Physico Chemical Principles & Applications of Fluorine

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Introduction

Molecular & Cellular Imaging is "*the in-vivo characterization and measurement of biological processes at the cellular and molecular level*" and aims to image molecular abnormalities associated with diseases and to monitor cell assemblys such as macrophages or stem cells.

MRI (magnetic resonance imaging), PET, and optical imaging are currently the modalities of choice, which can be used in the living system. Among the different non-invasive modalities, MRI has a number of characteristics that makes it an ideal candidate: (1) an extraordinary 3-dimensional capabilities, (2) a superb spatial and temporal resolution and (3) a high safety profile. In addition, MRI appears very attractive, since current MRI protocols already provide anatomic, functional and biochemical information in excellent image quality. Combining this high spatial resolution/high contrast imaging modality with specific MR imaging agents is current focus in many research laboratories, in particular small animal imaging centers, worldwide.

Molecular and cellular MRI is principally performed by using (actively or passively) targeted exogeneous MRI-contrast agents. However, so far, since MRI compared to other imaging techniques is traditionally a low sensitivity modality (micromolar range!), a major objective of current research is (1) to increase the sensitivity and (2) to detect unambiguously target structures beyond the resolution limit of current MRI techniques. Therefore, an arsenal of different techniques and approaches, which allow for a "background-free" identification and quantitative imaging of the labeled structures, is currently being developed. This teaching course will focus on one of the „mainstream" approaches based on fluorinated (^{19}F) labels for (quantitative) cell tracking.

The ^{19}F-MRI-cell tracking approach in short

The major application in the field of cellular MRI is the visualization and tracking of stem cells and macrophages. In standard approaches using (super-) paramagnetically labelled compounds cells appear as hypo/hyper intense regions in situ with the entire anatomy of the investigated object as background signal, which makes an unambiguous identification cells in vivo difficult or even impossible, especially if the cell biodistribution is completely unknown. An alternative and more selective approach, eliminates the background signal of existing "standard-methods" and has in addition the potential for cell number quantification [1-4]. This method applies cells labelled ex vivo or in vivo with ^{19}F nanoparticles, which are then

administered to the animal, and tracked using ^{19}F-MRI. The ^{19}F-signal from the fluorinated nanoparticles yields the spatial distribution of the labelled cells only, since the lack of detectable endogenous fluorine atoms in the body assures the absence of any background signal. Thus, the use of ^{19}F-nuclei enables direct detection rather than the indirect detection necessary for contrast agents, thus avoiding the need for pre-scans and removing localization ambiguity. This allows to track the cell biodistribution in a more systemically fashion. In combination with anatomical ^{1}H-MRI with exquisite spatial resolution, these "hot spot" ^{19}F-images can be superimposed on anatomical ^{1}H images generated with the same scanner and in the same imaging session, which allows to place the labelled cells in anatomical context

However, because of the inherent limitations in the available signal-to-noise ratio (SNR) of MRI, sensitivity is a key concern. Currently, the minimum number of detectable ^{19}F-spins per voxel is of the order of 10^{18} spin per voxel which corresponds to 2 x 10^{5} labeled cells per voxel @ 11.7T. In summary, the ^{19}F-MRI-approach selectively images only the labeled cells and a conventional ^{1}H image places the cells in their anatomical context.

Why ^{19}F-MRI and why PFC's?

^{19}F-MRI has been demonstrated in various applications since the first in vitro study of ^{19}F-MR imaging in 1977 [5], where the use of ^{19}F compounds as 'tracer substance' in a sample was also foreseen. ^{19}F has several properties, see Table, that make it an perfect agent for use as an MRI tracer:

(1) high relative sensitivity (83% of ^{1}H)
(2) 100% natural abundance
(3) resonance frequency close to that of ^{1}H, which potentially allows to use existing ^{1}H imaging hardware for ^{19}F-MRI
(4) broad chemical shift (interesting for cell population imaging)
(5) not detectable background signal
(6) unique capability to directly determine the absolute quantity of ^{19}F atoms

Table: MRI properties of the ^{1}H- and ^{19}F- nuclei

Nuclei	Gyromagnetic Ratio (MHz/T)	Spin quantum number	Natural abundance (%)	Relative sensitivity
^{1}H	42.6	½	99	1.0
^{19}F	40.0	½	100	0.83

The following list comprises some major requirements for a ^{19}F compound usable as a ^{19}F-tracer for cell labeling.

(1) biologically inert & chemically stable & stable in aqueous environments
(2) label should be suitable for cell labeling & cell uptake should be high*
(3) high ^{19}F density per molecule is desired*
(4) ^{19}F compound should have short T1 and long T2*

*Requirements 2-4 should be fulfilled for sensitivity reasons!

In the past perfluorocarbons (PFCs) have been shown to fulfill most of these requirements and thus established as compounds for ^{19}F-MRI, since PFCs are nontoxic, biologically stable, not metabolized and provide a high payload of ^{19}F nuclei. For biological applications, PFCs are typically emulsified into a (spheric) nanoparticle (sizes: <100 nm to several hundreds of nanometers). Such a PFC nanoparticle comprises a liquid PFC core encapsulated by a lipid monolayer, resulting in a high concentration of ^{19}F atoms (100 M). Because of size reason intravenously administrated PFC nanoparticles do not leak out of intact vasculature. Instead, PFC nanoparticles are removed from the blood stream primarily by macrophage endocytosis. Blood half-life of PFC nanoparticles varies with particle size with a typical value of 2–12 h.

In vivo cell tracking using 19F-MRI of intracellularly labeld PFC nanoparticles

Recently it was demonstrated that ^{19}F MRI can be used as a powerful method for quantitative trafficking of stem cells in vivo (1). In this study, PFC nanoparticles were internalized by stem cells and after local injection or systematic delivery, the biodistribution of the labeled cells could be specifically detected by ^{19}F-MRI at 11.7 T. In addition, since cells can be labeled with multiple types of PFC (i.e., PFOB and PFPE) nanoparticles with distinct chemical shift signatures, different cell populations can be distinguished by ^{19}F-MRI (6)

More recently, ^{19}F-MRI of PFC nanoparticles has been used to detect cardiac and cerebral ischemia (7). It was shown that intravenously administrated PFC nanoparticles were actively internalized by circulating macrophages, which showed an progressive accumulation in the corresponding inflammatory areas. This trafficking could be depicted via combined ^{19}F/^{1}H-MRI. In addition, it was demonstrated, that PFCs can serve as MRI contrast agent for the early and sensitive detection of transplant rejection (8) and for the prognostic and quantitative assessment of pulmonary inflammation (9) by in vivo ^{19}F-MRI.

In general, studies are well-suited for cell tracking using ^{19}F MRI, where a large numbers of cells are highly concentrated, because the dense localization of the cells results in a high cell density and thus ^{19}F density per voxel. Thus, in general systemic cell transfers are more challenging because the label might not accumulate locally at sufficient concentrations for imaging (i.e. the number of ^{19}F nuclei/voxel might not cross the detection threshold). However, because the ^{19}F images are overlaid on high resolution ^{1}H anatomical scans, the resolution and SNR of the ^{19}F images can be much lower, thus potentially increasing the numbers of cells per voxel over the sensitivity limit.

Short Summary

Molecular and cellular MRI is challenging, mainly because of lack in sensitivity and specificity. In this teaching session ^{19}F-MRI as one potential „problem solver“ for cell tracking is introduced. The main hurdle is sensitivity (i.e. detection of ^{19}F tracers within a reasonable time frame to allow clinical use). However, this approach has still to gain a lot from improved imaging hardware, imaging sequences and reconstruction techniques, label development, and cell labeling. The most fundamental MR-related physical and methodological aspects will be explained in this teaching session, at least superficially!

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