Absolute Quantitation of Deoxymyoglobin Concentration in Human Hand FDI Muscle In Vivo Using NMR Signal Injection Method

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Synopsis

Absolute quantitation using a synthetic signal injection method was performed to determine the dynamically changing deoxymyoglobin concentrations for human hand FDI (first dorsal interosseous) muscle in-vivo. Prior to the in vivo signal acquisitions, synthetic signals were designed and calibrated on actual ^{1}H NMR signals from phantom solutions with known deoxymyoglobin concentrations. The time course change of deoxymyoglobin concentrations for the human hand FDI muscle was then determined by the concentration references generated by the synthetic signals. Deoxymyoglobin concentration was reached to its maximum value (about 80 μM) about 6 minutes after inducing an ischemic condition to the muscle.

Introduction

Absolute quantitation for in vivo studies using NMR has been difficult to achieve due to some intrinsic problems. One of the problems is that many diagnostic methods using external concentration references do not guarantee the identical loading condition for both a calibrated signal and an actual in vivo signal. Also, the proper selection of a chemical for a calibration phantom is often difficult because the resonance frequency of a calibration signal for the phantom should be adequately shifted from the main signal for the quantification within a sweep width of a spectrum.

In the present study the dynamic absolute quantitation was attempted with a time resolution of about 50 seconds to determine the change of deoxymyoglobin concentrations in human hand FDI muscle in vivo. Instead of employing any extra custom built hardware as proposed by Akoka et al (1,2), a second waveform generator built in a MR spectrometer was utilized to generate an exponentially decaying signal that was afterwards mixed with an RF signal produced from a decoupler channel. The synthetic signals obtained from phantom solutions were calibrated against solutions of known concentrations and were designed to match the line-width of the solution signals. The sample loading condition for the calibration acquisitions was matched with that for the in vivo signal acquisitions by controlling the modified ionic strength in calibration solutions. The modified ionic strength was developed by taking into account the mobility of ions in addition to their ionic strength.

Methods

^{1}H NMR spectra were obtained using a surface coil of 2 cm diameter on a 4.7 T magnet. A synthetic FID was generated after calculating a required line shape based on characteristics such as area under the peak, line-width, phase and the corresponding frequency offset of the actual signal. All calculations were performed and stored before executing the pulse sequence of the absolute quantitation. Based on the calculations, a synthetic FID was formalized by using a wave form generator along with a second RF channel.

Four phantom solutions with different deoxymyoglobin concentrations (105, 157, 370 and 483 μM) were prepared after matching the coil loading conditions with that for an in vivo subject. Each deoxymyoglobin phantom was prepared by dissolving horse myoglobin crystals in 50 mM sodium phosphate buffer (a mixture of Na_2HPO_4 and NaH_2PO_4 with pH=7.0), reducing the solution with sodium dithionite ($Na_2O_4S_2$) and adding sodium chloride (NaCl) to adjust the ionic strength to match in vivo loading conditions.

Results and conclusions

Figure 1 displays ^{1}H NMR spectra for the highest in vitro concentration (483 μM) of deoxymyoglobin serially obtained using a second RF power at 20 dB with its linear power level varying from 2000 to 4000 with a step-size of 200 (from the bottom to the top spectra). As linear power level increases, its corresponding synthetic signal area (appearing at about 6 ppm) increases, while the same signal area is consistently shown for the actual deoxymyoglobin signals (appearing at about -4ppm) throughout all spectra. ^{1}H NMR spectra for the other phantoms (370, 157, and 105 μM) were acquired in a similar manner. For each phantom the linear power corresponding to the intersection of the regressions, computed as area under the peak as a function of varying RF power levels, for the synthetic and real deoxymyoglobin peaks was used to calculate a new linear power level "normalized" to 20 dB. Deoxymyoglobin concentrations were then plotted as a function of the normalized linear power levels at 20 dB, yielding a regression equation, which was then used to predict the in vivo deoxymyoglobin concentration (Fig.2).

Based on the concentration references obtained from the calibration data shown in Fig.2, the time course change of deoxymyoglobin concentrations was obtained for human FDI muscle in vivo (Fig.3). Three stages of the experimental protocol for the serial ^{1}H NMR spectra are resting period, ischemic stage and recovery period as shown in Fig.3. The ischemia was induced by rapid inflation of a pressure cuff applied to the upper arm at 55 mmHg above systolic blood pressure. The response was characterized by an exponentially increasing deoxymyoglobin concentration during ischemia followed by a rapid drop in signal upon release of the cuff. In agreement with previous reports, the maximum concentration of about 80 μM was reached about 6 minutes into the ischemic period.

References

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2. Akoka S, Barantin L, Trierweiler M. Concentration measurement by proton NMR using the ERETIC method. Anal Chem 1999;71:2554-2557.

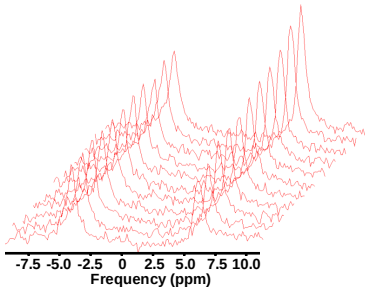

Figure 1.^{1}H NMR spectra for the d-Mb (deoxymyoglobin) concentration of 483μM.

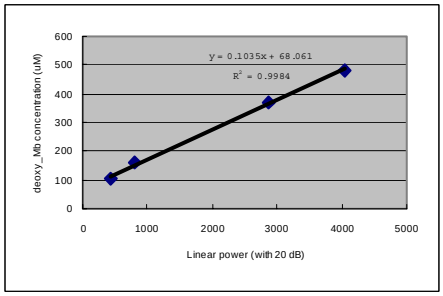

Figure 2. Linear relaxtionship between d-Mb concentrations and their corresponding power levels.

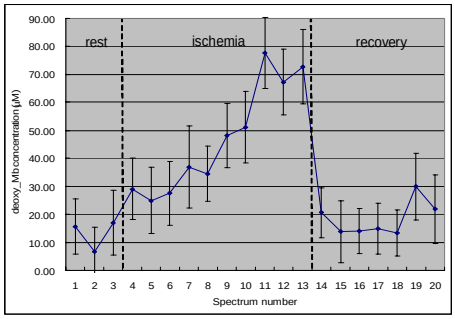

Figure 3. Dynamic changes of d-Mb concentrations for a human FDI hand muscle in vivo.