A RELAXOGRAPHIC ANALYSIS OF MULTI-COMPARTMENTAL WATER EXCHANGE IN ISOLATED PERFUSED RAT HEARTS

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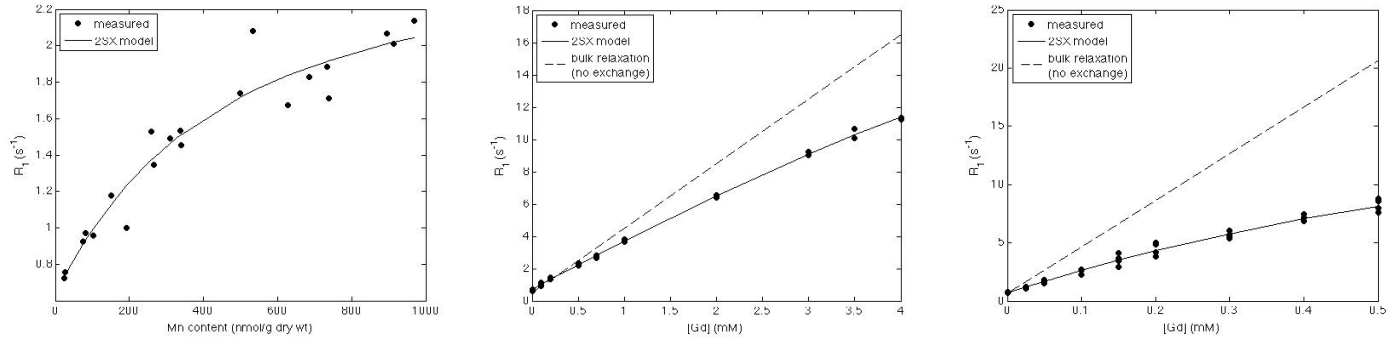
Introduction In T_1-weighted imaging, it is common to assume a linear relationship between the longitudinal relaxation rate ($R_1=1/T_1$) of the tissue and the local concentration of contrast agent (CA). The effective R_1 will however depend on the difference in relaxation rate between compartments, and to which extent the water in other compartments can access the CA through equilibrium water exchange. Without any CA added most tissues are in the fast-exchange limit (FXL) [1], and the assumption of a linear relationship between R_1 and [*CA*] is valid. However, when adding a compartment specific CA the system will depart from the FXL, and the properties of the effective R_1 will change non-linearly with increasing [CA]. Equilibrium water exchange can be investigated taking advantage of the difference in relaxation rates between tissue compartments with varying [CA], known as 'relaxography' [1]. We have performed a relaxographic analysis of multi-compartmental equilibrium water exchange in perfused isolated rat hearts using an intravascular (Gadomelitol), an extracellular (GD-DTPA-BMA) and an intracellular (Mn^{2+}) contrast agent.

Materials and Methods Perfused rat hearts were made cardioplegic (KHBB solution containing 20 mM KCl) to avoid motion artifacts, and NMR relaxography was performed *ex vivo* (Maran Ultra, Resonance Instruments Ltd, 23 MHz, 37°C). T_1 was measured along the long axis of the perfused heart using a 1D profile-Saturation Recovery method. T_1 values were thereafter selected from a 5 mm thick slice in the center of the hearts, where the influence from surface pooling was found to be insignificant. In experiments with Mn^{2+} the hearts were perfused for 5 minutes with KHBB solution containing the desired concentration of $MnCl_2$ (wash in), followed by perfusion with pure KHBB solution for 15 minutes (wash out). The system was switched to cardioplegic buffer and T_1 was measured at different flow rates (0-10 ml/min). In experiments with Gd-DTPA-BMA or Gadomelitol, cardioplegic KHBB solutions containing desired concentrations of the respective contrast agents were introduced, followed by subsequently measurements of T_1 at different flow rates. In between each concentration, the hearts were recovered from the cardioplegic state by perfusion with regular KHBB solution for some minutes. Equilibrium water exchange was analyzed using the two-site water exchange (2SX) model [1].

Results The obtained relaxation decays were mono-exponential. No effects from varying flow rate were observed. A flow rate of 4 ml/min was analyzed in more detail. As seen in the relaxivity plots below, only R_1 obtained with ic Mn^{2+} seems to reach a plateau value, indicative of the system entering the slow-exchange regime, whereas the other two systems remain in the fast-exchange regime. The results from iv and ec CA show similar values for water exchange rate across the cellular and vascular membrane, contrary to previous studies [2]. However, the exchange rate across the cell membrane obtained using ic Mn^{2+} is lower compared to results obtained using ec Gd-DTPA, but in correspondence with results obtained in excised non-perfused rat hearts [3]. This intriguing result indicates that Mn^{2+} is somehow spatially localized in the ic compartment, and is less accessible for ec water. The obtained volume fractions are in correspondence with values found in other studies of isolated cardioplegic rat hearts [4].

$MnCl_2$ **Gd-DTPA-BMA** **Gadomelitol**



Relaxivity plots obtained using different CA's are shown in the figure above (flow rate = 4 ml/min). The solid lines represents 2SX model fits to the observed data. For Gd-DTPA-BMA and Gadomelitol the curves (dotted lines) corresponding to bulk relaxation rates are shown for comparison.

CA	$R_{1\text{-}o}$ (s^{-1})	r_1 (mM^{-1} s^{-1})	$R_{1\text{-}i}$ (s^{-1})	τ_o (s)	p_o	τ_i (s)	f (Hz)
Mn^{2+}	0.37	40	1.36	0.44	0.71	0.18	8
Gd-DTPA-BMA	0.49	4	1.45	0.13	0.66	0.04	33
Gadomelitol	0.31	40	1.12	0.07	0.53	0.06	31

Results from the 2SX analysis are presented in the table to the left. Manganese content in dry tissue was determined using flame atomic absorption spectroscopy. The r_1 for ic Mn^{2+} (mM^{-1} s^{-1}) is estimated using a wet/dry weight ratio of 10 [4], a density of 1.0 and an ic water fraction of 0.29. The r_1 for Gd-DTPA/BMA and Gadomelitol in buffer solution was measured in independent experiments, and was kept constant in the 2SX analysis. The subscript 'o' refers to the compartment on the 'outside' of the respective membranes. The water exchange rate is given by $f = 1/\tau_i + 1/\tau_o$.

Conclusion We have determined water exchange rates across membranes in perfused isolated rat hearts together with volume fractions of the intracellular, intravascular and extracellular compartments. The exchange rate across cell- and vascular membranes was found to be equal in values. Intriguingly the water exchange from ic to ec compartments was found to be more restricted using Mn^{2+}, indicating an intracellular spatially localization of this substance.

References [1] Landis CS, *et al. Magn Reson Med,* 1999; **42**(3): 467. [2] Donahue KM, *et al. J Magn Res Imag.* 1997; **7**:102. [3] Seland JG, *et al. Magn Reson Med*, 2007; **58**: 674. [4] Clarke K, *et al. Magn Reson Med,* 1994; **32**(2):181.