INTRODUCTION

Intra-articular fractures, such as tibial plateau and pilon fractures, often hasten the progression of joint arthritis, a phenomenon known as post-traumatic arthritis (PTA). Previously hypothesized pathogenic mechanisms for PTA have been broadly categorized as alterations in cartilage cells and matrix, alterations in joint biomechanics, and alterations in systemic factors. We hypothesized that disruption of synovial fluid (SF) as a lubricant is an additional pathogenic mechanism. When a fracture damages the articular surface, the containment of SF is disrupted at the fractured cartilage. Acutely, the joint space is infiltrated by a variety of substances, including blood, fat, and bone marrow, which may affect SF function. Lubricant molecules in normal SF that provide a reduction in friction and wear at the articulating cartilage surface include hyaluronan (HA) and proteoglycan 4 (PRG4).

The objectives of this study were to compare the injury SF from patient joints afflicted with acute tibial plateau or pilon fracture with normal SF in terms of (1) biochemical composition, including HA and PRG4 concentrations, and (2) friction-lowering boundary lubrication function. In addition, the possible biochemical basis for impaired lubrication function was assessed by correlating friction coefficient and lubricant concentration.

METHODS

Lubricant Solutions. Following IRB-approved human subject protocols, SF was obtained from subjects with intra-articular fractures, either tibial plateau (Plat-hSF, n=5, age 48±14 yrs) or pilon (Pilon-hSF, n=5, age 41±14 yrs) fractures, who had granted informed consent. During the initial surgery of a staged treatment regimen in which an external fixator was applied to maintain fracture length and allow the soft tissue to rest, SF was aspirated from the affected joint and also from the contralateral knee joint (NL-hSF, n=8, age 45±15 yrs) which appeared normal on X-ray. These surgeries were performed within 30 days of the injury date. In addition, normal bovine SF (NL-bSF) was obtained pooled from forelimbs of ~10 adult animals. All SF were clarified of cells and debris by centrifugation (3,000–10,000 g, 30–60 min, 4°C), and the resultant samples were stored at ~70°C before subsequent analysis.

Biochemical Analysis of Putative Boundary Lubricants. Portions of Plat-hSF, Pilon-hSF, NL-hSF, and NL-bSF samples were assayed for the concentrations of total protein as well as the putative lubricant molecules HA and PRG4. Total protein was quantified with the BCA assay (Pierce). HA was quantified with an ELISA-like assay. PRG4 was quantified after digestion of HA by S. Hyaluronidase and then Western Blot, using Ab directed against either the human (AbCam) or bovine (3-A-4) molecules, with standards of PRG4 that were purified from conditioned medium of human and bovine cartilage explants.

Friction Test of Boundary Lubrication. Portions of Plat-hSF, Pilon-hSF, NL-hSF, and NL-bSF samples were analyzed for kinetic coefficient of friction as a measure of boundary lubrication function in a cartilage-on-cartilage articulation test. Friction tests were performed as described previously, using pairs of osteoarticular cylinders from normal adult bovine knee joints. Phosphate buffered saline (PBS) was also tested as a negative control lubricant. Pairs of test cylinders were bathed for 16 h at 4°C in a lubricant sample to which protease inhibitors had been added. Then, articular surfaces, bathed in lubricant, were apposed, preconditioned, compressed to 18%, allowed to stress-relax, and then tested for friction in the boundary mode at an effective sliding velocity of 0.3 mm/s. Friction coefficient was calculated from the measured torque and equilibrium axial load.

Statistical Analysis. Data are expressed as mean±SEM. ANOVA was used to assess effects of lubricant solution on parameters with Tukey post hoc tests for comparisons between injury and control groups. Friction coefficient data was log-transformed because of the large variation between groups. The relationship between friction coefficient and individual biochemical constituents were analyzed by linear regression against the log-transformed lubricant concentrations.

RESULTS

The biochemical composition of SF varied with joint injury (p<0.01, Fig. 1). Relative to that in NL-hSF, total protein concentration (Fig. 1A) was higher for Pilon-hSF (+159%, p<0.01), tended to be higher for Plat-hSF (+25%, p=0.58), and exhibited marked variability. In contrast, relative to levels in NL-hSF, the concentrations of HA (Fig. 1B) and PRG4 (Fig. 1C) lubricants were markedly lower in both Plat-hSF and Pilon-hSF (~50% to ~90%, each, p<0.001).

Friction coefficients of SF also varied with joint injury (Fig. 2A). Friction coefficient was similar for NL-hSF and NL-bSF (0.023 vs 0.024, p=1.00), indicating that adult bovine articular cartilage substrate can be lubricated by (human) NL-hSF. The friction coefficient of SF was increased by joint injury, averaging 0.058 for both Plat-hSF and Pilon-hSF, a value +160% higher than that of NL-hSF (each, p<0.001).

Linear regression analysis showed certain relationships between mechanical and biochemical SF properties. Friction coefficient correlated negatively with HA concentration (r^2=0.62, p<0.001, Fig. 2B), with friction coefficient being relatively high when HA concentration was relatively low. The correlation between friction coefficient and PRG4 exhibited a similar trend but did not reach statistical significance (r^2=0.49, p=0.12).

DISCUSSION

The present study identifies marked alterations in the lubricant composition and lubrication function of SF from patients afflicted by two types of intra-articular fracture and in the initial stage of treatment. The time course of altered SF lubricant function remains to be fully established, as does the possible return of SF to a more normal state with fracture and joint healing. If the observed lack of SF lubrication function contributes to the progression of cartilage deterioration during the early post-trauma time period, therapies to restore SF lubricant could alleviate or delay the progression to PTA.

REFERENCES


ACKNOWLEDGMENTS OTA, HHMI, and NIH for funding. UCSD Orthopaedic Surgeons for synovial fluid. Megan Blewis for purified PRG4.