Bupivacaine vs. Ropivacaine: Looking at the chondrotoxic effects of intraarticular anesthetics in osteoarthritic joints

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Abstract

Objectives: Intraarticular injections of corticosteroids used in combination with local anesthetics are commonly used in orthopedic practice for osteoarthritic pain relief. Many orthopedists question whether these anesthetics have chondrotoxic effects on human articular cartilage. The purpose of this meta-analysis is to examine the chondrotoxic effects of local anesthetics on osteoarthritic human articular cartilage and to determine whether ropivacaine has fewer chondrotoxic effects than bupivacaine.

Methods: Studies were found using PubMed with the search terms, “Ropivacaine,” “Bupivacaine,” and “Chondrocytes.” Initially 20 studies were found. Several studies were eliminated using the following excluding factors: human studies, published in last 10 years, English language and inclusion of ropivacaine and bupivacaine. The final studies were chosen based on their relation to our clinical question.

Results: Studies show that concentrations less than 0.75% ropivacaine and concentrations of 0.25% bupivacaine or less have fewer chondrotoxic effects on osteoarthritic cartilage. Studies show that chondrotoxicity increases with time after exposure and increases from ropivacaine to bupivacaine.

Conclusion: Both ropivacaine and bupivacaine are chondrotoxic to human cartilage in a time-dependent, drug-dependent, and concentration-dependent manner. Future studies are needed to observe results for longer durations and investigate whether chondrotoxicity is potency-dependent in order to conclude that ropivacaine is a safer option than bupivacaine.

Introduction

Osteoarthritis (OA) is a degenerative joint disease caused by cartilage degeneration, bone exposure, and synovial hypertrophy. Over time, the joint surface is subject to bony sclerosis, osteophyte formation and bone cyst formation. Osteoarthritis is the most common joint disorder in the United States. Obesity, female gender, age greater than 40, repetitive movement, and overuse of a joint are known risk factors of the development of osteoarthritis. The most common presenting symptom of OA is progressively worsening achy joint pain. The pain is generally exacerbated by use of the joint and may be partially relieved by rest. Crepitus and reduced range of motion are also common presentations. 1

Currently, there are no pharmacologic therapies available that prevent the progression of joint damage in osteoarthritic patients. Instead, the goals of therapy for a patient suffering from osteoarthritis include: pain and inflammation control, improved quality of life, minimized disability, and educating the patient of their important role in their disease management. 1 The therapeutic treatments for OA include both nonpharmacological and pharmacological agents.

Nonpharmacological treatments include exercise programs, weight loss, and patient education. These interventions are normally introduced before pharmacological treatment. Once nonpharmacological management has been proven ineffective, patients may begin treatment with oral and topical analgesics. Regarding patients with persistent moderate to severe symptoms that fail management with analgesics alone, intraarticular injections of a combined corticosteroid and local anesthetic may be indicated.

Intraarticular injections of corticosteroids are a commonly practiced therapy for osteoarthritis. Injection of corticosteroids can alleviate pain and inflammation of a joint. Low dose corticosteroid injections have been proven to be very successful in relieving OA symptoms by decreasing inflammation within the joint capsule. Conversely, at high doses, corticosteroids have been shown to have detrimental effects to the soft tissue structures within a joint. 2 For this reason, intraarticular corticosteroid are normally only indicated as an adjuvant therapy for OA patients. 3 Many times corticosteroids injections will be used short term for therapeutic relief in order to delay the time until total joint arthroplasty will be needed. The current recommended corticosteroid agent is triamcinolone acetonide at standard doses of 40 mg for a large joint such as a knee or shoulder. 1 The recommended frequency is four corticosteroid injections or less every year, spaced at least 4 to 6 weeks apart. 2
Local anesthetics are often used in combination with corticosteroids for intraarticular injections of osteoarthritic joints. A survey by the American College of Rheumatology found that approximately two-thirds of rheumatologists in the United States generally diluted glucocorticoid injections with a local anesthetic. Local anesthetics are customarily used in order to act as a diluting agent for the crystalline suspension of the corticosteroids. Other indications of local anesthetics are to provide additional temporary analgesia and to eliminate the possibility of referred pain.

Contraindications to local anesthetic use in intraarticular injections include sensitivity reactions, a known coagulopathy, or a local sepsis. Cardiovascular and central nervous system toxicities are the most well-known and worrisome adverse effects of local anesthetic. In the case of intraarticular injections, these adverse reactions are uncommon, since the agent is localized to the joint capsule. However, it is possible to reach high systemic levels if the amount of local anesthetic exceeds the recommended maximum dose or if the injection is accidentally misplaced intravenously into the systemic circulation.

Bupivacaine is currently the most commonly used intraarticular local anesthetic. Ropivacaine and lidocaine are two other commonly used local anesthetics. Bupivacaine is a member of the amino amide class of local anesthetics and a part of the piperidoloxylidide group. Ropivacaine’s onset ranges from 15 to 30 minutes. The onset of bupivacaine is relatively longer compared to a fast acting local anesthetic, like lidocaine, whose effects are almost immediate. However, a benefit of bupivacaine is its long duration, ranging from 5 to 12 hours of action. The current recommendations, according to the American Family Physician, is to use 0.25 to 0.5 percent bupivacaine or 1 percent lidocaine mixed with an equal volume of corticosteroid for intraarticular injections of arthritic joints.

Recently, experimental studies have suggested that local anesthetics may be toxic to articular cartilage. Many of these studies have focused on bupivacaine specifically. The results of these studies have indicated that bupivacaine does indeed have a dose-dependent and time-dependent toxicity to chondrocytes. This prompts the question of whether bupivacaine should remain the local anesthetic agent of choice in intraarticular joint injections.

Ropivacaine is a newer local anesthetic agent and may be a promising alternative to bupivacaine for intraarticular injections. Like bupivacaine, ropivacaine is a long-acting local anesthetic agent. The onset and duration of action of ropivacaine are relatively identical to that of bupivacaine. Ropivacaine is also a member of the amino amide class of local anesthetics and a part of the piperidoloxylidide group. The difference between ropivacaine and bupivacaine is a variation in the alkyl group of the two molecules. This small molecular variation makes ropivacaine less lipid soluble, thus causing it to diffuse less readily than bupivacaine into the systemic circulation. Overall, ropivacaine has fewer cardiovascular and central nervous system toxic effects, which allows for higher maximum doses of ropivacaine to be used. Recent studies have also shown that ropivacaine may have a lower toxic effect than bupivacaine on articular cartilage.

Currently, there is a lack of definitive data on which local anesthetic is the best choice to be used in combination with corticosteroids for intraarticular injections. This meta-analysis aims to compare experimental studies on the chondrotoxic effects of bupivacaine and ropivacaine. This will help us to determine which local anesthetic agent and which dose and duration of that agent is least harmful to articular cartilage.

**Clinical Scenario**

Mrs. OA is a 52 year old woman suffering from osteoarthritis of her right knee. The pain is described as constant and worsening. She has attempted weight loss, activity modifications, nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and topical analgesics, but has experienced no relief of symptoms. The orthopedist discusses combined corticosteroid and anesthetic intraarticular injections with Mrs. OA as a next step option. The orthopedist recently heard about potential toxic effects of local anesthetics on chondrocytes. He wants to know which anesthetic and concentration has the least chondrotoxic effects in order to prevent further damage to Mrs. OA’s knee.
Clinical Question
Population: 40-65 year olds with osteoarthritic joint pain
Intervention: Bupivacaine
Comparison: Ropivacaine
Outcome: The toxic effects on articular chondrocytes

In 40 to 65 year olds with osteoarthritic joint pain, what are the toxic effects of bupivacaine as compared to ropivacaine on articular chondrocytes?

Methods
Pub Med was searched on September 15, 2016, using the search terms “Bupivacaine,” “Ropivacaine,” and “Chondrocytes.” The database yielded 20 articles relating to these search terms, and no duplicates were present. Articles within the past 10 years that were of the English language, involved humans, and included both ropivacaine and bupivacaine were considered. Records that did not meet these requirements were excluded and 20 articles remained. The remaining articles were assessed for eligibility through a process of elimination. Of the remaining articles, all experiments were found to be performed in vitro, (performed outside of living organisms and in culture dishes). Experiments showing the effects of local anesthetics directly have not been performed yet. We excluded articles that combined anesthetics with other medications, such as corticosteroids. Meta-analyses and studies that did not answer our clinical question were removed. For example, some studies compared local anesthetics to other alternatives, such as opioids and magnesium, whereas we are comparing local anesthetics to each other. Three articles were left after the process of elimination. The three articles all tested bupivacaine and ropivacaine, used first pass chondrocytes for their experiments, used saline as a control, and used P values to compare their results (Figure 1).

The selected articles evaluated their results using several types of statistical analyses, including the two-tailed student T test and the ANOVA with the Bonferroni post hoc test, the Dunnett T post hoc test, and the Tukey Honestly Significant Difference post hoc test. The student T test is a way to test relationships between two subjects by comparing results to the mean in a positive and negative direction. Test results are compared in two directions, and therefore, look at the overall significant difference between two subjects.

Another test used was the analysis of variance, also known as ANOVA. ANOVA determines whether a significant difference is present between the means of two or more groups or variables. A one
way and two way ANOVA were utilized during the studies where appropriate. The one way is typically used when the study is comparing different conditions of one independent variable. The two way is utilized when there are two independent variables. Overall, the ANOVA allows researchers to test more than one hypothesis at the same time. However, it does not specifically tell which groups differed. The post hoc tests, Bonferroni, Dunnett, and Tukey, are utilized in order to inform researchers which groups significantly differed during the study. Overall, through the use of these analyses, P values were calculated in order to determine statistical significance of the data. Piper et al. and Grishko et al. defined statistical significance as less than 0.05, whereas Breu et al. defined it as less than 0.01.

Results

Study #1

Study Objective
To assess the chondrotoxic effects of mepivacaine, ropivacaine, and bupivacaine.

Study Design
The study was performed as an in vitro experiment. Various techniques of flow cytometry and fluorescence microscopy were used to measure the effects of mepivacaine, ropivacaine, and bupivacaine on cell viability, apoptosis, and necrosis of chondrocytes. The study sample was made up of harvested osteoarthritic cartilage from 4 patients, ages 42 to 62 years old, who were undergoing total joint arthroplasty.

Flow cytometry was one technique that was used to measure chondrocyte viability, apoptosis, and necrosis. Chondrocytes from each of the four subjects were exposed to 1 mL of bupivacaine, ropivacaine, and bupivacaine at different concentrations for 1 hour. The concentrations of the three anesthetics used were; bupivacaine 0.03125%, 0.0625%, 0.125%, 0.25% and 0.5%; ropivacaine 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, and 0.75%; mepivacaine 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, and 2%. Control cells were exposed to normal saline for 1 hour. The Annexin-V-Fluos Staining Kit was used to identify apoptotic, necrotic, and viable cells at 24 hours and 96 hours after local anesthetic treatment. The percentage of viable, apoptotic, and necrotic cells was then calculated at 24 hours and 96 hours using an analysis software called, FloJo.

Fluorescence microscopy was used to measure cell viability. Chondrocytes from the four subjects were treated with 1 mL of bupivacaine 0.5%, ropivacaine 0.75%, and mepivacaine 2% for 1 hour. Control cells were exposed to normal saline for 1 hour. The cells were stained with 4 uM calcine AM and 2 uM ethmidium homodimer for 2 hours. They were then imaged with fluorescence microscopy and scored for viability. The cell viability was measured at 1 hour, 24 hours, 4 days, and 7 days after exposure to the local anesthetics.

Fluorescence microscopy was also used to measure apoptosis activity of the chondrocytes. Chondrocytes from the four subjects were treated with 1 mL of bupivacaine 0.5%, ropivacaine 0.75%, and mepivacaine 2% for 1 hour. Control cells were exposed to normal saline for 1 hour. Using fluorescence microscopy, the cell morphology and capsase activity were measured to determine the degree of apoptosis. The degree of apoptosis of the chondrocytes was measured at 24 hours and 48 hours after exposure to local anesthetics.

The researchers also conducted another experiment specifically focusing on the effects of local anesthetics to osteoarthritic cartilage compared to intact cartilage. This study used exclusively femoral articular cartilage, which was harvested from only two out of the original four subjects. Five cores of intact cartilage and five cores of osteoarthritic cartilage were used from each of the two subjects. The chondrocyte viability of the intact cartilage was compared to the osteoarthritic cartilage after exposure to 5 mL of bupivacaine 0.5%, ropivacaine 0.75%, and mepivacaine 2% for 1 hour. Control cells were
exposed to normal saline for 1 hour. The cartilage cores were stained with 4 uM calcein AM and 2 uM ethidium homodimer for 24 hours and were then imaged with fluorescence microscopy to analyze chondrocyte viability. The cartilage cores were examined at 24 hours and 96 hours after exposure to the local anesthetic.

**Study Results**

For the purposes of this review we will be focusing only on the results that include bupivacaine and ropivacaine.

The flow cytometry results demonstrated that both bupivacaine and ropivacaine showed time-dependent and concentration-dependent cytotoxic effects on human chondrocytes. At 24 and 96 hours after bupivacaine treatment, there was a statistically significant decrease in the number of viable cells, \( P < 0.0001 \), an increased fraction of apoptotic cells \( P < 0.0001 \), and an increase in necrotic cells \( P < 0.0001 \) (Figure 2).

In regards to the chondrocytes exposed to the ropivacaine treatment, chondrocyte viability was not significantly reduced at 24 hours after treatment \( P > 0.01 \). However, 96 hours later, a decrease in viability \( P = 0.0067 \) and an increase in necrosis \( P = 0.0015 \) were noticed compared with the control. Concentrations of ropivacaine <0.75% did not demonstrate a reduction in viability. When comparing the different anesthetics to each other, the results showed that equipotent concentrations of local anesthetics caused differences in viability and necrosis. Viability rates were higher after ropivacaine 0.75% treatment compared with an equipotent concentration of bupivacaine 0.5% \( P = 0.0087 \). The rate of apoptotic and necrotic cells was also higher in bupivacaine 0.5% than in the ropivacaine 0.75% \( P = 0.0021 \) and \( P = 0.0009 \) (Figure 2).

Chondrocyte viability was further assessed using a live–dead staining. The results revealed that the number of dead cells and cell detritus increased in a time-dependent and substance-dependent manner. Bupivacaine treatment demonstrated a higher chondrotoxicity compared to ropivacaine (Figure 3).

Caspase activity was determined to distinguish whether apoptosis or necrosis

*Figure 2. Dose-response curves of different concentrations of bupivacaine and ropivacaine. Chondrocyte apoptosis (A) and necrosis (N) was measured using flow cytometry at time intervals of 24 hours and 96 hours after a 1-hour exposure.*

*Figure 3. The effects of bupivacaine 0.5% (1), ropivacaine 0.75% (2), and saline control (3) on chondrocyte viability at 24 hours after a 1-hour exposure using live–dead staining. The nuclei of dead cells is stained red, and the viable chondrocytes are stained green. The number of dead cells increased from ropivacaine to bupivacaine.*
was responsible for chondrotoxicity of local anesthetics. The cells exposed to bupivacaine 0.5% exhibited extensive chondrocyte apoptosis after 24 hours and an increased amount of cell fragments. When compared with the bupivacaine group, the fraction of apoptotic cells was distinctly lower after treatment with ropivacaine 0.75%.

Osteochondral cores of intact and osteoarthritic human cartilage were analyzed and compared for chondrotoxicity with local anesthetic treatment. Intact and osteoarthritic cartilage exposed to bupivacaine 0.5% for 1 hour exhibited extensive chondrocyte necrosis after 24 hours. Cell damage was worse in osteoarthritic compared with intact cartilage. The effects of ropivacaine 0.75% on the intact osteochondral cartilage were similar to that of the control saline solution. However, ropivacaine treatment of osteoarthritic cartilage resulted in an increased chondrocyte death rate. The samples were also measured four days after treatment and were compared to the results after 24 hours. No differences in death rates or cellular density were visible in the intact cartilage. Conversely, the osteoarthritic cartilage revealed signs of increased cell death. The bupivacaine treated sample resulted in substantial chondrocyte necrosis at the surface and subsurface with the nearly no viable cells. The ropivacaine treated samples, however, showed occasional necrotic cells along the surface layer of the osteochondral cores.

In conclusion, the results of the different experiments demonstrated a similar course of the dose–response curves among all samples (Figure 2). The results show that both ropivacaine and bupivacaine demonstrate time and concentration dependent toxic effects to chondrocytes. Toxic effects were shown to be greater with bupivacaine treatment compared to ropivacaine treatment. Bupivacaine was also shown to have a postponed onset of cytotoxicity. Additionally, it was determined that immediate cell death after anesthetic treatment was mainly due to necrosis followed by apoptosis. Cell damage was also observed to be worse in osteoarthritic compared with intact cartilage.

**Study #2**

*Apoptosis and Mitochondrial Dysfunction in Human Chondrocytes Following Exposure to Lidocaine, Bupivacaine, and Ropivacaine. Grishko, PhD et al.*

**Study objective**

To investigate the effects of lidocaine, bupivacaine, and ropivacaine on human chondrocyte viability and mitochondrial function in vitro and to characterize the type of cell death elicited following exposure.

**Study Methods**

The study was performed as an in vitro experiment. The sample population consisted of cartilage obtained from both femoral condyles and tibial plateaus of patients with osteoarthritis who were an average of 53 ± 16 years old and undergoing total knee replacement. The chondrocyte cultures were generated as a mixed population of damaged cartilage, in order to reflect osteoarthritic cartilage as a whole.

The cultures were exposed to the different concentrations of lidocaine, ropivacaine, and bupivacaine for one hour. Lidocaine concentrations of 2%, 1%, and 0.5%; bupivacaine 0.5% and 0.25%; and ropivacaine 0.5% and 0.2% were used. Control cultures were exposed to a saline solution under the same conditions. After 60 minutes, cells were removed and placed in a normal culture medium to allow time to recover for 24, 72, and 120 hours. The cultures were placed in tetrodotoxin or Earle’s Balanced Salts formulation media to evaluate for mitochondrial DNA repair and damage, ATP synthesis, the induction of apoptosis, or changes in concentrations of specific mitochondrial proteins.

Flow cytometry, using the ApoScreen Annexin V apoptosis kit, was utilized to identify apoptotic, necrotic, and viable cells. Samples were analyzed by a FACSDiva flow cytometry machine. The results were studied and recorded.

To further evaluate the involvement of apoptosis in chondrocyte death, the cultures were exposed to lidocaine, bupivacaine, or ropivacaine. The appearance of apoptosis was evaluated by the observation of condensed and fragmented nuclei following DAPI staining at 24, 72, and 120 hours after treatment. Western blot analysis with antibodies against caspase 3 and caspase 9 was also used to determine whether
caspase activation was involved in the initiation of apoptosis. Anti-actin antibody was used to ensure equal loading of protein samples.

The damage to mitochondrial DNA was studied at 60 minutes after treatment with the local anesthetics at their different concentrations. The DNA was extracted from primary chondrocyte cultures, isolated, and prepared. DNA damage was evaluated as the number of DNA breaks per 16.6-kb fragment. Break frequency was determined using the Poisson expression.

ATP levels in the cells were studied using the ATP bioluminescence assay kit. The cultures were studied three hours after a sixty minute exposure to the local anesthetic concentration.

Western blot was used to analyze changes in mitochondrial proteins. Mitochondrial subunit III of cytochrome-c oxidase was isolated and evaluated. This subunit is one of thirteen proteins encoded by mitochondria. The levels of this subunit change in occurrence with mitochondrial dysfunction.

**Study Results**

For the purposes of this review we will only be focusing on the results of this experiment that included bupivacaine and ropivacaine.

Chondrocyte culture results were interpreted at 24, 72, and 120 hours with flow cytometry (Figure 4). At 24 hours, a detectable but not significant decrease in chondrocyte viability was seen after one hour exposure of 0.5% bupivacaine. Exposure of 0.25% bupivacaine and 0.5% and 0.2% ropivacaine did not show any decrease in cell viability when compared to saline. Flow cytometry 24 hours after exposure also showed that all treatments caused cell necrosis with no significant increase in apoptotic cells. At 120 hours from exposure, all concentrations of bupivacaine and ropivacaine, except for 0.2% ropivacaine, significantly decreased chondrocyte viability (p<0.05) and increased the number of apoptotic cells. The use of DAPI staining also demonstrated cell apoptosis due to the presence of condensed and fragmented apoptotic nuclei.

Further evidence of apoptosis and possible mitochondrial damage was demonstrated from the caspase activation-cleavage assays. The elevation in caspase 3 cleavage confirmed that apoptosis occurs after exposure to local anesthetics, while the elevation in caspase 9 cleavage indicated that mitochondrial dysfunction is associated with apoptosis. The mitochondrial DNA Southern blot analyses and ATP bioluminescence assays also showed evidence of mitochondrial damage as a cause of apoptosis. After one hour of exposure, all local anesthetics were shown to cause mitochondrial DNA damage to chondrocytes. 0.5% bupivacaine was shown to significantly decrease ATP levels (P < 0.05), while all concentrations of ropivacaine had no significant effect on ATP levels.

Overall, this study demonstrated that both ropivacaine and bupivacaine are chondrotoxic to osteoarthritic cartilage and can cause cell apoptosis. Results highly suggest that apoptosis is induced by mitochondrial dysfunction due to damage of its DNA.

**Study #3**

*Comparison of Ropivacaine and Bupivacaine Toxicity in Human Articular Cartilage. Piper et al.*

**Study Objective**

To determine whether 0.5% bupivacaine is chondrotoxic to human articular cartilage and whether 0.5% ropivacaine is a less toxic alternative.
**Study Design**

This study was performed at the San Francisco VA Medical Center in San Francisco, California. It was implemented in vitro using normal human articular cartilage from five patients total. The five patients consisted of three that were undergoing hip hemiarthroplasty for treatment of a femoral neck fracture and two that were undergoing total knee replacement for treatment of a distal femoral tumor. Cartilage samples were obtained from the femoral head for those with the femoral neck fracture and the tibial plateau for those with the distal femoral tumor. Cartilage explants and chondrocyte cultures were then prepared from the samples.

To prepare the cartilage explants, a punch biopsy was used to collect fifteen cartilage explants from each bone sample. The explants were all placed into a culture plate with media. The plate was incubated at 37 degrees Celsius with 5% CO2, and samples were used for experimentation within 48 hours of collection.

Chondrocyte cultures were prepared using the remainder of the bone cartilage. The cartilage was first digested using hyaluronidase and sterile collagenase at 37 degrees Celsius in order to break up the cartilage and obtain chondrocyte cells. Chondrocytes were plated in monolayer culture in fresh media and were used one week later for experimentation. Before the cells were used, chondrocytes were observed under the microscope to make sure they were differentiated and ready for experimental use. Only first passage chondrocytes were re-plated. The plate was incubated at 37 degrees Celsius with 5% CO2, and culture medium was changed every three to four days.

Once the cultures and explants were prepared, they were all divided into three treatment groups, including 0.5% bupivacaine, 0.5% ropivacaine, and 0.9% normal saline (control). All treatment groups underwent the same protocol for the experiment. Culture mediums were aspirated, and 200 microliters of each treatment were added to separate wells. The samples were incubated at 37 degrees Celsius with 5% CO2 for thirty minutes. Samples were then aspirated, replaced with new culture medium, and incubated for 24 hours.

Chondrocyte viability in cartilage explants was assessed within three of the five patients, one from femoral head and two from tibial plateau, while viability in cultured chondrocytes was assessed within all five patients. Cell viability in cartilage explants was measured using the LIVE/DEAD Cell Viability/Cytotoxicity Assay and in cultured chondrocytes using the CellTiter-Glo Luminescent Cell Viability Assay. The LIVE/DEAD Cell Viability/Cytotoxicity Assay allows live and dead cells to be differentiated under fluorescent microscopy. Three sections were collected from each explant sample and were exposed to 60 μL of 1-μM calcein AM/1 μM ethidium homodimer-1 solution for 30 minutes. Cells were visualized under fluorescent microscopy, and photographs were taken at 5x magnification. Live and dead cells were counted in a 1 mm x 1 mm x 100 μm area using a computer software program. The CellTiter-Glo Luminescent Cell Viability Assay measures cell viability through the use of a homogenous mixture that causes cell lysis and production of luminescence based on the amount of ATP present. The cultured chondrocytes were exposed to the reagent and luminescence was measured. The data was standardized by dividing the measured luminescence of the bupivacaine and ropivacaine treated cells by the luminescence of the controlled saline cells.

**Study Results**

For this study, all data was analyzed using the two tailed student T test. The level of significance was defined as P < 0.05. Results from the LIVE/DEAD Cell Viability/Cytotoxicity Assay in cartilage explants showed 94.4% +/- 9% live chondrocytes after treatment with ropivacaine, 78% live chondrocytes after treatment with bupivacaine, and 95.8% +/- 5.7% live chondrocytes after treatment with normal saline solution. Chondrocyte viability was significantly greater in cartilage explants treated with 0.5% ropivacaine as compared to 0.5% bupivacaine (p=0.0004). However, treatment with ropivacaine showed no difference in chondrocyte viability when compared to treatment with normal saline (p=0.6). Cartilage explants treated with ropivacaine and normal saline showed live cells predominantly from the articular surface to a depth of 1 mm, while cartilage explants treated with bupivacaine showed dead cells predominantly from the articular surface to a depth of 0.5 mm (Figure 5).
Results from the CellTiter-Glo Luminescent Cell Viability Assay in cultured chondrocytes showed 63.9% +/- 10% viability after treatment with ropivacaine, 37.4% +/- 12% viability after treatment with bupivacaine, and 100% viability after treatment with 0.9% normal saline.

Chondrocyte viability was significantly greater in cultured chondrocytes treated with 0.5% ropivacaine as compared to 0.5% bupivacaine (p<0.0001). Cultured chondrocytes treated with normal saline had significantly higher chondrocyte viability than those treated with ropivacaine or bupivacaine (p<0.0001).

The study demonstrated that short term treatment with 0.5% bupivacaine is toxic to intact human articular cartilage and cultured chondrocytes. Meanwhile, 0.5% ropivacaine is shown to be toxic to just cultured chondrocytes. Overall, 0.5% ropivacaine is shown to be significantly less chondrotoxic to human articular cartilage than 0.5% bupivacaine. Therefore, ropivacaine may be a safer intra-articular anesthetic for the orthopedic world.

**Critiques and Limitations of Studies**

Overall, Breu et al. was a great representation of our clinical question. To begin, the sample population consisted of human articular cartilage harvested from 4 patients in the age range of 42 – 62 years old. This is an accurate representation of the population in our clinical question. Bupivacaine and ropivacaine were both included in this study, and the results of these two anesthetics were compared. The study also compared the effects of different concentrations and time intervals of both drugs, which accurately addresses the intervention and comparison of our clinical question. Another great aspect of this study was its consistency and reproducibility. The researchers did an excellent job documenting the specific methods of their experiment. The articular cartilage used for all aspects of the experiment was prepared and collected in a standardized approach. The study also used more than one test to determine the results. Flow cytometry is a common test used for the assessment of chondrocyte viability. However, membrane damage can occur during the cell preparation of flow cytometry. Breu et al. addressed this limitation and stated that it could over estimate viability. Therefore, the experimenters also analyzed viability rates and apoptosis markers with cell-staining methods. The results of the cell staining methods indicated a reduced proportion of viable cells compared with flow cytometry. This was subsequently addressed in the results and conclusions of the study. The compared results of the tests used in this study demonstrated consistency and accuracy. This is an inadequacy that was present in the other two studies since they did not compare the test used and did not address this limitation in their paper. At the end of Breu et al., the authors clearly defined that there are no conflicts of interest within their experiment. Additionally, it was stated that the ethics of this experiment were approved by the IRB.

A unique and advantageous aspect of Breu et al. was that it included an experiment comparing the effect of bupivacaine and ropivacaine on chondrocytes of both intact and osteoarthritic cartilage. The other two studies used in this analysis varied in that aspect. Grishko et al. used only samples of osteoarthritic cartilage, and Piper et al. used samples of intact cartilage.

The second study we looked at, Grishko et al., did a fairly good job of representing our clinical question. The sample population of this study consisted of articular cartilage from both femoral condyles and tibial plateaus of patients with osteoarthritis, who were an average of 53 ± 16 years old and undergoing total knee replacement. This is an accurate representation of the population in our clinical question. However, one limitation to the sample population is that the number of samples was not included in the article. Like Breu et al., this study also compared the effects of different concentrations
and time intervals of both drugs, accurately addressing the intervention and comparison of our clinical question. The study also demonstrated reproducibility with specific methods and clearly demonstrated results. The researchers used multiple methods of measuring the effects of the anesthetics on the chondrocytes. All of the experiments found the same results, portraying reproducibility. The researchers also stated that there was no external funding source to support the study, which reduces the possibility of bias.

Piper et al. was the third study used in our analysis, and while it was a good study, it was the least comparable to our clinical question. The sample population used in this study consisted of five patients. Three of the five patients had femoral neck fractures and two had distal femoral tumors. The articular cartilage used in this experiment was intact cartilage that was absent from damage or degeneration. This differs from our clinical question, which focuses on the effects of ropivacaine and bupivacaine on osteoarthritic cartilage. The sample population was also inconsistent. The researchers did not state the age group or gender they were focusing on and did not include any population inclusion or exclusion criteria. Also, the results of the study only looked at cartilage explants from three out of the five patients, but did not state why the other two patients were excluded. Another limitation to the study is that this experiment only used one concentration of bupivacaine and ropivacine and measured the chondrocyte viability at only one point in time. Our clinical question was specifically focusing on the concentration and time dependent effects of anesthetics on chondrocyte viability.

There were also strengths to Piper, et al.’s study. One strength was that this study included and compared the results of both ropivacaine and bupivacaine. Another strength of the study was that it used a standardized tissue collection procedure approved by the University of California at San Francisco for collecting cartilage. Cartilage was prepared under sterile conditions, and all cartilage was inspected for damage or degeneration before the study. When performing the experiment, the same protocol was used to treat each cartilage explant and chondrocyte culture. Overall, the study’s results were highly reproducible between experiments and showed significant differences between experimental groups.

One of the major limitations for all three of our studies was that they were all conducted in vitro. Due to this, the clinical impact of these results remains unclear. In order to accurately represent the chondrotoxic effects of bupivacaine and ropivacaine, an in vivo experiment would need to be conducted. A huge drawback of in vitro experiments is that all laboratory conducted experiments are prone to common sources of lab error. Another disadvantage within all the studies was the small sample sizes. A small sample size increases the probability of a type II error. A type II error occurs when the null hypothesis is false but fails to be rejected by the results of the study. This happens when no difference is found between the control and the study groups. However, in reality, a difference does exist within a larger representative population. This limitation is understandable given it may be difficult for researchers to recruit a large number of subjects that are willing to donate their articular cartilage. Also, all of the studies did a poor job delineating the histories of the patients that were involved. There was no mention of any possible comorbidities or significant past medical histories. Due to the older age range of the sample population in these studies, it is likely that the patient have multiple comorbidities that could affect the results of the study.
Table 1. Limitations of the studies.

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<th>Breu et al.</th>
<th>Grishko, PhD et al</th>
<th>Piper et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro experiment</td>
<td>In vitro experiment</td>
<td>In vitro experiment</td>
</tr>
<tr>
<td>Small population size</td>
<td>Small population size</td>
<td>Small population size</td>
</tr>
<tr>
<td>No past medical history of patients</td>
<td>No past medical history of patients</td>
<td>No past medical history of patients</td>
</tr>
<tr>
<td>The type of articular cartilage is not specified</td>
<td>Number of patients not specified</td>
<td>Age range of population not specified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Used intact (non-osteoarthritic) articular cartilage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Used only one concentration of each anesthetic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Measured the results at only one time interval</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obscure data used in figures</td>
</tr>
</tbody>
</table>

Table 2. Advantages of the studies.

<table>
<thead>
<tr>
<th>Breu et al.</th>
<th>Grishko, PhD et al</th>
<th>Piper et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized tissue collection</td>
<td>Standardized tissue collection</td>
<td>Standardized tissue collection</td>
</tr>
<tr>
<td>Population age range (42 – 62 years old)</td>
<td>Used osteoarthritic articular cartilage</td>
<td>Specified the source of articular cartilage: knee joint</td>
</tr>
<tr>
<td>Included experiments with both osteoarthritic and intact articular cartilage</td>
<td>Population age range (53 ± 16 years old)</td>
<td></td>
</tr>
<tr>
<td>Compared different concentrations of ropivacaine and bupivacaine</td>
<td>Compared different concentrations of ropivacaine and bupivacaine</td>
<td></td>
</tr>
<tr>
<td>Measured the results at multiple time intervals</td>
<td>Measured the results at multiple time intervals</td>
<td>Specified the source of articular cartilage: knee joint</td>
</tr>
<tr>
<td></td>
<td>Specified the source of articular cartilage: knee joint</td>
<td></td>
</tr>
</tbody>
</table>

Discussion:

Local anesthetics are chondrotoxic to human cartilage in a time dependent, drug dependent, and concentration dependent manner. Both ropivacaine and bupivacaine demonstrate toxic effects on human articular cartilage through the induction of cell apoptosis. Our studies have demonstrated that some concentrations of ropivacaine and bupivacaine are less chondrotoxic than others.
Breu, et al. showed that both ropivacaine and bupivacaine had significant chondrotoxic effects on human articular cartilage. They also demonstrated statistically significant differences in chondrotoxicity when comparing equal potencies of bupivacaine and ropivacaine. Ropivacaine was shown to be less chondrotoxic than bupivacaine overall. Significant cytotoxicity also occurred in a time dependent manner. Bupivacaine showed a significant increase in apoptotic and necrotic cells after 24 and 96 hours of exposure, while ropivacaine showed a significant increase in necrotic cells after 96 hours. Cell apoptosis was seen with the use of both anesthetics when analyzing caspase activity. However, apoptotic fragments were higher with use of bupivacaine in comparison to use of ropivacaine.

Grishko, et al. demonstrated a statistically significant decrease in cell viability for all concentrations of ropivacaine and bupivacaine after 120 hours except for 0.2% ropivacaine. A significant decrease in cell viability was not seen after 24 hours with 0.5% and 0.25% bupivacaine and 0.5% ropivacaine, but a detectable decrease was seen in 0.5% bupivacaine. Cell death was shown to be caused by cell apoptosis when analyzing caspase activity.

Piper, et al. found that chondrocyte viability was significantly greater in ropivacaine than in bupivacaine after 24 hours, demonstrating that 0.5% bupivacaine is more chondrotoxic than 0.5% ropivacaine. Chondrocyte viability was significantly greater when treated with normal saline in comparison to ropivacaine or bupivacaine.

All of the studies concluded that both bupivacaine and ropivacaine have chondrotoxic effects on human articular cartilage (Table 3). Breu, et al. concluded that the chondrotoxic effects were time dependent and concentration dependent. Breu, et al., Grishko et al., and Piper, et al. found that ropivacaine is less chondrotoxic than bupivacaine. They all also found that concentrations of ropivacaine less than 0.75% showed no significant chondrotoxic effects after 24 hours. Grishko et al. and Breu et al. found that 0.25% bupivacaine had no significant chondrotoxic effects after 24 hours. However, Grishko et al. did find that these concentrations of ropivacaine and bupivacaine had significant toxic effects at 120 hours. Both Grishko et al. and Breu et al. also showed that local anesthetics cause cell death through apoptosis.

Even though results were reproducible between studies, variations in the populations make it difficult to come to a conclusion. Table 3 demonstrates the similarities and differences of the three studies. It is difficult to compare Breu et al. and Grishko et al. to Piper, et al. since both Breu et al. and Grishko et al. performed the experiment on osteoarthritic cartilage while Piper, et al. performed the experiment on intact cartilage. The population sizes were also very small for all of the studies, which can lead to type 2 error. Although Breu et al. and Grishko et al. found similar results, they measured statistical significance with different P value limits, which affects their significant findings. Unlike Breu et al., Grishko et al. and Piper et al. did not use equipotent concentrations of ropivacaine and bupivacaine when comparing the two in their studies. Therefore, their results may be unreliable for forming a conclusion. All of the studies were also performed in vitro, which makes them less applicable to real life scenarios.

At this time, we cannot confidently conclude which concentrations of bupivacaine and ropivacaine are the safest and whether ropivacaine is a better alternative to bupivacaine until further studies are performed. Future studies should be performed in vivo in order to observe the chondrotoxic effects on humans directly and to understand the pharmacokinetics of the anesthetics. Future studies should also focus on comparing several equipotent concentrations of ropivacaine and bupivacaine in order to conclude which anesthetic is definitively less toxic. With Grishko et al. finding significant toxic effects after 120 hours, future studies should focus on measuring chondrotoxic effects of ropivacaine and bupivacaine at longer durations.

When applying results to our clinical scenario, studies show that orthopedists should refrain from using high concentrations of local anesthetics due to their extensive chondrotoxic effects and due to the correlation found between chondrotoxicity and drug concentration. Since osteoarthritic patients already have significant cartilage damage, it’s best for providers to refrain from causing any further damage. Breu et al. further investigated the effects of local anesthetics on osteoarthritic cartilage versus intact cartilage and found that cellular death rates were significantly higher in osteoarthritic cartilage.
**Table 3. Comparison of subjects, methods, and statistical significant findings between the three studies.**

<table>
<thead>
<tr>
<th></th>
<th>Breu, et al. (Study 1)</th>
<th>Grishko, et al. (Study 2)</th>
<th>Piper, et al. (Study 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo or in vitro</strong></td>
<td>In vitro</td>
<td>In vitro</td>
<td>In vitro</td>
</tr>
<tr>
<td><strong>Number of patients</strong></td>
<td>4</td>
<td>N/A</td>
<td>5</td>
</tr>
<tr>
<td><strong>Patient population</strong></td>
<td>42-62 year olds</td>
<td>53 +/- 16 years old</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>OA or intact cartilage</strong></td>
<td>OA</td>
<td>OA</td>
<td>Intact</td>
</tr>
<tr>
<td><strong>Bone used</strong></td>
<td>N/A</td>
<td>Femoral condyles and tibial plateaeus</td>
<td>3 from femoral head 2 from tibial plateau</td>
</tr>
<tr>
<td><strong>Drug concentrations</strong></td>
<td>1 mL Bupivacaine 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5% 1 mL Ropivacaine 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 0.75%</td>
<td>Bupivacaine 0.5%, 0.25% Ropivacaine 0.5%, 0.2%</td>
<td>200 µL 0.5% Bupivacaine 200 µL 0.5% Ropivacaine</td>
</tr>
<tr>
<td><strong>Exposure Time</strong></td>
<td>1 hour</td>
<td>1 hour</td>
<td>30 minutes</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>Saline</td>
<td>Saline</td>
<td>Saline</td>
</tr>
<tr>
<td><strong>Statistical Significance</strong></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td><strong>Flow Cytometry</strong></td>
<td>At 24 hours and 96 hours Concentrations &lt; 0.75% ropivacaine and 0.25% bupivacaine showed no cytotoxic effects. 0.75% ropivacaine &gt; 0.5% bupivacaine in cell viability. Significant cytotoxicity seen after 24 and 96 hours with ropivacaine and 96 hours with bupivacaine.</td>
<td>At 24 hours detectable but not significant decrease in cell viability with 0.5% bupivacaine. No decrease with 0.25% bupivacaine and 0.5% and 0.2% ropivacaine. At 120 hours only 0.2% ropivacaine did not have significant decrease in cell viability</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>LIVE/DEAD cell staining</strong></td>
<td>At 24 hours Bupivacaine &gt; ropivacaine in chondrotoxicity Cell viability was higher in equipotent concentrations of ropivacaine vs. bupivacaine</td>
<td>N/A</td>
<td>At 24 hours 0.5% ropivacaine &gt; 0.5% bupivacaine in cell viability. No significant difference in viability b/w ropivacaine and normal saline</td>
</tr>
<tr>
<td><strong>Caspase activity</strong></td>
<td>0.5% bupivacaine &gt; 0.75% ropivacaine in cell apoptotic fragments</td>
<td>Increase in caspase 3 and caspase 9 cleavage demonstrated apoptosis</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Cell Titer-Glo Luminescent Cell Viability Assay</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>At 24 hours 0.5% ropivacaine &gt; 0.5% bupivacaine in cell viability. Normal saline &gt; bupivacaine and ropivacaine in cell viability</td>
</tr>
</tbody>
</table>
**Conclusion:**

Intraarticular injections of corticosteroids combined with local anesthetics is a commonly practiced treatment for patients suffering from osteoarthritis. Bupivacaine is one of the most commonly used anesthetics for this orthopedic problem. The current recommendations, according to the American Family Physician, is to use 0.25 to 0.5 percent bupivacaine mixed with an equal volume of corticosteroid for intraarticular injections of arthritic joints.\(^{3,4}\)

Recent experimental studies have revealed evidence that local anesthetics, like bupivacaine, may have dose and time dependent toxic effects on chondrocytes. Additionally, the studies suggest that ropivacaine, a newer anesthetic, may be a possible alternative to bupivacaine. With further investigation, three analogous studies were selected to compared the effects of bupivacaine and ropivacaine on chondrocytes. The combined results of these three studies demonstrated that both ropivacaine and bupivacaine show time and concentration dependent toxicity to chondrocytes. Specifically, the results of these studies show that concentrations less than 0.75% ropivacaine and concentrations of 0.25% bupivacaine or less have fewer chondrotoxic effects on osteoarthritic cartilage.

Ropivacaine and bupivacaine are two very similar local anesthetic agents. Both drugs are long-acting agents with similar onsets and durations of anesthesia. Both drugs are also a part of the amide group of anesthetics and have a very similar chemical make-up. However, there are a few differences in these drugs. For one, ropivacaine diffuses less readily than bupivacaine into the systemic system. Therefore, ropivacaine is known to have fewer cardiovascular and central nervous system toxic effects. Bupivacaine, on the other hand, is more potent than ropivacaine, which allows it to reach the same therapeutic level as ropivacaine at lower concentrations. Another major benefit is that bupivacaine is far more inexpensive than ropivacaine. The average cost of a ropivacaine is generally two to three times more than bupivacaine. Ropivacaine's cost is about $6.20, while the average cost of bupivacaine is $2.30 for equivalent dose.

In conclusion, both bupivacaine and ropivacaine demonstrate toxic effects to chondrocytes at respective doses. This is an important revelation that clinicians should be wary of when choosing a local anesthetic and its respective concentration. Both drugs have shown their own particular advantages and disadvantages. At this point, we are unable to make a clear recommendation as to which local anesthetic agent and concentration would be the best choice for an intraarticular injection.

In order to further expand this research and more accurately portray the clinical relevance of these results, an in vivo study would need to be performed. Furthermore, the study should be performed using multiple concentrations of both ropivacaine and bupivacaine at equal potencies in order to compare the toxic effects of these drugs on chondrocytes in greater detail. These expansions will offer a more solidified recommendation for the proper protocol for intraarticular injections.

**Acknowledgements:**

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References: