Probe Development: Biophysics and Validation

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MR offers a remarkable set of anatomical, functional and metabolic readouts that find extensive use in biomedical research and clinical diagnostics. The nuclear imaging techniques like PET and SPECT as well as optical imaging approaches, however, have a much higher intrinsic sensitivity than MR. This explains why these techniques are presently preferred for molecular imaging applications, in which one aims to obtain information on the expression levels of molecular markers or the activity of specific metabolic or signaling pathways. Making MRI and/or MRS suitable for use in these novel molecular imaging applications, is a challenging but potentially very rewarding task. This presentation will describe the state-of-the-art of target-specific MRI contrast agents and in particular focus on the biophysics of MRI signal and contrast generation as well as the validation of the imaging probes that are in use. Molecular imaging obviously starts with the choice of the most suitable target that can serve as a hallmark of the process of interest. For many disorders molecular players that take part in the disease process have been identified and a broad range of ligands capable of binding to these so-called disease biomarkers has been developed. The ligands come in many different versions, including antibodies, antibody fragments, proteins, peptides, low-molecular weight peptidomimetics and aptamers. The ligand of choice depends, among others, on target location and nature, ligand cost and size, specificity of the ligand-target interaction and the risk of immunological effects. There are many options to conjugate MRI contrast agents to these ligands and the ultimate choice again depends on many factors, among which target location and expression level are the most dominant.

Most of the imaging agents that are under development for applications in molecular MRI are indirectly detected via their effect on the magnetic properties of tissue water. Examples include Gd-based paramagnetic agents and FeO-based superparamagnetic contrast materials. Gd^{3+}-chelates are mainly used as T_1-shortening agents and are most effective in case of rapid exchange of water between the primary coordination sphere of the Gd^{3+}-ion and the bulk of the water in the imaging voxel. The efficacy of T_1-shortening agents is expressed as the relaxivity r_1 [$mM^{-1}.s^{-1}$]. Extrapolation of test tube data on r_1 to the *in vivo* setting may be complicated by compartmentation effects, when the agent is inhomogeneously distributed in the imaging voxel and confined to a relatively small volume fraction. This situation has been described for both low-molecular weight Gd-agents [1] and for Gd-containing nanoparticles [2]. Although this sensitivity to water exchange kinetics provides interesting opportunities to assess the cellular environment with the use of Gd^{3+}-agents, this situation may complicate the quantification of the local contrast agent concentration as a measure of target expression. FeO-based nanoparticles (NPs) are most often detected via their pronounced effect on the T_2- and T_2*-relaxation time. The T_2–shortening effect is governed by so-called outer sphere relaxation, which describes dephasing of the hydrogen nuclear spins of water through diffusion in the inhomogeneous field created by the FeO NPs. The mechanisms underlying T_2 relaxation mechanisms exerted by FeO NPs are reasonably well understood and strongly depend on their size as compared to the characteristic water diffusion lengths. This property forms the basis for the detection of the clustering of engineered FeO NPs as a measure of the local activity of specific enzymes [3]. Several other approaches are being taken to tackle some of the drawbacks of the traditional T_1- and T_2-based detection of (super)paramagnetic agents. Chemical Exchange Saturation Transfer (CEST) agents provide on/off opportunities, as they are detected as a reduction of the free water signal only upon off-resonance saturation while having no significant effect in its absence [4]. The CEST agents provide a pool of protons that are shifted as compared to the resonance of the bulk water protons and are in chemical exchange with bulk water. The CEST agents are available both as exogenous contrast materials (*e.g.,* LipoCEST agents [5]) and in a quasi-endogenous version in the form of polypeptides, which are rich in exchangeable protons and are constitutively expressed by genetically engineered cells [6]. The advantage of the latter CEST probes is that they are nonparamagnetic; the disadvantage, however, is that they require genetic manipulation, making them in particular suited for the tracking of exogenously implanted cells. A final class of contrast agents that are explored for use as target-specific probes are fluorinated compounds for F-19 MRI/MRS detection [7]. F-19 MR is a hot-spot technique as the endogenous levels of F-19 are negligible.

There are several ways to validate target-specific imaging agents. A first step is to test their target specificity using well-controlled *in vitro* systems, including cultures of mammalian cells that abundantly express the target. As a control for agent specificity, one can use a non-functional targeting ligand, or resort to blocking of the target by pre-incubation with excess non-labeled ligand. Validation is greatly supported in case the contrast agent also contains a fluorescent moiety for (confocal) fluorescence microscopy. The optical label also offers opportunities for intravital microscopy, with which targeting kinetics and specificity can be probed, be it with a limited field-of-view and penetration depth. Also combinations of MRI with complementary imaging techniques, including SPECT and PET, may be attractive. The high sensitivity of nuclear techniques and their quantitative nature make them ideally suited to determine, for example, the whole-body bio-distribution of targeted imaging agents.

The final portion of the presentation is devoted to *in vivo* applications of target-specific contrast agents. The emphasis will be on targeted nanoparticles [8,9], which can be prepared in a wide range of sizes and equipped with a high contrast agent payload. NPs also offer multimodality imaging, using additional labels for complementary optical and/or nuclear imaging. Many NP concepts enable incorporation of drugs for targeted, image-guided therapy. For size reasons, NPs have primarily been used for detecting intravascular markers, as well as extravascular markers in case of enhanced vascular permeability, such as in tumors [10], atherosclerosis [11] and myocardial infarction [12]. The factors affecting the target-to-background ratio and thus the sensitivity and specificity of agent detection will be described. Depending on the type of agent, biocompatibility may be an issue, which is addressed with the use of toxicity assays, gene profiling studies, etc. The clinical translation of the novel agents represents a major challenge. This especially holds for Gd-based agents, in particular in the light of the NSF debate.

References

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