

Lidocaine Exhibits Dose- and Time-Dependent Cytotoxic Effects on Bovine Articular Chondrocytes In Vitro

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Background: Intra-articular lidocaine is commonly used.

Purpose: This study was conducted to determine whether short-term exposures to 1% and 2% lidocaine are toxic to articular chondrocytes, whether this is due to pH, and whether an intact articular surface is protective.

Study Design: Controlled laboratory study.

Methods: Fresh bovine articular chondrocytes in alginate bead cultures were treated with 1% or 2% lidocaine or buffered saline (pH 7.4, 7.0, and 5.0) for 15, 30, or 60 minutes. Chondrocytes were then analyzed for viability by flow cytometry 1 hour, 1 day, and 1 week later. Bovine osteochondral cores with and without the superficial 1 mm of cartilage removed were submerged in either 0.9% saline (pH 7.4) or in 1% or 2% lidocaine for 30 minutes and assessed for viability using fluorescent microscopy.

Results: Chondrocyte viability decreased after just 15-minute exposures to 1% lidocaine. Longer exposures to 1% and 2% lidocaine further reduced chondrocyte viability. Chondrotoxicity of 2% lidocaine was greater than 1% lidocaine. There was no difference in chondrocyte viability after exposures to saline solutions of pH 7.4, 7.0, or 5.0. An intact articular surface did not affect lidocaine's chondrotoxic effects.

Conclusion: Results show dose- and time-dependent cytotoxic effects of lidocaine on bovine articular chondrocytes. Reduction of pH alone did not decrease chondrocyte viability, and the intact articular surface was not protective.

Clinical Relevance: Although lidocaine chondrotoxicity was less than previously reported with bupivacaine, these observations suggest that local anesthetics as a class of drugs may negatively affect articular cartilage.

Keywords: cartilage; lidocaine; cytotoxicity; local anesthetics

Perioperative pain control after arthroscopic surgery and use of local anesthetics in joint injections are important parts of orthopaedic practice. Intra-articular single bolus injections and continuous pain pumps containing local anesthetics are frequently used clinically. Recently, published clinical case reports have suggested potential adverse effects of continuous intra-articular infusions of bupivacaine on articular cartilage.¹⁸ Both in vitro and in vivo studies have shown cytotoxic effects of bupivacaine on chondrocytes.^{1,7} However, a variety of other local anesthetics, most

commonly lidocaine, are also used within the joint with little information regarding potential effects of these agents on articular chondrocytes.

Lidocaine and bupivacaine are both members of the amide group in the local anesthetic family. Lidocaine's duration of action is approximately one half that of bupivacaine and with lower reported systemic toxicities.¹⁶ Lidocaine has been effective in perioperative pain control after arthroscopic surgery. Multiple studies have shown intra-articular injections of lidocaine to have high success rates when used as a local anesthetic for knee arthroscopy.^{3,4,9,10,14,17} Based on patient comfort levels and systemic toxicity data, recommendations for bolus 20-mL intra-articular injections of lidocaine at concentrations 1% or greater for knee arthroscopy have been made.³

Because chondrocyte loss has been implicated in the development of osteoarthritis,⁶ it is important to determine whether lidocaine may also have cytotoxic effects on articular chondrocytes. This study was conducted to determine whether short-term exposures to 1% and 2% lidocaine are

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toxic to articular chondrocytes, whether any chondrotoxicity is due to the lower pH of lidocaine solutions, and whether an intact articular surface is protective.

METHODS

Fresh bovine chondrocytes and osteochondral tissues were used for this study. All flow cytometry experiments were conducted with bovine chondrocytes within 3-dimensional alginate bead constructs. The effects of intact articular surface experiments were conducted with fresh osteochondral cores.

Alginate Bead Cultures

Bovine knees obtained within 4 hours of slaughter were dissected to expose the articular cartilage. Articular chondrocytes were isolated from the cartilage by enzymatic digestion and were encapsulated in alginate beads at a density of 4×10^6 cells per milliliter as described by Masuda et al.¹⁵ Beads were kept in a tissue culture incubator at 37°C/5% CO₂ in chondrocyte growth medium consisting of Dulbecco's Modified Eagle's Medium/Medium F-12 (50:50; Invitrogen, Grand Island, NY) plus 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen) for 1 week before lidocaine exposure.

Treatment Groups

After 1 week of culture, alginate beads were segregated into 15 groups consisting of 10 beads each. Experimental groups were immersed in 1 mL of either 1% or 2% single-dose preservative-free lidocaine HCL (pH 5.0-7.0) (AstraZeneca) or buffered saline solution at pH 5.0 or pH 7.0, and control groups were placed in 1 mL of sterile 0.9% saline (pH 7.4) (Invitrogen) for 15, 30, or 60 minutes. Beads were then washed and reincubated in chondrocyte growth media. Cell viability was assayed at 3 time points (1 hour, 24 hours, and 1 week) by flow cytometry as described below. Each experiment was repeated 9 times using freshly isolated preparations of bovine articular chondrocytes within 3-dimensional alginate cultures from 3 different animals. Experimental group viabilities were compared with control group actual viability.

Flow Cytometry

At designated time points, 10 beads from each treatment group were removed for labeling using a Vybrant Apoptosis Assay Kit #3 (Molecular Probes, Eugene, Ore). Alginate beads were dissolved in sodium citrate, and recovered chondrocytes were collected by centrifugation. The cells were washed and resuspended in 400 μ L of 1X Annexin Binding Buffer. To 100 μ L of each suspension, 5 μ L of Alexa Flour 488 Annexin V and 1 μ L of propidium iodide were added to stain the cells. The cells were incubated at room temperature for 15 minutes, and then 400 μ L of 1X Annexin Binding Buffer was added. Samples were analyzed by a FACSDiva flow cytometry machine (Beckson Dickinson, Franklin Lakes, NJ) to identify

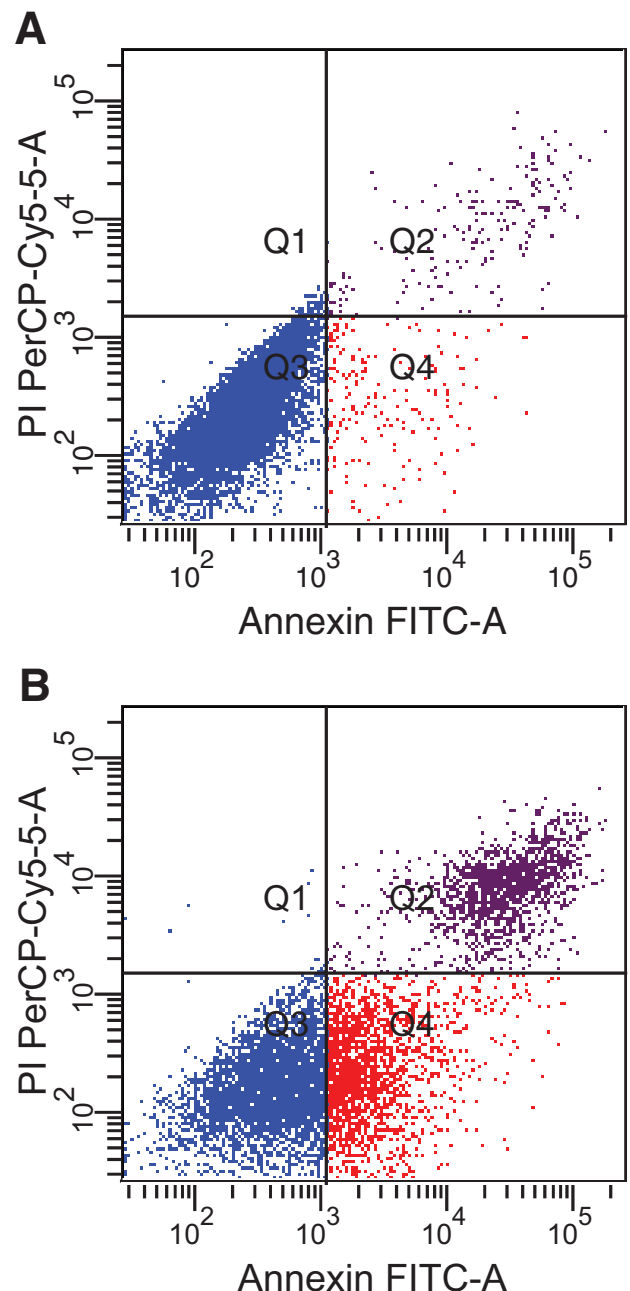


Figure 1. Scatter plots 1 hour after 15-minute exposures to saline pH 7.4 (A) and 1% lidocaine (B) reveal a significant decrease in cell viability ($P < .05$). Propidium iodide fluorescence (ordinate) is plotted against Annexin V fluorescence (abscissa). Quadrant 2 (Q2) depicts necrotic cells, quadrant 3 (Q3) depicts live cells, and quadrant 4 (Q4) depicts apoptotic cells.

Annexin V- and propidium iodide-positive cells. Live cells were not labeled with either stain, apoptotic cells had only Annexin V staining, and necrotic cells were stained with both Annexin V and propidium iodide. Figure 1 shows an example of a flow cytometry scatter plot 1 hour after 15-minute exposures to 1% lidocaine and saline pH 7.4 (quadrant 2 shows necrotic cells, quadrant

TABLE 1
Percentage of Live, Dead, and Apoptotic Chondrocytes After Lidocaine Exposure Compared to Saline at pH 7.4^a

Exposure Time, min	15			30			60		
	% Live	% Dead	% Apoptotic	% Live	% Dead	% Apoptotic	% Live	% Dead	% Apoptotic
1 hour after treatment									
Saline pH 7.4	87.0	5.1	8.0	81.7	8.3	9.5	79.7	10.7	9.3
1% lidocaine	77.1	11.8	10.6	76.2	12.6	10.9	66.1	21.7	11.8
2% lidocaine	68.5	18.8	12.3	65.9	23.6	10.0	28.5	60.4	10.7
24 hours after treatment									
Saline pH 7.4	82.3	7.7	8.3	83.0	6.7	8.9	79.2	9.3	9.7
1% lidocaine	55.2	19.0	24.9	46.4	26.3	26.4	53.2	22.1	23.9
2% lidocaine	22.9	56.8	19.9	25.1	50.9	23.4	19.5	57.7	22.5
1 week after treatment									
Saline pH 7.4	77.3	9.2	11.5	76.4	9.8	11.9	76.0	11.1	11.6
1% lidocaine	59.2	12.9	24.5	51.6	24.8	20.3	53.9	19.3	23.6
2% lidocaine	21.5	28.3	47.5	11.5	18.8	57.3	17.3	25.1	55.5

^aData are reported after 15-, 30-, and 60-minute exposures to saline pH 7.4 and 1% lidocaine and 2% lidocaine at 1-hour, 24-hour, and 1-week time points after treatment.

3 shows viable cells, and quadrant 4 shows apoptotic cells). For statistical comparisons between saline and lidocaine treatments, data were analyzed by 1-way analysis of variance followed by Bonferroni *t* test (using GraphPad software, GraphPad Software Inc, San Diego, Calif) with significance set at $P < .05$.

Bovine Cartilage Core Exposure to Lidocaine

An 8-mm Mitek coring device (DePuy Mitek, Norwood, Mass) was used to cut 36 osteochondral cores from the trochlear groove of 2 different freshly slaughtered bovine calves (18 cores per animal per experiment). Cores were randomly assigned to 6 groups of 3 cores each; 3 groups (9 cores) were left intact ("top on"), and 3 groups (9 cores) had a 1-mm slice taken off the most superficial layer with a sharp scalpel ("top off"). Both top-on and top-off groups were further divided into 3 experiment groups (3 cores in each group): 0.9% saline pH 7.4, 1% lidocaine, or 2% lidocaine. Cores were immersed into their respective treatment groups for 30 minutes. They were then washed and returned to chondrocyte growth medium for 24 hours and imaged after live-cell/dead-cell fluorescent staining (Molecular Probes, Invitrogen, Carlsbad, Calif). Osteochondral cores were centered within the field under $\times 4$ magnification. One depth measurement was taken at this center point, 1 measurement was taken to the right of the center point approximately midway to the lateral edge, and 1 measurement was taken to the left of the center point approximately midway to the opposite lateral edge. These 3 measurements were averaged and analyzed by 1-way analysis of variance followed by Bonferroni *t* test (using GraphPad software) with significance set at $P < .05$.

RESULTS

Flow cytometry data are included in Table 1.

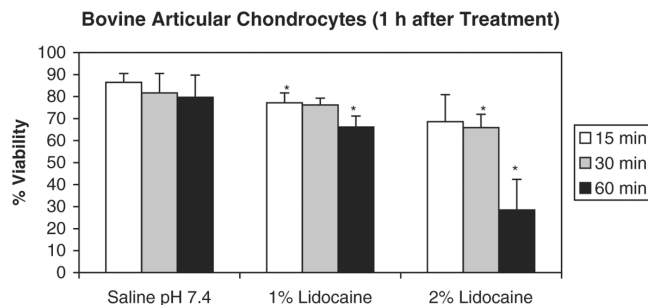


Figure 2. Chondrocyte viability 1 hour after exposure to saline pH 7.4 or 1% and 2% lidocaine. Data are given as mean \pm SD. * $P < .05$ versus saline (pH 7.4) control.

Effects of Lidocaine

Flow cytometry revealed that chondrocyte viability decreased to $77\% \pm 4.5\%$ by 1 hour after 15-minute exposure to 1% lidocaine, representing a significant reduction compared with $87\% \pm 4\%$ viability in saline (pH 7.4) controls ($P = .0009$). Experimental group viabilities were compared with control group (saline pH 7.4) actual viabilities. One hour after 60-minute exposures to 1% and 2% lidocaine, chondrocyte viability was further reduced to $66\% \pm 5\%$ ($P = .028$) and $28\% \pm 13\%$ ($P = .031$), respectively, compared with saline (pH 7.4) controls (Figure 2). The chondrotoxicity of 2% lidocaine was greater than that of 1% lidocaine ($P = .037$).

After exposures to 2% lidocaine, further reduction in chondrocyte viabilities to 25% or less ($11\%-25\% \pm 9\%-27\%$) was observed 1 day and 1 week after 15-, 30-, and 60-minute exposures ($P < .05$) compared with saline (pH 7.4) controls. One day and 1 week after exposures to 1% lidocaine, cell viabilities decreased to $46\%-59\% \pm 9\%-24\%$ ($P < .05$) compared with saline (pH 7.4) controls. At the 1-day and 1-week time points, no differences were observed in chondrocyte viabilities between the different lidocaine

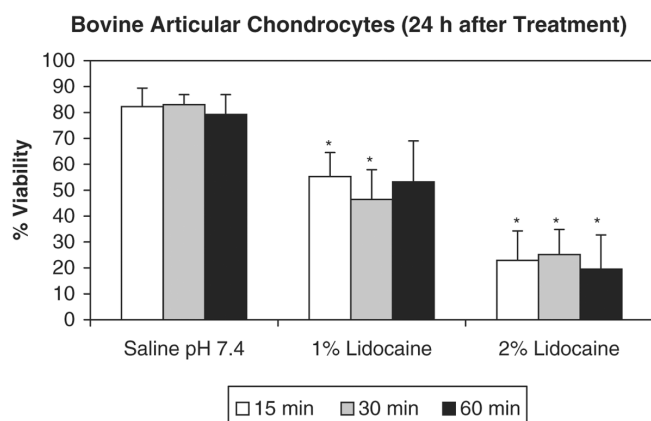


Figure 3. Chondrocyte viability 24 hours after exposure to saline pH 7.4 or 1% and 2% lidocaine. Cell viability continued to decrease for both 1% and 2% lidocaine, with chondrotoxicities of 2% lidocaine greater than those of 1% lidocaine after all exposure times. Data are given as mean \pm SD. * $P < .05$ versus saline (pH 7.4) control.

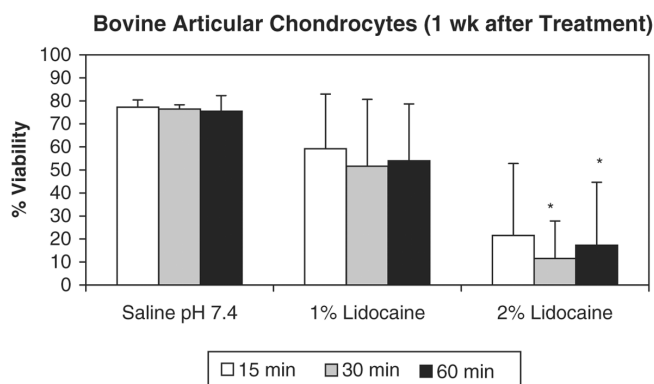


Figure 4. Chondrocyte viability 1 week after exposure to saline pH 7.4 or 1% and 2% lidocaine. Cell viabilities decreased with greater time after lidocaine exposures ($P < .05$), and higher chondrocyte death was seen after exposures to 2% lidocaine than after 1% lidocaine ($P < .05$). Data are given as mean \pm SD. * $P < .05$ versus saline (pH 7.4) control.

exposure times of 15, 30, and 60 minutes ($P > .05$). However, cell viabilities decreased with greater time after lidocaine exposures ($P < .05$), and higher chondrocyte death was seen after exposures to 2% lidocaine than after exposure to 1% lidocaine ($P < .05$) (Figures 2-4).

Effects of pH

There was no difference in chondrocyte viability after 15-, 30-, and 60-minute exposures to saline solutions of pH 7.4, 7.0, or 5.0 ($P > .05$). Chondrocyte viability ranged from $79\% \pm 8\%$ to $86\% \pm 4\%$ for all exposure times through 1 week after exposure to 0.9% saline (pH 7.4) and to buffered saline solutions (pH 7.0 and pH 5.0) (Figure 5 shows viability 1 hour after exposures).

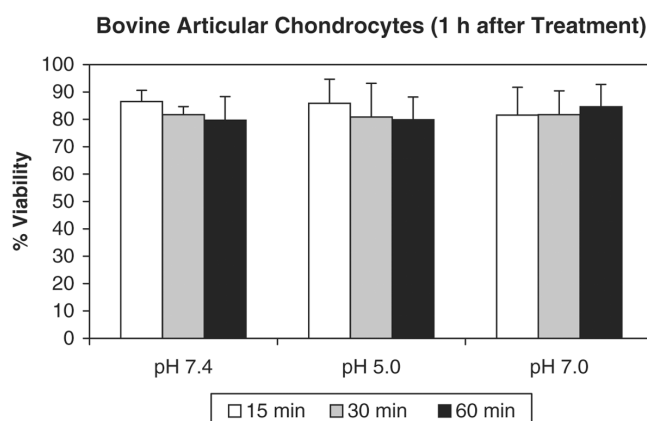


Figure 5. Chondrocyte viability 1 hour after exposure to 0.9% saline (pH 7.4) and buffered saline solutions of pH 7.0 and pH 5.0. There was no difference in chondrocyte viability after 15-, 30-, and 60-minute exposures ($P > .05$). Equivalent results were obtained 24 hours and 1 week after treatment groups.

TABLE 2
Depth of Cell Death in Articular Cartilage With and Without Intact Articular Surfaces^a

	Surface On	Surface Off
Saline pH 7.4	62.6 \pm 18	71.4 \pm 21
1% lidocaine	185.89 \pm 24 ^b	179.5 \pm 22 ^b
2% lidocaine	196.5 \pm 25 ^b	191.1 \pm 20 ^b

^aThe mean depth (micrometer) of cell death after 30-minute exposures to saline (pH 7.4) and 1% and 2% lidocaine solutions was similar ($P > .05$) for bovine cartilage with and without intact articular surfaces.

^bComparison was made between saline (pH 7.4) and lidocaine (1% and 2%) groups with significance set at $P < .05$.

Effects of the Intact Articular Surface

Increased chondrocyte necrosis was observed in osteochondral cores exposed to 1% and 2% lidocaine for 30 minutes compared with osteochondral cores exposed to saline (pH 7.4; $P \leq .0004$). Cell death was primarily observed in chondrocytes close to the surface, in contact with the lidocaine (Figure 6). The depth of chondrocyte death was similar for intact and nonintact articular surfaces ($P = .316$). However, an increase in depth of chondrocyte death from $62.6 \pm 18 \mu\text{m}$ in saline (pH 7.4) to $185.9 \pm 24 \mu\text{m}$ ($P < .0001$) in 1% lidocaine and $196.5 \pm 25 \mu\text{m}$ ($P < .0001$) in 2% lidocaine was observed in the top-on group, and an increase in depth of chondrocyte death from $71.4 \pm 21 \mu\text{m}$ in saline (pH 7.4) to $179.5 \pm 22 \mu\text{m}$ ($P < .0001$) in 1% lidocaine and $191.1 \pm 20 \mu\text{m}$ ($P < .0001$) in 2% lidocaine was observed in the top-off group (Table 2). No significant difference between 1% and 2% lidocaine or between top-on and top-off groups was observed ($P > .05$).

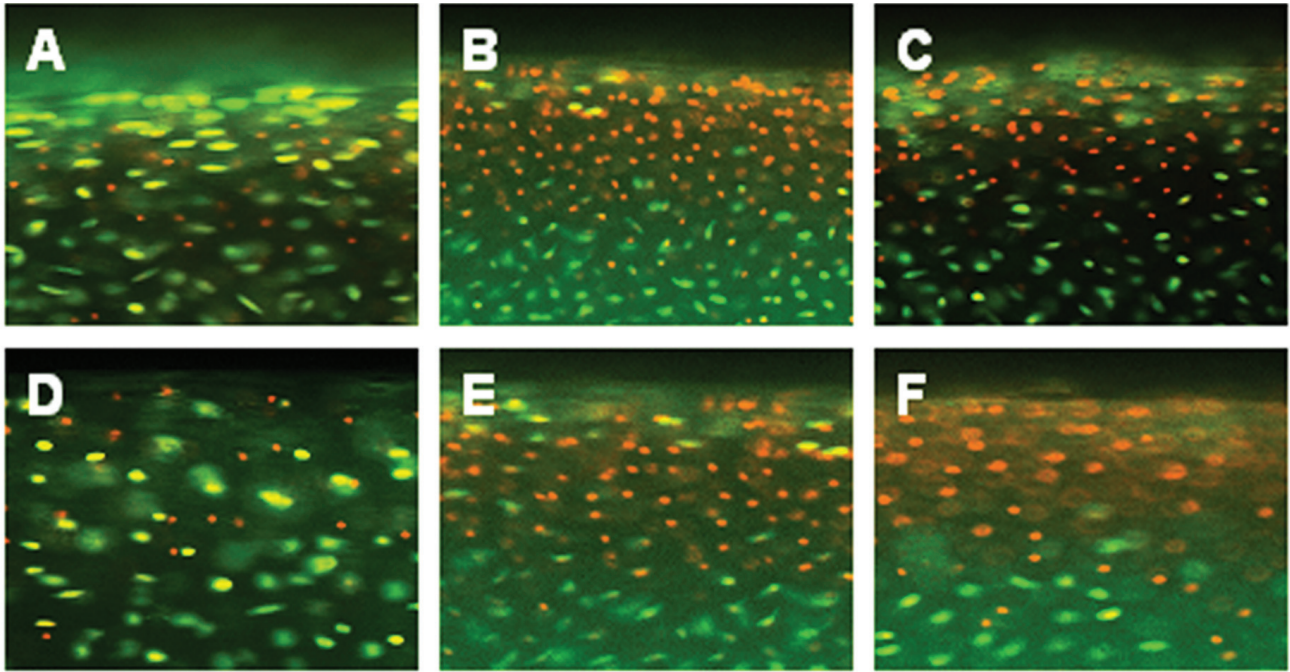


Figure 6. Confocal images of bovine articular osteochondral cores stained with CMFDA and PI. Green cells are live, and red cells are dead. Intact articular surfaces of osteochondral cores are depicted in images A-C, and cores with the superficial 1 mm removed are depicted in images D-F. Saline-treated controls show primarily live cells throughout (A + D). Exposure to 1% lidocaine (B + E) and 2% lidocaine (C + F) shows more extensive chondrocyte necrosis. The depth of chondrocyte death was similar for intact and nonintact articular surfaces ($P = .316$).

DISCUSSION

This study shows that lidocaine has dose- and time-dependent cytotoxic effects on bovine articular chondrocytes and bovine articular cartilage in vitro. Although the observed chondrotoxicities were less than what has been reported for bupivacaine,¹ a reduction in chondrocyte viability was observed 1 hour after 15-minute exposure to 1% lidocaine. Cell viability progressively declined through the 1-week study period. These data suggest that even a 15-minute lidocaine exposure “terminally stressed” chondrocytes, leading to eventual cell death. Chondrotoxicity of 2% lidocaine was greater than that of 1% lidocaine at all time points, and a progressive decrease in chondrocyte viability to a low of 11% was observed 1 week after 30-minute exposures to 2% lidocaine. Because the single-dose preservative-free lidocaine used in this study is stored at a pH range of 5.0 to 7.0, we also tested chondrocyte viability after exposures to saline solutions at pH 5.0 and pH 7.0. The observed chondrotoxicity was not due to lower pH, as no reduction in chondrocyte viability was seen after exposures to buffered saline solutions in this pH range.

In contrast to results with bupivacaine, the intact articular surface did not have an apparent protective effect against lidocaine cytotoxicity. Chu et al¹ demonstrated that depth of cell death was less for cartilage with intact articular surfaces after exposures to bupivacaine, suggesting that the articular surface may provide a partial protective barrier to bupivacaine. In this study, we found a

similar increase in depth of chondrocyte necrosis after exposure of cartilage with and without intact articular surfaces to lidocaine. With a molecular weight of 234.34 g/mol, lidocaine is smaller than bupivacaine is (molecular weight, 342.9 g/mol) and may therefore have greater potential to penetrate the intact articular surface.

Recently published in vitro and in vivo studies have demonstrated that bupivacaine is cytotoxic to articular chondrocytes.^{1,7} The dose- and time-dependent cytotoxic effects of lidocaine on chondrocytes suggest that there may be a class effect of local anesthetics on cartilage. The longer acting local anesthetics, such as bupivacaine, with more documented systemic toxicities¹⁶ showed greater chondrotoxicity than did the shorter acting lidocaine. The cytotoxic effects of local anesthetics on various cell types (neurons, muscle) have been shown previously.^{5,11} Although necrosis occurs through disruption of the cell membrane, additional mechanisms of action include disruption of the mitochondrial transmembrane potential leading to programmed cell death. Grousselle et al⁸ showed that local anesthetics with the highest lipid solubility, when applied outside a cell, can reach the mitochondria and collapse mitochondrial transmembrane potential. They also showed potential restoration of mitochondrial transmembrane potential after removal of the local anesthetic. Additional studies need to be conducted to identify the drug-, dose-, and time-dependent effects of different local anesthetics on chondrocyte cell and mitochondrial membrane potentials, with emphasis on identifying the conditions in which cellular functions can be restored.

The potential for using chondroprotective agents to improve chondrocyte recovery after injury is of high clinical interest. The data showed high percentages of both necrotic and apoptotic cells after lidocaine exposures. Chu et al² have shown potential protective effects of growth factors and antiapoptotic agents such as insulin-like growth factor 1 (IGF-1) and c-Jun N-terminal kinase inhibitor on chondrocyte metabolism after thermal stress. Tonomura et al¹⁹ reported that treatment of chondrocytes with glutamine protected the cells from heat stress and NO-induced apoptosis. Van Offel et al²⁰ have demonstrated that therapeutic doses of bisphosphonates prevent dexamethasone-induced growth retardation and apoptosis of chondrocytes. These studies show a potential for chondroprotective and antiapoptotic therapies to assist chondrocytes in recovering from various environmental stresses, including exposure to local anesthetics.

Limitations of this study were that this was an in vitro study using bovine articular chondrocytes and bovine articular cartilage. Freshly isolated bovine chondrocytes were immediately encapsulated in 3-dimensional alginate constructs. Consistent with prior studies,^{1,13} a certain percentage of cells do not survive the isolation and manipulation process. We chose immediate encapsulation as opposed to encapsulation after monolayer culture to maintain chondrocytes as close to their native state as possible. Bovine chondrocytes in an alginate bead construct are a 3-dimensional culture model permitting quantitative studies of chondrocytes and differ from articular cartilage in several respects. Alginate is a natural hydrogel that allows for a more uniform distribution of chondrocytes than does articular cartilage and permits recovery of cells from suspension culture for flow cytometry. The matrix properties differ from the extracellular matrix of articular cartilage and, as such, the diffusion of lidocaine through alginate may differ from that of articular cartilage. Bovine tissues, however, offer the advantage of permitting obtainment of sufficient quantities of healthy cartilage from several individuals of uniform age. As this study was performed using chondrocytes obtained from healthy bovine cartilage, the results may be different with human cartilage, especially human cartilage after joint injury or in the presence of degenerative changes. This in vitro study additionally permitted precise quantification of cell viability using flow cytometry, a method that cannot be used in vivo.

Although in vitro results cannot be directly extrapolated to in vivo situations, the observed dose- and time-dependent cytotoxic effects of lidocaine on articular chondrocytes and articular cartilage in vitro raise several discussion points regarding intra-articular use of lidocaine. The exposure times used in this study (15, 30, and 60 minutes) were selected to model the potential effects of a single large-volume injection (30-60 mL) of lidocaine into a joint before arthroscopic surgery. Smaller volume injections (5-10 mL) are also commonly given alone or in combination with other agents such as corticosteroids. As Mankin and Conger¹² have previously shown a detrimental effect of

intra-articular cortisone in vivo, combination injections such as corticosteroid-lidocaine injections potentially subject chondrocytes to 2 different pharmacological stressors.

Another clinical situation in which lidocaine may be administered intra-articularly is through a postoperative continuous pain pump. In this scenario, chondrocytes may be exposed to local anesthetics for as long as 24 to 48 hours. The dose- and time-dependent detrimental effects of lidocaine on chondrocytes and on articular cartilage shown in this study support using the lowest concentrations and volumes of lidocaine for the shortest period of time to achieve clinical treatment goals. Further in vivo studies designed to model each potential clinical scenario are needed.

CONCLUSION

This study shows dose- and time-dependent cytotoxic effects of lidocaine on bovine articular chondrocytes in vitro. Reduction of pH alone did not decrease chondrocyte viability. After lidocaine exposure to osteochondral cores, increased chondrocyte death was seen in the superficial layers of articular cartilage. Intact articular surfaces have no apparent protective effects against lidocaine. Although the chondrotoxicity was less than previously reported with bupivacaine,¹ these data suggest a negative class effect of local anesthetics on articular chondrocyte viability.

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