Evaluation of the Time- and Dose-dependent Cytotoxic Effects of Lidocaine on Bovine Nucleus Pulposus Cells in vitro

Introduction:
The aim of this study was to study if lidocaine exhibits a toxic effect on bovine-derived nucleus pulposus (NP) cells in an in vitro study. Provocative discography is utilized by some physicians in the evaluation of back pain that may be discogenic in origin, and some variations of the technique suggest infiltration of lidocaine into the outer annulus fibrosus prior to intradiscal injection of contrast agent. In the evaluation of morphologically normal discs adjacent to abnormal discs, some practitioners utilize intradiscal lidocaine to block the abnormal disc prior to evaluation of the adjacent level. There have been recent reports noting a toxic effect of lidocaine and bupivacaine to articular chondrocytes. Although the mechanism has not been fully described, prior studies have indicated a tight coupling between the ionic channels of chondrocytes and proliferation.

In contrast to diarthrodial joints with vascularized synovium, the intervertebral disc relies completely on diffusion for nutrient transport. Consequently, it is hypothesized that any intradiscal injection of anesthetic will have a significantly greater residence time, as both metabolism and clearance will be markedly slower. Nucleus pulposus cells remain to be completely characterized in terms of phenotype and cellular markers. Although NP cells are a distinct cell population, they share many phenotypic traits with chondrocytes, and consequently, there is reason for concern regarding possible lidocaine toxicity.

Methods:
Cell Harvest: Fresh bovine tails were obtained from a local abattoir and transported on ice. Tissue from nine intervertebral discs (3 tails, 3 discs/tail) was pooled and digested for 1 hour with Pronase (0.4 %) at 37 °C. At the end of Pronase digestion the NP tissue was washed 3 times with sterile media, and then digested for 9 hours with Collagenase-P (0.25 %). Cells were filtered with a cell strainer sieve filter and centrifuged at 1000 rpm for 10 minutes, and then washed 3 times. Each of the monolayer culture experiments was done in triplicate (3 wells/treatment group)

Establish Toxicity: NP cells were plated at 7.5x10^4/well in 24 well plates and cultured for 18 hours at 37 °C in Dulbecco’s Modified Eagle Media (DMEM) supplemented with 10 % fresh bovine serum (FBS). Media was exchanged for either control media (3:4 dilution complete DMEM with HBSS), 0.5 % lidocaine (1:4 dilution of 2% lidocaine with DMEM), or 0.25% lidocaine (1:4 dilution 1% lidocaine with DMEM). Once the media was exchanged, the cells were incubated for 24 hours at 37 °C/5% CO2, media changes were performed at the sample 24 hour timepoint.

Dose Response: Bovine NP cells were plated at 7.5x10^4/well in 24 well plates and cultured for 18 hours at 37 °C in Dulbecco’s Modified Eagle Media (DMEM) supplemented with 10 % fresh bovine serum (FBS). Media was then exchanged for either control (3:4 dilution media with HBSS) or DMEM with a lidocaine concentration of 0.5%, 0.05%, 0.005%, or 0.0005%. Cells were cultured for 28 hours at 37 °C/5% CO2, and then MTT assays were performed.

Time Response: For the time-dependent experiment, the initial media (DMEM with 10% FBS) was exchanged for control media (3:4 dilution media with HBSS) or DMEM with a lidocaine concentration of 0.5 %, 0.05%, 0.005%, or 0.0005% and then MTT assays were performed.

Alginic Beads: NP cells were suspended in alginate beads with a cell concentration of 2 million cells per mL of 1.2% alginate. Cells were matured within the alginate beads for nine days; media was changed every 2-3 days. Beads were treated with either control media, or 0.5%, 0.05%, 0.005% Lidocaine in DMEM. After a treatment period of 24 hours, the beads were stained with both CellTracker Green (5-chloromethylfluorescein diacetate) and propidium iodide (PI). The beads were then imaged with both confocal laser scanning microscopy and fluorescent microscopy.

Cell Morphology: NP cells were plated at 5x10^4/well in a 96 well plate and cultured for 18 hours at 37 °C in Dulbecco’s Modified Eagle Media (DMEM) supplemented with 10 % fresh bovine serum (FBS).

Results Section:
NP cells treated for 24 hours with 0.5% and 0.25% lidocaine had 14 %/−.04% viable and 12.6 %/−.2% viable cells, respectively, relative to control (p<0.05) by MTT assay.
NP cell viability after 28 hour treatment with 0.5%, 0.05%, 0.005%, 0.0005% lidocaine was 16 /−.17%, 72.9 /−.7.4%, 99.2 /−.4.4%, 103 /−2.1 %, respectively. (p<0.05)
NP cells treated with 0.5% Lidocaine had 53.9 /−.2.8 %, 57.6 /−3.7%, 33.8 /−.8 %, and 16 /−1.7% cell viability at 1, 2, 6, and 28 hours post-treatment, respectively, relative to control. (p<0.05 at all timepoints)
NP cells treated with 0.05% Lidocaine had 105 /−7.4 %, 118 /−5.8%, 91.1 /−1.6 %, and 72.9 /−7.4% viable cells at 1, 2, 6, and 28 hours post-treatment, respectively, relative to control. (p<0.05 at 2 hour and 28 hour timepoint.) Changes in cellular morphology at 6 hours post-lidocaine exposure demonstrated a clear dose response. Imaging, both confocal and fluorescent, of the beads demonstrated a dose-dependent decrease in cell viability with increasing lidocaine exposure.

Discussion:
There appears to be a time- and dose-dependent cytotoxic effect of lidocaine on bovine NP cells in ex vivo culture, both monolayer and three-dimensional matrix culture. Cytotoxic effects were confirmed by MTT assay, fluorescent staining, and changes to cell morphology at doses much less than that currently used in clinical practice.

The limits of monolayer culture are recognized, particularly with respect to potential dedifferentiation and loss of phenotype of cells that are maintained in monolayer or passaged. To address we suspended cells from the same population as that used in the monolayer experiments in alginate beads, which has been shown to redifferentiate cells that have lost phenotype in monolayer culture, and the three-dimensional matrix scaffold more closely resembles the matrix of the intervertebral disk.

While caution must be taken in extrapolating the effects of these culture experiments, this data does indicate a definite adverse pharmacologic effect. While this effect may be attenuated in vivo, the presence of a clear toxicity in monolayer culture at a dose of 0.05% Lidocaine (1/4000° that used in clinical intradiscal injections) suggests cause for concern.

Given the emerging data on the toxicity of lidocaine and other local anesthetics of the amino amide group to articular chondrocytes, and findings of this study regarding the toxicity of lidocaine to NP cells in monolayer and three-dimensional alginate matrix culture, there may be cause for concern regarding the intradiscal injection of lidocaine in clinical practice.

References: