Rapid Transmembrane Exchange of Hyperpolarized ^{13}C-Urea: Pathology-Methods Development Using Erythrocytes

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Introduction: Human erythrocytes (red blood cells; RBCs) have high plasma-membrane permeably to urea; the exchange rate between the intra- and extracellular compartments is comparable to that of water (1). This property is surmised to be to avoid membrane rupture when RBCs pass through the medulla of the kidney where there exists very high urea concentrations. Whether this situation pertains to other cell types including neoplastic cells has not been fully explored; and if a membrane-flux-measuring method for urea could be developed it might serve as a test of cell pathology, *in vivo*. The ^{13}C NMR spectrum of urea shows partial resolution of the resonances from the molecules inside and outside RBCs (2). However, the low NMR-receptivity allows measurement of transmembrane exchange under only equilibrium conditions and cannot give estimates of the initial influx rate. The development of dynamic nuclear polarization (DNP) (3) of aqueous solutions, enhancing the signal ~10,000-fold, has opened up the possibility of recording metabolic and membrane transport fluxes on the 1-second timescale. Thus sequential ^{13}C NMR spectra of hyperpolarized ^{13}C-urea can be used to estimate the zero-*trans* transport of this solute. We have developed a kinetic model that enables us to estimate the rate constants for urea transport as well as taking into account relaxation of magnetization of the hyperpolarized nuclear spins.

Methods: RBCs were obtained from healthy donors (GP and PWK), washed in hypertonic (583 mol kg^{-1}) saline solution containing 10 mM glucose; and CO was bubbled through the RBC suspension for 15 min. 2 mL of packed RBCs (Ht ~85%) were added to a 10-mm o.d. NMR tube. The spectrometer was operated at 400.13 MHz for ^{1}H, and 100.61 MHz for ^{13}C, and the temperature was regulated at 298 K. For the hyperpolarization step, ^{13}C urea was dissolved in water to a concentration of 8 M, and 15 mM OX063Me radical, as well as 3 mM $GdCl_3$ were added to facilitate the polarization transfer (4). The hyperpolarization was performed using a microwave frequency of 94.112 GHz for ~1 h. The dissolution of the hyperpolarized solid was done into hypertonic PBS buffer to obtain a final urea concentration of 60 mM. After dissolution, 2 mL of the hyperpolarized urea solution was rapidly injected into the NMR tube containing the RBC suspension after which the ^{13}C NMR spectra were recorded at 1 s intervals.

Results: The addition of urea in a solution containing only urease showed all the various chemical reactants: urea, CO_2, H_2CO_3 and HCO_3^-. In the presence of RBCs, we could distinguish the intra- and extracellular urea due to a small difference in their chemical shifts. Figure 1 shows the biochemical pathway for the reactions in the presence of RBCs and urease, as well as the experimental results. We note that the conversion of CO_2 into HCO_3^- was very fast, preventing the detection of chemical intermediates.

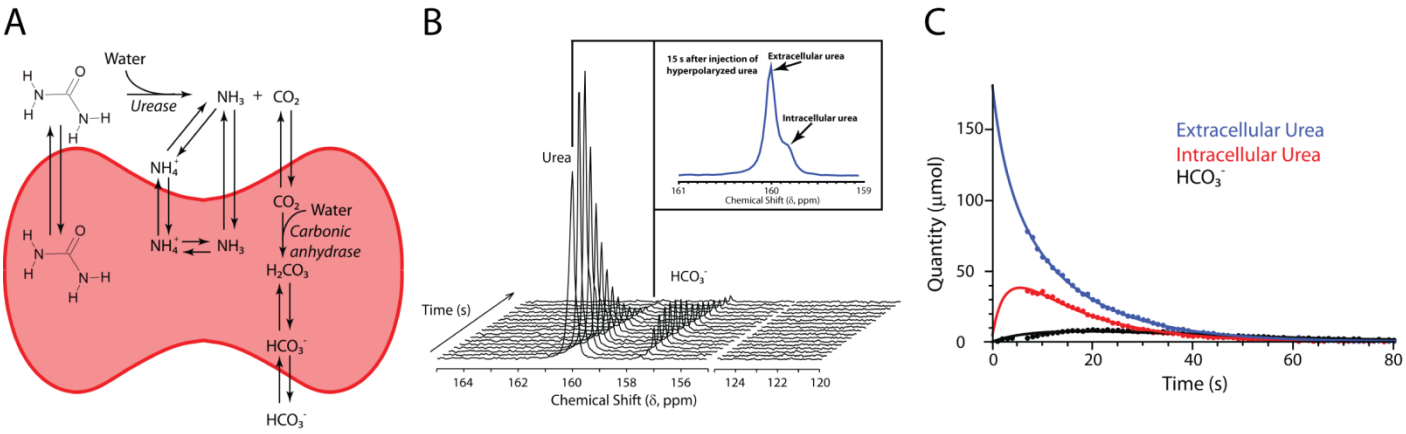

Fig.1: Conversion of urea into bicarbonate in the presence of RBCs and urease. (**A**) pathway for this biochemical reaction scheme; (**B**) stacked plot of the reaction after adding hyperpolarized ^{13}C-urea with the inset showing the chemical shift difference between intra- and extracellular urea; and (**C**) fitting of the NMR signals according to a kinetic model that takes into account the very fast conversion of the urea into bicarbonate.

Discussion: We have developed an extensive mathematical model to fit the zero-*trans* transport data of urea in RBCs. The model takes into account the restrictions due to the short lifetime of hyperpolarized molecules: T_1 decay and detection of only part (the hyperpolarized fractions) of the metabolites. Our approach has given accurate estimates of kinetic parameters in this simple case. While the full model can be used to fit the reaction of urea with urease or in the presence of RBCs, we have to use a simplified model for the reaction with RBCs and urease due to the very fast reaction mediated by carbonic anhydrase. We will extend this study to other metabolites as well as neoplastic cells, *in vitro* and *in vivo*.

References:

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