Effects of Caffeine on Injured and Uninjured Intervertebral Discs in a Whole Organ Culture Model

Stricklin OE, Stannard JT, Raines BT, Stoker AM, Cook JL
Comparative Orthopaedic Laboratory, University of Missouri, Columbia, Missouri

Veterinary Research Scholars Program
University of Missouri

www.columc.missouri.edu

Introduction

Intervertebral disc (IVD) disorders resulting in pain are prevalent, but the mechanisms of IVD degeneration are not fully understood. A recent study by Stannard JT *et al* demonstrated decreased cell viability and glycosaminoglycan (GAG) content in injured rat tail IVDs within 7 days of exposure to nicotine. Caffeine shares similar physiologic effects to nicotine, although different structurally. 85% of US citizens consume at least one caffeinated beverage daily. Due to the prevalence of caffeine consumption and the similarity to nicotine, the effects of caffeine on IVD pathology at the cellular and tissue level are of extreme interest.

Objective

To compare the effects of caffeine on injured and uninjured IVDs.

Hypothesis

Caffeine will be associated with a marked loss of cell viability of injured IVD explants compared to Day 0 control and uninjured explants.

Methods

All procedures were approved by the IACUC and the animals used were euthanized for reasons unrelated to this study.

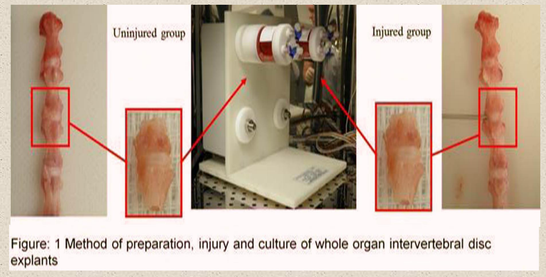

Figure: 1 Method of preparation, injury and culture of whole organ intervertebral disc explants

Tissue Harvest: Tails were collected from 22 skeletally-mature Sprague Dawley rats (n=22). Discs were harvested aseptically and soft tissue was dissect from the caudal vertebrae. Explants (n=190) consisted of the cranial body half, endplate, IVD, endplate, and caudal body half (Figure 1).

Tissue Culture: Explants were cultured for 21 days in a rotating wall vessel bioreactor; each unit contained 100 mL of DMEM culture media supplemented with ITS, Pen\Strep\Amphotericin B, L-glutamine, L-ascorbic acid, MEM non-essential amino acids, sodium pyruvate, and caffeine at the appropriate concentration. Media were changed every 7 days, and tissue harvests occurred on days 7, 14, and 21.

Treatment Groups: Explants were randomly assigned to one of two treatments: injured (n=90) or uninjured (n=100). To simulate injury, a 20-gauge needle was inserted into the nucleus pulposus and 0.5 mL was aspirated. Within treated groups, explants were randomly assigned to one of four sub-groups: control (CON) (n=28/total), low caffeine (LCAF) (5 mg/L) (n=24/uninjured, 32/injured), moderate caffeine (MCAF) (10 mg/L) (n=24/uninjured, 31/injured), and high caffeine (HCAF) (15 mg/L) (n=24/injured, 29/injured).

Cell Viability: Viability of IVD cells was subjectively assessed for discs harvested on days 0, 7, 14, and 21 using fluorescent microscopy. Calcien-AM and ethidium homodimer stains were used to stain live and dead cells respectively. Cell counting was performed using custom in house software.

Extracellular Matrix: Glycosaminoglycan (GAG) and collagen contents were determined using a DMMB and hydroxyproline assay, respectively.

Statistical Analysis: Statistical significance was evaluated with SigmaPlot® using students t test for data between groups with significance set at $p<0.05$.

Conclusions

- **There is a dose-dependent effect of caffeine on the degeneration of viable IVD cells with increasing caffeine levels, regardless of injury.**
- **IVD injury causes disc cell counts to significantly decrease over a period of 21 days.**
- **Increasing caffeine exposure correlates with decreased IVD proteoglycan concentration.**

Acknowledgments: Funded by The Comparative Orthopaedic Laboratory and a gift from Zoetis Animal Health.

Results

Cell Viability

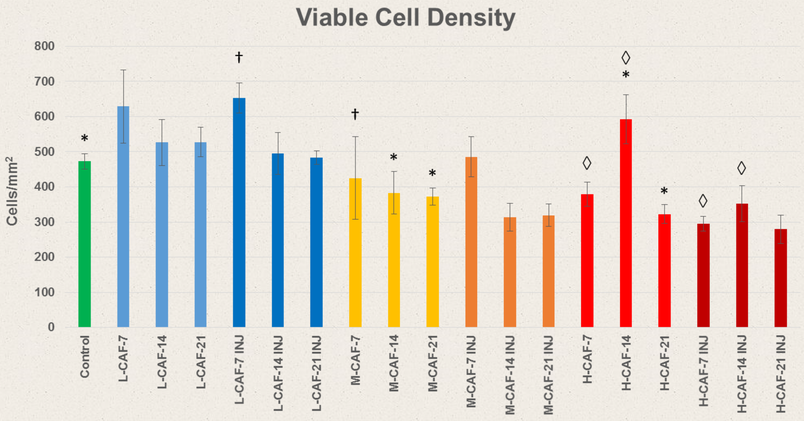

Figure 1 – Cell viability was significantly higher in CON compared to D14 and D21 MCAF and HCAF (*), regardless of injury. LCAF7 INJ had significantly greater cell viability than LCAF14 and LCAF21 INJ, and the same occurred in the MCAF INJ group (†). HCAF7 INJ and HCAF 14 INJ had significantly lower cell viability than HCAF7 and HCAF14, respectively (◊).

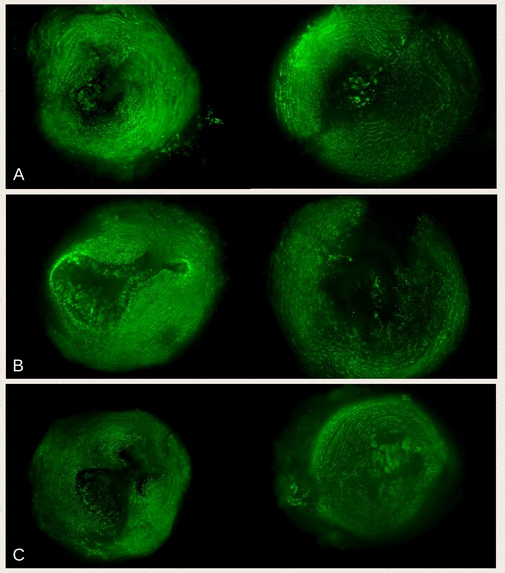

Figure 2A HCAF21 uninjured sample (left) compared to HCAF21 INJ (right).
Figure 2B – MCAF21 uninjured sample (left) compared to MCAF21 INJ (right).
Figure 2C – LCAF14 uninjured sample (left) compared to LCAF14 INJ (right).

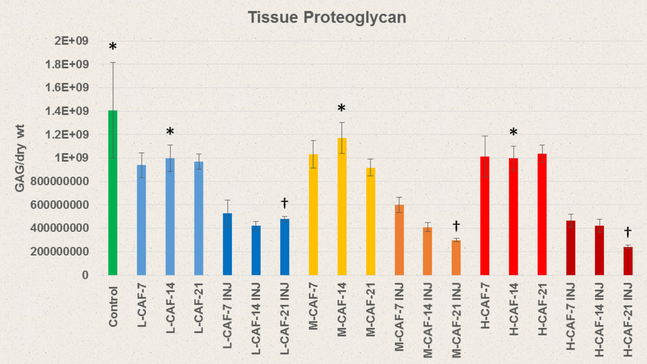

Figure 3: All uninjured groups (*) had significantly ($p<0.05$) higher proteoglycan concentration/mg than the injured and control groups. LCAF21 INJ was significantly greater than MCAF21 INJ was significantly greater than HCAF21 INJ (†), demonstrating a dose-dependent effect of caffeine to decrease proteoglycan concentration/mg.

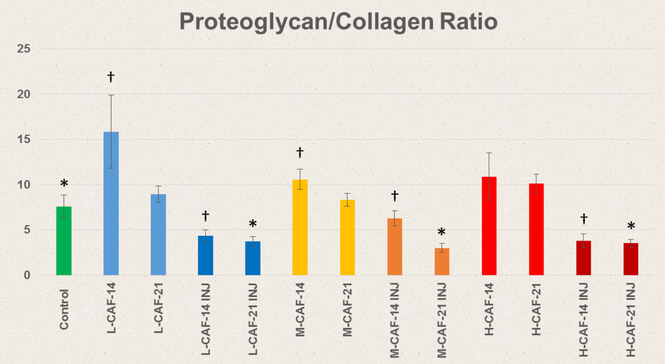

Figure 4: All day 21 injured groups had a significantly lower GAG:COL ratio than the Control group (*). All injured groups, with the exception of HCAF14 INJ, had a significantly lower ratio compared to the corresponding uninjured groups (†).

Discussion

To our knowledge, this is the first study to investigate the effects of caffeine on injured and uninjured IVDs using a whole organ *in vitro* model. Low to moderate levels of caffeine do not appear to affect the average viable cell density (VCD) regardless of treatment. At high levels, caffeine significantly decreases disc VCD over time. Interestingly, VCD appears to be greater than the Day 0 controls for all uninjured groups after 7 days. VCD also appears to be reduced in the moderate to high caffeine groups over time. Tissue GAG content significantly decreased in injured treatment group as compared to uninjured treatment for all groups. The injured treatment group also saw a significant dose-dependent decrease in tissue GAG at day 21. GAG/COL ratio was significantly lower for injured treatment group as compared to uninjured. The data supports the theory that caffeine consumption may adversely affect injured IVDs in culture. Further exploration of the impacts of caffeine on larger whole organ explants *in vitro* and *in vivo* are necessary to understand the exact nature of the impact of caffeine on the IVD.