A Three-Compartment T_1-Relaxation Model for Intracellular Contrast Agents: Implications for Molecular MR Imaging

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Introduction: One of the major goals of molecular MRI is to detect receptor expression on endothelial cells lining blood vessel lumens, e.g. to detect angiogenesis or atherosclerosis. Often, a Gd-based contrast agent (CA) conjugated with a targeting ligand is intravenously injected and allowed to accumulate at the desired site by ligand-receptor binding. Hence, the receptor is indirectly detected by the CA-induced relaxation enhancement. A complicating factor may be that an internalizing receptor may convey the ligand with the CA into the cell. Since the effectiveness of a Gd-based CA depends crucially on equilibrium water exchange kinetics, the relaxation enhancement may be "quenched" by CA sequestration in intracellular compartments [1].

Aim: The goal of this work is to develop a model describing the effective longitudinal relaxation rate constant (R_1) for ^1H_2O in three compartments experiencing possible exchange, and to apply this model to explain the effective R_1 [CA]-dependence of internalized CA.

Model: Fig. **1** shows the model voxel enclosing a cell with three kinds of compartments into which CA may be distributed: extracellular, cytosolic, and/or intravesiclar (e.g. endosomal, organellar, etc.), indicated with subscripts e, i and n, respectively. The effective R_1, determined by the different compartmental ^1H_2O R_1 values and the equilibrium exchange of water between the compartments, was calculated using a modified Bloch-McConnell equation in matrix notation $d\mathbf{M}/dt = \mathbf{XM} + \mathbf{C}$, with $\mathbf{M}$ the three compartment magnetization vector and $\mathbf{X}$ the exchange matrix [2,3]. An effective R_1 was calculated using the signal intensity for the inversion recovery sequence $S = (\mathbf{I} - 2\, e^{TI\, \mathbf{X}})\, \mathbf{M_0}$, which was also used experimentally. Input parameters are: the volumes of the compartments (v_e, v_i, v_n), the water mole fractions in the different compartments (p_e, p_i, p_n), the fraction of macromolecules 1-fw, the number of endosomes (n_n), the mean water lifetime in the compartments (τ_e, τ_i, τ_n), and the ^1H_2O relaxation rate constants for the different compartments (R_{1e}, R_{1i}, R_{1n}) in the absence of exchange, which were varied to simulate the presence of contrast agent using a relaxivity value r_1=4.2 $mM^{-1}s^{-1}$. Note that not all parameters are independent. [CA] is the effective concentration of CA of the whole voxel. Direct exchange between e and n is assumed negligible.

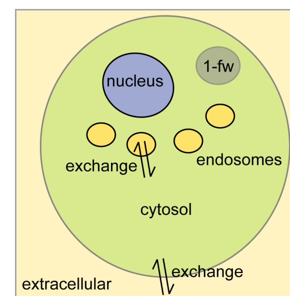

Figure 1: Model voxel containing cell with three kinds of compartments into which CA may be distributed.

Results: With the model, several possible scenarios for the internalized CA fate can be evaluated. Fig. **2A** shows the ^1H_2O R_1 [CA]-dependence for CA internalization in the cytosol (blue) or into one endosomal compartment (red). The saturation of the [CA]-dependence when CA is endosomally sequestered is illustrative for quenching due to limiting transendolemmal water exchange (between n and i). In Fig. **2B,** the number of endosomes (n_n) each with a constant [CA_n] is varied. Many small endosomal vesicles (blue curve) result in less quenching than do a few large endosomes (red line). This is no doubt due to the higher surface to volume ratio of the small endosomes (and, thus, smaller τ_n value). Fig. **2C** displays ^1H_2O R_1 for varying [CA_n] in n_n = 200 small or n_n = 5 large endosomes with the same total volume fraction, v_n. Again the relaxation rate constant is quenched at

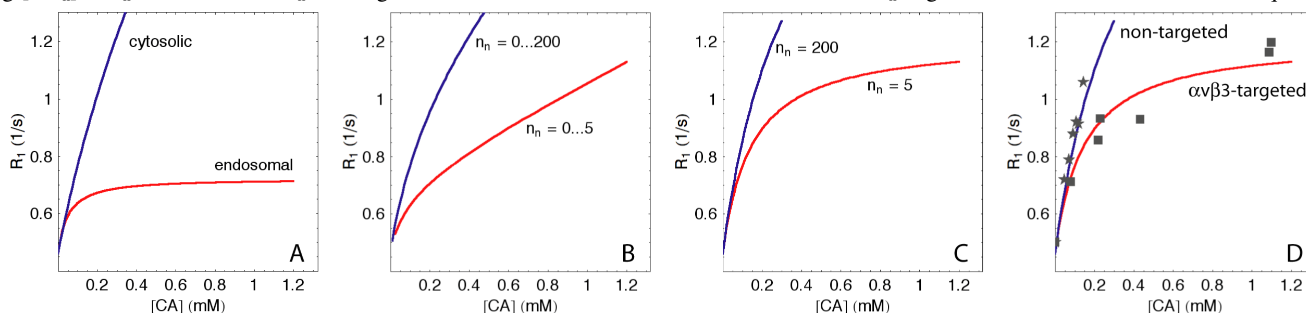

Figure 2: Longitudinal ^1H_2O relaxation constant R_1 as function of [CA]. (**A**) CA present in the cytosol (blue) or in one endosomal vesicle (red). (**B**) Variation of the number of CA-filled endosomes; n_n = from 0 to 200 small endosomes (blue) or n_n = from 0 to 5 large endosomes (red). The value of [CA_n] is constant. (**C**) CA present in n_n = 200 small (blue) or n_n = 5 large endosomes (red). The value of [CA_n] is increased. (**D**) The panel C (red and blue) curves match experimental data very well. (✶) Experimental ^1H_2O R_1 of endothelial cells incubated with non-targeted liposomes, which are internalized into many small endosomes. (■) Experimental R_1 of endothelial cells incubated with αvβ3-targeted RGD-liposomes. The internalizing αvβ3 receptor conveys CA into a limited number of large endosomes. *Initial parameters*: v_e=0.3, v_i=0.6, v_n=0.1, fw=0.8, p_e=0.375, p_i=0.5, p_n=0.125, τ_e=1.1 s, R_{1e}=0.4 s^{-1}, R_{1i}=0.5 s^{-1}, R_{1n}=0.5 s^{-1}.

higher concentrations mostly for the large endosomes. Finally, Fig. **2D** shows a comparison between the model and experimental data. Human endothelial cells were incubated with paramagnetic (Gd-containing) liposomes. Fluorescence microscopy revealed that non-targeted liposomes accumulated in many small vesicles (probably endosomes) distributed uniformly throughout the cytosol, while liposomes conjugated with RGD-peptide, a ligand targeted towards αvβ3 (a widely used receptor in molecular imaging of e.g. angiogenesis [4]), resulted in accumulation of the CA in a limited number of large vesicles. Quenching was observed experimentally for the αvβ3-targeted liposomes (■) but much less for the non-targeted liposomes (✶), which is matched by our model very well.

Discussion & conclusions: We have presented a model for the ^1H_2O longitudinal relaxation rate constant of three compartments with exchange, which allows for calculations of the effect on R_1 of contrast agent internalized within intracellular compartments. The quenching of ^1H_2O R_1 due to limited water exchange could be simulated and the model matched experimental *in vitro* data very well. The model may be used to evaluate the design of CAs that evade endosomation and therefore quenching, which is highly desirable for *in vivo* applications of targeted contrast agents.

[1] Terreno *et al. MRM* **55**, 491 (2006); [2] Spencer *et al. JMR* **142**, 120 (2000); [3] Li *et al. MRM* **54**, 1351 (2005) [Erratum, *MRM* **55**:1217(2006)]; [4] Winter *et al. Cancer Res.* **63**, 5838 (2003).