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Factors affecting paramagnetic contrast enhancement in synovial fluid: effects of electrolytes, protein concentrations, and temperature on water proton relaxivities from Mn ions and Gd chelated contrast agents☆

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SUMMARY

Introduction: Protein and electrolyte concentration of synovial fluid (SF) varies with the type of underlying arthritis. These characteristics can be utilized by magnetic resonance technology to provide a potentially significant diagnostic modality through quantitative assessments of inherent water relaxation rates and their response to contrast agents.
Methods: We evaluated the effect of a classic "*in vitro*" contrast agent, the Mn ion, and a common "*in vivo*" gadolinium based contrast agent, gadopentetate dimeglumine, on the water relaxation times of solutions with biochemical compositions simulating different types of arthritis along with similar studies of SF obtained from patients.
Results: The results demonstrate how protein and electrolyte concentrations play a significant role in the response of water relaxation to the Mn ion but much less so to chelated gadolinium contrast agents used clinically.
Discussion: A major challenge remains to develop paramagnetic agents with less toxicity than the Mn ion but with similar properties that can then serve as a tool to determine protein concentrations through imaging and thereby assist in the diagnosis of inflammatory arthrides and evaluation of therapeutic regimens.

Introduction

Normal synovial fluid (SF) is an ultrafiltrate of plasma. Plasma proteins are delivered to the articular tissues by the microcirculation and removed by lymphatic drainage. Factors affecting the SF protein concentration include not only the molecular radius of each protein but also the abundance of synovial microvessels and transsynovial kinetics. Net intraarticular synthesis or catabolism of proteins also plays a role[1]. The varying types of arthritis' differentially affect these factors, such that by biochemical analysis, SF from patients with rheumatoid arthritis (RA) has a greater protein concentration than that from normal subjects and patients with Osteoarthritis (OA)[2]. Similarly, electrolyte compositions and concentrations in SF also vary with disease state. These factors influence the water longitudinal and transverse relaxation rates, R1 and R2 respectively, associated with SF signal intensities in magnetic resonance imaging (MRI) and also play an important role in how these relaxation times respond to MRI contrast agents. In this work, the effect of a classic "*in vitro*" contrast agent, the Mn ion, and a common "*in vivo*" gadolinium (Gd) based contrast agent (Magnevist) on the water relaxations times of solutions with biochemical compositions mimicking those in various types of SF are examined along with similar studies of SFs extracted from subjects. There have been previous contrast agent relaxation studies in which the biochemical environment of specific tissues is simulated including studies designed to examine the effects of Gd-DTPA2− on articular cartilage relaxation times[3] and the more general case of macromolecular content on the efficacy of water

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relaxation from Gd contrast agents[4]. To our knowledge however, effects of the native biochemical milieu within SF on the efficacy of clinical and research type contrast agents to alter relaxations times has not been examined.

Materials and methods

Four separate experiments were performed in order to characterize how protein concentration and composition, temperature and electrolyte composition within SF type solutions and actual SF samples influences the efficacy of the Mn ion or a common Gd contrast agent in altering water proton relaxation rates. The design of each experiment is described below followed by a description of the MR acquisitions and data analyses common to all four experiments.

Experiment 1

Effects of protein concentration on R1 and R2 relaxation rates in the presence of the manganese ion were examined in the absence and presence of normal SF electrolytes. Protein solutions consisting of dissolved bovine serum albumen (BSA, Sigma–Aldrich, St Louis, MO) from 0.063 to 0.632 mM were prepared in stock solutions of distilled water with added concentrations of either 0.3 or 0.5 mM $MnCl_2$. Identical solutions but with the added electrolytes sodium, potassium, chloride, bicarbonate, lactate and calcium electrolytes at concentrations mimicking normal SF, as shown in the second column of Table I, were also prepared.

Experiment 2

Effects of temperature on R1 and R2 relaxation rates and their sensitivity to the manganese ion in solutions mimicking normal (N) and RA SFs were examined. Solutions with concentrations of 0.316 mM and 0.630 mM BSA were used to represent the N and RA SFs, respectively, and titrated with $MnCl_2$ from concentrations of 0.0 to 0.5 mM. R1 and R2 measurements on these solutions were performed at room temperature ($\sim$ 22°C) and at approximately 37°C by heating the solutions in a 37°C water bath and scanning them within a Styrofoam container to retain heat during the imaging experiments. Temperature drops during scanning were estimated to be less than 2°C.

Experiment 3

Effects of both Mn ions and the common gadolinium (Gd) contrast agent (Magnevist@, active ingredient gadopentatate dimeglumine, Bayer HealthCare Pharmaceuticals, Inc. Wayne, NJ) on R1 and R2 relaxation rates in simulated synovial solutions mimicking normal (N) and RA chemical compositions were examined. For this purpose, solutions were prepared according to the compositions provided in Table I and included not only dissolved BSA but also α and β globulins Cohn Fraction IV-4, human, and γ globulins Cohn Fraction II, III, human, in addition to hyaluronic acid sodium salt, sodium chloride, potassium chloride, sodium bicarbonate, lactic acid sodium salt and calcium chloride dehydrate. These solutions were prepared in two sets. To one set, $MnCl_2$ was added at increasing doses to achieve a final dilution from 0.0 to 0.5 mM. To the second set, the Gd contrast agent was added in gradually increasing amounts to achieve final dilutions from 0.0 to 3.0 mM Gd. Control solutions with only hyaluronic acid and electrolytes at normal and RA levels were also prepared and titrated similarly with $MnCl_2$ and Gd. The normal and RA control solutions, without the proteins, are referred to as NHE and RAHE, respectively, and normal and RA solutions with the proteins are labelled NPHE and RAPHE, respectively.

Table I
Simulated SF constituents[2]

SF constituents	Simulated normal SF concentration	Simulated RA SF concentration
Hyaluronic acid	0.32 gm/dl	0.115 gm/dl
BSA	1.176 gm/dl	1.76 gm/dl
α and β globulins	0.55 gm/dl	1.39 gm/dl
γ globulins	0.38 gm/dl	1.05 gm/dl
Na^{+1}	133.79 mmol/l	132.26 mmol/l
K^{+1}	3.5 mmol/l	3.5 mmol/l
Cl^{-1}	106 mmol/l	106 mmol/l
HCO_3^{-1}	23 mmol/l	23 mmol/l
Lactate	1.6 mmol/l	5.7 mmol/l
Ca^{+2}	2.1 mmol/l	2.1 mmol/l

Experiment 4

SF was obtained from four patients who had been previously diagnosed with RA, Pigmented Villonodular Synovitis (PVNS), Psoriatic Arthritis (PSORI) and OA. These fluid specimens had been previously obtained after appropriate informed consent was obtained and were stored at −20°C prior to evaluation. Relaxation rates as functions of $MnCl_2$ and Gd-DTPA concentration were measured in all four samples except for the OA specimen sample for which only $MnCl_2$ measurements were made due to the limited quantity of fluid available for that study. The absolute protein concentrations of the four SFs were provided in gm/dl by the clinical laboratory at the Brigham and Women's hospital using standard assay methods.

Common to all four experiments were the relaxation rate measurements of the various solutions. For these measurements, 1 ml samples of specifically prepared fluids in polypropylene test tubes were imaged using a 1.5 T imager (Signa; GE Medical Systems, Milwaukee, WI) with a quadrature head coil. A single

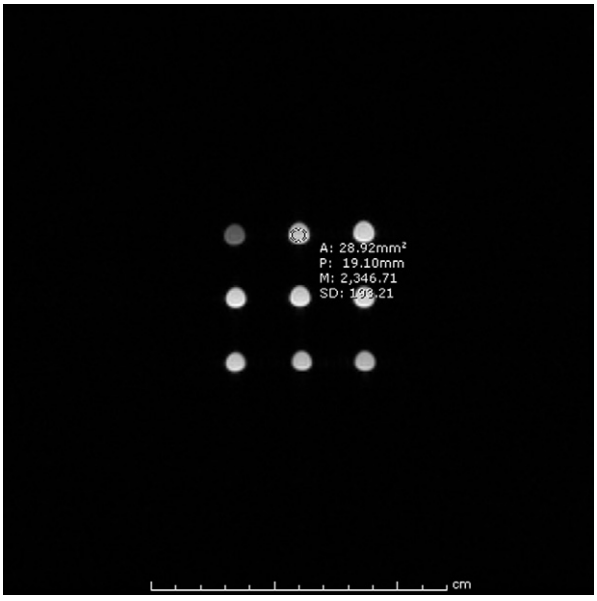

Fig. 1. Typical image (TR/TE = 1200/ms, three echo FSE sequence) of nine vials doped with varying levels of Gd contrast agent (0–4 mm Gd) from which signal intensity measurements are made from individual ROI's like that shown for the central vial, top row, where A = area of ROI, P = position of the coronal slice from isocenter, M = mean and SD = standard deviation of the signal intensity from the pixels within the ROI. Signal intensities extracted from the vials as a function of TR and TE are used to calculate the T1 and T2 values for each sample, as described in the text.