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STUDY OF APOPTOSIS IN DISC CELLS FROM PATIENTS WITH SCOLIOSIS

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ABSTRACT

OBJECTIVES: To assess the fate of disc cells in patients with scoliosis of the spine; to evaluate the distribution of apoptosis in the discs affected by scoliosis and to investigate the expression and distribution of Fas receptor / Fas ligand in scoliotic discs.

DESIGN: Surgically obtained intervertebral discs from scoliosis patients were examined by immunohistochemical staining of p85 PARP fragments and DNA nick end labeling method (TUNEL) to assess the fate of disc cells in the discs of scoliosis patients. Fas receptor and Fas ligand were evaluated by means of immunohistochemical staining and RT-PCR to demonstrate the existence of apoptosis in the scoliotic disc tissue, with special reference to the degree of expression of these indicators of apoptotic cells in the scoliotic discs.

BACKGROUND It has been reported that no DNA fragmentation is observed in normal discs with the TUNEL method. However, expression of both Fas receptor and Fas ligand was revealed in the disc cells of herniated lumbar disc tissue by immunohistochemical staining and the DNA nick end labeling method (TUNEL), respectively. It is known that in addition to genetic factors, scoliotic discs also have an important role in the development and advantage of the disease. As a control disc, the expression of Fas ligand has been reported in scoliotic discs with an immunohistochemical method. However, as far as we are aware, there are no earlier detailed reports of apoptosis in scoliotic discs.

MATERIALS & METHODS: A total of 70 intervertebral discs, including annulus, nucleus and endplate of each, were surgically obtained from 16 scoliosis patients (8 female and 8 male, age range 10–48 years, mean 20.67 years). Nine patients had idiopathic scoliosis and seven, neuromuscular scoliosis. The specimens were divided into two groups by patient age: group I (GI=younger than or equal to 15 years; *n*=8) and group II (GII=older than 15 years; *n*=8). DNA fragmentation was analyzed by TUNEL assay and cleavage of the caspase substrate PARP1 by immunohistochemical detection with the p85 fragment. The total and positive cells were counted and photographed in five high-power fields (HPF; 400×) and then statistically analyzed. The expression of Fas receptor and Fas ligand were evaluated with immunohistochemical staining, after which the total and positive cells were counted in five HPF (400×) and confirmed by RT-PCR with Fas receptor/FasL primer.

RESULTS: Positive cell staining of both DNA fragments and p85 fragments was found in the nucleus, annulus, and endplate of each disc from all scoliosis patients with TUNEL and immunohistochemical methods. The percentage of positive cells in GII was higher than that in GI with PARP, and this was confirmed by the result of TUNEL. In the PARP results, higher expression was found in apex discs than in discs one or two levels away from the apex. There was more expression of Fas receptor and Fas ligand in children than in adults and adolescents, especially in the endplate. M staining of Fas receptor/Fas ligand was found in the apex discs than in those two or more levels away from the apex, and especially in the endplate. The highest expression of all factors, DNA fragments, p85 fragments, and Fas receptor/Fas ligand were located in the nucleus, and the lowest, in the annulus. There was stronger expression of the Fas receptor and Fas ligand in 4 cases in which disc RNA was studied with RT-PCR, which confirms the results obtained by immunohistochemistry.

CONCLUSION: Expression of DNA fragments, p85 fragments and Fas receptor with Fas ligand was found in the annulus, nucleus and endplate of intervertebral discs from all the scoliosis patients studied, indicating that apoptosis had occurred or was in progress in all scoliotic discs. The apoptosis in the scoliotic discs was affected both by age and by the biomechanical load caused by deformation. The most severe apoptosis was found in the nucleus of the scoliotic discs, which indicated a relationship between apoptosis and degeneration of the discs. As the inductive gene, Fas receptor and Fas ligand co-existed in the scoliotic discs, and the high expression of these suggested that apoptosis in scoliotic discs is subject to a Fas receptor-/Fas ligand-mediated process. The different expression of Fas receptor/Fas ligand in the endplates of discs from patients in the two age groups indicates that the endplate is more sensitive to apoptosis. The biomechanical effects on the apex discs are presumably also the result of differential expression of the Fas receptor in the endplates of apex discs.

Keywords: Apoptosis, Scoliosis, Intervertebral disc, Idiopathic scoliosis, Neuromuscular scoliosis, Degeneration, Biomechanics