Solid-State 1H and 31P MRI Detects Changes in Bone Mineralization and Water Content in OVX Rat Bone in Response to Treatment with Alendronate

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Introduction

We hypothesize that hormone loss following menopause results in decreased degree of mineralization of bone (*DMB*) and increased water content. Since compressive strength of bone is largely attributed to the bone's mineral content, *DMB* is a potential contributor to fracture risk. The antiresorptive agent alendronate (ALN) is known to reduce bone resorption rate in a dose-dependent manner resulting in an increase in *DMB* (1) to a level similar to that in healthy premenopausal women. However, presently there is no non-invasive, non-destructive modality to measure *DMB* and thus monitor the changes in *DMB* during treatment. Here, we investigate the potential of 3D solid-state magnetic resonance imaging (*SSI*) of ^{31}P (*^{31}P MRI)* and ^{1}H (*^{1}H MRI)* of cortical bone in a rat model of osteoporosis to monitor the effect of ALN treatment. Toward this goal, we obtained 3D *SSI radial* images of ^{31}P and ^{1}H at 9.4 T (162 MHz and 400 MHz for ^{31}P and ^{1}H respectively) in the excised rat femur. For validation, other measures of *DMB* were also quantified, including µ-CT density, phosphorus content by ^{31}P solution NMR (*^{31}P NMR)* and ash weight by gravimetry.

Materials and methods

The extremely short T_2 (~100 *µs* and ~200 *µs* for ^{31}P and ^{1}H respectively), precludes signal detection by conventional spin-warp imaging. For this work, a 3D radial projection reconstruction sequence was designed with acquisition followed immediately after excitation, similar to (2, 3). In order to capture the center of *k*-space before a significant portion of the signal has decayed, ramp sampling was incorporated. The FID was sampled from 2,626 gradient directions on a series of equally spaced parallel rings in 65 azimuthal-angle increments so as to be uniformly distributed on a sphere (3). The long T_1 of ^{31}P (~90 s) necessitates an initial preparatory pulse in order to bring the spin system close to steady state (4). The FOV was 17 (mm) ^{3} and 15 (mm) ^{3}, respectively, for ^{31}P and ^{1}H. Images were reconstructed by standard 3D FFT following regridding (5). Scan times were 88 min and 44 min for ^{31}P and ^{1}H *respectively*. The pulse sequence was implemented on a 9.4*T* vertical-bore superconducting NMR system equipped with standard 100G/cm gradients (DMX-400, Bruker Instruments, USA), allowing for a ramping time of 100 *µs* in conjunction with a saddle-type rf coil for ^{1}H and a custom-made solenoidal coil for ^{31}P (2.7 *cm* long x 1.2 *cm* diameter). Reference capillaries containing known concentrations of K_2HPO_4 and GDTPA were co-imaged in order to quantify bone phosphorus and water respectively as wet weight %. Representative ^{1}H and ^{31}P images for one of the specimens are given in Figure 1.

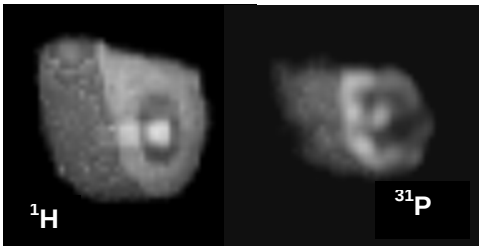

Figure 1. Translucent volume rendered images of ^{31}P and ^{1}H obtained from solid state imaging after off line reconstruction.

Quantitative µ-CT was performed jointly with K_2HPO_4 solutions of varying concentrations for calibration (MS-8, GE Medical Systems, formerly EVS Corp., London, Ontario, Canada). Seven hundred twenty-one 2D views were collected at 0.5^0 increments, corresponding to one full rotation of the specimen, and the data reconstructed with the manufacturer's cone-beam reconstruction program. Scan time was 2 hrs yielding a final voxel size of 32x32x64 μm^3. Water content was also obtained after drying the specimens at 100 ^{0}C for 48 hrs and ash content was obtained by heating the same specimens at 600 ^{0}C for 24 hrs. Finally, the ashed specimens were dissolved in 2ml of 1.2 mM HCl and diluted 4 times to obtain high-resolution NMR spectra in conjunction with a reference capillary containing a known concentration of methylene diphosphonate.

Thirty-six female four-month old rats were randomly divided into four groups. Twenty-seven were ovariectomized to induce osteoporosis and divided into untreated ovariectomized (*OVX*), OVX treated with 5µg/kg/day ALN (*ALN*) and OVX treated with 25µg/kg/day ALN (*ALN1*). Finally, the remaining nine animals were sham operated to serve as baseline control group (*NO*). All animals were fed standard chow and water *ad libitum* for fifty days after which they were euthanized. Cortical bone specimens of ~1 *cm* length were harvested from the right femur for *SSI* and specimens of 2 cm length from the left femur for µ-CT. Plate-like specimens of 10 mm x 4 mm x cortical thickness from the right femur were cut for gravimetry and solution-state ^{31}P NMR.

Results and Conclusion

Phosphorus content is significantly increased in the high-dose (*ALN1*) group relative to the *OVX* group for both *SSI* ^{31}P MRI and ^{31}P *NMR* (Figure 2a, both p<0.0001). A similar trend is seen for the low-dose (*ALN*) group but the associations are not significant. Similarly, µ-CT *DMB* and ash content are increased upon treatment (p<0.05). Increased mineral content is paralleled by a decrease in water quantified by ^{1}H *MRI* in line with the water content measured by gravimetry (both p<0.0005, Figure 2b). The data indicate that *in situ* MRI of intact cortical bone allows detection of subtle treatment effects on mineralization and water content. Further our results shed new light on the effects of antiresorptive treatment on bone quality. Quantitative solid-state MRI methods may be applicable *in vivo* in laboratory animals and possibly in humans.

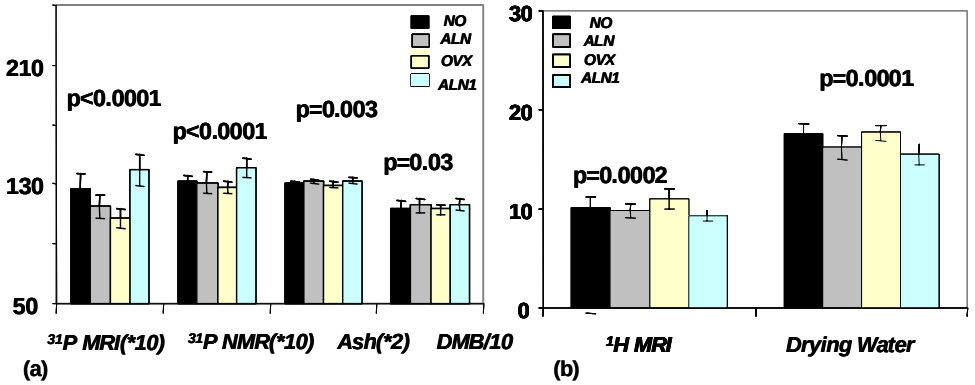

Figure 2a. Group differences showing significantly increased measures of mineralization by *SS* ^{31}P *MRI* (wt. %), ^{31}P *NMR* (wt.%), ash (wt.%) and *DMB* (mg/cm^3) in *ALN1* compared to *OVX* indicating recovery of mineralization upon treatment; 2b) Group differences showing decreased bone water by *SS* ^{1}H *MRI* (wt.%) and drying in *ALN1* relative to *OVX*. P-values refer to comparison between *OVX* and *ALN1*; some of the other comparisons are significant as well.

References: 1) Rodan G, *Bone* 1997; **20**:1– 4. 2) Wu Y, et al. *Calcif Tissue Int* 1998; **62**:512–518. 3) Glover G H, et al. *JMRI* 1992; **2**; 47-52. 4) Anumula S, et al. *Magn Reson Med* 2006; **56**:946–952. 5) Greengard L, et al. *Siam Review* 2004; 46:443–54.

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