Induced Cell Lysis By Ultrasound Contrast Agents And Its Potential For Chondrosarcoma Treatment

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Introduction: Chondrosarcoma is a rare, cartilage-like neoplasm that is currently untreatable except by surgical resection. There have been frequent attempts that ultrasound-induced cancer cell treatment using ultrasound contrast agents (UCA) which are gas-filled microbubbles typically used for ecogenicity in medical sonography since inertial or stable cavitational effects are expected depending on the strength of ultrasonic pressure.[1,2] Here, we evaluated the effect of commercial UCA, Optison, on human chondrosarcoma cells (hCSCs) and bovine normal chondrocytes (bNCs) viability in suspension with 1 MHz continuous or tone burst ultrasound. In addition, the effect of UCA with continuous ultrasound on bNCs viability in articular cartilage was also evaluated.

Methods: Osteochondral explants harvest: Mature bovine knee was obtained and 2 cm x 2 cm explants including the central loaded area from tibial plateau were prepared. The explants were then rinsed by Hank’s Balanced Salt Solution (Invitrogen Life Technologies) and culture in 45% Dulbecco’s modified Eagle’s medium (DMEM) and 45% Ham’s F12 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml Amphoterican B at 37°C, 5% CO2 and O2. Cell suspension: Forty thousand hCSCs or bNCs containing 10% Optison, commercially available (GE Healthcare) ultrasound contrast agents, were suspended in 200 ul centrifuge tube. Ultrasound generation: Either continuous or pulsed (1,000 tone burst per second) ultrasound are generated using Direct-digital synthesis function generator (4040B, BK Precision), Power amplifier (310L, ENI) and 1 MHz immersible transducer (Ultrasonic S-Lab). The intensity of continuous ultrasound was evaluated as 225 mW/cm2 by radiation force balance. Prepared cell suspensions were exposed to continuous ultrasound or 1,000 tone bursts per seconds with various output cycles for each trigger for 10 minutes. Osteochondral explants containing 10% Optison with culture media were sealed with non-porous polyethylene flat plastic bag and were stimulated for 20 minutes with continuous ultrasound and cultured for additional 24 hours. Viability assay: After ultrasound exposure, cells or explants were stained with 1μM Calcein-AM, a live cell indicator, and 1 μM Ethidiumhomodimer-2, a dead cell indicator for 30 minutes in the same culture condition. Hematocytometer was performed to count live or dead cells from cell suspensions and confocal microscopy was performed to analyze the depth-dependent chondrocyte death in articular cartilage. Statistical analysis: Student’s T-test was performed using statistical analysis package, SPSS (IBM Corporation, New York) and the level of significance was set at p<0.05.

Results: Continuous ultrasound exposure with 10% Optison in both hCSCs and bNCs suspension resulted in significant cell death. However, no significant cell death was observed in both control groups containing 10% Optison without continuous ultrasound exposure (Fig. 1).

The effect of pulsed ultrasound on bNCs viability was evaluated in suspension containing 10% Optison and the results showed that cell viability was dependent on number of output cycles per each tone burst wave (Fig. 2).

Post 24 hours of cell viability in osteochondral explants exposed to continuous ultrasound with 10% Optison was evaluated. Confocal microscopy revealed depth-dependent chondrocyte death in articular cartilage. Zonal analysis of chondrocyte viability showed that cell death was the most significant in superficial zone, however, chondrocyte death was gradually decreased from superficial to deep zone (Fig. 3).

Discussion: In this study, we evaluated the effect of continuous and pulsed ultrasound with 10% Optison on cell viability in suspension or in osteochondral explants. Continuous ultrasound with 10% Optison resulted in significant cell death for both hCSCs and bNCs and pulsed ultrasound with 10% Optison resulted in gradual bNCs death in explants. Therefore, these findings may support future study into the localized ultrasound-induced chondrosarcoma treatment in articular cartilage with UCA.

Significance: One of the most effective treatment methods for chondrosarcoma is to resect by surgery. However, direct surgical resection of chondrosarcoma should not be available when chondrosarcoma develops to invade into cartilage in joints. Since few decades, low-intensity ultrasound has been studied as a tool for physical therapy and its application was extended to cancer treatment with frequent reports that induced cancer cell lysis by UCA.[1,2] In this study, we evaluated that continuous ultrasound exposure with UCA resulted in significant hCSCs and bNCs death in suspension and bNCs death in explants. In addition, bNCs viability was dependent on number of cycles in pulsed ultrasound. Therefore, these findings could support future study that the therapeutic treatment of chondrosarcoma in situ in joints by ultrasound stimulation.

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