Introduction: Apoptosis is genetically-controlled physiological, active cell death required for the maintenance of the individual in contrast with necrosis, which is passive cell death caused by excessive external irritation. The purpose of our study was to investigate apoptosis involved in intervertebral disk growth and degeneration by examining the appearance of apoptosis in the lumbar disks of mice.

Materials and Methods: Male ICR mice aged 1 day, 1, 3, 7, or 15 weeks were used, comprising 10 animals of each age. The tissue was fixed by perfusion with 4% paraformaldehyde, after which the lumbar spine was removed and further fixed by immersion in the same fixative for 24 hours. The resected specimens were then decalcified with EDTA and embedded in paraffin. Hematoxylin and eosin (H&E)-stained specimens were prepared. Apoptotic cells were detected by Tunnel method and transmission electron microscope (TEM). The specimens for TEM were fixed in 2.5% glutaraldehyde, decalcified with 10% EDTA solution, and dehydrated. The specimens were then embedded by epoxy resin and examined under a TEM.

Results: In the H&E stained specimens at 1 day old and 1 week old, the nucleus pulposus was made up primarily of notochordal cells, and the boundary between the annulus fibrosus and the growth plate was indistinct (Fig.1A). From 3 weeks, the annulus fibrosus and the epiphyseal cartilage layer corresponding to the epiphyseal secondary ossification center became more clearly distinguishable with age. There were few notochordal cells at 1 day and 1 week old, with a mean of 59.8 cells at 1 day old and 75.2 cells at 1 week old. The number increased sharply to 195.6 at 3 weeks of age, then decreased with age thereafter. In specimens from 1-day-old and 1-week-old animals, the nucleus pulposus comprised only notochordal cells. From 3 weeks of age, these were replaced by hyaline cartilage from the inner layer of the annulus fibrosus (Fig.1C,E). The region replaced by hyaline cartilage from the inner layer of the annulus fibrosus became more extensive with age.

In the Tunnel-stained nucleus pulposus specimens, there were only a few positive cells at 1 day old. From 1 week old, apoptotic notochordal nucleus pulposus cells were stained yellowish brown. Only 4% of cells were apoptosis-positive at 1 day old. At 3 weeks, 42% of cells were apoptosis-positive. At 7 weeks, this had increased to 69% and at 15 weeks, there was a slight decrease to 56% (Fig.1B,D,F).

Under TEM, the cells in the nucleus pulposus at 1 week of age had large nuclei dotted with chromatin and there was dense glycogen in the cell (Fig.2A). From 3 weeks of age, TEM revealed apoptosis characterized by chromatin condensation and fragmentation of the nuclei. These findings became more extensive with age (Fig.2B).

Discussion: Apoptosis in bone and cartilage tissue can be broadly divided into a programmed phenomenon associated with development and growth, or a decline in the function of the cell itself accompanying the ageing of the individual. Lotz et al. noted destruction of the annulus fibrosus, increased apoptosis, and a decrease in type II collagen in the coccygeal disks of mice after disk compression [1]. Gruber et al. also noted apoptosis in normal intervertebral disks in humans, and after considering the mechanism responsible for the onset of disk degeneration, concluded that further research is needed to determine how apoptosis within the intervertebral disk is promoted or inhibited [2]. It has been reported that blood circulation from the vertebral and annular routes generally plays an important role in the supply of nutrients to intervertebral disk tissue, and disturbance of this blood flow is considered an important cause of disk degeneration. Apoptosis is generally said to occur via the processes, with the apoptotic cell finally being removed through phagocytosis by macrophages from the blood vessels and local phagocytes. However, our research shows that in the intervertebral disk where there is no blood circulation, macrophages do not infiltrate the nucleus pulposus. It is therefore unclear by what process apoptotic notochordal cells are removed and replaced by hyaline cartilage. More research will be needed to elucidate this further.


Fig.1. Histological (A,C) and Tunnel (B,D) study. A,B: at 1 day old, C,D: at 15 weeks old. (Arrows: hyaline cartilage)

Fig.2: TEM of nucleus pulposus cells. A. at 1 week, B. at 7 weeks (Apoptotic cell).