Measurement of water apparent diffusion coefficient in rabbit cortical bone with pulsed gradient NMR

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Introduction

The rate-limiting step for the transport of nutrients to and removal of waste products from osteocytes in cortical bone is diffusion[1]. Bulk water – approximately 10% by volume – fills the structure of interconnected pores (the lacunocanalicular system) of the bone matrix, which forms the network allowing communication between Haversian canals and the osteocytes[2]. Diffusion therefore is highly restricted. Quantitative bone water diffusion data, which would help elucidate fluid transport mechanisms, is scarce. Fernandez *et al* reported the apparent diffusion coefficient (ADC) of osteoid water using a deuterium-proton NMR exchange technique[3]. This approach, however, requires immersion of the bone in D_2O and measurement of the kinetics of H_2O as it migrates into the surrounding D_2O bath. Direct (*in situ*) measurement of the ADC by pulsed gradient spin-echo techniques is complicated by the extremely short transverse relaxation time of bone water (~250 μs)[3]. Here we report the osteoid water ADC in rabbit cortical bone as measured by a pulsed gradient stimulated echo spectroscopy sequence at 400 MHz using a home-built gradient/RF coil set capable of generating amplitudes up to 50 T/m and gradient pulse widths of 140 μs at peak amplitude.

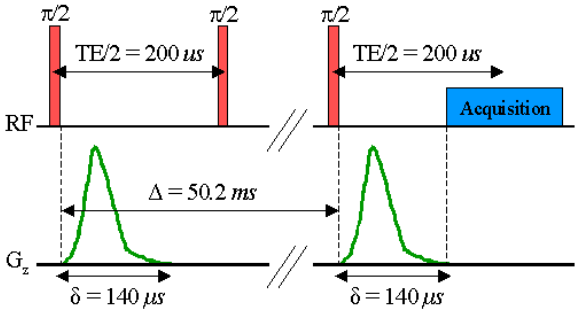

Fig. 1 Pulsed gradient stimulated echo sequence. The G_z gradients shapes are the actually waveforms observed from the amplifier. Experimental parameters: 64 pts, RF pulse width = 15 μs, TR = 1 *s*, TE = 400 μs, NEX = 64, SW = 200 kHz, scan time = 67 *s*.

Materials and Methods

The short T_2 (250 μs) but relatively long T_1 (500 *ms*) of bone water naturally suggest the use of a stimulated echo pulse sequence (Fig. 1). The short T_2 along with highly restricted diffusion put stringent demands on the gradient system in terms of gradient pulse duration and peak amplitudes. The experiments were performed with a custom-built 50 T/m z-gradient/solenoidal RF coil set interfaced to a 9.4 T commercial spectrometer and micro-imaging system (Bruker DMX 400 with Micro2.5 gradients and BAFPA40 amplifiers)[4]. A gradient duration of δ=140 μs (including rise and fall times) was achieved by control parameters available within the Bruker scan control software. The non-analytic shape of the gradient waveform required the *b-value* to be computed numerically (using $b = \gamma^2 \int_0^{TE} (\int_0^t \mathbf{G}(t')dt')^2 dt$) based on the actual amplifier output monitored on an oscilloscope (shown in Fig. 1) and captured via Labview™ software.

To test the technique the ADC of water was determined with a spin echo sequence (Δ = 4.75 *ms*, δ = *140* μs) using gradient pulse amplitudes of 0-47 T/m, and numerically calculating the *b-values*. The ADC of water calculated from the echo attenuation plot agreed with literature values and with the ADC measured by a spin echo sequence (Δ = 28 *ms*, δ = 1 *ms*) employing the standard Bruker x-gradient (data not shown). Three cortical bone specimens (*approx.* 1×2×12 mm^3) were harvested from the right tibial shaft of three healthy 6-month-old skeletally mature New Zealand White rabbits. The long axis of the specimen, corresponding to the longitudinal direction of the tibia, was placed perpendicular to the gradient axis. The bone water ADC was measured with the stimulated echo sequence shown in Fig. 1 with z-gradients ranging from 27 to 47 T/m in six steps. The signal was normalized to that at 27 T/m, and the temperature was 19 °C.

Results and Discussion

Figure 2 shows a logarithmic plot of the attenuated echo from each bone specimen versus *b-value*. The echo signal was corrected for background contamination, as discussed below. The ADCs of the bone specimens along with the R^2 values of the linear fit are shown in Table 1. The plots show high linearity and the average ADC ($(3.59\pm0.36)\times10^{-7} cm^2/s$) is in excellent agreement with the reported value obtained by proton-deuteron exchange NMR ($(3.56\pm0.78)\times10^{-7}$ cm^2/s)[3]. As osteocytes, on the average, are located within 150 μm from the Haverisan canals, this ADC supports the reported mean transport time of small molecules through the lacunocanalicular system to be on the order of minutes[1,3].

A background signal from the epoxy resin (T_2 ~200 μs) surrounding the gradient/RF coil set, which was used to provide mechanical stability, contaminated the desired bone signal and had to be removed. An FID from the background after the third π/2 RF pulse masks the echo from bone water, but is dephased by the application of the second gradient pulse. A gradient amplitude of 27 T/m or higher was found to be necessary in order to observe a well-defined echo at TE. However, this signal still contains an echo arising from the background. Using a D_2O sample, echo intensities of this background signal were measured at the same gradient strengths and experimental parameters as for the bone water ADC measurements. The background echo amplitude was then subtracted from the echo amplitude measured in bone to provide a first-order correction.

Fig. 2 Normalized background-corrected echo attenuation plots for all three bone specimens. The average ADC is $(3.59\pm0.36)\times10^{-7} cm^2/s$.

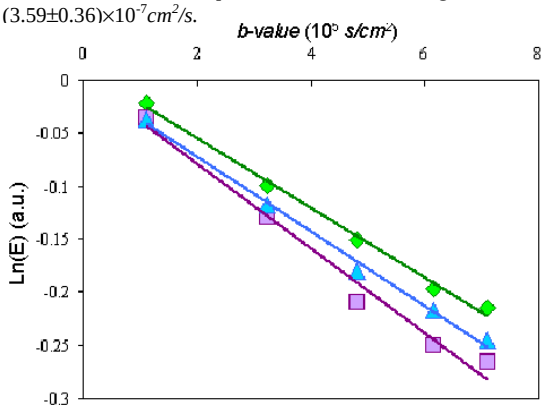

Table 1. ADC values for bone specimens

Specimen	ADC ($10^{-7} cm^2/s$)	R^2
1	3.28	0.994
2	3.98	0.981
3	3.15	0.995

Conclusion

The ADC of bone water measured is in excellent agreement with previously reported values obtained by proton-deuteron exchange NMR and further supports transport times of small molecules to and from osteocytes via diffusion to be on the order of minutes. This work shows the feasibility of measuring slow molecular diffusion involving spins of submillisecond transverse relaxation by means of a pulsed gradient stimulated echo sequence in conjunction with high-amplitude gradients allowing for extremely short gradient duration.

References: **1.** Tate, M.L., *et al*, *Bone* **22**:107-112 (1998). **2.** Martin ,R.B., *et al*, *Structure, Function, and Adaptation of Compact Bone*, Raven Press: New York (1988). **3.** Fernandez-Seara, M.A., *et al*, *Biophys. J.* **82**:522-9 (2002). **4.** Wright, A.C., *et al*, ISMRM 12th Scientific Meeting, Kyoto, Japan, 2004, p. 741.