PhD thesis

Diffusion and relaxation in heterogeneous media - Magnetic resonance study of model biological systems

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Scientific background

Diffusion MR Imaging plays a vital role in the diagnosis of the pathologies of Central Nervous System, kidney, liver and lymph-nodes. In comparison of the healthy tissues with the pathological tissues, translocation of water molecules on the length scale of the cells reveals the changes in water transport, geometrical and spatial reorganization of tissues. Data on relaxation provides information about the solute concentration and the nature of the solute molecules.

One of the highlights of diffusion weighted imaging (DWI) is the diagnosis of vascular diseases of the brain: stroke, ischemia. The fact that diffusion weighted and T2 weighted images do not correlate shortly after the onset of stroke has not been clarified by molecular explanation for years. Several plausible mechanisms may provide explanation. On the tissue level, evolution of cytotoxic oedema, later on increased level of water content in tissues are plausible mechanisms. On the microscopic level, water translocation from extracellular to intracellular compartments, cell membrane permeability changes, extracellular molecular crowding, increasing level of osmotically active water in the intracellular space, increased intracellular tortuosity may play vital role in the MR signal intensity. These mechanisms may also be cause and effect.

Several animal experiments suggest in-vivo model. In order to provide microscopic explanation, less complex, and more controlled experimental models are to be proposed. To clarify complex physiological processes and the related MR imaging findings, tissue models provide a viable solution.

Several theoretical and simulation model is available to explain diffusion and relaxation properties of heterogeneous biological systems. The cross-references between these models, also the limitations in validity of the individual models are often overlooked. The models originate from diverse disciplines, geophysics, polymer physics and quantum chemistry. The choice of the proper model provide the opportunity for the explanation of the complex physiological events, also it offers the basis of new diagnostic techniques and optimization of existing ones. The validity range of the theoretical models is precisely set by the time-, and length scale of the MR experiments.

Motivation and goal

Initially motivated by the evaluation problems of MRI findings related to stroke, the main goal of this study was to identify and quantify the relevant parameters and their role in

influencing the apparent diffusion coefficient (ADC) and T2 relaxation time. The subject of the study consisted of model polymer solutions and cell suspensions. The goals of theoretical modeling of the experimental systems are the following: a) test the validity range of models; b) predict ADC and T2 changes based on validated models; c) build a coherent picture of time-, and length scale in order to support field radiologists. In the focus of the experiments were heterogeneous systems (consisting of intracellular and extracellular space as well), and their diffusion and relaxation parameters. The effect of the quality of the intra-, and extracellular space was investigated.

Proteolysis mode experiments

The diffusion and relaxation properties of water in a homogenous sample are determined by the interactions between the solute and the solvent (water) molecules. Molecular events (protein degradation, polymerization), and concentration changes may occur simultaneously in physiological processes. The independent effect of these events are in the focus of these experiments.

Molecular crowding experiments

The proteins in red blood cells (RBC) consist of 95% hemoglobin. Mammalian erythrocytes differ in the amount of their hydrophilic amino acids. Also the interaction between the water and their intracellular proteins are different. The goal of these experiments was to compare extracellular space-free RBC samples regarding their ADC and osmotic behavior. In comparison with the experimental results on the polymer solutions, the effect of the cell membrane in diffusion was to be evaluated.

Analysis of the effect of intracellular (IC) and extracellular(EC) space

The effect of the EC and IC solute molecules in RBC samples was studied. The effect of systematically modified EC space concentration and the nature of EC space solute molecule, also the concentration of the IC space effects the diffusion and relaxation of the samples. The goal was to identify and apply a theoretical model that properly describes the experimental findings. These findings and their theoretical models yield plausible explanation of complex physiological processes in heterogeneous systems, on the time-, and length-scale of the MR experiments.

Modification of the intracellular space and its effects

The experimental findings and theoretical models imply that in-vivo intracellular events can be indeed detected by macroscopic, voxel size MR imaging measurements. The experiments with cell-suspensions evaluate the feasibility of detection of intracellular events. Jurkat cell line was utilized in an experiment series, where O-glycolisation and its effect on diffusion and

relaxation was studied. O-glycolisation modifies the intracellular proteins in several pathological processes: diabetes, tumor genesis, apoptosis, reperfusion following ischemia. Another experiment series was carried out on HeLa cell suspensions, where the cell population was synchronized by application of thymidine. These experiments aimed the feasibility of detection of cell cycle phases by MR diffusion and relaxation experiments. These latter experiments served as a proof of concept study for further diagnostic technique.

Methods

MR techniques

The intracellular crowding experiments were carried out by MR imaging technique, the effects of EC/IC space, intracellular modification and experiments on solutions were carried out by MR spectroscopy technique. All measurements were performed on a Varian ^{UNITY}INOVA 400 WB spectrometer (Varian Inc., Palo Alto, CA, USA), with a bore magnet of width 89 mm, vertical setup 9.4 T (Oxford Instruments Ltd., UK). Imaging was performed with a 35 mm inner diameter hollow multinuclear probe with Litz Volume coil and built-in actively shielded gradient system, up to 350 mT/m. Spectroscopy was performed with a 5 mm inner diameter ID-PFG type (Varian) probe. ADC was measured by PFGSE (Pulsed Field Gradient Spin Echo) sequence, T2 was measured by CPMG (Carr-Purcell-Meiboom-Gill) sequence, T1 was measured inversion recovery sequence. ADC, T2, T1 was derived from exponential signal decay by linear fitting with Varian built-in post-processing software packages.

Experiments with solutions

Aqueous solutions of various solutes were prepared for the proteolysis model experiments. To test the effect of weight of the solute molecules, PEG (Polyethilene-glycol: HO - $(CH_2\text{-}CH_2\text{-}O\text{-})_n$ - H) was used. To test the effect of the biological polymers, albumin and lysozyme was used. To test the effect of peptides of various sizes, peptone solutions were used.

RBC suspensions

Human and camel erythrocytes and their suspensions were used in these experiments. The effect of the intracellular space was studied by osmotically altered RBC suspensions and also by comparison of RBC suspensions of various species. The effect of the extracellular space was tested by applying various suspension media, where solute concentration and the nature of solute molecules were systematically altered.

Intracellular modifications

The dynamic equilibrium in O-glycolization of intracellular proteins was modified. The increased glycolized protein content was monitored, the diffusion and T2 relaxation properties of 50% suspension of the modified cells were determined in MR experiments. Synchronization of HeLa cells was achieved by application of thymidine. The diffusion and relaxation properties of suspension of HeLa cells in various cell cycle phases were compared.

Theoretical modeling of heterogeneous systems

The goal is to compare Effective Medium Theory (EMT) model and experimental findings. The relationship between the parameters of the heterogeneous system, derived from EMT, is rewritten in a relationship between relative parameters, where all parameters can be measured easily, although these parameters are not independent pair-wise. The coherence between experimental findings and this theoretical relationship between dimensionless parameters was investigated. Furthermore, for the interpretation of the behavior of T2 relaxation time, diffusion-corrected volume weighting was used, and its validity investigated.

Results

Proteolysis model – homogenous biological model

Based on the experimental findings related to ADC and T2, the following statements can be made.

- ADC is influenced mainly by the concentration of the solute molecules, the molecular weight has little effect. In a wide range of the concentration, there is a linear relation between ADC and concentration. ADC proved to be a good indicator of concentration and a poor indicator of the solute molecular weight.
- In a wide range of concentration, there is a linear dependence between the diffusion coefficient of the solute and solvent molecules. In the higher regime of concentrations a deviation from linear behavior is observed. This deviation is explained by the interaction between solute molecules.
- In a wide range of concentration T2 relaxation time appears to be independent from concentration (emphasized in experiments with biologically relevant solute molecules). T2 appears to be strongly dependent on the solute molecular weight, also on the individual characteristics of solute molecules. T2 relaxation time proved to be a good indicator of the nature of the solute molecules.
- Changes in ADC and T2 can be related to physiological processes. A possible explanation is provided by the experimental findings for the uncorrelated behavior of these parameters:

- The processes involved in ischemia induce changes in the spectral (molar distribution of molecular weight) composition of solute molecules, thus inducing T2 changes. It can be rightly expected, that T2 changes reflect proteolytic processes.
- It can be further assumed, that changes in ADC reflect concentration changes (oedema).

These results were presented at "Magyar Neuroradiológiai Társaság XIV. Kongresszusa és Továbbképző Kurzusa" and "CNS Injury és a Magyar Idegsebészeti Társaság XVII. Kongresszusa" (see publication 3., 4.).

Intracellular crowding

The experiments on human and camel erythrocytes are in accordance with the diffusion experiments on polymer solutions. The diffusion coefficients of the various hemoglobin solutions are very close at the same concentration. The differences in hemoglobin hydrophilicity have little effect on the diffusion. The increase in hemoglobin concentration leads to the decrease of the water diffusion. The hydrophilicity of the IC space has effects on the ADC only indirectly, i.e. through the influence in the intracellular water content.

The effect of the cell membranes reveals in the non-mono-exponential signal decay in the MR diffusion experiment, the effect of concentration reveals both in the slow and the fast component. These experimental findings emphasize the fact, that emergence of modes in signal decay does not necessarily reflect the existence of physically separate compartments.

These results were published in Cell Biology International (see publication 2.).

Independent analysis of IC and EC spaces – heterogeneous biological systems

EMT proved to be a proper theoretical model. The derived, new relationship between dimensionless parameters proved to be a very useful evaluation tool, the experiments simplified to MR experiments and straightforward mass measurements. Based on the experimental findings and the theoretical modeling, the following statements can be made, valid in a wide range of the intracellular space volume fraction (these results were published in Magnetic Resonance Imaging, see publication 1.):

- The measured diffusion coefficient does not depend on the nature of the extracellular space solutes; ADC depends linearly on the EC solute concentration, in good accordance with the experiments on polymer solutions.
- The IC water content determines the ADC value of the sample, according to the EMT model. The dependence is asymmetric for the hypo-, and hyper-osmotic regimes. This

finding of other studies was supported by new argumentation, also independent experimental findings was achieved.

- In a wide range of the EC solute concentration, T2 relaxation time proved to be independent of concentration, also solute molecule weight was found to be irrelevant.
- T2 relaxation time proves to be strongly influenced by IC water content, in good accordance with the diffusion-corrected volume weighting. This model, in contrast to the simple volume weighting, describes the behavior of the T2 relaxation time as a function of the IC volume fraction. This model is valid for ergodic systems.
- Related to the MR diagnostics of ischemia, the following statements can be made:
  - The changes in the extracellular space are less relevant than expected.
  - It is questionable that the cell swelling, induced during cytotoxic oedema, will lead to changes in the ADC.
  - The longer T2 relaxation times refer to cytotoxic oedema.

Intracellular modifications

The detection of changes in intracellular properties - related to normal and pathological processes – proved to be feasible in 50% cell suspensions by MR measurements. The modified equilibrium in intracellular O-glycolized protein content led to significant ADC changes. However, no T2 relaxation time change was observed.

Also the changes of the intracellular space during normal cell cycle phases were detected by MR diffusion measurements. Apparently no T2 changes followed in contrast with the expectations.

The publishing of the results is in progress.

Future research opportunities

The evaluation of theoretical models and experimental findings suggest further research and application opportunities.

- Study of the ischemic processes in cell-biological models and animal models,
- Optimizing Diffusion Tensor Imaging MR technique, further clarify interpretation methods,
- In-vivo, quantitative evaluation of cell-synchronizing effect of cytotoxic chemotherapy.

Publications on the subject

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Effects of intra- and extracellular space properties on diffusion and T2 relaxation in a tissue model.
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2. Peter Bogner, Attila Miseta, Zoltan Berente, Attila Schwarcz, Gyula Kotek, Imre Repa:
Osmotic and diffusive properties of intracellular water in camel erythrocytes: effect of hemoglobin crowdedness.
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Conference materials on the subject

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4. Gy.Kotek, Z.Berente, Zs.Vajda, A.Schwarcz, P.Bogner:
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Other publications

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Implanted deep brain stimulator and 1.0-Tesla magnetic resonance imaging.
Journal of Magnetic Resonance Imaging. 2006 Dec; 24(6):1409-12.