Cytotoxicity of Local Anesthetics on Human Mesenchymal Stem Cells

Ruyan Rahnama, BA, Miqi Wang, BA, Alexis C. Dang, MD, Hubert T. Kim, MD, PhD, and Alfred C. Kuo, MD, PhD

Investigation performed at the Department of Orthopaedic Surgery, University of California San Francisco, San Francisco, and the San Francisco Veterans Affairs Medical Center, San Francisco, California

Background: Local anesthetics are frequently delivered intra-articularly to provide perioperative pain control. Previous studies have shown that the commonly used drugs lidocaine, ropivacaine, and bupivacaine can be toxic to human chondrocytes. The present study was conducted to determine whether the toxic effects of local anesthetics on human chondrocytes also extend to human mesenchymal stem cells.

Methods: Human mesenchymal stem cells from three healthy donors were grown in tissue culture and exposed to the following anesthetic treatments for sixty minutes: (1) 1% lidocaine, (2) 2% lidocaine, (3) 0.25% bupivacaine, (4) 0.5% bupivacaine, (5) 0.2% ropivacaine, and (6) 0.5% ropivacaine. The cells were then allowed to recover for twenty-four hours in regular growth media, and viability was measured with use of fluorescent staining for live cells or a luminescence assay for ATP content.

Results: The live cell counts and ATP content were correlated ($r^2 = 0.79$), and 2% lidocaine was found to be significantly more toxic than all doses of bupivacaine and ropivacaine. Treatment with 1% lidocaine resulted in significantly fewer live cells (49%) compared with the control, and the live cell count was also significantly less than that for the other anesthetics. However, the ATP level in the 1% lidocaine group was not significantly lower than those in the other groups. Bupivacaine and ropivacaine did not exhibit significant differences in toxicity compared with the control or with each other.

Conclusions: Ropivacaine and bupivacaine had limited toxicity in human mesenchymal stem cells. However, lidocaine could significantly decrease mesenchymal stem cell viability. Since other studies have shown ropivacaine to be less toxic to chondrocytes than bupivacaine, ropivacaine may be a safer intra-articular anesthetic.

Clinical Relevance: Mesenchymal stem cells likely play a key role in healing following surgical procedures such as microfracture and ligament reconstruction. If local anesthetics are used following joint surgery, selection of an agent with low toxicity toward mesenchymal stem cells, such as ropivacaine, may maximize tissue healing potential.

Local anesthetics are frequently delivered into joints to provide postoperative analgesia. The intra-articular delivery of local anesthetics after arthroscopy through single injections or through continuously acting pain pumps and indwelling catheters has been shown to substantially minimize postoperative pain and maintain joint motion[1-4]. Such use of local anesthetics is a common practice worldwide. In one survey of all orthopaedic units associated with the Swedish Arthroscopic Society, all but one of thirty-seven practices used intra-articular local anesthesia for postoperative pain relief following knee arthroscopy[5].

Despite their efficacy in pain control, local anesthetics may also have detrimental effects. Numerous studies have linked their use to the development of chondrolysis, a complication characterized by the rapid destruction of articular cartilage[6-8]. Notably, shoulder arthroscopy patients diagnosed with rapid onset of glenohumeral chondrolysis routinely have a history of intra-articular infusion of bupivacaine or lidocaine[8]. Consistent with these clinical findings, numerous in vivo and in vitro studies have shown that common local anesthetics such as bupivacaine, ropivacaine, and lidocaine can be toxic to chondrocytes. Such toxicity depends on the specific anesthetic, duration of exposure, and dose administered[9-13]. For example, 0.5% ropivacaine induced less cell death after a thirty-minute exposure in vitro compared with 0.5% bupivacaine in both

Disclosure: None of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of any aspect of this work. None of the authors, or their institution(s), have had any financial relationship, in the thirty-six months prior to submission of this work, with any entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. Also, no author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

isolated human articular chondrocytes and intact articular cartilage[13].

Although the chondrotoxicity of local anesthetics has been well studied, the effects of these drugs on reparative cells within a joint are unknown. Many orthopaedic procedures, including tendon repair, ligament reconstruction, and surgical marrow stimulation procedures such as microfracture, depend on patient healing responses. Mesenchymal stem cells likely play a key role in these processes. Under appropriate conditions, these cells can form bone, fat, tendon, and cartilage[14,15]. These regenerative cells are found in multiple tissues, including bone marrow, adipose, and synovium[14]. In addition to the healing resulting from the action of endogenous mesenchymal stem cells, surgical administration of exogenous mesenchymal stem cells may also enhance tissue regeneration[16].

We hypothesized that the toxicity of local anesthetics on chondrocytes would also extend to mesenchymal stem cells. If so, local anesthetics could both directly injure tissues within a joint and decrease the ability of tissues to heal. In this in vitro study, we evaluated the effects of commonly used doses of lidocaine, ropivacaine, and bupivacaine on the viability of mesenchymal stem cells from three healthy donors. Our goal was to identify treatments that minimized toxicity.

Materials and Methods

Mesenchymal Stem Cell Monolayer Culture

Commercially available bone-marrow-derived human mesenchymal stem cells (Lonza, Walkersville, Maryland) from a twenty-year-old man (Lot 8F354), a thirty-year-old man (Lot 0F4452), and a twenty-two-year-old woman (Lot 0F3825) were plated in monolayer culture in high-glucose DMEM (Dulbecco's modification of Eagle's medium) (Mediatech, Manassas, Virginia) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah) and 1% antibiotic/antimycotic solution (Hyclone) on tissue-culture-treated polystyrene at a density of 5000 cells/cm^2.

The cells were grown at 37°C in a humidified 5% CO_2 incubator until 90% confluent, were trypsinized, and were expanded until the fifth or sixth passage. They were then seeded onto ninety-six-well plates at a density of 2500 cells/well (8000 cells/cm^2) and cultured for forty-eight hours, leading to 90% confluence prior to experimental treatment.

Treatment Groups

Mesenchymal stem cell cultures were subdivided into six replicate wells per condition and exposed to one of the following drug dilutions: (1) 2% lidocaine, pH 6 (APP Pharmaceuticals, Schaumburg, Illinois), (2) 1% lidocaine, pH 6, (3) 0.5% bupivacaine, pH 6 (Hospira, Lake Forest, Illinois), (4) 0.25% bupivacaine, pH 6, (5) 0.5% ropivacaine, pH 5 (APP Pharmaceuticals), and (6) 0.2% ropivacaine, pH 5. The higher concentration of each anesthetic was used as provided by the manufacturer. The supplied solutions were preservative-free, with sodium chloride (to adjust osmolarity) and sodium hydroxide or hydrochloric acid (to adjust pH) added by the manufacturer. Control groups were treated with 0.9% saline solution (APP Pharmaceuticals), which was also used to prepare the lower concentration of lidocaine and of bupivacaine from the higher concentration. The 0.2% ropivacaine solution was used as provided by the manufacturer or diluted from 0.5% ropivacaine with 0.9% saline solution.

The cell culture media was aspirated, and 200 μL of the appropriate treatment solution was added to each well. Cells were incubated in the treatment solution for sixty minutes at 37°C in a humidified 5% CO_2 incubator, washed in 1× phosphate-buffered saline (PBS) solution, and returned to the incubator in fresh culture media. Mesenchymal stem cell viability was measured twenty-four hours later.

Assessment of Viability

Live Cell Counts

The cell culture media was gently aspirated from each well, and viable mesenchymal stem cells were stained with a LIVE/DEAD Viability/Cytotoxicity Kit (Life Technologies, Grand Island, New York). Briefly, 100 μL of a 1:2000 dilution of calcein AM (acetomethoxy) in PBS was added to each well. Cells were incubated for forty-five minutes at room temperature and then visualized with use of an Axiovert 200M fluorescent microscope (Carl Zeiss Microscopy, Thornwood, New York) with a fluorescein filter. Digital photographs of the center of each well were taken at 5× magnification. The cell viability in each field was quantified by superimposing a grid onto the digital image in Photoshop (Adobe Systems, San Jose, California), and calcein-stained live cells, which exhibit green fluorescence, were counted. The number of dead cells was also quantified in a subset of the experiments by staining with ethidium homodimer-1 and visualization with use of a rhodamine filter. Ethidium-stained dead cells and calcein-stained live cells were counted, and the percentage of live cells for each treatment was calculated.

ATP (Adenosine Triphosphate) Content

The ATP content in treated samples was measured with use of the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, Wisconsin) according to the manufacturer's instructions. Briefly, the CellTiter-Glo buffer and the CellTiter-Glo substrate were combined to form the CellTiter-Glo reagent. The reagent was added to the culture media in each well in a 1:1 ratio, mixed well, and incubated for ten minutes at room temperature. Luminescence was then measured with use of a Wallac 1420 VICTOR^2 Multilabel Counter (PerkinElmer, Waltham, Massachusetts).

Statistical Analysis

Live cell counts from the three independent trials, calculated as a percentage of the control, were analyzed together with use of mixed-model analysis of variance (ANOVA), with the specified drug concentration as the fixed factor and trial as a random factor. Live cell count results are reported for each drug concentration as the least-squares mean (the calculated best-fit value across all trials) and the standard error. ATP content results are reported for each of the three individual donors as the mean luminescence (the CellTiter-Glo output) and the standard error. Differences in ATP content among treatment groups were assessed for each donor with use of ANOVA. Results for the three donors were combined with use of mixed-model ANOVA, with the specified drug concentration as the fixed factor and donor as a random factor. ATP content results are reported as the least-squares mean luminescence and the standard error. Because multiple comparisons were made, the significance of differences among treatments was calculated with use of the Tukey honestly significant difference test; a p value of <0.05 was considered significant. Statistical analysis was performed with use of JMP (version 9.0; SAS Institute, Cary, North Carolina).

Source of Funding

This work was performed with funding from the Department of Orthopaedic Surgery of the University of California San Francisco and with resources of the Veterans Affairs Medical Center, San Francisco, California. There was no external funding source.

Results

Comparison of Cell Viability Measured with the Live Cell Count, Percentage of Live Cells, and ATP Content

In a pilot experiment, mesenchymal stem cells from one of the donors (the twenty-year-old male) were exposed to each of the six anesthetic conditions, and viability was determined with each of three methods: (1) live cell count, (2) percentage of live cells, and (3) ATP content. Six replicates were performed for each of the three evaluation methods for each condition. Linear regression yielded an r^2 value of 0.84 between the live cell count and the percentage of live cells and an r^2 value of 0.79 between

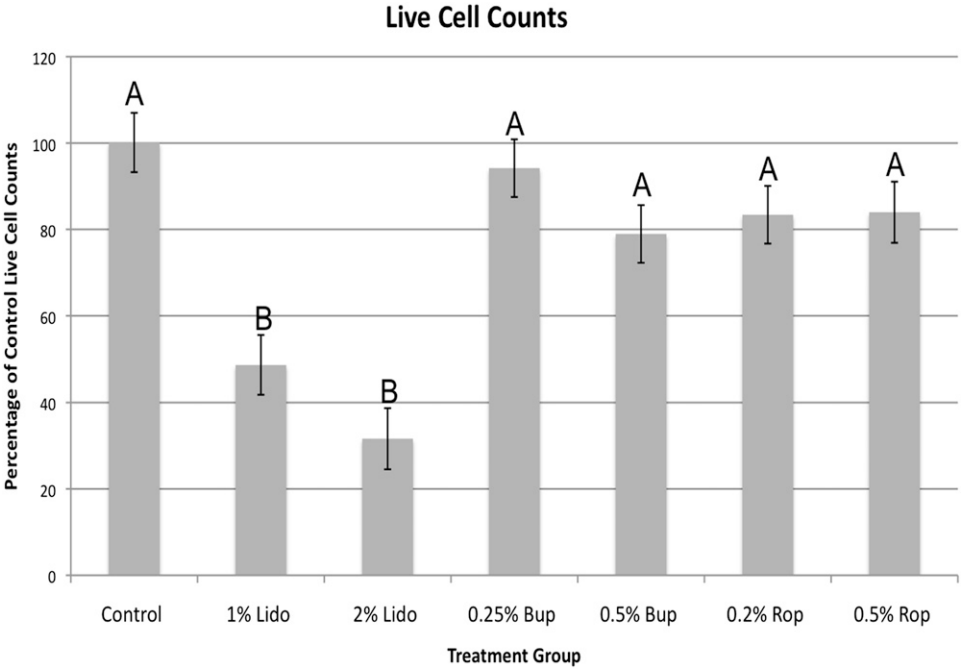

Fig. 1

The number of live human mesenchymal stem cells present twenty-four hours after a sixty-minute treatment with 0.9% normal saline solution (Control), 1% and 2% lidocaine (Lido), 0.25% and 0.5% bupivacaine (Bup), and 0.2% and 0.5% ropivacaine (Rop). Three separate experiments were performed in each treatment group. The bar heights represent the least-squares mean of the number of live cells relative to the control, and the error bars represent the standard error of the mean. The results for treatments labeled with different letters (e.g., A and B) differed significantly from each other ($p < 0.05$), whereas treatments labeled with the same letter did not.

the live cell count and the ATP content. Given the strength of these correlations, we evaluated viability with use of the live cell count and the ATP content for this donor and with use of only the ATP content for the other two donors.

Live Cell Counts

The number of live cells remaining after treatment with local anesthetic was determined with use of mesenchymal stem cells from the twenty-year-old male donor. Treatment with 0.25% and 0.5% bupivacaine in three independent trials reduced the live cell count to 94% and 79%, respectively, of the value in the control group. Treatment with 0.2% and 0.5% ropivacaine reduced the live cell count to 83% and 84%, respectively, of the value in the control group. No difference among these four groups was significant. Treatment with both concentrations of lidocaine, however, resulted in a significant reduction in the live cell count compared with all other groups. Treatment with 1% lidocaine reduced the live cell count to 49% of the value in the control group ($p < 0.0001$), and 2% lidocaine reduced it to 32% ($p < 0.0001$) (Figs. 1 and 2).

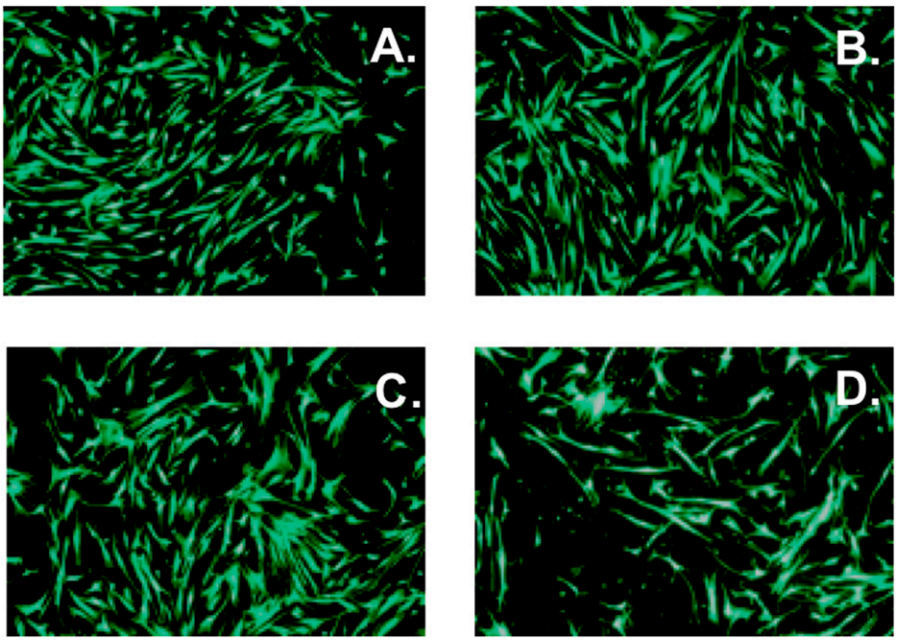

Fig. 2

Fluorescent microscopy images of human mesenchymal stem cells stained with calcein AM (acetomethoxy) twenty-four hours after a sixty-minute treatment with 0.9% normal saline solution (Control) (**Fig. 2-A**), 0.5% bupivacaine (**Fig. 2-B**), 0.5% ropivacaine (**Fig. 2-C**), and 1% lidocaine (**Fig. 2-D**) (×5).

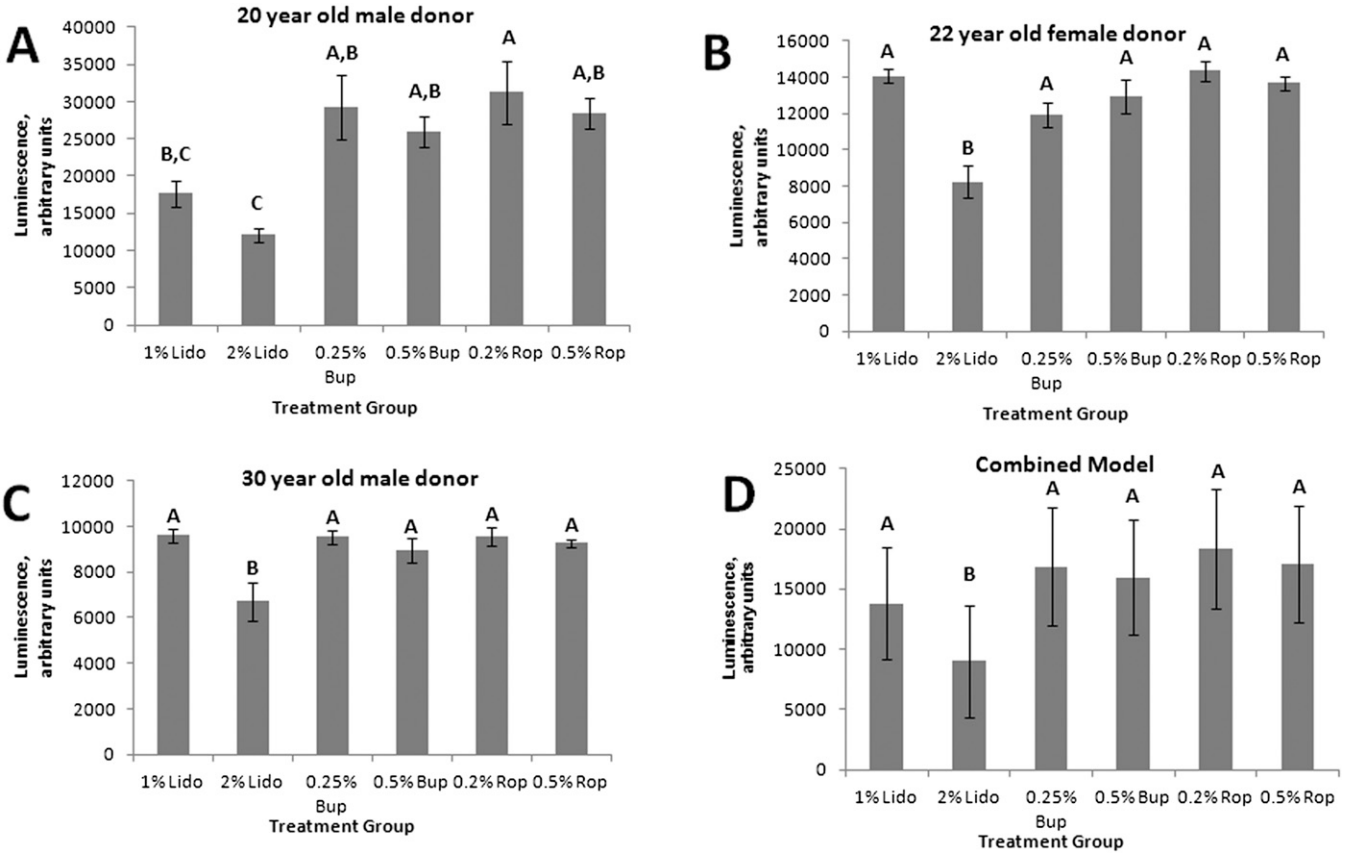

Fig. 3

Human mesenchymal stem cell viability twenty-four hours after a sixty-minute treatment with 1% and 2% lidocaine (Lido), 0.25% and 0.5% bupivacaine (Bup), and 0.2% and 0.5% ropivacaine (Rop). Viability was assessed on the basis of luminescence due to adenosine triphosphate (ATP), which was measured with use of a CellTiter-Glo assay. The bar heights represent the least-squares mean for each treatment, and the error bars represent the standard error of the mean. Results are presented for three individual donors (**Figs. 3-A, 3-B, and 3-C**) and for a model combining the three donors (**Fig. 3-D**). The results for treatments labeled with different letters (e.g., A and B) differed significantly from each other ($p < 0.05$), whereas treatments labeled with the same letter did not.

ATP Content

As an alternative, independent measure of cell viability, cellular ATP content after local anesthetic treatment was determined with use of mesenchymal stem cells from each of the three donors (the twenty-year-old man, the twenty-two-year-old woman, and the thirty-year-old man). The results for each donor as well as the results of a model combining information from all three donors are shown in Figure 3. In all four cases, 2% lidocaine treatment significantly reduced viability compared with treatment with either ropivacaine or bupivacaine. In the combined model, the ATP content in the 2% lidocaine group was only 49% of that in the 0.2% ropivacaine group. For the twenty-year-old donor, the ATP content in the 1% and 2% lidocaine groups was only 56% and 39%, respectively, of that in the 0.2% ropivacaine group ($p < 0.05$). However, the difference between the 1% lidocaine group and the other treatment groups was not significant for the other two donors or for the combined model. No significant differences were found between the ropivacaine and bupivacaine groups.

Discussion

The chondrotoxicity of local anesthetics including bupivacaine, ropivacaine, and lidocaine in both human and animal models has been widely reported. However, to our knowledge, the effects of these drugs on human mesenchymal stem cells have not been previously addressed. Given the likely role of these cells in tissue healing following orthopaedic procedures, the toxicity of local anesthetics on these cells may be clinically important[14]. The present study indicated that bupivacaine and ropivacaine at the commonly used 0.5% concentration were significantly less toxic than a concentration of lidocaine with equivalent anesthetic potency. These in vitro results suggest that intra-articular administration of lidocaine may have detrimental effects on mesenchymal stem cell populations.

The relative toxicities of bupivacaine, ropivacaine, and lidocaine on mesenchymal stem cells differed from their toxicities on human chondrocytes. Previous in vitro and in vivo studies have shown that bupivacaine is more toxic to cartilage cells than ropivacaine and lidocaine[11,13,17]. Specifically, Piper and Kim directly compared the toxicity of 0.5% ropivacaine with that of 0.5% bupivacaine and found that although ropivacaine

did induce cell death in human chondrocytes, it was significantly less chondrotoxic than bupivacaine[13]. The observed cytotoxicity differences between chondrocyte and mesenchymal stem cell populations suggest a differential effect of local anesthetics on different cell types.

The inherent differences in physical and chemical properties among these drugs likely contribute to the differences in their relative toxicities on different cell types. Bupivacaine and ropivacaine are highly lipophilic molecules, whereas lidocaine is only slightly lipophilic[18,19]. Treatment of human leukemia cells with these anesthetics induces different patterns of cell death. Bupivacaine and ropivacaine predominantly cause necrosis, whereas lidocaine induces DNA fragmentation and apoptosis (programmed cell death)[19]. Studies of human T-cell lymphoma and neuroblastoma cell lines showed that lidocaine treatment leads to apoptosis but ropivacaine does not. These differences may be due to differential activation of caspases, which are proteolytic enzymes that play key roles in apoptosis[20,21]. The three anesthetics may also differentially affect inflammation. Bupivacaine induces nitric oxide synthase-2 activity in rat glial cells and astrocytes but ropivacaine does not[22]. This pathway is normally activated as part of an inflammatory response, suggesting that bupivacaine toxicity may be attributable in part to the production of nitric oxide during ongoing inflammation. In contrast, ropivacaine has anti-inflammatory properties[13].

The relative potencies of lidocaine, bupivacaine, and ropivacaine were accounted for in our comparisons among the drugs. Bupivacaine and ropivacaine are highly potent anesthetics with long-lasting effects, whereas lidocaine is a moderately potent anesthetic with shorter effects[23]. Specifically, bupivacaine and ropivacaine are approximately four times more potent anesthetics than lidocaine[24]. Therefore, the effects of 0.5% bupivacaine and 0.5% ropivacaine on pain should be similar to that of 2% lidocaine, but with a longer duration of action and less toxicity on mesenchymal stem cells. Although the CellTiter-Glo assay for ATP content was slightly less sensitive to differences among conditions than the live cell count, similar trends were found with both methods of analysis.

One limitation of the present study is the fact that the experiments were performed in vitro. Further experiments are needed to assess the in vivo effects of these drugs on mesenchymal stem cell viability. However, the experimental conditions in the present study (growth of cells in tissue culture to yield a monolayer in the fifth or sixth passage) are commonly used in studies of mesenchymal stem cells[25,26], and such cells are defined in part by their ability to adhere, proliferate, and differentiate into mesenchymal tissues under such in vitro conditions[27]. Another limitation is the fact that viability measurements were only performed after sixty minutes of anesthetic treatment. This duration of exposure was chosen on the basis of a previous study of the pharmacokinetics of bupivacaine after intra-articular injection into the knee[28]. The absorption of this anesthetic from the joint into the bloodstream is rapid, with the peak blood concentration being reached within the first hour after injection. We are not aware of similar data for lidocaine or ropivacaine. We did not test for time dependence or for potential early-acting toxicity in the drug treatments. The effect of local anesthetic treatment on the differentiation potential of mesenchymal stem cells is also of interest in future experiments. Although cells treated with bupivacaine and ropivacaine in the present study appeared to be metabolically and morphologically normal, the drugs may subtly affect differentiation patterns. To study these effects, mesenchymal stem cells treated with varying classes and doses of local anesthetic can be assayed for the ability to form bone, cartilage, and fat.

In conclusion, in contrast to the toxicity observed in human and bovine chondrocytes, commonly used concentrations of ropivacaine and bupivacaine appeared to have limited toxicity on human mesenchymal stem cells. In contrast, lidocaine significantly decreased mesenchymal stem cell viability. Although these experiments are limited by their in vitro nature, these data suggest that ropivacaine and bupivacaine may represent better options with respect to mesenchymal stem cell health. Taking into further account the chondrotoxicity of bupivacaine and the chondrolysis associated with postoperative bupivacaine infusions, ropivacaine may be the safest of these three choices for intra-articular analgesia. ■

NOTE: The authors thank Dr. Eva Grotkopp for statistical assistance and Linsey Jacobs and Dr. Sonja Limburg for technical assistance.

Ruyan Rahnama, BA
Miqi Wang, BA
Alexis C. Dang, MD
Hubert T. Kim, MD, PhD
Alfred C. Kuo, MD, PhD
Department of Orthopaedic Surgery,
University of California San Francisco,
San Francisco Veterans Affairs Medical Center,
4150 Clement Street Box 112,
San Francisco, CA 94121

References

1. Chirwa SS, MacLeod BA, Day B. Intraarticular bupivacaine (Marcaine) after arthroscopic meniscectomy: a randomized double-blind controlled study. Arthroscopy. 1989;5(1):33-5.

2. Katz JA, Kaeding CS, Hill JR, Henthorn TK. The pharmacokinetics of bupivacaine when injected intra-articularly after knee arthroscopy. Anesth Analg. 1988 Sep;67(9):872-5.

3. Middleton F, Coakes J, Umarji S, Palmer S, Venn R, Panayiotou S. The efficacy of intra-articular bupivacaine for relief of pain following arthroscopy of the ankle. J Bone Joint Surg Br. 2006 Dec;88(12):1603-5.

4. Yamaguchi K, Sethi N, Bauer GS. Postoperative pain control following arthroscopic release of adhesive capsulitis: a short-term retrospective review study of the use of an intra-articular pain catheter. Arthroscopy. 2002 Apr;18(4):359-65.

5. Brattwall M, Jacobson E, Forssblad M, Jakobsson J. Knee arthroscopy routines and practice. Knee Surg Sports Traumatol Arthrosc. 2010 Dec;18(12):1656-60. Epub 2010 Sep 21.

6. Anderson SL, Buchko JZ, Taillon MR, Ernst MA. Chondrolysis of the glenohumeral joint after infusion of bupivacaine through an intra-articular pain pump catheter: a report of 18 cases. Arthroscopy. 2010 Apr;26(4):451-61.

7. Busfield BT, Romero DM. Pain pump use after shoulder arthroscopy as a cause of glenohumeral chondrolysis. Arthroscopy. 2009 Jun;25(6):647-52.

8. Scheffel PT, Clinton J, Lynch JR, Warme WJ, Bertelsen AL, Matsen FA 3rd. Glenohumeral chondrolysis: a systematic review of 100 cases from the English language literature. J Shoulder Elbow Surg. 2010 Sep;19(6):944-9. Epub 2010 Apr 24.

9. Chu CR, Coyle CH, Chu CT, Szczodry M, Seshadri V, Karpie JC, Cieslak KM, Pringle EK. In vivo effects of single intra-articular injection of 0.5% bupivacaine on articular cartilage. J Bone Joint Surg Am. 2010 Mar;92(3):599-608.

10. Chu CR, Izzo NJ, Coyle CH, Papas NE, Logar A. The in vitro effects of bupivacaine on articular chondrocytes. J Bone Joint Surg Br. 2008 Jun;90(6):814-20.

11. Karpie JC, Chu CR. Lidocaine exhibits dose- and time-dependent cytotoxic effects on bovine articular chondrocytes in vitro. Am J Sports Med. 2007 Oct;35(10):1621-7. Epub 2007 Jul 30.

12. Lo IK, Sciore P, Chung M, Liang S, Boorman RB, Thornton GM, Rattner JB, Muldrew K. Local anesthetics induce chondrocyte death in bovine articular cartilage disks in a dose- and duration-dependent manner. Arthroscopy. 2009 Jul;25(7):707-15.

13. Piper SL, Kim HT. Comparison of ropivacaine and bupivacaine toxicity in human articular chondrocytes. J Bone Joint Surg Am. 2008 May;90(5):986-91.

14. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol. 2004 Apr;36(4):568-84.

15. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science. 1999 Apr 2;284(5411):143-7.

16. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. J Orthop Res. 2007 Jul;25(7):913-25.

17. Piper SL, Kramer JD, Kim HT, Feeley BT. Effects of local anesthetics on articular cartilage. Am J Sports Med. 2011 Oct;39(10):2245-53. Epub 2011 Apr 22.

18. Grouselle M, Tueux O, Dabadie P, Georgescaud D, Mazat JP. Effect of local anaesthetics on mitochondrial membrane potential in living cells. Biochem J. 1990 Oct 1;271(1):269-72.

19. Onizuka S, Yonaha T, Tsuneyoshi I. Local anesthetics with high lipophilicity are toxic, while local anesthetics with low pka induce more apoptosis in human leukemia cells. J Anesth Clin Res. 2011;2:1.

20. Boselli E, Duflo F, Debon R, Allaouchiche B, Chassard D, Thomas L, Portoukalian J. The induction of apoptosis by local anesthetics: a comparison between lidocaine and ropivacaine. Anesth Analg. 2003 Mar;96(3):755-6, table of contents.

21. Perez-Castro R, Patel S, Garavito-Aguilar ZV, Rosenberg A, Recio-Pinto E, Zhang J, Blanck TJ, Xu F. Cytotoxicity of local anesthetics in human neuronal cells. Anesth Analg. 2009 Mar;108(3):997-1007.

22. Feinstein DL, Murphy P, Sharp A, Galea E, Gavrilyuk V, Weinberg G. Local anesthetics potentiate nitric oxide synthase type 2 expression in rat glial cells. J Neurosurg Anesthesiol. 2001 Apr;13(2):99-105.

23. Tomin J, Zivanov-Curlis J, Popovic D, Glogovac S, Basic D. Differences in local anesthetic effects of optically active isomers of local anesthetic compounds. Biotechnol Biotechnol Eq. 2006;20:9-14.

24. Adams HR, editor. Veterinary pharmacology and therapeutics. 8th ed. Ames, IA: Iowa Stae University Press; 2001. p 346.

25. Bian L, Zhai DY, Tous E, Rai R, Mauck RL, Burdick JA. Enhanced MSC chondrogenesis following delivery of TGF-β3 from alginate microspheres within hyaluronic acid hydrogels in vitro and in vivo. Biomaterials. 2011 Sep;32(27):6425-34. Epub 2011 Jun 8.

26. Cooke ME, Allon AA, Cheng T, Kuo AC, Kim HT, Vail TP, Marcucio RS, Schneider RA, Lotz JC, Alliston T. Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. Osteoarthritis Cartilage. 2011 Oct;19(10):1210-8. Epub 2011 Jul 23.

27. Penfornis P, Pochampally R. Isolation and expansion of mesenchymal stem cells/multipotential stromal cells from human bone marrow. Methods Mol Biol. 2011;698:11-21.

28. Katz JA, Kaeding CS, Hill JR, Henthorn TK. The pharmacokinetics of bupivacaine when injected intra-articularly after knee arthroscopy. Anesth Analg. 1988 Sep;67(9):872-5.