DECREASED DIFFUSION AS A RESULT OF PERFUSION BLOCK IN THE OVINE LUMBAR SPINE: A FUTURE MODEL FOR DISC DEGENERATION

van der Werf, MJ; Lezuo, P; Maissen, O; Ito, K
AO Research Institute, Davos, Switzerland
keita.itob@aofoundation.org

INTRODUCTION:
Intervertebral disc degeneration is believed to play an important role in low back pain, one of the most costly health care problems in western civilization. To study the degenerative process in vivo, various animal models have been developed: external static loads, endplate or annulus fibrosus damage, or enzymatic degradation of the extracellular matrix. They all produce disc degeneration in a relative short term and as a result of structural damage. In most humans however, disc degeneration is a very slow process which often has no clear structural cause.

The cells inside the disc rely on diffusion through the endplate for nutrition and removal of waste products. Although correlation has been found between occlusion of endplate vascular openings and disc degeneration, causality has never been demonstrated in intact discs. To study this, a model for nutrient insufficiency induced disc degeneration is being developed. In this model the blood supply to the endplates is disrupted and a titanium foil is inserted to prevent vascular regeneration. The method of blocking the major nutritional route results in inhibited perfusion of the cartilaginous endplate and diffusion into the disc.

METHODS:
These animal experiments were approved by the veterinary authority of Canton Graubünden, Switzerland. Four sheep were anaesthetized and the anterior lumbar spine exposed. A 1 cm wide thin slot was sawn into the vertebrae parallel and adjacent to the endplates overlying the nuclear region of the L2-3 and L4-5 discs. After Ti-foils were inserted into the slots, the inhalation gas mixture was changed from O2 with 2 to 3% isoflurane to 70% N2O and 30% O2. At 5 min intervals, intranuclear O2 and N2O concentrations were measured amperometrically, in triplicate. 5 minutes pre-mortem, 25,000 IU of heparin were injected intravenously. Post-mortem, 50,000 IU of heparin in 1 l of normal saline was injected through the cannulated abdominal aorta to prevent blood clotting (aortic ligation proximally and at both femoral arteries distally). The vertebral vasculature was then infused with 800-1000 ml normal saline with 5% Procion red.

Spinal motion segments were dissected, cryopreserved in liquid nitrogen and freeze substituted in acetone. Following embedding in MMA, mid-sagittal thick sections were cut, ground to approx. 100 µm thickness, polished and examined with a fluorescent microscope. Quantification of the density of patent endplate capillary buds was done semi-automatically with an image analysis program and custom macro. Significance between control and blocked discs was tested by t-test.

RESULTS:
The O2 diffusion measurements showed a drop in [O2] after switching the anesthesia gasses. The disc received less O2 and the drop over time was clearly shown in figure 1a. There was a trend that the control discs showed a slower drop in [O2] than the blocked discs.

The N2O diffusion measurements showed a clear inhibition of transport with the block (Fig. 1b). After 35 minutes the [N2O] had increased more than 3.5 fold in the control discs. In the blocked discs the increase was much lower.

The histology showed that the perfusion block left many vascular buds unperfused (Fig. 2). In the control discs there were 3.4 ± 2.3 perfused capillary buds per mm of endplate, 3.87 ± 2.59 in the anterior region and 2.99 ± 1.94 posteriorly. In the blocked discs this was 1.31 ± 1.12 for the whole disc (p < 0.001 compared to control), but only 0.56 ± 0.43 in the anterior region and 2.07 ± 1.10 (p < 0.001) posteriorly.

DISCUSSION & CONCLUSIONS:
In this study N2O was used as a tracer for nutrient diffusion into the disc. It demonstrated that it was possible to partially block the diffusion of N2O into the disc. A reason for the lack of a significant difference in O2 concentrations between blocked and control discs could be generally low cellular activity in discs. The discs were saturated with O2 at the beginning of the operation, and the drop in O2 was slow in both blocked and control discs.

REFERENCES:

ACKNOWLEDGEMENTS:
The authors would like to thanks S. Ohashi, P. Heil, I. Gröngöröft, C. Sprecher, D. Arens, J. Urban and S. Smith for technical assistance. Funding was received from the AO Foundation, Switzerland.

AFFILIATED INSTITUTIONS FOR CO-AUTHORS:
* Eindhoven University of Technology, The Netherlands

Paper No: 1234