Chondrotoxicity of Liposomal Bupivacaine in Articular Chondrocytes: Preliminary Findings

CPT K. Aaron Shaw, MC USA*; CPT Peter C. Johnson, MC USA†; CPT Steve Zumbrun, MS USA‡; Augustine H. Chuang, PhD†; Craig D. Cameron, DO*

ABSTRACT Objective: The chondrotoxicity of local anesthetics has been previously recognized. Recent introduction of a liposomal formulation of bupivacaine has been found to significantly improve postoperative pain control but its effect on chondrocyte viability has yet to be investigated with this new formulation. We sought to assess the in vitro chondrotoxicity of liposomal bupivacaine. Methods: Chondrocytes were isolated from articular cartilage from fresh stifle joints and grown in culture medium. Cultured chondrocyte-derived cells (CDCs) were treated with 0.9% normal saline solution, 0.5%, 0.25%, and 0.13% bupivacaine and ropivacaine, 1.3% liposomal bupivacaine for 1 hour. Following treatment, cells were washed and incubated in media for 23 hours. The CDCs were then harvested and viability was assessed by flow cytometry using SYTOX green dead cell stain. Results: Treated CDCs demonstrated a dose-response effect for chondrocyte viability when treated with bupivacaine, ropivacaine, and liposomal bupivacaine. Liposomal bupivacaine demonstrated the highest chondrocyte viability following treatment. Ropivacaine demonstrated higher chondrocyte viability than bupivacaine. Conclusion: Following 1 hour of treatment, liposomal bupivacaine demonstrated the highest chondrocyte viability. Chondrocyte viability was inversely proportional to anesthetic concentration.

INTRODUCTION

Intra-articular infusions of local anesthetic agents are common procedures performed by the practicing orthopedic surgeon. Various agents, such as bupivacaine, lidocaine, and ropivacaine, have been used in conjunction with a steroid for degenerative arthritis treatment or for postoperative pain management. Bupivacaine is the most commonly used agent, as well as the best studied in orthopedic practice. Despite its common use, bupivacaine has been associated with toxic effects to articular chondrocytes, resulting in histopathologic changes similar to those of osteoarthritis. Chu et al identified the chondrotoxicity of 0.5% bupivacaine to articular chondrocytes in vitro. Piper and Kim assessed the chondrocyte viability of 0.5% bupivacaine as well and 0.5% ropivacaine, finding that ropivacaine was significantly less chondrotoxic than bupivacaine using an in vitro analysis of human chondrocytes. Despite this data, no clinical reports of chondrolysis following single intra-articular injection use have been reported, rather chondrolysis has been associated with continuous intra-articular pain catheter infusions. Recently, a multivesicular liposomal formulation of bupivacaine has been designed and introduced for clinical use. This liposomal formulation has been shown in clinical studies to demonstrate prolonged analgesia, up to 72 hours following infusion, potentially eradicating the need for continuous intra-articular infusions. Clinical results after local infiltration of surgical wounds have identified liposomal bupivacaine as a safe and effective technique for postoperative pain management. Additionally, it has been shown to result in significantly lower cumulative pain scores and decreased opioid consumption compared with standard bupivacaine.

Given its demonstrated efficacy, liposomal bupivacaine use in periarticular infusion warrants further investigation. While its parental formulation is a known chondrotoxic agent, the effect of the liposomal derivation on chondrocyte viability should be investigated before in vivo use. The purpose of this study was to perform an in vitro assessment of chondrocyte viability following exposure to liposomal bupivacaine.

MATERIALS AND METHODS

Analysis of chondrocyte viability was performed according the protocol described by Piper and Kim. Articular cartilage was harvested from fresh bovine stifle joint. Cartilage was isolated and processed under sterile conditions. The articular cartilage was removed from bone with a sterile scalpel and digested in sterile 0.2% hyaluronidase (Sigma-Aldrich, St. Louis, Missouri) for 20 minutes at 37°C followed by sterile 0.1% collagenase (Sigma-Aldrich) for four to 6 hours at 37°C. Following digestion, chondrocytes were plated in monolayer culture with fresh media into a 75-cm² flask at a density of 10⁴ cells/cm². Twenty-four hours before experimental treatment, culture specimens were visualized under phase microscopy.
to verify a cell morphology consistent with differentiated chondrocytes (Fig. 1), and the cells were replated into a 6-well Falcon plate at a density of $10^5$ cells per well. Only first-passage chondrocytes will be used. Cultured chondrocytes were maintained in high-glucose Dulbecco’s Modified Eagle Medium, 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% Fungizone and were kept in an incubator at 37°C with 5% CO₂. The medium was changed every 3 or 4 days.

**Experimental Groups**

Cultured chondrocyte-derived cells (CDCs) were subdivided into 1 of 4 treatment groups consisting of 0.9% normal saline solution (Baxter, Deerfield, Illinois), 0.5% bupivacaine (Hospira, Lake Forest, Illinois), 0.5% ropivacaine (Fresenius Kai, Lake Zurich, Illinois), and 1.3% liposomal bupivacaine (Pacira Pharmaceuticals, Parsippany, New Jersey). All samples were treated according to the same protocol. Specifically, culture medium was aspirated and 200 μL of the treatment solution was added to each well. Samples were incubated in 5% CO₂ at 37°C for 1 hour and the treatment solution was aspirated and fresh culture medium was added. Samples were returned to the incubator, and chondrocyte viability was measured after a 24 hour incubation period.

**Chondrocyte Viability Analysis**

Following the incubation period, culture medium was aspirated from the wells, CDCs were washed once with 1X phosphate-buffered saline solution and detached from the wells with 0.25% Trypsin/0.53 mM EDTA (ATCC, Manassas, Virginia). One milliliter of cell suspension was placed into flow cytometry tubes and 1 μL of SYTOX green dead cell stain (Invitrogen, Carlsbad, California) was added to each tube and mixed in the dark for 20 minutes at room temperature. Samples were assessed with flow cytometry using an Accuri C6 Flow cytometer (BD Biosciences, San Jose, California) at a collection rate of 100 μL/min and fluorescence emission detected using a 530/30 bandpass filter. Emission data were used to determine the chondrocyte viability, recorded relative to amount of chondrocyte death. Experimental groups were compared using student t-tests. The effective concentration of the liposomal bupivacaine was calculated, in milligrams, based on the solution concentration and the pharmacokinetic profile of liposomal bupivacaine reported as demonstrating 3% free bupivacaine.²⁰ Statistical significance was predetermined as $p < 0.05$.

**RESULTS**

Chondrocyte viability is summarized in Table I based on treatment group. Testing demonstrated a relative lack of chondrotoxicity for the liposomal bupivacaine group (4.8% ± 1.9 nonviable cells), whereas the bupivacaine and ropivacaine groups demonstrate significant levels of chondrocyte death.

When compared against the untreated control CDC’s, Figure 2, there was no significant difference in the percentage of nonviable cells ($p = 0.12$) for 1.3% liposomal bupivacaine. The bupivacaine and ropivacaine treatment groups, demonstrated significantly lower chondrocyte viability ($p < 0.05$) when compared against liposomal bupivacaine.

<table>
<thead>
<tr>
<th>Treatment Solution</th>
<th>Nonviable Cells (%)</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>1.9</td>
<td>0.06</td>
</tr>
<tr>
<td>1.3% Liposomal Bupivacaine</td>
<td>4.8</td>
<td>1.9</td>
</tr>
<tr>
<td>0.5% Bupivacaine</td>
<td>32.05</td>
<td>9.1</td>
</tr>
<tr>
<td>0.5% Ropivacaine</td>
<td>23.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Chondrocyte derived cells visualized under phase microscopy at 100 × magnification.

**FIGURE 2.** Result of experimental protocol demonstrating comparative chondrotoxicity of liposomal bupivacaine, bupivacaine, and ropivacaine following 1 hour exposure. Bup = bupivacaine; Rop = ropivacaine; Lip. Bup = Liposomal Bupivacaine.
In calculating the effective concentration of the liposomal bupivacaine treatment, we determined there to be 0.39 mg of bupivacaine in comparison to 5 mg in the standard bupivacaine group.

DISCUSSION
The chondrotoxicity of local anesthetic agents has been well recognized in the literature. Reports of chondrolysis following anesthetic administration through intra-articular infusion pumps have been made for both the shoulder and knee.\(^1\,\,^8\) From these early reports, many in vitro analysis have been conducted, indicating the influence of the specific anesthetic,\(^1\,\,^4\,\,^5\,\,^6\,\,^11\,\,^12\,\,^14\,\,^18\,\,^21\,\,^22\) anesthetic pH,\(^23\) presence of synovial fluid,\(^21\,\,^24\) solution preservative,\(^23\) and combination with epinephrine.\(^23\)

In vivo animal studies investigating the long-term effects of bupivacaine on chondrocyte viability showed that exposure resulted in decreased proteoglycan synthesis and content 3 months following exposure.\(^25\) As well as decreased cell density at 6 months but no significant difference in viability in comparison to controls.\(^26\)

Similar to previous studies,\(^5\,\,^6\) we found that bupivacaine was more chondrotoxic than ropivacaine. When including the liposomal formulation of bupivacaine, it was found to exhibit the least chondrotoxicity after short-term exposure. This finding could be the result of the delayed release of the bupivacaine from the liposomes resulting in an overall decrease in exposure to the chondrocytes, demonstrated in the difference in effective bupivacaine concentration, 5 mg bupivacaine versus 0.39 mg liposomal bupivacaine. The peak concentration of bupivacaine released from liposomal bupivacaine has been demonstrated within the first hour after administration,\(^27\) however, as we did not perform as assay at the conclusion of the treatment exposure period, we were unable to incorporate this analysis into the effective bupivacaine concentration. Extrapolating from previous studies,\(^23\) the more alkaline pH, ranging from 5.8 to 7.4 per product insert, could additionally be diminishing the chondrotoxicity as it has been previously shown that a pH <5 results in chondrotoxicity.

Liposomal bupivacaine has seen growing use in orthopedic surgery, particularly in arthroplasty. Previous studies have demonstrated that a single administration can provide decreased postoperative pain intensity scores, decreased narcotic consumption, decreased length of stay, and reduced hospital costs.\(^28\,\,^29\) Additionally, it has been found to be safe with no reports of cardiac adverse effects and no wound complications.\(^30\)

This study has several limitations. As an in vitro analysis utilizing bovine chondrocyte, these cells may not represent human chondrocyte response to the treatment solutions. Additionally, the cultured chondrocytes have the tendency to transform into mesenchymal cells following prolonged exposure. Most importantly, this study focused on the short-term treatment effects of liposomal bupivacaine. Previous studies have identified that this liposomal formulation allows for sustained release of bupivacaine, detectable in the blood for up to 96 hours following a single administration with the peak in bupivacaine release documented within the first hour after exposure.\(^22\) The lack of quantification of the bupivacaine concentration at the completion of the study precluded us from performing a more detailed analysis of chondrotoxicity relative to the effective bupivacaine concentration. Ongoing research is needed to characterize the long-term treatment effects of liposomal bupivacaine.

CONCLUSION
Single dose exposure of 1.3% liposomal bupivacaine demonstrates minimal chondrocyte toxicity following 1 hour of treatment, with significantly less chondrotoxicity in comparison to 0.5% ropivacaine and bupivacaine. Further research is needed to assess the long-term viability of chondrocytes following exposure to liposomal bupivacaine as well to perform in vivo testing.

REFERENCES
9. Chu CR, Izzo NJ, Papas NE, Fu FH: In vitro exposure to 0.5% bupivacaine is cytotoxic to bovine articular chondrocytes. Arthroscopy 2006; 22(7): 6939.