Assessment of Chemotherapeutic Effects Using Intracellular Sodium Weighted MRI

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Introduction
The significance of intracellular sodium in cellular metabolism in tumors as a measure of cytotoxicity is potentially important as a clinical indicator of response to cancer therapy. Assessing the signal from intracellular sodium using magnetic resonance imaging (MRI) and/or spectroscopy has been hindered by the large extracellular sodium signal detected with conventional single quantum technique. Other approaches such as use of chemical shift reagents or multiple quantum filtering to detect intracellular sodium also suffer serious shortcomings [1-3]. Malignant tumors have been generally shown to have elevated intracellular sodium [Na], and the chemotherapeutic treatment itself kills tumor cells, which elevates [Na], even further [4-6]. We have recently developed an intracellular sodium imaging technique that is based on the inversion recovery sequence [7]. In this study we apply this technique to monitor the chemotherapeutic effects in nude transgenic mice propagated with human cancer tumor.

Method
Intracellular and extracellular sodium NMR signals are here differentiated according to their longitudinal relaxation time T1. Intracellular sodium weighted imaging is achieved in a relatively simple and robust way through the inversion recovery nulling (IR) of the extracellular sodium [7]. For sodium imaging, we employed a 3D gradient echo based IR sequence. TE was 2.5 ms, TR 100 ms, FOV 50 mm, slice thickness 3 mm, and acquisition matrix 64x64x12. An inversion time of 25 ms was chosen and used, which has been shown to null the extracellular sodium signal effectively [7]. Imaging was performed on a high field 4.2 T whole body system with a small birdcage quadrature coil (50 mm ID) and a high strength Bruker head gradient insert (3 G/cm). Human prostate tumor line PC3 was propagated in nude transgenic mice (30 gram) through cell injection. Mice were anesthetized with xylazine/ketazine cocktail and positioned on a customized mouse holder during data acquisition so that their position could be reproduced during different imaging sessions. Fig. 1 shows the proton image, and sodium image with and without IR nailing at the same slice location where tumor and kidneys are visible. Note that two reference tube phantoms at bottom left and right were permanently mounted on the mouse holder for signal normalization purpose. They were made of 200 mM NaCl solution and Ficoll (30 and 40%, respectively). Mice were treated with 0.2 mL of 2 mg/mL VP-16, a known antineoplastic drug, through injection via femoral veins. For control mice, 0.2 mL of saline solution (0.9% NaCl) was injected instead of VP-16.

Results
Twelve mice were used in this study, ten treated with VP-16, two as controls. Mice were imaged immediately prior to the injection or baseline, 24 hrs and 48 hrs after. Tumors were subsequently excised and pathology verified. Their sizes were between 0.3 mL to 0.9 mL. The acquisition times with and without IR nulling were 24 min and 6 min, respectively. Fig 2 shows the general increase of IR (intracellular sodium) signal after the chemotherapy (filled circle - VP-16 treated; unfilled circle - controls). On average, it increases by 40% 24 hrs after chemotherapy and this increase drops to 28% after 48 hours. IR signal intensity was measured from the value of the highest peak of the pixel intensity histogram in the tumor area. It was then normalized to the smaller reference tube phantom on the bottom right, and further normalized to the intensity at baseline or prior to injection. No apparent signal changes were seen in tumor in the images acquired with no IR nalling; no changes in non-tumor areas including kidneys in IR nulled images. We confirmed this general trend of intracellular sodium elevation after chemotherapy by cultured PC3 cell studies using Na+ sensitive dye fluorescent measurements. These dye studies showed that intracellular sodium levels increased as early as 2 hours after chemotherapy, continued to increase for about 24 hours by approximately 13 mM, and then subsequently declined. We also applied this intracellular sodium imaging to examine the chemotherapeutic effects of another known antineoplas, Taxotere, as well as in another prostate tumor line DU145. Similar changes were found.

Discussion
Changes in [Na] have been described in a variety of biological systems during normal and pathophysiological events relative to chemotherapy including movement through the cell cycles, apoptosis, necrosis, metabolic suppression, and transformation from normal to neoplastic tissue [4-6]. These [Na] changes occurs within minutes to hours in response to alterations in transmembrane flux or subcellular sequestration. We have demonstrated that intracellular sodium weighted MRI can be used for rapid monitoring of chemotherapeutic efficacy in real time may significantly contribute to patient management by providing important information regarding the activity of a drug in a patient. However, important questions remain to be answered for quantitative assessment. Study is presently under way to refine the quantitation technique and examine how extracellular and intracellular sodium levels are related to the tumor type and the stage of tumor.

References