Introduction: The intervertebral disc is the largest avascular structure in the body. The disc consists of at least two distinct regions, the inner nucleus pulposus (NP) with high aggrecan content, surrounded by the more collagenous annulus fibrosus. At the embryonic stage, mesenchymal cells accumulate around the funicular notochord, and the notochord separates into the segments during growth to form the nucleus pulposus. In human, notochordal cells in the NP disappear during adolescence and are replaced by cartilaginous NP cells. Recently, many researchers have been attempting to achieve restoration and regeneration by biological procedures in patients with intervertebral disc degeneration. However, it is still unknown whether the process of change from notochordal to non-notochordal cells is one of early signs of intervertebral disc degeneration. Therefore, comparison of morphology and metabolic activity among these cells is considered important for determination of the type of transplanted cells to use for regeneration of intervertebral discs. In this study, we used notochordal cells of rat and rabbit, as well as non-notochordal cells of bovine. We reviewed differences of morphology of these groups and whether proteoglycan metabolism of nucleus pulposus cells varied among these groups in vitro.

Methods: Histologically, we examined the specimens of NP among each animal using light microscopy, confocal laser scanning microscopy and transmission electron microscopy. And cells were isolated from the NP of lumbar discs of 15 weeks rats, 6 months rabbits and bovine caudal discs of 18-24 month. They were cultured for 5 days in alginate beads of 4 million cells/ml in DMEM containing 6% FBS under 21% O2 with medium osmolality of 400mOsm. Cell viability was determined by manual counting using trypan blue, lactate production was measured enzymatically [1] and glycosaminoglycan (GAG) accumulation was measured using a DMB assay [2].

Results: Notochordal cells were observed in rats and rabbits discs under light microscope. The cells were generally large (cell diameter: 20-30µm), and there were very few small, chondrocyte-like cells in the rat and rabbit. On the other hand, cartilaginous-like NP cells were observed in bovine discs (Figure 1A-C). Transmission electron micrographs of rat and rabbit NP cells, many electron-lucent inclusions were contained in the nucleus cells (Figure 1D, E). And in rat and rabbit NP cells, tight junctions were shown between cells (Figure 2).

The cell viability rate was more than 80% during culture in each group. The lactate production/million cells of NP shows that rat and rabbit cells produced much more lactate compared with containing non-notochordal bovine cells (Figure 3A). The time course of GAG accumulation in cultures shows that bovine NP cells produced about 100µg/ml of GAG after culture for 5 day (20µg/ml/day). Rat and rabbit NP cells produced about 8 and 3 times more GAG than bovine, respectively. (Figure 3B) GAG production per million cells was also higher for rat and rabbit than bovine.

Discussion: Notochordal cells are observed in rats, mice, rabbits, cats, and, pigs throughout life, but in human only up to adolescence. On the other hand, cartilaginous-like NP cells are observed in bovines, sheep, and goats, and also in humans aged 4 years or older [3]. The number of disc cells is said to decrease with aging. It is unknown, however whether this decline is caused by differentiation of notochordal cells into non-notochordal cells, apoptosis, or lack of nutrients from the end plate. It is possible that the disappearance of notochordal cells may trigger intervertebral disc degeneration. In the future, further studies will be required to promote the development of biological therapy for intervertebral disc degeneration. It is said that a 7-10% content of GAG [4] is required to promote to obtain regenerated intervertebral disc tissue with enough strength for clinical application. According to the results of

Reference

Discussion

Figure 1: Histological findings, a lot of electron-lucent inclusions were observed in rat and rabbit discs, on the other hand no inclusion was observed in bovine discs.

Figure 2: Transmission electron micrograph of rabbit NP cell. Tight junctions were shown between cells.

Figure 3: Left graph shows lactate productions/million cells/24Hrs was higher for rat and rabbit than bovine. Right graph shows GAG accumulation/million cells among rat, rabbit and bovine nucleus pulposus cells. Rat and rabbit GAG production was higher than bovine.