Supraphysiologic temperature sensitizes bovine articular chondrocytes to bupivacaine-induced cell death

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Objectives
Physicians currently use intra-articular bupivacaine injections after closing up arthroscopically repaired joints to reduce immediate post-operative pain. However, some physicians have recently reported irreversible articular cartilage degeneration in patients receiving these injections. The objective of this in-vitro study is to determine the effects of temperature on chondrocyte viability and to determine the effects of bupivacaine in combination with different temperatures on chondrocyte viability.

Methods
Normal bovine articular chondrocytes were cultured in medium containing 10% fetal bovine serum (FBS) in a 5% CO2 humidified incubator at 37°C. Approximately 1x10⁶ cells per milliliter of phosphate-buffered saline (PBS) were incubated at 37°C, 40°C, 42°C, 45°C, 47°C and 50°C, for 15, 30 and 60 minutes, respectively. To test effects of bupivacaine under different temperatures, the cells were incubated at 37°C, 45°C and 50°C for either 30 or 60 minutes. Immediately before being taken out of the water baths, each group was treated with either 0.5 ml of saline or 0.5 ml of 0.5% bupivacaine hydrochloride (Hospira, Inc., Lake Forest, IL). The cells were incubated at room temperature for 60 minutes and then stained with the LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen) for 20 minutes at room temperature. The percentages of live and dead cells were determined by flow cytometry using a BD LSRII analyzer. The means and standard deviations (error bars) of triplicate samples were calculated and compared using the Student’s t test (two tails). P<0.05 was considered statistically significant.

Results
When bovine articular chondrocytes were exposed to temperatures from 45 °C to 50 °C for 15 minutes, there was significantly more cell death compared to chondrocytes at 20 °C and 37 °C (p < 0.05). When the chondrocytes were exposed to temperatures of 40 °C for 30 minutes, there was significantly more death than at 20 °C and 37 °C. When the cells were exposed to 50 °C for 60 minutes, the cell death rate was almost three times of the cells exposed to 20 °C (Figure 1). When the cells were incubated at 37°C for either 30 minutes or 60 mintues, there was no significant difference in cell death rates between the groups treated with bupivacaine and those treated with saline. When the cells were incubated at 45 °C and 50 °C for either 30 minutes or 60 mintues, there was significantly more cell death in the bupivacaine-treated groups compared to the saline-treated groups (p < 0.05) (Figure 2).

Conclusions
This study suggests that articular chondrocytes are more sensitive to bupivacaine-induced cell death when the cells are at a temperature higher than 37 °C, indicating that intra-articular injection of bupivacaine immediately following some heat-generating joint surgeries may cause more damage to the articular cartilage. This study is limited by the fact that it is an in-vitro study.