Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 13
Sofia • 2008

Degenerative Changes in the Human Intervertebral Discs. Histochemical Study

B. Landzhov, L. Stokov, B. Vladimirov*, A. Bozhilova-Pastirova, W. Ovtscharoff*

Department of Anatomy and Histology, Medical University, Sofia
**Department of Orthopaedy and Traumatology, Medical University, Sofia*

The object of this study was to examine histochemically some of the causes leading to disc degeneration. Our results suggest that nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), which is a marker for nitric oxide synthase (NOS) and an indirect marker for nitric oxide (NO), is linked with the degenerative changes in the intervertebral disc (IVD).

Key words: intervertebral disc, degeneration, NADPH-d.

Introduction

NO is a messenger molecule that is synthesized from l-arginine. It is a result from the metabolism of the cells and activates many pathways by diffusing across membranes. It is synthesized from three different enzymes: inducible (iNOS), endothelial (eNOS) and neuronal (nNOS).

Materials and Methods

The obtained material was from individuals between 21 and 70 years old. We examined 10 controls from cadavers and 12 surgical specimens from patients with disc disease. Our histochemical study was to demonstrate histochemically the NADPH-d.

Results and Discussion

Our findings suggest that NADPH-d reactivity cells were more over in the discs with degenerative changes than normal. The lamellas in annulus fibrosus (AF) get more disorganized, and the connection between AF and cartilage and plates (CEP) disrupts. Many authors report that there is a correlation between proinflammatory mediators and the production of NO [1, 2, 4, 5, 6, 7, 10].

The quantity of the positive NADPH-d cells increase with the increase of degenerative changes in the disc. It is not clear enough this relationship. NADPH-d expression is strongest in the chondrocytes in 50-65 years old individuals (Fig. 1). Differences between expressions of NADPH-d positive cells in different parts of the disc are viewed. The quantity of these chondrocytes in the outer AF (Fig. 2) is greater than those in the inner region (Fig. 3). It is not clear whether this is a result of degeneration or it is a process of normal chondrocyte maturation [11]. On the other hand, many NADPH-d positive cells are observed on the border of the vascular canals (Fig. 4). This NO may come basely from endothelium. The basely production of NO comes from newly formed blood capillaries and granulate tissue around them [3].

The number of positive cells increases with ageing and degeneration respectively. The quantity of NO depends on the types of degenerative diseases [12].

Fig. 1. Old IVD, outer AF (× 250)

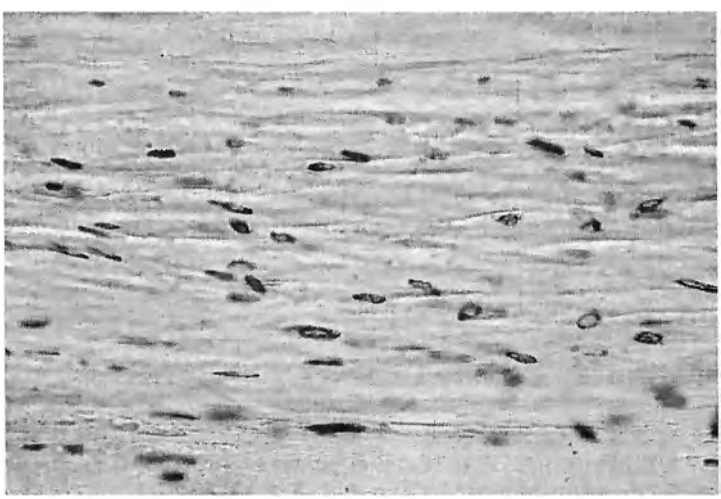

Fig. 2. Old IVD, outer AF (× 500)

Fig. 3. Old IVD, inner AF (× 250)

Fig. 4. Old IVD, outer AF (× 500)

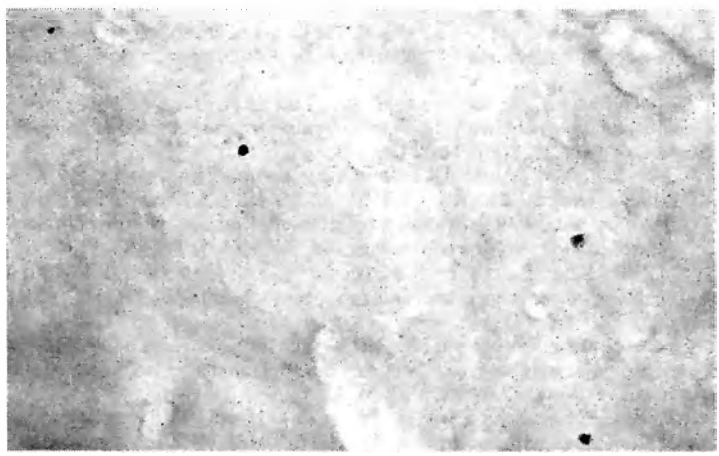

Fig. 5. Young IVD, AF (× 500)

The number of NADPH-d reactivity cell is least in the groups of 25-35 years old individuals (Fig. 5). The increased number of NOS-positive chondrocytes in young individual is a result of chondrocytes maturation [11]. The material from degenerative discs showed greater number of positive cells than normal control discs. With this we confirm that NO is a molecule that regulate the cell metabolism and increase its quantity parallel with the increase of cyclic tensile stretch [8, 9] and disc degeneration.

Conclusion

Degenerative changes in IVD are one of the main factors for appearance of low back pain. Often the degeneration starts as a result of abnormal mechanical load. This strength is related with changes in the cells, proteoglycans and collagen fibers, all intercellular and extracellular structures.

References

1. Burke, J. G., R. W. Watson, D. Conhyea, D. McCormack, F. E. Dowling, M. G. Walsh, J. M. Fitzpatrick. Human nucleus pulposis can respond to a pro-inflammatory stimulus. — Spine, **28**, 2003, No24, 2685-2693.
2. Furusawa, N., H. Baba, N. Miyoshi, Y. Maezawa, K. Uchida, Y. Kokubo, M. Fukuda. Herniation of cervical intervertebral disc: immunohistochemical examination and measurement of nitric oxide production. — Spine, **26**, 2001, No10, 1110-1116.
3. Hashizume, H., M. Kawakami, H. Nishi, T. Tamaki. Histochemical demonstration of nitric oxide in herniated lumbar discs. A clinical and animal model study. — Spine, **22**, 1997, No10, 1080-1084.
4. Kang, J. D., H. I. Georgescu, L. McIntyre-Larkin, M. Stefanovic-Racic, W. F. Donaldson 3rd, C. H. Evans. Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. — Spine, **21**, 1996, No3, 271-277.
5. Kang, J. D., H. I. Georgescu, L. McIntyre-Larkin, M. Stefanovic-Racic, C. H. Evans. Herniated cervical intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. — Spine, **20**, 1995, No22, 2373-2378.
6. Kang, J. D., M. Stefanovic-Racic, L. A. McIntyre, H. I. Georgescu, C. H. Evans. Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. — Spine, **22**, 1065-1073, No10.
7. Kohyama, K., R. Saura, M. Doita, K. Mizuno. Intervertebral disc cell apoptosis by nitric oxide: biological understanding of intervertebral disc degeneration. — Kobe J. Med. Sci., **46**, 2000, No6, 283-295.
8. Liu, G. Z., H. Ishihara, R. Osada, T. Kimura, H. Tsuji. Nitric oxide mediates the change of proteoglycan synthesis in the human lumbar intervertebral disc in response to hydrostatic pressure. — Spine, **26**, 2001, No2, 134-141.
9. Rannou, F., P. Richette, M. Benallaoua, M. Francois, V. Genries, C. Korwin-Zmijowska, M. Revel, M. Corvol, S. Poiraudeau. Cyclic tensile stretch modulates proteoglycan production by intervertebral disc annulus fibrosus cells through production of nitrite oxide. — J. Cell Biochem., **90**, 2003, No1, 148-157.
10. Taskiran, D., M. Stefanovic-Racic, H. Georgescu, C. Evans. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleu-kin-1. — Biochem. Biophys. Res. Commun., **200**, 1994, 142-148.
11. Teixeira, C. C., H. Ischiropoulos, P. S. Leboy, S. L. Adams, I. M. Shapiro. Nitric oxide-nitric oxide synthase regulates key maturational events during chondrocyte terminal differentiation. — Bone, **37**, 2005, No1, 37-45.
12. Watanabe, T., S. Kato, K. Sato, K. Nagata. Nitric oxide regulation system in degenerative lumbar disease. — Kurume Med J., **52**, 2005, No1-2, 39-47.