Chondrolysis After Continuous Intra-Articular Bupivacaine Infusion: An Experimental Model Investigating Chondrotoxicity in the Rabbit Shoulder

Andreas H. Gomoll, M.D., Richard W. Kang, B.S., James M. Williams, Ph.D., Bernard R. Bach, M.D., and Brian J. Cole, M.D., M.B.A.

Purpose: Postoperative pain pumps are increasingly used to deliver a continuous infusion of local anesthetic into the surgical wound or the joint. Recently, there have been concerns that the use of such devices may be associated with chondrotoxicity and even cases of chondrolysis in the shoulder. An experimental model is presented that investigates potential chondrotoxic effects of a continuous intra-articular infusion of bupivacaine in the rabbit shoulder. **Methods:** We divided 30 rabbits into 3 groups that received continuous infusions of either saline solution, bupivacaine, or bupivacaine with epinephrine into the glenohumeral joint over a period of 48 hours. Animals were killed after 1 week, and osteochondral and synovial samples from the glenohumeral joint underwent analyses with confocal microscopy for live/dead cell assay, metabolic sulfate uptake assessment, and conventional histologic analysis. **Results:** Infusion of bupivacaine with epinephrine and without epinephrine decreased sulfate uptake by 56% ($P = .009$) and 50% ($P = .02$), respectively, when compared with saline solution; cell viability decreased by 20% ($P = .08$) and 32% ($P = .02$), respectively. Histologic analysis yielded significantly worse scores for bupivacaine infusion with epinephrine ($P = .004$) and without epinephrine ($P = .02$). The results for bupivacaine with or without epinephrine were not significantly different. **Conclusions:** Continuous intra-articular infusion of bupivacaine with and without epinephrine led to significant histopathologic and metabolic changes in articular cartilage. **Clinical Relevance:** Bupivacaine showed profound chondrotoxic effects in an experimental model that closely followed the current clinical application of postoperative pain pumps. The results caution against the use of such devices in applications for smaller joints with minimal clearance or dilution as a result of hematoma, where continuous exposure of cartilage to bupivacaine is expected. **Key Words:** Chondrolysis—Chondral damage—Bupivacaine—Pain management—Shoulder arthroscopy.

Increasingly, orthopaedic procedures are being performed on an outpatient basis, requiring adjustment of traditional perioperative pain management protocols. In response to concerns over narcotic pain medication, pain pumps were developed for the continuous delivery of local anesthetic agents, commonly bupivacaine,[1,2] into the wound or affected joint. This infusion is generally continued for 48 hours after a procedure, a period when severe pain is commonly observed.[3]

However, bupivacaine has been associated with significant side effects, including immediate and delayed hypersensitivity reactions,[4] systemic toxicity,[5] myotoxicity,[6-8] and most significantly, chondrotoxicity.[9] Exposure to a single intra-articular injection of bupivacaine has been shown to cause histopathologic changes in articular cartilage[9] that are similar to those associated with the development of osteoarthritis.[10,11]

Musculoskeletal side effects of bupivacaine in clinical practice have not been rigorously evaluated; how-

From the Department of Orthopedic Surgery, Rush University Medical Center, Rush Medical College (A.H.G., R.W.K., B.R.B., B.J.C.), Chicago, Illinois; and Department of Anatomy, Rush University Medical Center, Rush Medical College (J.M.W.), Chicago, Illinois, U.S.A.

Supported by a research grant from the Arthroscopy Association of North America and an Alpha Omega Alpha Carolyn L. Kuckein Student Research Fellowship. The authors report no conflict of interest.

Address correspondence and reprint requests to Brian J. Cole, M.D., M.B.A., Department of Orthopedic Surgery, Rush University Medical Center, 1725 W Harrison St, Suite 1063, Chicago, IL 60612, U.S.A. E-mail: bcole@rushortho.com

0749-8063/06/2208-6169$32.00/0
doi:10.1016/j.arthro.2006.06.006

ever, recently, there has been increasing concern that the use of continuous intra-articular bupivacaine infusion via pain pumps may be associated with the development of chondrolysis of the shoulder[12] and ankle joint.[13] In contrast, 1-time injections of bupivacaine into various joints have not been associated with detrimental long-term clinical effects.

Our practice is seeing increasing numbers of referred cases of rapid-onset glenohumeral arthritis after shoulder procedures in young patients. Often, the only common denominator is the placement of a postoperative intra-articular pain catheter with infusion of bupivacaine for 48 hours, when other suspected causes of chondrolysis such as infection or thermal necrosis have been excluded. Although the exact incidence has not been established, chondrolysis is a rare complication, with devastating consequences including the subsequent rapid development of osteoarthritis. We hypothesized that prolonged exposure of articular cartilage to a bupivacaine infusion would result in depressed function or death of chondrocytes. To our knowledge, no experimental model exists to evaluate the histopathologic effects of continuous intra-articular bupivacaine infusion. The purpose of this study was to investigate these effects in an experimental setup that follows the clinical application of pain pumps as closely as possible to provide a better understanding of whether bupivacaine can initiate chondral changes that may potentially lead to chondrolysis.

METHODS

Study Subjects

This study was conducted after review by our institution's animal review board and in accordance with established animal care protocols. All procedures were performed in male New Zealand white rabbits with a mean weight of 3.8 kg (SD, 0.15 kg; range, 3.5 to 4.1 kg). This weight is considered to be equivalent to an age of 4 to 6 months, which is when skeletal maturity occurs.[14] Radiographs confirming growth plate closure were obtained.

A total of 30 rabbits were randomized into 3 groups: group 1 rabbits acted as controls with infusion of normal saline solution with a pH of 5.9 (Baxter Healthcare, Deerfield, IL), group 2 received bupivacaine hydrochloride (0.25%) with a pH of 5.7 (Abbott Laboratories, Abbott Park, IL), and group 3 received bupivacaine hydrochloride (0.25%) and epinephrine (1:200,000) with a pH of 4.0 (Hospira, Lake Forest, IL).

Surgical Procedure

Each rabbit was induced with an intramuscular injection of ketamine (40 mg/kg), acepromazine (1 mg/kg), and xylazine (5 mg/kg) and subsequently intubated and ventilated with isoflurane in oxygen. Pain control was achieved preoperatively with buprenorphine (0.01 to 0.03 mg/kg), given either intramuscularly or subcutaneously, and postoperatively every 10 to 12 hours or as needed for pain. Antibiotic prophylaxis was provided by subcutaneous injection of cefazolin (22 mg/kg) at the time of operation and then followed by daily injections for 48 hours.

After standard preparation and draping, the glenohumeral joint was exposed through a 2-cm incision over the dorsolateral aspect of the left shoulder, and a soft polyurethane catheter was introduced into the joint under direct visualization. The capsule and rotator cuff musculature were closed around the catheter, leaving approximately 5 mm of catheter inside the joint. The suture flanges on the catheter were secured to the soft tissues of the proximal humerus with 3-0 Vicryl (Ethicon, Somerville, NJ). The free end of the catheter was then tunneled subcutaneously toward the midline to externalize it away from the incision and was sutured to the skin. Wound closure was performed in a subcutaneous and subcuticular fashion. The catheter was connected to a small ($<$30 g), self-contained infusion disk, and the rabbit was then placed into a jacket that protected the incision and housed the disk.

Postoperative Considerations

The infusion disk was set to run for 48 hours at a constant flow rate of 210 μL/h. The flow rate was based on proportional weight, because no data exist regarding the human-equivalent dosing of intra-articular bupivacaine in a rabbit shoulder model or the capsular volume of the rabbit glenohumeral joint. On the basis of the assumption that the flow rate is 4.16 mL/h in an average human weighing 70 kg, the proportional infusion rate for a 3.5-kg rabbit was calculated as 0.21 mL/h.

The rabbits were allowed unrestricted ambulation, food, and water in individual cages and were monitored closely for postoperative complications. Once the infusion disk had delivered its volume after 48 hours, it was disconnected and the catheter was cut, tied off, and internalized under the skin in a sterile fashion. At 7 days after the index operation, and 5 days after catheter closure, the rabbits were killed through an intravenous overdose of pentobarbital after

sedation with acepromazine (1 mg/kg) and ketamine (25 mg/kg). All incisions were re-explored to ensure that the catheter tips had remained in the correct intra-articular location.

Histopathologic Analyses

Sulfate Uptake: Sulfate uptake is a standard measure of cartilage proteoglycan metabolism[15] and serves as an indication of cartilage anabolism. Shavings of articular cartilage from the humeral head were retrieved and incubated for 4 hours with Na $^{35}SO_4$ (50 μCi/mL) in 3 mL of Ham's F-12 medium (Invitrogen Corp., Carlsbad, CA) containing 10% fetal calf serum with gentamicin (50 μg/mL) in sterile 12-well plates at 37°C under an atmosphere of 95% air/5% carbon dioxide. Subsequently, the samples were rinsed in brief with fresh medium and placed in non–radioisotope-containing medium for an additional 24 hours of incubation. The samples were then fixed for 4 days with 10% neutral buffered formalin containing 0.5% cetylpyridinium chloride, followed by measurement of radioisotope activity, expressed in counts per minute per wet weight of cartilage. The mean wet weight of the cartilage was 2.01 $\pm$ 1.37 mg. The ratio of activity between the infused and control shoulders was then calculated for each animal and averaged for each group. Thus in these analyses lower scores represent less metabolic activity for the infused versus control shoulders.

Confocal Live/Dead Cell Assay: Full-thickness sections of articular cartilage, including the subchondral bone, of 2 mm in width were obtained from the midsection of the humeral head and incubated with the live cell stain at 37°C under constant stirring/shaking, following a previously established protocol.[16] This initial solution consisted of sterile phosphate buffer containing 4-μmol/L calcein acetoxymethyl ester (Molecular Probes, Eugene, OR). After 1 hour, 8-μmol/L ethidium homodimer (Molecular Probes) was added to the solution for an additional hour to label dead cells. Thereafter the samples were removed from the liquid medium, embedded, and sectioned (5 μm). By use of confocal laser microscopy (488-nm excitation) (Fluoview IX70 confocal laser microscope; Olympus, Melville, NY), 2 blinded and independent observers calculated the ratio of live to dead cells. The results were averaged between observers for each animal and side. The final live/dead cell score was calculated as the ratio between the infused and control sides; thus a lower ratio indicates a smaller proportion of live cells for the infused shoulder than for the control shoulder.

Table 1. *Histologic Grading System*[17]

	Points
Articular surface	
Normal	1
Focal fibrillation	2
Moderate fibrillation	3
Widespread fibrillation (>50% of total surface)	4
Complete loss of cartilage	5
Safranin O staining	
Normal	1
Slight decrease	2
Moderate decrease	3
Severe decrease	4
No staining	5
Clone formation	
None	1
1-5 cells/low-power field (×10)	2
6-15 cells/low-power field (×10)	3
16-25 cells/low-power field (×10)	4
>25 cells/low-power field (×10)	5
Cellularity	
Normal	1
Slight focal decrease	2
Moderate decrease	3
Widespread decrease (>50% of cells)	4
Complete loss of cells	5
Synovial membrane	
No inflammation	1
Mild inflammation (increase in cell lining thickness)	2
Moderate inflammation (increase in cell lining thickness and presence of inflammatory cells)	3
Marked inflammation (increase in cell lining thickness and marked increase in inflammatory cells)	4

Histologic Analysis: Synovial tissue and coronal sections of articular cartilage obtained from the humeral head were procured for histologic evaluation. Each sample was fixed in 10% neutral buffered formalin and decalcified in aqueous formic acid (22%)/sodium citrate (10%), after which it was processed for paraffin embedding. The specimens were sectioned (4 to 6 μm), placed on glass slides coated with Vectabond (Vector Laboratories, Burlingame, CA), and stained with H&E for routine histologic analysis. In addition, osteochondral sections of the humeral head were stained with safranin O to evaluate the presence of matrix proteoglycans.

Samples of articular cartilage and synovium were assessed with a modified Mankin grading scale for 5 aspects of histopathologic changes[17] (Table 1). This analysis was performed by 2 independent and blinded observers, whose results were averaged. The scores for each of the 5 histopathologic measures were then added to produce a compound histologic grade for

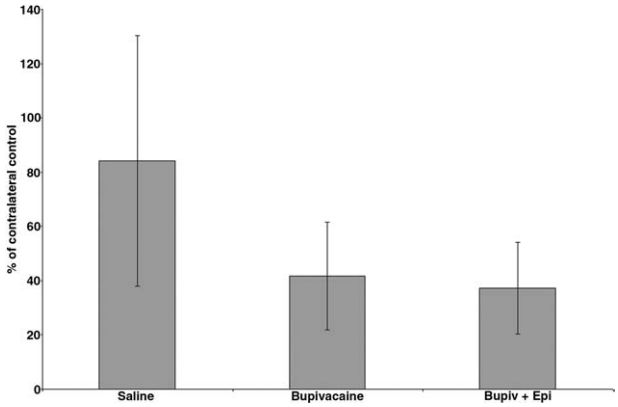

FIGURE 1. Results of sulfate uptake measurements in samples infused with saline solution, bupivacaine without epinephrine, and bupivacaine with epinephrine (Bupiv + Epi). The results are expressed as the relative difference between activity readings from samples of the control and infused sides. Data are given as mean ± SD.

each specimen (which could range from 5 to 24, with higher scores representing worse histopathologic changes).

Statistical Analysis

Normally distributed variables were analyzed with 1-way analysis of variance, with statistically significant results subsequently undergoing a Tukey multiple comparisons post hoc test. Variables that were not normally distributed were analyzed by use of the Kruskal-Wallis test. Statistically significant results were then further evaluated by a Bonferroni-adjusted 2-sided Mann-Whitney test for pairwise comparisons. Significance levels were set at $P < .05$.

RESULTS

We excluded 1 animal each from group 1 (saline solution) and group 2 (bupivacaine) because of catheter malfunction, leaving 9 animals in group 1 and in group 2 and 10 animals in group 3 (bupivacaine with epinephrine) for analysis.

Sulfate Uptake

Within groups, saline solution decreased sulfate uptake by 16% in the infused side compared with the control side; the decrease was 58% and 63% for bupivacaine alone and bupivacaine with epinephrine, respectively. Analysis of variance revealed significant differences among these 3 groups ($P = .005$). Subsequent Tukey multiple comparisons testing was performed to detect differences between groups; bupivacaine infusion resulted in a 50% greater reduction in sulfate uptake than saline solution ($P = .02$) (Fig 1). Similarly, bupivacaine with epinephrine yielded a significant, 56% greater reduction in sulfate uptake than saline solution infusion ($P = .009$). No statistically significant differences were found when bupivacaine infusion with epinephrine and bupivacaine infusion without epinephrine were compared ($P = .6$).

Confocal Live/Dead Cell Assay

Within groups, saline solution infusion resulted in no significant difference in cell viability ($P = .8$) for the infused side compared with the contralateral, noninfused control side; bupivacaine infusion, however, reduced cell viability by 32%. This change in cell viability was statistically significant when compared with saline solution infusion ($P = .02$). Similarly, infusion with bupivacaine with epinephrine decreased cell viability by 20%; compared with the saline solution group, these results were not significant ($P = .08$). No statistically significant differences were found when bupivacaine infusions with or without epinephrine were compared ($P = .35$). Figure 2 shows representative confocal microscopy images of articular cartilage obtained from the noninfused control side (left), as well as after infusion with bupivacaine with epinephrine (right). Both samples contain red-staining, dead chondrocytes; however, the infused sample shows a larger number of dead cells, especially in the superficial layer.

Histologic Analysis

All 3 infusions led to statistically significant changes in the histologic score when compared with the contralateral, noninfused control side. For saline solution, this was predominantly a result of decreased proteoglycan content, whereas the other solutions affected all parameters of the histologic grading system. In comparing the 3 groups we found significant differences ($P = .007$, Kruskal-Wallis test). Specifically, significantly higher (worse) histologic scores were observed for bupivacaine with and without epinephrine, as compared with saline solution infusion ($P = .004$ and .03, respectively), whereas no significant differences were found between the bupivacaine groups ($P = .2$) (Figs 3 and 4).

DISCUSSION

Our investigation showed significant histopathologic and functional changes in articular cartilage after

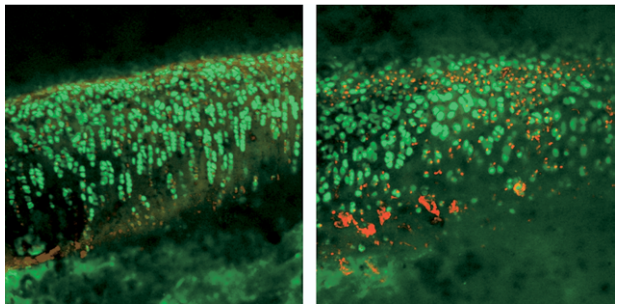

FIGURE 2. Confocal microscopy images depicting articular cartilage from control side (left) and after exposure to bupivacaine with epinephrine (right). Live cells stain green, whereas dead chondrocytes appear red. (Calcein-acetoxymethylester and ethidium homodimer, original magnification ×10.)

infusion of bupivacaine with or without epinephrine. The study design closely simulated the current clinical practice of continuous intra-articular infusion of local anesthetic after shoulder surgery[18] in an experimental animal that is well established in cartilage research for the study of cellular effects.[14]

Conventional histologic analysis showed changes in both cartilage and synovial membrane consistent with significantly greater histopathologic changes after infusion of bupivacaine with or without epinephrine, as compared with saline solution. Functional investigations included a confocal live/dead cell assay, which provides a direct assessment of cell viability at the time of tissue harvest, as well as sulfate uptake as a measure of cartilage metabolism, which serves as an indicator of chondrocyte activity. Both tests showed significant detrimental changes in articular cartilage after infusion of bupivacaine with or without epinephrine; confocal laser microscopy showed that treatment with bupivacaine with epinephrine and bupivacaine without epinephrine reduced cell viability by 20% and 32%, respectively. More importantly, sulfate uptake, as an indirect measure of proteoglycan synthesis, was suppressed even further, by 56% and 50%, respectively, compared with saline solution. The comparatively larger reduction in sulfate uptake than in cell viability suggests that even cells that survived after bupivacaine infusion remained at a decreased metabolic state 5 days after cessation of the infusion.

Our results confirm prior investigations of bupivacaine toxicity. Dogan et al.[9] described inflammatory cell infiltration, hypertrophy, and hyperplasia of the synovial membrane after intra-articular injections of bupivacaine into the rabbit knee. Other investigators have shown significant damage to skeletal muscle after exposure to bupivacaine.[6-8] Further research showed decreases in $^{35}SO_4$ uptake with both saline solution and bupivacaine exposure,[19] although the effects of saline solution were only transient.[20]

The low apparent incidence of chondrolysis may seem surprising, considering the profound detrimental effects of bupivacaine shown in this study, as well as the increasing number of pain pumps used in clinical practice. However, no rigorous clinical evaluation of bupivacaine and chondrolysis has been conducted, and sporadic cases are likely to go unreported. Furthermore, because one would expect a quiescent interval between the inciting event on the cellular level and the development of clinically apparent chondrolysis, it may be several years before an increase in chondrolysis can be observed in clinical practice. It is also possible that the usually small postoperative hematoma after arthroscopic shoulder procedures such as instability repairs, which would otherwise dilute the infusion, in combination with the relatively low glenohumeral joint volume, places the shoulder joint at increased risk. Interestingly, single-shot bupivacaine injections have been used in the knee without clinically apparent chondrotoxic effects for many years. We believe that this noteworthy difference can potentially be explained by the thinner cartilage[21,22] in the shoulder and exposure to a continuous infusion, whereas bupivacaine after knee surgery is usually applied as a 1-time injection. Similarly, 1-time injections into the glenohumeral joint, though much less common, have also not been associated with progressive joint degeneration.

One limitation of our study, which it shares with most animal models, should be considered; although we were able to show the detrimental effects of bupivacaine on the cellular and tissue level in a rabbit model, it remains to be determined whether human cartilage is equally susceptible and whether these histopathologic and functional changes result in the subsequent development of rapidly progressive osteoarthritis. However, because chondrocyte density in human articular cartilage is much less than that in the rabbit (1.7% *v* 12.2% for the medial femoral condyle)[23] and each chondrocyte maintains an approximately 8- to 10-fold larger area of surrounding matrix,[23] one would expect chondrocyte death to have a larger effect on cartilage health in humans than in the rabbit model. Several studies have established a strong link between chondrocyte impairment or death and the development and severity of osteoarthritis in various animal models, as well as in humans.[24-29] This association is especially concerning because a large number of shoulder procedures are performed in younger

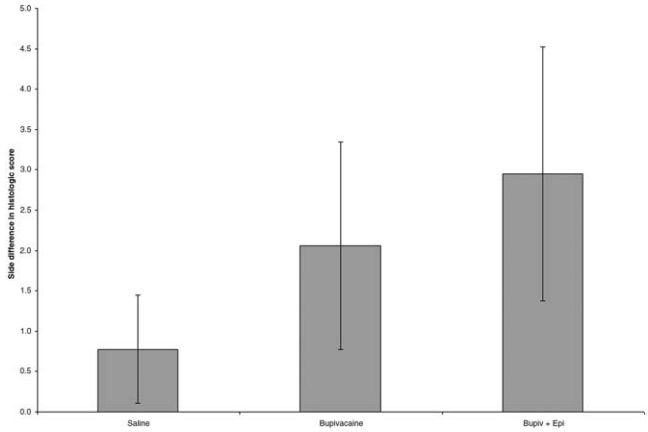

FIGURE 3. Results of conventional histologic examination of cartilage and synovial samples, presented as difference in histologic score between control and infused sides. Data are given as mean ± SD. (Bupiv + Epi, bupivacaine with epinephrine.)

patients, whose long life expectancy can be expected to amplify even small insults to articular cartilage, with its extremely limited capacity for self-healing. Because epidemiologic study of chondrolysis in humans will require an extremely large sample size because of the low incidence and prevalence of this condition, additional studies in a larger animal model with longer-term follow-up, as well as in vitro studies with continuous exposure of human cartilage to bupivacaine, are necessary to provide further understanding.

CONCLUSIONS

Continuous intra-articular infusion of bupivacaine with and without epinephrine led to significant histopathologic and metabolic changes in articular cartilage. In particular, bupivacaine showed profound chondrotoxic effects in an experimental model that closely followed the current clinical application of postoperative pain pumps. The results caution against the use of such devices in applications for smaller joints with minimal clearance or dilution as a result of hematoma, where continuous exposure of cartilage to bupivacaine is expected.

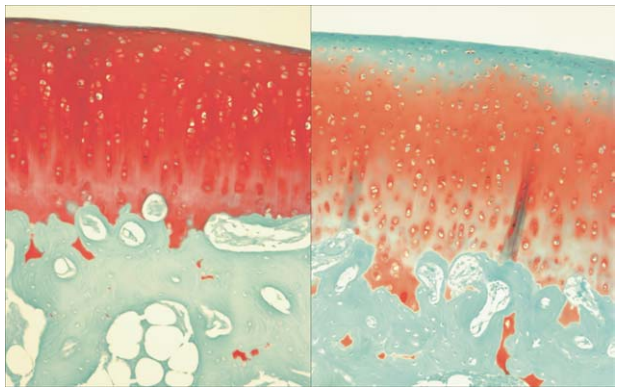

FIGURE 4. Safranin O–stained, fast-green articular cartilage samples from control side (left) and after exposure to bupivacaine with epinephrine (right). There is less intense red staining in the infused sample (right), indicative of a loss of glycosaminoglycan matrix within the cartilage. (Original magnification ×40.)

Acknowledgment: The authors thank Dr. Charles Beck, Carol Pacione, Susan Shott, Sarah Gitelis, Adam Kramer, and Vamsi Singaraju for their assistance on the project.

REFERENCES

1. Park JY, Lee GW, Kim Y, Yoo MJ. The efficacy of continuous intrabursal infusion with morphine and bupivacaine for postoperative analgesia after subacromial arthroscopy. *Reg Anesth Pain Med* 2002;27:145-149.
2. Tetzlaff JE, Brems J, Dilger J. Intraarticular morphine and bupivacaine reduces postoperative pain after rotator cuff repair. *Reg Anesth Pain Med* 2000;25:611-614.
3. Tuominen M, Pitkanen M, Rosenberg PH. Postoperative pain relief and bupivacaine plasma levels during continuous interscalene brachial plexus block. *Acta Anaesthesiol Scand* 1987; 31:276-278.
4. Craft DV, Good RP. Delayed hypersensitivity reaction of the knee after injection of arthroscopy portals with bupivacaine (Marcaine). *Arthroscopy* 1994;10:305-308.
5. Liguori GA, Chimento GF, Borow L, Figgie M. Possible bupivacaine toxicity after intraarticular injection for postarthroscopic analgesia of the knee: Implications of the surgical procedure. *Anesth Analg* 2002;94:1010-1013.
6. Hall-Craggs EC. Early ultrastructural changes in skeletal muscle exposed to the local anaesthetic bupivacaine (Marcaine). *Br J Exp Pathol* 1980;61:139-149.
7. Johnson ME, Jones GH. Effects of Marcaine, a myotoxic drug, on macromolecular synthesis in muscle. *Biochem Pharmacol* 1978;27:1753-1757.
8. Schultz E, Lipton BH. The effect of Marcaine on muscle and non-muscle cells in vitro. *Anat Rec* 1978;191:351-369.
9. Dogan N, Erdem AF, Erman Z, Kizilkaya M. The effects of bupivacaine and neostigmine on articular cartilage and synovium in the rabbit knee joint. *J Int Med Res* 2004;32:513-519.
10. Lukoschek M, Schaffler MB, Burr DB, Boyd RD, Radin EL. Synovial membrane and cartilage changes in experimental osteoarthrosis. *J Orthop Res* 1988;6:475-492.
11. Van Lent PL, Van den Hoek AE, Van den Bersselaar LA, et al. In vivo role of phagocytic synovial lining cells in onset of experimental arthritis. *Am J Pathol* 1993;143:1226-1237.
12. Hansen B, Beck C, Townsley R. Post-arthroscopic shoulder chondrolysis with associated intraarticular pain pump catheter use. Presented at the Annual Meeting of the American Academy of Orthopaedic Surgeons, Chicago, IL, March 2006.
13. Bojescul JA, Wilson G, Taylor DC. Idiopathic chondrolysis of the ankle. *Arthroscopy* 2005;21:224-227.
14. Reinholz GG, Lu L, Saris DB, Yaszemski MJ, O'Driscoll SW. Animal models for cartilage reconstruction. *Biomaterials* 2004;25:1511-1521.
15. Masuda K, Shirota H, Thonar EJ. Quantification of 35S-labeled proteoglycans complexed to alcian blue by rapid filtration in multiwell plates. *Anal Biochem* 1994;217:167-175.

16. Grogan SP, Aklin B, Frenz M, et al. In vitro model for the study of necrosis and apoptosis in native cartilage. *J Pathol* 2002;198:5-13.
17. Williams JM, Ongchi DR, Thonar EJ. Repair of articular cartilage injury following intra-articular chymopapain-induced matrix proteoglycan loss. *J Orthop Res* 1993;11:705-716.
18. 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;18:359-365.
19. Nole R, Munson NM, Fulkerson JP. Bupivacaine and saline effects on articular cartilage. *Arthroscopy* 1985;1:123-127.
20. Bulstra SK, Kuijer R, Eerdmans P, van der Linden AJ. The effect in vitro of irrigating solutions on intact rat articular cartilage. *J Bone Joint Surg Br* 1994;76:468-470.
21. Cohen ZA, McCarthy DM, Kwak SD, et al. Knee cartilage topography, thickness, and contact areas from MRI: In-vitro calibration and in-vivo measurements. *Osteoarthritis Cartilage* 1999;7:95-109.
22. Graichen H, Jakob J, von Eisenhart-Rothe R, Englmeier KH, Reiser M, Eckstein F. Validation of cartilage volume and thickness measurements in the human shoulder with quantitative magnetic resonance imaging. *Osteoarthritis Cartilage* 2003;11:475-482.
23. Eggli PS, Hunziker EB, Schenk RK. Quantitation of structural features characterizing weight- and less-weight-bearing regions in articular cartilage: A stereological analysis of medial femoral condyles in young adult rabbits. *Anat Rec* 1988;222:217-227.
24. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. *Arthritis Rheum* 1998;41:1266-1274.
25. Adams CS, Horton WE Jr. Chondrocyte apoptosis increases with age in the articular cartilage of adult animals. *Anat Rec* 1998;250:418-425.
26. Kuhn K, D'Lima DD, Hashimoto S, Lotz M. Cell death in cartilage. *Osteoarthritis Cartilage* 2004;12:1-16.
27. Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ, Galdo F. Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. *Arthritis Rheum* 1998;41: 284-289.
28. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. *Arthritis Rheum* 1998;41:1632-1638.
29. Kim HA, Lee YJ, Seong SC, Choe KW, Song YW. Apoptotic chondrocyte death in human osteoarthritis. *J Rheumatol* 2000; 27:455-462.