The Effects Of Drug-Induced Changes To The Vertebral Endplate Vasculature On Diffusion Into The Nucleus Pulposus And Annulus Fibrosus In Vivo.

Sarah E. Linley¹, Joshua Peterson¹, Rosemarie Mastropolo¹, Gregory Salamone², James Lawrence, MD, MBA³, Luciana Lopes, PhD⁴, Jeffrey C. Lotz, PhD⁵, Eric H. Ledet, PhD¹.

¹Rensselaer Polytechnic Institute, Troy, NY, USA, ²University of Buffalo, Buffalo, NY, USA, ³Albany Medical College, Albany, NY, USA, ⁴Albany College of Pharmacy, Albany, NY, USA, ⁵UCSF, San Francisco, CA, USA.

Disclosures:

Introduction: The intervertebral disc is avascular and relies on diffusion from the microvasculature in the adjacent subchondral bone to receive nutrients and expel waste products. A reduction in diffusion can lead to ischemia, hypoxia, and ultimately degeneration of the disc. Understanding how altering the vertebral endplate vasculature affects diffusion into the disc may aid in elucidating the contribution of nutrition to degeneration, and may also suggest therapeutic strategies to slow or reverse the degenerative cascade. In this study, two drugs were selected to alter the vertebral endplate vasculature - nimodipine and nicotine. Nimodipine is a calcium channel antagonist that has been shown to enhance endplate vascularity.[1] Nicotine has been shown to contribute to disc degeneration and reduced endplate vascularity.[2] The purpose of this study was to quantify the effects of drug-induced changes to the vertebral endplate vasculature on diffusion into the intervertebral disc in vivo.

Methods: Following IACUC approval, 9 skeletally mature New Zealand white rabbits were randomized into 3 groups of 3 animals each: 8 weeks daily nimodipine treatment (2.5 mg/kg, subcutaneous injection), 8 weeks daily nicotine treatment (10.5mg/24hrs, transdermal patch), and a control group which received no drug treatments. At the conclusion of the drug treatment regimen, animals were administered the small molecule MRI contrast agent gadodiamide (0.3 mmols/kg, IV) and euthanized 10 minutes later. Excised lumbar spine motion segments were imaged using post- contrast enhanced 7T MRI. T1 constants were quantified in the nucleus pulposus (NP) and annulus fibrosus (AF) as a quantitative measure of gadodiamide diffusion into the tissues. T2 constants were quantified in the nucleus pulposus as a measure of disc health. Lumbar motion segments were routinely processed for histology, and stained via the rapid Heidenhain stain for visualization of red blood cells. Serial images of the subchondral bone adjacent to the annulus and nucleus were obtained at 20x magnification to quantify vessel area, vessel number, vessel distance from the cartilage endplate, and percentage of the endplate in direct contact with the microvasculature.

Results: Nimodipine treatment resulted in a significant (mean 8.4%) increase in gadodiamide diffusion into the NP as compared to control. Representative histology images adjacent to the NP from each treatment group are illustrated in Figure 1. No difference in the number of vessels in the nimodipine group compared to control was seen, but vessel area was increased by a mean 37%, and the normalized length of vessel contact with the cartilage endplate was also significantly increased by a mean 78%. Nimodipine treatment resulted in a mean 10.9% increase in transport into the posterior AF, but this was not associated with changes in vessel number or area. A trend towards increased diffusion into the anterior annulus was observed in the nimodipine group, which was associated with an increase in vessel area by a mean 49%. There was no detectable difference in diffusion into the NP between nicotine and control groups. Despite this, a mean 32% increase in vessel number and 57% increase in vessel area were observed in the subchondral bone adjacent to the NP in the nicotine group compared to control. However, vessel distance from the cartilage endplate was increased in the nicotine group by an average of 20µm. Nicotine treatment also caused a mean 18.2% decrease in the NP T2 constant compared to control. In the posterior AF, a mean 11.9% increase in diffusion was observed in the nicotine group compared to control, associated with an increase in vessel number in the adjacent subchondral bone. A trend towards increased diffusion into the anterior AF was also observed in the nicotine group. In the subchondral bone adjacent to the anterior AF, nicotine treatment increased vessel number and vessel area by 30% and 61%, respectively.

Discussion: Transport into the intervertebral disc is complex and influenced by many factors. The observed increase in diffusion into the NP in the nimodipine group was associated with increases in vessel area and vessel contact with the cartilage endplate, but not with an increase in vessel number. Nicotine significantly altered endplate vasculature adjacent to the NP, but did not influence transport into the disc. This may have been due to the observed increase in vessel distance from the endplate, or due to reduced nucleus proteoglycan content (reduced T2 constant). Nicotine and nimodipine both increased transport into the posterior annulus to a greater extent than the anterior annulus, yet drug induced changes to the subchondral vasculature were primarily observed adjacent to the anterior annulus. Our results suggest a correlation between drug-induced changes to the vasculature and transport into the nucleus pulposus, however, our results were inconclusive for the annulus.

Significance: Our results illustrate the potential of drug-induced alterations to the vertebral endplate vasculature to affect small
molecule diffusion into the intervertebral disc in vivo. As diffusion is often reduced in degenerated discs, drugs that could selectively target and enhance the subchondral vasculature to enhance diffusion provide a promising therapeutic strategy to slow or reverse disc degeneration.

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Figure 1. Representative histology images of the subchondral bone adjacent to the NP in the control group (A), the nimodipine group (B), and the nicotine group (C). Images taken at 40X, scale bar = 50µm. NP = nucleus pulposus, BN = subchondral bone, EP = cartilage endplate, V = microvessel.

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