL-positive cells in the spinal cord of the rabbit model. B. The statistical data are shown as apoptotic index (the average number of TUNEL-positive cells per hundred spinal cord cells ± SD). Surgical (C6-C7, C7-C8) versus sub-adjacent (C4-C5, C9-C10) levels. *Significant difference was found between experimental group and control group.

4.7) compared to controls (P = 0.001) but adjacent segments remained with a similar apoptotic rate as our 4-week time point (P = 0.0004). Compared to adjacent sections, surgical sections had fewer TUNEL-positive spinal cells. The mean number of TUNEL-positive spinal cells increased over this 6 weeks period.

BM-MSCs reduce apoptosis in treated rabbits

At week 6, we evaluated the effect of BM-MSC transplantation on apoptosis of spinal cord cells. Apoptosis was rarely observed at the defective spinal cord region around transplanted BM-MSCs (**Figure 6**). **Figure 6A** shows the

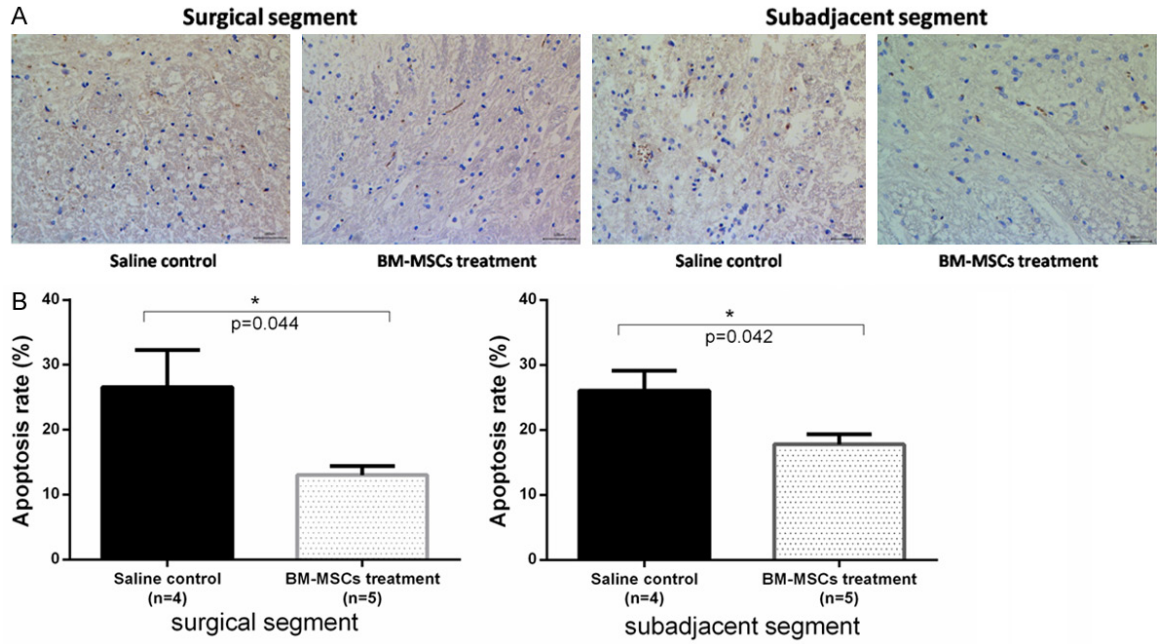

Figure 6. Effects of BM-MSC transplantation on spinal cell apoptosis in rabbit syringomyelia-associated scoliosis model. (A) TUNEL-positive cells in the spinal cord of the BM-MSC treatment group and the control group (× 200 magnification). Statistical data are shown in (B) (Quantification was performed on three sections per animal). There were significant differences between the groups ($P < 0.05$, t-test). Data (means ± SD) represent the rate of TUNEL-positive cells.

representative images of TUNEL assay in spinal tissues. Rate of apoptosis of spinal cells in sub-adjacent segments in BM-MSCs group and saline control group was 26.6 ± 5.5% and 13.0 ± 3.4%, respectively. Apoptosis rate of spinal cells in surgical segments in BM-MSC group and saline control group was 26.1 ± 6.1% and 17.8 ± 3.5%, respectively. Number of TUNEL-positive cells was significantly lower in BM-MSCs transplantation group compared with saline-injection group ($P = 0.04$). These results suggest that MSCs have an impact on apoptosis of spinal cells in a rabbit model.

BM-MSCs administration reduces syringomyelia and scoliosis occurrence

After the significant effects of BM-MSCs transplantation in spinal cell apoptosis, syringomyelia and scoliosis occurrence was evaluated at postoperative weeks 4, 6, 8, and 12 (**Table 3**). Consistent with apoptosis results at week 6, occurrence of syringomyelia and scoliosis decreased (16.7% and 16.7%, respectively) in rabbits treated with BM-MSCs compared with those animals in the control group (23.5% and 29.4%, respectively). We also observed a decrease at postoperative week 8. No evident differences of incidence rate of syringomyelia and scoliosis were observed between BM-MSC-treated or saline-rabbits by the end of our experiment (postoperative 12-week). These results suggest that BM-MSCs administration reduced incidence of syringomyelia as well as scoliosis in kaolin induced rabbit models.

Discussion

Syringomyelia is a condition of the spinal cord from trauma, malformation, etc. Scoliosis is a three-dimensional spinal deformity. Coexistence of scoliosis and syringomyelia has usually been found in patients before the age of 10 years [9-11]. The concomitant rate of scoliosis in syringomyelia has been reported as 25%-85% [12]. Currently, the cause of syringomyelia or idiopathic scoliosis is unclear. An ideal animal model has always played a key role in researching pathogenesis and treatment of human disease. Many scoliosis and syringomyelia animal models have been developed in recent years. A number of methods have induced scoliotic deformity across many animal species such as resection of ribs, dorsal rhizot-

Table 3. Summary of incidence of syringomyelia and scoliosis in rabbit models after BM-MSCs administration

	Postop time				
	Week 4	Week 6	Week 8	Week 10	Week 12
SM	0	16.7% (3/18)	33.3% (6/18)	66.7% (12/18)	66.7% (12/18)
Scoliosis	0	16.7% (3/18)	38.9% (7/18)	55.6% (10/18)	61.1% (11/18)
SM & scoliosis	0	5.3% (1/18)	22.2% (4/18)	33.3% (6/18)	38.9% (7/18)

SM, syringomyelia.

omy, unilateral tethering, or injecting botulinum toxin [13]. Methods that establish an experimental porcine model of early-onset scoliosis have also been developed by use of a radiopaque ultra-high molecular weight polyethylene posterior spinal tether [14]. Barrios et al. reported that temporary interpedicular tethering at the thoracic spine induces severe scoliotic curves in pigs [15]. However, commonly used methods still have some disadvantages including complicated preparation and high mortality rates, etc. In 2005, Lee et al. attempted to establish a rat model of syringomyelia by intraparenchymal injection of kaolin into the rat cervical spinal cord. They further noted that large numbers of macrophages were recruited from bone marrow in kaolin-induced rat syringomyelia [16]. Wong et al. reported that they created a posttraumatic syringomyelia model using excitotoxic amino acid and kaolin-injection [17]. More recently, in a study by Mohrman et al., rats injected with quisqualic acid and kaolin were also observed to develop syringomyelia [18]. In our study, we developed an animal model for scoliosis and syringomyelia in rabbits using kaolin administration. Twelve weeks after syrinx induction, a relatively higher incidence of scoliosis (58.8%) and syringomyelia (64.7%) was observed. Syrinx and scoliosis were found in 64.7% and 58.8% of our experimental animals. Syringomyelia-associated scoliosis appeared in 41.2% of the animals, suggesting that kaolin-administration could be an optimal choice for induction of scoliosis and syringomyelia.

A better understanding of underlying molecular mechanisms associated with syringomyelia or scoliosis formation will unveil new targets for treatment and possibly the future prevention of morbidity [19]. The cause of scoliosis and syringomyelia is believed to be multifactorial [20]. Increased apoptosis has always been thought to be a driving factor in development and progression of many bone diseases and has been reported in intervertebral discs of patients with adolescent idiopathic scoliosis [21]. The role of apoptosis in scoliosis and syringomyelia remains unclear due to the relatively few number of studies performed. Bao et al. observed cell apoptosis and necrosis in herniation of the cerebellar tonsil in Chiari I malformation complicated with syringomyelia patients and suggested that apoptosis might play a role in development of syringomyelia [22]. Karner et al. reported that increased apoptosis suggests a common pathophysiology for adolescent idiopathic scoliosis [23]. These related studies have provided evidence indirectly supporting the idea that apoptosis might be involved in the pathogenesis of syringomyelia and scoliosis. In our present study, apoptosis of spinal cord tissues from surgical segments and adjacent segments of the rabbit model were evaluated by TUNEL method. Apoptosis indexes of spinal cells in both the surgical and adjacent segments of rabbit models were significantly higher than that of the control. Levels of apoptosis of spinal cells increased along the observation period (postoperative 6-week). Apoptosis of spinal cord tissues prior to manifestation of syringomyelia and scoliosis suggests a cellular etiology for both diseases.

In past years, several potential treatments for syringomyelia or scoliosis have been evaluated by many research groups [24, 25]. MSCs can differentiate into different cell types such as osteoblasts, chondrocytes and myoblasts, fibroblasts, adipocytes, and oligodendrocytes [26]. Based on the results of Hanetal [27], about 115 differently expressed proteins were found in MSCs of patients with degenerative scoliosis and the abnormality of MSCs in DS may be associated with the pathophysiology of scoliosis. Application of MSC-based therapy to supplement traditional surgical therapy shows

great promise for treatment of spinal cord injuries and degenerative disc disease [28-30]. Vaquero et al. reported that injection of MSCs in the syrinx of posttraumatic syringomyelia is safe and is associated with clinical and neuroimaging improvement. They concluded that cell therapy is a new approach to posttraumatic syringomyelia and even for idiopathic syringomyelia [31]. In this study, we evaluated the therapeutic potential of BM-MSCs transplantation in treatment of scoliosis/syringomyelia in a rabbit model. Our results demonstrate that, in addition to decreased levels of apoptosis of spinal cells, BM-MSCs administration could decline morbidity of syringomyelia/scoliosis when injected before occurrence of clinical symptoms (postoperative week 2), compared with control rabbits. This indicates that BM-MSCs may be attributable to a partial blocking of occurrence of syringomyelia/scoliosis and that apoptosis might be a factor contributing to development of syringomyelia/scoliosis.

One limitation to our study was that we failed to investigate effects of spinal cord cell apoptosis on syringomyelia/scoliosis occurrence in a rabbit model. Association between apoptosis and incidence of syringomyelia/scoliosis remains unclear. This should be investigated in the future.

In summary, a kaolin-induced syringomyelia-associated scoliosis rabbit model was established. In postoperative periods, we noticed an increase of apoptosis in surgical segments and adjacent segments of spinal cord. We found that prenatal BM-MSCs transplantation could decrease spinal tissue apoptosis and reduce incidence of syringomyelia and scoliosis in models. This study supports the concept of using BM-MSCs transplantation to treat scoliosis and syringomyelia before occurrence of clinical symptoms.

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Disclosure of conflict of interest

None.

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