Clinical CT Contrast Agents Alter the Mechanical State of Articular Cartilage

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INTRODUCTION: Knee arthrography is a common technique to assess cartilage lesions. Although Magnetic Resonance Imaging (MRI) arthrography is most common, Computed Tomography (CT) arthrography is an alternative for patients for whom MRI might be contraindicated. A conventional CT arthrogram involves direct injection of an iodinated contrast agent into the synovial joint followed by a CT scan. Even though CT arthrography is a widely accepted as a reliable clinical technique, protocols vary substantially and involve contrast agents at full strength or diluted to variable concentrations in anesthetics or saline, based primarily on local preference rather than considerations of effects on articular tissues. This study compared the mechanical and cytotoxic effects of different dilutions of Omnipaque 350 (Iohexol), an iodinated contrast agent (CA), on articular cartilage tissue explants.

METHODS: Articular cartilage plugs were isolated from the femoral condyles of immature bovine stifles using an 8mm biopsy punch and trimmed to 2mm thickness, keeping the surface layer intact. Explants were stored at -20°C in Phosphate Buffered Saline (PBS) with protease inhibitors until mechanical testing. On the day of testing, samples were thawed for 30 minutes in a 37°C bath and trimmed to a final diameter of 6mm, then randomly assigned to one of 4 conditions: 100% CA, 50% CA/H2O, 50% CA/PBS, and PBS. Mechanical Testing: Samples (n=3/group) were placed inside a rubber confining ring and tested on an Instron 5940 microtester using a 10N load cell and a custom made indenter with a hemispherical tip (radius 1.2mm). Each sample was pre-loaded to 0.02% in PBS and loaded at a rate of 0.002mm/s to 15% strain followed by an unloading step at the same rate and then a 180s recovery period (~8min per loading cycle). After 3 cycles in PBS, the bath was changed to the assigned solution and the sample was indented for an additional 10 cycles to monitor CA equilibration. The bath was subsequently returned to PBS and the sample was tested for another 10 cycles to monitor recovery. Viability: Tissue viability was assessed using a LIVE/DEAD assay kit on 3 freshly harvested samples per group after exposure to the CA solutions for 1 hour. Analysis: The peak force from each cycle was normalized by the peak force value of the third initial cycle in PBS. A biexponential equation, with a decaying and a growing component, was used to model the normalized peak force data to find the time constants governing the equilibration process. Data were analyzed by one-way ANOVA followed by Bonferroni post-hoc test for pairwise comparisons. Results are presented as mean +/- SEM.

RESULTS: Peak force in the control (PBS) group did not vary (Fig 2A, 2B), indicating that the testing protocol itself did not damage the tissue. Peak force data showed an overall long-term swelling and stiffening trend for all CA groups, while only the 100% CA group showed an initial deswelling (Fig 2A). The peak force data for recovery equilibrium show a deswelling trend for all CA groups (Fig 2B). The first equilibrium time constant $\tau_1$ describing the initial transient was significantly lower for the 100% CA group (1.00 +/-0.45s) compared to the 50% CA/H2O group (5.29 +/-0.95s). This trend was reversed for the recovery equilibrium, with $\tau_1$ significantly greater for the 100% CA group (5.79 +/-0.78s) compared to the 50% CA/H2O group (2.95 +/-0.23s). There were no differences among groups for $\tau_2$, which describes the long-term equilibrium transient (Fig 2C). At cycle 8 of CA equilibration, the normalized peak forces in 100% CA and 50% CA/H2O groups were significantly greater than in PBS controls, and was significantly greater for 50% CA/H2O than for 50% CA/PBS (Fig 2D). At the cycle 8 of recovery, the normalized peak force in 100% CA remained significantly greater than in PBS control, with no other significant differences among groups. LIVE/DEAD staining showed viable cells throughout the tissues, with no qualitative differences between groups (Fig 3).

DISCUSSION: Although all examined CA solutions were hyperosmolar, the long-term trend with CA equilibration was consistent with an osmotic swelling (rather than deswelling) response. As the neutral CA could theoretically reach intramuscular concentrations comparable to the bath, this swelling response is likely due to the reduced (or absent) levels of ions in the CA solutions. Interestingly, the 100% CA group exhibited a minimal deswelling response consistent with the high osmolality of Omnipaque 350 (541 mOsm/L). The competition between the initial effects of high Iohexol concentration and the minimal ion concentration in the 100% CA solution produced the complex transient response seen in this group. The substantial increase in peak force seen after 8 cycles of equilibration was mostly (but not fully) reversed after 8 cycles of recovery, suggesting that there may be lingering effects of clinical contrast agent injection well after the arthrography procedure has been completed. Fortunately, these mechanical effects were not accompanied by notable changes in cell viability. Nevertheless, these results indicate that clinical contrast agent solutions may alter the physical properties of articular cartilage. As these changes predominantly effect the cartilage surface, rapid return to weight-bearing (or CA use in new weight-bearing diagnostic procedures) might place patients at risk of superficial cell or tissue injury. Although the results presented here focus solely on the articular cartilage of the knee, we expect similar outcomes for the articulating surfaces of other joints that routinely undergo CT arthrography. These results suggest that physical effects of intra-articular solutions should be evaluated in the development of clinical practice guidelines for CT arthrography.

SIGNIFICANCE/CLINICAL RELEVANCE: Our results show that exposure to iodinated contrast agents results in an immediate change to the osmotic environment of the cartilage. These results highlight the need for the establishment of robust clinical protocols to prevent damage to the cartilage surface when the tissue is most susceptible to loading.


FIGURES:

Figure 1: Experiment set-up

Figure 2: Normalized peak force data (circles) and biexponential fit (solid line) for (A) contrast agent equilibrium and (B) recovery in PBS. (C) Equilibrium time constants from biexponential fit. (D) Normalized peak force data for cycle 8.

Figure 3: Representative images from LIVE/DEAD staining (A) 100% Iohexol, (B) 50% Iohexol/Water, (C) 50% Iohexol/PBS, and (D) PBS