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Inhibition of Chondrocyte and Synovial Cell Death After Exposure to Commonly Used Anesthetics

Chondrocyte Apoptosis After Anesthetics

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Background: An intra-articular injection of local anesthetics is a common procedure for diagnostic and therapeutic purposes. It has been shown that these agents are toxic to articular cartilage and synovial tissue in a dose- and time-dependent fashion, and in some cases, they may lead to postarthroscopic glenohumeral chondrolysis (PAGCL). However, the role of apoptosis in cell death is still unclear, and the potential role of apoptosis inhibition in minimizing chondrocyte and synovial cell death has not been reported.

Purpose: (1) To quantify the degree of apoptotic cell death in chondrocytes and synovial cells exposed to local anesthetics, and (2) to determine whether caspase inhibition could reduce cell death.

Study Design: Controlled laboratory study.

Methods: Human chondrocytes and synovial cells were expanded in vitro and exposed to normal saline, 0.5% bupivacaine, 0.5% ropivacaine, 1% lidocaine, or 1:1000 epinephrine for 90 minutes. Apoptosis was then detected at 1, 3, 5, and 7 days after exposure using terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick-end labeling (TUNEL) and immunohistochemistry. Apoptosis was then inhibited using the pan-caspase inhibitor z-vad-fmk. Results were normalized to normal saline controls and analyzed by generalized regression models and pairwise confidence intervals.

Results: Analysis of cumulative chondrocyte apoptosis relative to controls after anesthetic exposure demonstrated more than 60% cell death with 0.5% bupivacaine and 1:1000 epinephrine. The greatest chondroprotective effect of caspase inhibition occurred with 0.5% ropivacaine. Similarly, in synovial cells, epinephrine was also very cytotoxic; however, 1% lidocaine induced the most apoptosis. Synovial cells exposed to 0.5% ropivacaine were again most sensitive to protective caspase inhibition.

Conclusion: Local anesthetics induce chondrocyte and synovial cell apoptosis in a time-dependent fashion, with peak apoptosis occurring 5 days after exposure. Both chondrocytes and synovial cells are most sensitive to caspase inhibition after exposure to 0.5% ropivacaine.

Clinical Relevance: Apoptosis inhibition may be an effective strategy in minimizing chondrocyte and synovial cell death after exposure to anesthetics. Further investigation is clinically warranted.

Keywords: arthritis; apoptosis; anesthetics; chondrolysis; synovial cells; cartilage; shoulder; caspase; inhibition; PAGCL

References:

1. Intra-articular injections of local anesthetics may be used as a procedure to enhance postoperative analgesia.\(^{18,19,40,41}\)

2. These agents are used in isolation or in conjunction with vasoconstrictors to enhance their effect and minimize bleeding during surgery. Studies have indicated that the use of these agents may decrease perioperative pain and reduce postoperative narcotic requirements.\(^{19,40}\) Additionally, in the outpatient setting, local anesthetics are routinely injected into joints for diagnostic or therapeutic purposes.

3. Previous in vitro studies have shown that local anesthetics such as lidocaine and bupivacaine can have cytotoxic...
and Institutional Review Board. Normal human synovial cells were extracted from approximately 1 cm³ of synovial explant tissue by collagenase digestion overnight and cultured in high-glucose DMEM, 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% Fungizone in an incubator at 37°C with 5% CO₂. Synovial tissue was obtained from patients undergoing elective arthroscopic surgery for shoulder subacromial impingement before the activation of the pump, which normally circulates normal saline into the joint during surgery. Cells were subsequently expanded to passages 3 to 6. For testing, pooled cells were plated in a monolayer culture in a 6-well culture plate at a density of 100,000 cells per well.

**Chondrocyte Viability Analysis: LIVE/DEAD Assay**

Chondrocyte cultures were subdivided into 4 treatment groups. Each group was exposed to 1 of the following: (1) 0.9% normal saline solution (Hospira, Lake Forest, Illinois), (2) 0.5% bupivacaine (Hospira), (3) 0.5% ropivacaine (APP Pharmaceuticals, Schaumburg, Illinois), and (4) 1% lidocaine (APP Pharmaceuticals). All samples were treated according to the same protocol. Specifically, culture medium was aspirated, and 200 μL of each treatment solution was added to each well. Cell viability was assessed after short-term exposure to local anesthetics with use of the LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen, Carlsbad, California) after 30, 45, 60, 75, 90, 105, and 120 minutes of exposure. Briefly, cells were incubated in 2 μM calcein AM and 4 μM EthD-1 solution diluted in phosphate-buffered saline (PBS) (Gibco) at room temperature in the dark for each exposure duration. Cells were then viewed with a fluorescent microscope (Zeiss, Thornwood, New York), and digital photographs were taken at 5× magnification. Cells were quantified using ImageJ Software (National Institutes of Health, Bethesda, Maryland) to determine chondrocyte viability at the 7 time points.

**Apoptosis Analysis: Anesthetic Exposure**

Chondrocyte and synovial cell cultures were subdivided into the same treatment groups as above as well as (5) 1:1000 epinephrine USP (Hospira). Because the viability analysis above indicated that 90 minutes of anesthetic exposure resulted in at least 50% cell death, the same duration of anesthetic exposure was chosen for further apoptosis analysis. After the incubation of chondrocytes and synovial cells for 90 minutes, the anesthetic was aspirated, and fresh medium was added to the plates. Apoptosis was analyzed at 1, 3, 5, and 7 days after exposure.

**Apoptosis Analysis: Caspase-3 Staining**

Antiacivated caspase-3 antibody (Promega, Madison, Wisconsin) was used as a marker of apoptosis. At 1, 3, 5, and 7 days after exposure, cells were fixed in 10% neutral buffered formalin in 6-well plates for 25 minutes at room temperature. All assays were run in triplicate. The plates were washed 2 times for 5 minutes in PBS at room temperature.
The fixed cells were then permeabilized by incubation in PBS/0.2% Triton X for 5 minutes at room temperature. The plates were washed 3 times in PBS for 5 minutes. Then, 200 μL of blocking buffer was added (PBS/0.1% Tween 20 + 5% normal goat serum, Vector Labs, Burlingame, California) for 2 hours at room temperature in a humidified chamber. After 1 rinse in PBS, 100 μL of anti-activated caspase-3 antibody diluted 1:500 in blocking buffer was added and incubated overnight at 4°C. The following day, the plates were washed twice for 10 minutes in PBS, twice for 10 minutes in PBS/0.1% Tween 20, and twice for 10 minutes in PBS at room temperature in a humidified chamber. The plates were washed twice in PBS for 5 minutes, once in PBS/0.1% Tween 20 for 5 minutes, and once in PBS for 5 minutes. The plates were stained with DAPI Gold Anti-Fade (Invitrogen) and observed by fluorescent microscopy.

Apoptosis Analysis: TUNEL Assay

Chondrocyte and synovial cell cultures were maintained and treated with anesthetics as described above. Terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick-end labeling (TUNEL) assay (Millipore, Billerica, Massachusetts) was used as a second measure of apoptosis. Briefly, plates were fixed for 10 minutes in 1% paraformaldehyde. Plates were washed twice in PBS for 5 minutes and permeabilized in cold 2:1 ethanol:acetic acid. Cultures were then washed twice in PBS for 5 minutes and incubated in equilibrium buffer for 15 seconds. Diluted TdT (Millipore) was added, and plates were incubated at 37°C in the dark for 1 hour. Stop solution was added, and plates were rinsed with PBS. Cultures were stained with DAPI Gold Anti-Fade and observed by fluorescent microscopy.

Apoptosis Inhibition

The irreversible broad-spectrum pan-caspase inhibitor z-vad-fmk (CalBiochem, Billerica, Massachusetts) was added at 50 μM to chondrocyte and synovial cell cultures for the entirety of the 90-minute anesthetic exposure period for each group.23,30,36,42 The anesthetic and caspase inhibitor were then aspirated and replaced with fresh medium + 50 μM of the caspase inhibitor for 24 hours after exposure, after which the medium was aspirated and replaced with normal (caspase inhibitor–free) medium for the duration of the protocol.

Statistical Analysis

Statistical analysis was performed by a professional statistician. Generalized linear regression models were used to estimate the effects of condition and time and the interaction effects of condition and time. These models were then used to construct simultaneous 95% confidence intervals for the analysis of pairwise differences between conditions for the different outcomes. When the time-by-condition interaction terms were not significant, they were removed from the models. When the time-by-condition interactions were significant, the pairwise comparisons were constructed at a specific time point (eg, day 7), and the nature of the interactions was described. Analyses were conducted using the R statistical program (version 13.2, WU University, Vienna, Austria).

RESULTS

Chondrocyte Viability Analysis: LIVE/DEAD Assay

Short-term chondrocyte viability in response to varying durations of anesthetic exposure of 30 to 120 minutes was assessed with the LIVE/DEAD assay at 15-minute intervals (Figure 1). When considered across all time points, bupivacaine exposure resulted in the highest percentage of chondrocyte death, with a statistically significant treatment effect of 18.25% increased cell death versus normal saline controls and 14.8% increased cell death versus ropivacaine (P < .001).

Figure 1. Chondrocyte death as a function of anesthetic exposure time: LIVE/DEAD assay. Short-term chondrocyte viability in response to varying durations of anesthetic exposure of 30 to 120 minutes was assessed with the LIVE/DEAD assay at 15-minute intervals. Across all time points, bupivacaine exposure resulted in the highest percentage of chondrocyte death, with a statistically significant treatment effect of 18.25% increased cell death versus normal saline controls and 14.8% increased cell death versus ropivacaine (P < .001). Notably, the bupivacaine effect significantly increased with increasing time of exposure (Figure 1).

Chondrocyte Apoptosis Analysis: Caspase-3 Staining and TUNEL Assay

Analysis of the degree of chondrocyte apoptosis after 90 minutes of anesthetic exposure was conducted by immunohistochemistry and TUNEL analysis at 1, 3, 5, and 7 days after exposure. Compared with treatment with normal saline alone, across all time points, bupivacaine and lidocaine exposure resulted in statistically significant
TABLE 1

Chondrocyte Apoptosis Across All Time Points After 90-Minute Anesthetic Exposure With and Without CI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increased Apoptosis vs Normal Saline (TUNEL Assay), %</th>
<th>Increased Apoptosis vs Normal Saline (Antiactivated Caspase-3 Antibody Staining), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupivacaine</td>
<td>62.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bupivacaine + CI</td>
<td>22.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.8</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>62.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.4</td>
</tr>
<tr>
<td>Epinephrine + CI</td>
<td>24.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>47.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lidocaine + CI</td>
<td>17.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>45.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.8</td>
</tr>
<tr>
<td>Ropivacaine + CI</td>
<td>0.96</td>
<td>0.2</td>
</tr>
<tr>
<td>Normal saline + CI</td>
<td>0.58</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>CI, caspase inhibitor.

<sup>b</sup><i>P</i> < .001.

<sup>c</sup><i>P</i> < .01.

<sup>d</sup><i>P</i> < .05.

Figure 2. Chondrocyte apoptosis after anesthetic exposure with and without the caspase inhibitor: TUNEL assay. Chondrocytes were exposed to anesthetics for 90 minutes with and without the caspase inhibitor, and apoptosis was analyzed with TUNEL assays at 1, 3, 5, and 7 days after exposure. Across all time points, bupivacaine and epinephrine exposure resulted in the highest percentage of chondrocyte apoptosis. Ropivacaine exposure with caspase inhibition resulted in the greatest rescue effect from apoptosis.

Figure 3. Chondrocyte apoptosis after anesthetic exposure with and without the caspase inhibitor: antiactivated caspase-3 staining. Chondrocytes were exposed to anesthetics for 90 minutes with and without the caspase inhibitor, and apoptosis was analyzed with antiactivated caspase-3 antibody staining at 1, 3, 5, and 7 days after exposure. Bupivacaine and lidocaine exposure resulted in the greatest percentage of chondrocyte apoptosis across all time points, with 20% and 15.7% apoptosis, respectively (<i>P</i> < .001). Bupivacaine showed the greatest response to caspase inhibition across all time points at 16% (<i>P</i> < .001).

<sup>Using Tukey comparison of means. CI, caspase inhibitor.</sup>
TABLE 3
Synovial Cell Apoptosis Across All Time Points After 90-Minute Anesthetic Exposure With and Without CI*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increased Apoptosis vs Normal Saline (TUNEL Assay), %</th>
<th>Increased Apoptosis vs Normal Saline (Antiactivated Caspase-3 Antibody Staining), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupivacaine</td>
<td>94.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bupivacaine + CI</td>
<td>78.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>81.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epinephrine + CI</td>
<td>53.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>99.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lidocaine + CI</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>87.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ropivacaine + CI</td>
<td>3.35</td>
<td>10.8</td>
</tr>
<tr>
<td>Normal saline + CI</td>
<td>0.16</td>
<td>-5.4</td>
</tr>
</tbody>
</table>

*CI, caspase inhibitor.
<sup>b</sup>P < .001.
<sup>c</sup>P < .01.

**Figure 4.** Synovial cell apoptosis after anesthetic exposure with and without the caspase inhibitor: TUNEL assay. Synovial cells were exposed to anesthetics for 90 minutes with and without the caspase inhibitor, and apoptosis was analyzed with TUNEL assays at 1, 3, 5, and 7 days after exposure. All anesthetic exposures resulted in a greater than 80% apoptotic effect across all time points (P < .001). Ropivacaine showed the greatest response to caspase inhibition, with an 84% decrease in apoptosis (P < .001).

**Figure 5.** Synovial cell apoptosis after anesthetic exposure with and without the caspase inhibitor: antiactivated caspase-3 staining. Synovial cells were exposed to anesthetics for 90 minutes with and without the caspase inhibitor, and apoptosis was analyzed with antiactivated caspase-3 antibody staining at 1, 3, 5, and 7 days after exposure. Bupivacaine, lidocaine, and ropivacaine exposures resulted in greater than 30% apoptosis (P < .001). Lidocaine showed the greatest response to caspase inhibition, with a 32% reduction (P < .01).
increased rates of chondrocyte apoptosis by both TUNEL and antiactivated caspase-3 staining, while epinephrine and ropivacaine exposure resulted in statistically significant increased rates of chondrocyte apoptosis only by TUNEL (Table 1 and Figures 2 and 3). Time-by-condition comparisons demonstrated that all anesthetic exposures resulted in significantly more apoptosis at baseline compared with normal saline controls (day 1) and an increased apoptotic effect over time, with bupivacaine demonstrating the largest average daily increase in apoptosis (11.7%; \( P < .0001 \)) and ropivacaine demonstrating the smallest (6.1%; \( P < .01 \)). Bupivacaine and epinephrine demonstrated the most severe apoptosis-inducing effects relative to the other agents tested (Table 1).

**Chondrocyte Apoptosis Inhibition**

To examine the potential effect of caspase inhibition on chondrocyte and synovial cell apoptosis, immunohistochemistry and TUNEL analysis were again used to quantify apoptosis rates at 1, 3, 5, and 7 days after anesthetic exposure in the presence of the caspase inhibitor. When considered across all time points, the addition of the caspase inhibitor caused a statistically significant reduction in apoptosis for chondrocytes exposed to all agents relative to controls, with the largest effect visible with ropivacaine (44.9% reduction overall by TUNEL; \( P < .001 \)) and the smallest effect with lidocaine (30.2% reduction overall by TUNEL; \( P < .01 \)) (Table 2 and Figures 2 and 3). For all agents, levels of apoptosis were still elevated relative to saline controls.

**Synovial Cell Apoptosis Analysis: Caspase-3 Staining and TUNEL Assay**

Apoptotic synovial cell death after 90-minute anesthetic exposure was assessed using the same technique as for chondrocytes at 1, 3, 5, and 7 days after exposure. Synovial cells demonstrated a statistically significant increase in apoptosis in response to all anesthetic exposures versus normal saline controls by both TUNEL and immunohistochemistry, with lidocaine demonstrating the greatest effect consistently (Table 3 and Figures 4 and 5).

Time-by-condition comparisons using both methods were then conducted to assess baseline apoptosis rates and rates of change over time. By TUNEL, all anesthetic exposures resulted in statistically significant increased baseline apoptosis of more than 60% (day 1) but no significant changes in apoptosis rates over time versus normal saline controls. By immunohistochemistry, only epinephrine- and lidocaine-exposed synovial cells demonstrated statistically significant increased baseline apoptosis versus normal saline (27.6% \( P < .005 \) and 18.9% \( P < .05 \), respectively), while bupivacaine and ropivacaine demonstrated a significantly increased rate of apoptosis over time compared with normal saline controls (15% and 13%, respectively; \( P < .0001 \)). However, both assays did show a statistically significant increase in apoptosis overall with exposure to bupivacaine, epinephrine, lidocaine, and ropivacaine compared with normal saline controls (Table 3 and Figures 4 and 5).

**Synovial Cell Apoptosis Inhibition**

When considered across all time points, the addition of the caspase inhibitor caused a significant reduction in apoptosis for synovial cells exposed to ropivacaine (by TUNEL) and lidocaine (by immunohistochemistry), with the largest effect visible with ropivacaine (83.8% reduction overall by TUNEL; \( P < .001 \)) and the smallest effect with bupivacaine (15.9% reduction overall by TUNEL; \( P > .05 \)) (Table 4).

**DISCUSSION**

The goal of the present study was to confirm the known toxic effects of local anesthetics and epinephrine on chondrocytes and, for the first time, to explore whether similar patterns of toxicity exist for synovial cells exposed to the same conditions. After determining that apoptotic cell death was present in both cell lines after anesthetic exposure, we also wanted to explore whether apoptosis inhibition was effective in reducing cell death after toxic exposure. To our knowledge, this is the first study reporting on the presence of synovial cell apoptosis after anesthetic exposure and, furthermore, the first report demonstrating a successful reversal of both chondrocyte and synovial cell apoptosis with concurrent administration of a caspase inhibitor during anesthetic exposure.

One of the drawbacks of this study was that it utilized chondrocytes and synovial cells in a monolayer, which maximizes cell exposure to the condition being tested. However, it may not be representative of the clinical setting, where there is a continuous circulation of synovial fluid (in the outpatient setting) or saline (during arthroscopic surgery). The concentrations chosen for this present study were derived from established parameters in prior studies.22,41
Apoptosis can be induced through both intrinsic and extrinsic caspase pathways, both involving the activation of cellular caspases. Caspases are key enzymes involved in regulating the highly specific proteolytic cleavage of cellular proteins leading to cell death, and evidence suggests that caspase-3 may also be involved in DNA fragmentation. Individual caspases may be cell specific, making the use of a pan-caspase inhibitor, such as z-vad-fmk, a more potent inhibitor in a joint space with multiple cell types.\textsuperscript{20,30,36} We used 2 different assays to assess apoptosis: TUNEL and antiactivated caspase-3 staining. Although both assays confirmed an apoptotic effect in response to the agents tested, the percentages often differed with a given agent. This may reflect the variability in their detection, as TUNEL reflects DNA fragmentation and caspase-3 reflects the early execution phase of apoptosis. There also exist apoptotic pathways that are independent of caspase activation, an effect that would favor TUNEL detection of apoptosis. Also, TUNEL may detect some necrotic cell death, and this topic remains controversial.\textsuperscript{11}

Previous in vitro studies have described a clear time- and dose-dependent association of anesthetic exposure with reduced chondrocyte viability. Various in vitro models utilizing bovine chondrocytes, human chondrocytes, and canine chondrocytes have all shown that the exposure of chondrocytes to 0.5% bupivacaine resulted in greater than 95% cell death at varying times of exposure from 15 minutes to 2 hours.\textsuperscript{3,7,8} Lo et al\textsuperscript{32} also showed that 0.5% bupivacaine, 1% lidocaine, and 0.5% ropivacaine displayed a clear time- and dose-dependent increase in chondrocyte death in bovine articular cartilage explants; interestingly, this occurred even in the presence of epinephrine or with neutralization of pH. Additionally, Piper and Kim\textsuperscript{40} showed that 0.5% ropivacaine was significantly less chondrotoxic than 0.5% bupivacaine at 24 hours after a 30-minute exposure. Finally, Dragoo et al\textsuperscript{19} demonstrated a significant reduction in chondrocyte viability after a continuous infusion of 1% lidocaine, 0.25% bupivacaine, or 0.5% bupivacaine with or without epinephrine for 24, 48, or 72 hours using a pain pump in normal human articular chondrocytes in vitro.

A series of in vivo studies have also corroborated the finding of significant chondrocyte cytotoxicity associated with anesthetic agents. In a rabbit shoulder model, Gomoll et al\textsuperscript{25} created a continuous infusion of 0.25% bupivacaine with and without epinephrine for 48 hours using a pain pump in vivo. They noted significant reductions in sulfate uptake and cell viability and worse histological scores relative to controls at 7 days after exposure but found no significant differences in levels of toxicity with and without the presence of epinephrine. In a rat model investigating the long-term effects of a single intra-articular injection of 0.5% bupivacaine, Chu et al\textsuperscript{6} found a significant difference in cell density relative to controls at 1, 4, 12, and 24 weeks after exposure but no differences in cell viability or histological score.

The present study confirmed that among the agents tested—0.5% bupivacaine, 0.5% ropivacaine, and 1% lidocaine—the most cytotoxic was clearly bupivacaine, which caused more than 60% greater chondrocyte cell death relative to controls, with a direct linear relationship of increasing cell death with increasing duration of exposure, as previously described in prior studies.\textsuperscript{5,7,8,32} In addition, epinephrine seems to also be significantly more toxic relative to controls, although in the present study, it was administered in isolation as opposed to in conjunction with the anesthetic agents tested. It appears, on the basis of our findings, that epinephrine is not only toxic to chondrocytes and synovial cells but may also potentiate the effects of anesthetics, as has been suggested in the literature.\textsuperscript{28,50} Based on these findings, it may not be advisable to add epinephrine to normal saline used in arthroscopic surgery, even at concentrations as low as 1:1000 mL, despite the clinical advantage of minimizing bleeding during arthroscopic surgery. The greatest chondroprotective effect of caspase inhibition occurred with 0.5% ropivacaine, which is encouraging because this agent seems to be the least toxic to chondrocytes at baseline based on our findings and those of multiple prior authors.\textsuperscript{22,26,40,41}

In a similar fashion to chondrocytes, the present study indicates that the exposure of human synovial cells to commonly used anesthetics and epinephrine is consistently cytotoxic over time and that the effects appear to increase in a linear fashion up to 7 days after exposure. Interestingly, although 0.5% bupivacaine was most toxic to chondrocytes, in the case of synovial cells, 1% lidocaine was most toxic and caused the highest percentage of cells undergoing apoptotic cell death. However, similar to what we observed with chondrocytes, synovial cells exposed to 0.5% ropivacaine were most susceptible to caspase inhibition. These findings may support the concomitant administration of ropivacaine with a caspase inhibitor if a local anesthetic is warranted in the clinical setting to minimize both apoptotic chondrocyte and synovial cell death and thereby enhance cell viability over time.

The association between anesthetic exposure and chondrocyte death in the clinical setting is well characterized in humans, specifically the potential for profound chondrolysis or PAGCL after pain pump use, and has led to the recommendation that these devices are no longer used for postoperative analgesia in joints.\textsuperscript{1,2,4,27,44} Since the initial clinical report in 2004 by Petty et al\textsuperscript{39} of 1 case of PAGCL, there have been a series of subsequent reports in the orthopaedic peer-reviewed literature that draw an association between intra-articular pain pump use and the potential development of PAGCL.\textsuperscript{27,44,45-51,54} Although there appears to be a consistent relationship between intra-articular pain pump use and the potential development of PAGCL, other possible contributors including radiofrequency devices, anchors, iatrogenic injuries, thermal factors, and bioabsorbable implants have also been suggested.\textsuperscript{43,51}

For patients with PAGCL, many of whom are young, shoulder arthroplasty is the only currently reliable means of providing pain relief and functional improvement after nonoperative measures have failed.\textsuperscript{21,31,35,46} The clinical benefit of shoulder arthroplasty results both from resection of pathological articular cartilage as well as from capsulectomy and capsular release of pathological synovial tissue.
On the basis of clinical evidence in patients undergoing shoulder arthroplasty for PAGCL, there appears to be profound damage to both articular cartilage and synovial tissue associated with anesthetic exposure delivered by pain pumps. The current in vitro study demonstrates a convincing linear relationship between anesthetic exposure of synovial cells and increased cell death by both necrosis and apoptosis. This may explain why patients with PAGCL seem to present with more profound damage to synovial tissue relative to patients undergoing shoulder arthroplasty for primary osteoarthritis.

Apoptosis has been shown to play an important role in the progression of cartilage degeneration in arthritis as well as chondrocyte cell death in response to local anesthetics, specifically lidocaine. Apoptosis inhibitors are actively being researched as adjunctive agents in the administration of volatile anesthetics to decrease neuronal apoptosis, with promising results. However, this study represents the first application of the use of a caspase inhibitor to mitigate local anesthetic-induced chondrocyte and synovial cell apoptosis. By acting both through the inhibition of DNA fragmentation, as seen with TUNEL analysis, and proteolytic enzyme cleavage, as seen with both assays, apoptosis inhibition may be a potential adjunct to the administration of anesthetics into joints. Caspase inhibitors are also being investigated for clinical use and are currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials. The caspase-1 inhibitor VX-740 is currently in Food and Drug Administration (FDA) clinical trials.