Is Discography or Discoblock Safe for Human Intervertebral Disc cells?

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Introduction: Discography and discoblock are imaging procedures used to diagnose discogenic low back pain. However, a recent long-term clinical study reported that discs treated with either of this procedure still show degenerative changes [1]. Although it has been reported that needle puncture to the intervertebral disc (IVD) induces disc degeneration [2], reagents used for the procedure also may have harmful effects on IVD cells [3, 4]. The purpose of this study was to examine whether contrast agent and local anesthetics showed detrimental effects on 3D-cultured human nucleus pulposus (NP) cells.

Methods: This study was approved by the ethical committee at our institution. Healthy intact human NP samples were obtained from patients who underwent spinal fusion for adolescent idiopathic scoliosis. NP cells were cultured for one week in 3D-alginate bead composites. Cells were then exposed to Isovist®240 (iopromide; contrast agent), lidocaine (1% or 2%), or bupivacaine (0.25% or 0.5%) for 30, 60, or 120 min. Saline (0.9%) was used as a control. Following treatment, cells were incubated in normal culture medium with 24 h. Viable and non-viable cells were detected with calcein AM and propidium iodine (PI), respectively. Apoptotic cells were counted by a flow cytometer using PI and Annexin-V staining kits. Finally, western blot analyses were performed to determine the apoptotic pathways. Statistical analyses were performed using one-way ANOVA test followed by a Bonferroni’s post hoc test or by an unpaired Student’s t-test. P < 0.05 was considered statistically significant.

Results: The Isovist®240 did not affect NP cell viability, but local anesthetics significantly decreased cell viability in a dose-dependent manner (Fig 1). Furthermore, Isovist®240 did not induce NP cell apoptosis, but local anesthetics significantly increased the number of apoptotic cells. Bupivacaine treatment at 0.5 % showed the greatest increase in apoptotic cell death (Fig 2). NP cells treated with local anesthetics showed a dose-dependent increase in cleaved caspase-3 expression levels. Cleaved caspase-8 was increased in lidocaine-treated cells, whereas both cleaved caspase-8 and -9 were increased in bupivacaine-treated cells (Fig 3).
Fig1. A. Confocal laser scanning micrographs of human NP cells exposed to saline, contrast agent or local anesthetics for 60 minutes.
B. Viability of human NP cells exposed to reagents for 30, 60, 120 minutes. *P < 0.05 versus saline. (n=3). C. Viability of human NP cells exposed to saline or local anesthetics for 60 minutes. *P < 0.05. (n=3).
Fig 2. A. Scatterplots of flow cytometry 24 hours after 30, 60, and 120 minutes exposure to saline, contrast agent or local anesthetics.
B. Viability of human NP cells exposed to reagents for 30, 60, 120 minutes. *P < 0.05 versus saline. (n=5). C. The percentage of viable and apoptotic cells of human NP cells exposed to saline or local anesthetics for 60 minutes. *P < 0.05. (n=5).

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Fig 3. Western blot analysis of caspase-3, -8, -9, cleaved caspase-3, -8, -9 in human NP cells exposed to reagents for 60 minutes. Representative image of western blot analysis.
Discussion: The present study demonstrated that contrast agent does not affect NP cell viability, but local anesthetics, particularly bupivacaine, have harmful effects on NP cells and induce apoptotic cell death. The protein expression patterns associated with apoptosis were different between bupivacaine and lidocaine. Extrinsic pathway expression was increased in lidocaine-treated cells, whereas both extrinsic and intrinsic pathway expression were increased in bupivacaine-treated cells. NP cells subsequently underwent apoptotic cell death through mitochondrial involvement. These results indicate that bupivacaine detrimentally affects NP cells and induces apoptotic cell death. We are further investigating the long-term in vivo effects of bupivacaine on intervertebral disc degeneration.

Significance: The present study suggests that discoblock is not safe for human nucleus pulposus cells.

Acknowledgments:

References:

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