ORTHOVISC PROTECTS BOVINE ARTICULAR CHONDROCYTES AGAINST CELL DEATH INDUCED BY BUPIVACAINE UNDER SUPRAPHYSIOLOGIC TEMPERATURES


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Bupivacaine has long been used to reduce immediate post-operative pain following arthroscopic procedures on joint diseases. It has been known that supraphysiologic temperature (> 37 °C, up to 80 °C) can be attained inside the articular joint during some heat-generating arthroscopic procedures. Thus, it is possible that bupivacaine may be injected into the joint when the intra-articular temperature is supraphysiologic. Supraphysiologic temperature has been known to reduce chondrocyte viability. However, it is not known whether a combination of bupivacaine and supraphysiologic temperature would further increase cytotoxicity in articular chondrocytes, and if so, what measures could protect the chondrocytes against such cytotoxicity. The specific aim of this in-vitro study was to determine if Orthovisc, a bacterially derived sodium hyaluronate solution, could protect chondrocyte death induced by bupivacaine under supraphysiologic temperatures. Bovine articular chondrocytes suspended in phosphate-buffered saline (PBS) were incubated at 37 °C, 45 °C, and 50 °C for 1 h, and then treated with or without 0.25% or 0.5% bupivacaine hydrochloride (Hospira, Inc., Lake Forest, Illinois) for 1 h at room temperature. The chondrocytes were stained with a solution of 0.8 µM ethidium homodimer-1 and 0.8 µM calcein AM (acetoxymethyl ester) (from the LIVE/DEAD Viability/Cytotoxicity Kit, Molecular Probes, Eugene, Oregon) in PBS for 20 minutes in the dark at room temperature. The percentages of live and dead cells were obtained by counting 10,000 cells using a BD LSR II flow cytometry analyzer (Becton, Dickinson and Company, San Jose, CA). For cell viability at 6 and 24 h after treatment, the cells were washed three times with PBS to remove bupivacaine and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum. At each time point, the cells were stained with the LIVE/DEAD kit. Representative photomicrographs were taken with a fluorescence microscope (Nikon AZ100) equipped with a digital camera (Nikon DS-Qi1Mc) and NIS-Elements Basic Research 3.0 software (Nikon Instruments Inc., Melville, NY). We found that, 1 h after treatment, supraphysiologic temperatures (45 °C and 50 °C) caused 9.2% and 24.8% of chondrocyte death, respectively. Bupivacaine at 37 °C did not induce significant chondrocyte death, compared to the PBS control group. However, at 45 °C and 50 °C, 0.25% bupivacaine caused 21.2% and 72.4% of chondrocyte death, respectively; and 0.5% bupivacaine caused 44.3% and 61.5% of chondrocyte death, respectively. Addition of 1.5, 3.75 and 7.5 mg/ml Orthovisc (in concentration of hyaluronan) (Depuy Mitek, Inc., Raynham, MA) to the chondrocytes significantly inhibited chondrocyte death caused by 0.25% bupivacaine at 50 °C (P < 0.01). Addition of 1.5 mg/ml Orthovisc slightly inhibited chondrocyte death caused by 0.5% bupivacaine at 45 °C and 50 °C but did not reach statistical significance (P > 0.05). However, addition of 3.75 and 7.5 mg/ml Orthovisc significantly inhibited chondrocyte death caused by 0.5% bupivacaine at 45 °C and 50 °C (P < 0.05 and P < 0.01, respectively) (Figure 1). Orthovisc’s protective effects were still observed at 6 (Figure 2) and 24 h (Figure 3) after bupivacaine treatment at 45 °C. However, at 50 °C, Orthovisc could only delay the process of cell death caused by bupivacaine, but could not prevent the eventual chondrocyte death after 24 h. We concluded that bupivacaine under
supraphysiologic temperatures could cause articular chondrocyte death and Orthovisc could prevent chondrocyte death induced by bupivacaine at or below 45 °C. This finding implies that bupivacaine can be mixed with Orthovisc for intra-articular injection to prevent bupivacaine’s potential cytotoxicity.

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