Utility of Urea, a Passive Transport Marker, for Biomarker Studies of Arthritis

Introduction: The majority of studies of synovial fluid biomarkers rely upon joint lavage, particularly for the sampling of normal control joint fluid which is scarce and highly viscous. The goal of these studies was to test the hypothesis that urea measurements provide a means of correcting synovial fluid biomarker concentrations for dilutional effects introduced by both joint lavage and synovial membrane permeability to water and solutes with inflammation. Urea is a small molecule that is neither synthesized nor metabolized by joint tissues. The transport of urea across both normal and pathologic synovium in man is consistent with passive diffusion (1). We measured synovial fluid concentrations of lactate and glucose, two metabolites considered to be markers of synovial inflammation in both rheumatoid arthritis and osteoarthritis (2). The utility of urea as a normalization factor for these metabolites was evaluated in normal and inflamed canine joint fluids.

Materials and Methods: Serum and synovial fluid were obtained from 32 adult mongrel dogs (52 total joints), which were available from other unrelated studies. Procedures were approved by the Institutional Animal Care and Use Committee. We collected fasting blood for sera, and synovial fluid by needle aspiration, within 10 minutes after venipuncture. Synovial fluid was obtained, directly without lavage ("neat") from left elbows (n=19) and knees (n=22), and by lavage from the right elbows or knees (n=7). Lavage was carried out by intra-articular injection of 3 ml of normal saline, the needle was withdrawn, and the joint was manipulated through a full range of motion twelve times followed by aspiration of all obtainable fluid through a lateral patellar approach. To evaluate whether joint inflammation alters synovial fluid urea, 4 additional dogs underwent intra-articular injection with 0.5 ml of 4 mg/ml chymopapain in buffer (100 mM sodium phosphate, 50 mM EDTA, and 10 mM cystein hydrochloride) under anesthesia (Domitor and Antisedan).

Fasting serum and neat synovial fluid from the inflamed knees were obtained 24 hours later. Concentrations of urea, glucose and lactate were determined using a microdialysis analyser (CMA600). Data were analyzed by ANOVA and Use Committee. We collected fasting blood for sera, and synovial fluid by needle aspiration, within 10 minutes after venipuncture. Synovial fluid was obtained, directly without lavage ("neat") from left elbows (n=19) and knees (n=22), and by lavage from the right elbows or knees (n=7). Lavage was carried out by intra-articular injection of 3 ml of normal saline, the needle was withdrawn, and the joint was manipulated through a full range of motion twelve times followed by aspiration of all obtainable fluid through a lateral patellar approach. To evaluate whether joint inflammation alters synovial fluid urea, 4 additional dogs underwent intra-articular injection with 0.5 ml of 4 mg/ml chymopapain in buffer (100 mM sodium phosphate, 50 mM EDTA, and 10 mM cystein hydrochloride) under anesthesia (Domitor and Antisedan).

Fasting serum and neat synovial fluid from the inflamed knees were obtained 24 hours later. Concentrations of urea, glucose and lactate were determined using a microdialysis analyser (CMA600). Data were analyzed by ANOVA using StatView 4.5 with a p value < 0.05 considered significant.

Results: The mean synovial fluid/serum ratio of urea concentrations (urea ratio) from normal elbow joints aspirated neat was 1.054 +/- 0.035 SE. The urea ratio obtained from normal knee joints aspirated neat was 1.073 +/- 0.021 SE, a value statistically equivalent to the ratio from elbow joints. The mean urea ratio from these 41 combined normal joints was 1.06. This value is consistent with this small molecule partitioning evenly between the blood and synovial fluid; being neither synthesized nor degraded in normal joint tissues. Urea concentrations in the synovial fluid obtained by lavage were determined for each of 7 animals and the dilutional factor (DF) introduced by a 3 ml lavage was calculated using the derived constant 1.06 urea ratio as follows: DF = 1.06 x (serum urea/lavaged joint fluid sample). Correcting for the dilutional effect of lavage should yield final values with no significant difference from those drawn neat from companion joints. As expected, significantly lower levels of both lactate and glucose were found in lavaged synovial fluid compared to neat fluid (p<0.0001 and p=0.036, respectively) (Fig 1). Glucose and lactate levels corrected for dilutional effects of lavage using the urea ratio showed no statistically significant difference from those levels measured in samples drawn neat (p=0.7 and p=0.9, respectively). Finally, the urea ratio was explored in the context of joint inflammation. Intra-articular chymopapain injection produced knee effusions in all 4 animals within 24 hours. Interestingly, the urea ratio was significantly lower in these inflamed joints compared to normal non-effused joints (urea ratio of 0.87 +/- 0.07 SE compared to 1.06 +/- 0.04 SE, p=0.01). Furthermore, compared to normal joints, lactate concentrations were higher and glucose concentrations lower in the inflamed joints. We assume that urea is not metabolized by joint tissues and that the lower urea ratio in inflamed joints reflects a relative dilutional effect due to alterations in water or urea transport under conditions of inflammation. Thus, the urea ratio can be used in this circumstance to normalize for this effect. After normalization, only lactate remained significantly different compared to mean levels in normal joints.

Conclusion: Urea has been shown to be an excellent marker of passive synovial transport (1). We have established the existence of a fixed relationship between concentrations of synovial fluid urea and serum urea in normal elbow and knee joints. We demonstrate the utility of applying the urea ratio to correct for dilutional effects of lavage on concentrations of two small molecular markers of joint inflammation, lactate and glucose. The decreased urea ratio in inflamed joints could arise from an increased water content, a decreased urea content, or both, within the synovial space. An increased water fraction of synovial fluid could arise from synovial fluid hyperosmolarity produced by highly negatively charged glycosaminoglycans released from cartilage due to chymopapain. A decreased urea content of joint fluid could also be caused by altered permeability of inflamed synovium as a result of microvascular changes in the membrane. Under these circumstances, normalization of biomarker levels to urea would provide an indication of the change in a marker level after accounting for fluid fluxes and microvascular changes of synovium. When the urea ratio was used to normalize glucose and lactate concentrations for inflamed joints, only lactate remained significantly different from normal. In the chymopapain model presented here, it appears that glucose is not a reliable marker of inflammation and that these changes can be attributed to changes in fluid flow or tissue permeability alone. In contrast, lactate is increased in inflamed joints, even after accounting for changes in synovial fluid fluxes. Lactate likely increases as a result of tissue hypoxia associated with inflammation. Thus, urea is a reliable, and effective small molecule that can be used to ascertain the true values of various solutes and disease biomarkers in the face of joint lavage and changes in synovial permeability.


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