Intervertebral Disc Stability in Organ Culture: Rat versus Rabbit

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INTRODUCTION: In vitro organ culture studies of the intervertebral disc (IVD) have been commonly used to understand the biologic and mechanical pathways contributing to disc degeneration. In particular, small animal models such as rat and rabbit have many advantages over large animals in an organ culture standpoint. Temporal and spatial stability of cell viability and extracellular matrix content are deemed essential prerequisites in order to deduce meaning inferences on IVD biology. Culture conditions aside, previous data from our lab and others [1] indicate that IVD culture stability could also be species-dependent. The purpose of this study was to evaluate the stability of rat and rabbit IVDs in vitro and to compare morphologic differences between the two species when cultured at identical culture conditions.

METHODS: IVD organ culture: Young adult male Sprague-Dawley (SD) rats (280g) and New Zealand White rabbits (NZW) (3.6kg) were used in accordance with a protocol approved by the University of Iowa Animal Care and Use Facilities. Under sterile condition, animals were sacrificed and lumbar IVD motion segments were dissected from consecutive levels (L1-L6). Posterior elements and soft tissues were removed and cultured for 2 weeks. Proteoglycan content: IVDs isolated from vertebral body were weighed and digested with papain digestion buffer for 4 hours. The PG content was measured by colorimetric assay with Dimethylmethylene Blue (DMMB). The measures were normalized to the wet IVD weight. Histologic Analysis: The lumbar motion segments were fixed in 10% neutral-buffered formalin and then embedded in paraffin. Midsigittal sections were prepared and stained with Safranin-O and H&E. To identify cell apoptosis, TUNEL assay was performed using In Situ Cell Death Detection Kit. Collagen type II stain: According to a standard protocol, sections were digested by testicular hyaluronidase (1,600 units/ml) and then incubated with primary antibody (II-II 6B3) and goat anti-mouse secondary antibody (Alexa Fluor 568).

RESULTS: PG content showed a dramatic decrease in the rat IVD (Fig. 1). Similarly, we could observe a progressive PG loss in Safranin-O stain (Fig. 2a). However, the rabbit IVD showed no PG decrease during the culture (Fig. 1 and Fig 2b). Histologically, the rat IVD showed severe degenerative changes such as loss of notochordal cells, migration of cartilage endplate (CE) cells into the nucleus pulposus (NP), appearance of chondrocyte-like cells in the NP, and growth plate (GP) damage (Fig. 3). After 2-week organ culture, cell apoptosis and collagen type II positive deposits in the rat IVD were homogeneously distributed in the NP and the annulus fibrosus (AF) (Fig. 4). In contrast, the rabbit IVD showed apoptosis in the AF alone. In the rat IVD, the thick CE was only observed between the GP and the NP in the center. On the other hand, a thin CE, which has a couple of cell layers, was located between the thick epiphysis and the NP in the rabbit (Figure 5).

DISCUSSION: In this study, we evaluated the stability of the rat and rabbit IVDs in organ culture. Our results indicate that rat IVD shows remarkable PG decline and NP degeneration. In contrast, the rabbit IVD showed no PG loss with minor changes in the NP compared to the rat IVD. We believe the comparative stability differences between the two species could be morphologically induced. In addition, PG loss could be explained by short diffusion distance in the rat IVD. We conclude that the rabbit IVD is more stable in vitro organ culture system than the rat IVD and is a suitable small animal model for studying of degenerative changes in the disc. However, further study is warranted to understand the mechanism of PG loss and tissue stability in organ culture.


Figure 1. Temporal profile of proteoglycan content in cultured IVDs show that rabbit IVDs were able to maintain PG content for up to 2 weeks whereas the rat IVD showed decrease of PG as early as 3 days.

Figure 2. Safranin-O stain corroborates the PG content variations between rat and rabbit IVDs till 2 weeks in organ culture.

Figure 3. Histologic changes of the IVD in organ culture

Figure 4. Cell apoptosis and collagen type II (10x)

Figure 5. Morphologic differences between the rat and rabbit IVD