Spatially Resolved Quantification of Gadolinium(III)-Based Magnetic Resonance Agents in Tissue by MALDI Imaging Mass Spectrometry after In Vivo MRI

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Abstract: Gadolinium(III)-based contrast agents improve the sensitivity and specificity of magnetic resonance imaging (MRI), especially when targeted contrast agents are applied. Because of nonlinear correlation between the contrast agent concentration in tissue and the MRI signal obtained in vivo, quantification of certain biological or pathophysiological processes by MRI remains a challenge. Up to now, no technology has been able to provide a spatially resolved quantification of MRI agents directly within the tissue, which would allow a more precise verification of in vivo imaging results. MALDI imaging mass spectrometry for spatially resolved in situ quantification of gadolinium(III) agents, in correlation to in vivo MRI, were evaluated. Enhanced kinetics of Gadofluorine M were determined dynamically over time in a mouse model of myocardial infarction. MALDI imaging was able to corroborate the in vivo imaging MRI signals and enabled in situ quantification of the gadolinium probe with high spatial resolution.

Magnetic resonance imaging (MRI) provides high-resolution tomographic imaging with excellent soft tissue contrast. The ability of MRI to differentiate soft tissues is due to the high contrast in an MR image, which is the result of a complex interplay of numerous factors including the relaxation rate of water protons. The latter can be enhanced by the administration of a contrast agent. Small-molecular-weight paramagnetic gadolinium-based chelates are the workhorse in contrast-enhanced MRI and have led to remarkable improvements in sensitivity and specificity, better tissue characterization, and increased functional information. Highly stable gadolinium(III)-based chelates are currently used in clinical settings as reporters of blood flow and organ perfusion and improve tissue characterization. Novel targeted MR contrast agents can inform on cellular and molecular processes both in health and disease and are therefore important tools in molecular imaging. The quantification of specific biological and pathophysiological processes in vivo by contrast-enhanced MRI is based on the quantification of the MR signal induced by some kind of molecular imaging agent. Depending on their relaxivity these agents lead to a positive enhancement on T1 weighted MR images, which can be quantified either by calculating signal-contrast-to-noise ratios or T1/R1 relaxation rates. Given the various factors such as the local chemical environment and compartmentalization of the agent, the in vivo signal does not directly correlate with the actual concentration of the agent in situ. Because of this nonlinear relationship, both signal intensities as well as R1 values do not precisely inform about the actual concentration of a certain contrast agent within the tissue and the actual amount of a molecular contrast agent bound at the target site. To verify the specificity and kinetics of targeted MRI contrast agents, spatially resolved quantification of the actual local contrast agent concentration and correlation to the in vivo imaging signal would be necessary. Spatially resolved information about actual contrast agent concentrations in tissue ex vivo would allow evaluation of different semiquantitative methods of in vivo MRI by serving as a reference standard.

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry is a powerful tool for investigating the spatial distribution of endogenous and exogenous molecules such as tracers and contrast agents within biological systems through the in situ analysis of tissue sections. A previous study provided evidence that a specific gadolinium contrast agent, B22956/1, could be measured by MALDI imaging. In their proof-of-principle study the MALDI imaging signal-to-noise ratio of B22956/1 was compared to MRI enhancement in a time-course analysis of the B22956/1 signal in the liver of mice. The authors used dihydroxybenzoic acid (DHB) as a matrix for MALDI imaging which resulted in a B22956/1 ligand signal derived from a molecule representing the dissociation from B22056/1 during matrix...
Angewandte Communications

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Quantitative data were generated on the amount of free/dissociated gadolinium in homogenized liquid tissue samples by ICP-AES. In contrast to the work described above, in our study, we established cyanohydroxycinnamic acid (CHCA) as a matrix and demonstrated that full gadolinium(III)-based chelate complexes are detectable. We used the strength of MALDI imaging and the spatial resolution of mass spectrometry data to generate tissue-region-specific semi-quantitative data. We demonstrated tissue dynamics over time of a collagen-targeted MRI contrast agent (Gadofluorine M) in different tissue regions of the diseased mouse heart. The spatially resolved semi-quantitative MALDI imaging results were directly compared to the in vivo MRI signals. We furthermore describe for the first time the principle measurability of the molecule of four different gadolinium(III)-based contrast agents. Gadofluorine M (Bayer Healthcare, Berlin, Germany) is an amphiphilic experimental MRI contrast agent, composed of a Gd-DOTA derivative with a lysine backbone, a hydrophilic sugar moiety (mannose), and a perfluorinated lipophilic side chain, which has been shown to selectively bind to collagens, proteoglycans, and tenascin in animal models of atherosclerosis.

The prolonged in vivo enhancement kinetics of the agent was the primary rationale for evaluating this agent further by MALDI imaging. The analytical MALDI imaging setting for Gadofluorine M was optimized using ex vivo treated tissue according to a previously published protocol. In a first proof-of-principle study to test our MALDI imaging set up in situ, we administered Gadofluorine M (0.3 mmol kg⁻¹ body weight) through the tail vein in a mouse model of myocardial infarction to evaluate MALDI imaging for spatially resolved semi-quantitative MALDI imaging results were directly compared to the in vivo MRI signals. We furthermore describe for the first time the principle measurability of the molecule of four different gadolinium(III)-based contrast agents. Gadofluorine M (Bayer Healthcare, Berlin, Germany) is an amphiphilic experimental MRI contrast agent, composed of a Gd-DOTA derivative with a lysine backbone, a hydrophilic sugar moiety (mannose), and a perfluorinated lipophilic side chain, which has been shown to selectively bind to collagens, proteoglycans, and tenascin in animal models of atherosclerosis.

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results demonstrated that the application of MALDI imaging in the assessment of contrast agent kinetics in tissue is potentially feasible.

We designed another experiment to evaluate the comparability of the in vivo MRI signal intensity to semiquantitative ex vivo MALDI imaging mass spectrometric data (Figure 2A). Mice bearing a myocardial infarct for seven days were again administered with Gadofluorine M (0.3 mmol kg⁻¹ body weight through the tail vein) and imaging was performed at 6, 24, and 48 hours after injection of the contrast agent. Mice (n=2 for each time point) were sacrificed after MRI measurement and prepared for MALDI imaging and signal quantification. MRI signal-to-noise ratio and R1 was compared to semiquantitative MALDI imaging data of the very same mice. Signal-to-noise ratio decreased over time from 6 to 24 to 48 hours post injection as expected (infarct after 6 h: 25.9 ± 4.7; after 24 h: 12.9 ± 5.5; after 48 h: 6.8 ± 2.6; healthy myocardium after 6 h: 6.8 ± 0.7; after 24 h: 4.7 ± 0.4; after 48 h: 6.7 ± 4.7) while after 48 hours visually no contrast enhanced signal was detectable any more in the infarcted area. The R1 data followed as expected [infarct after 6 h: (0.007 ± 0.0003) s⁻¹; after 24 h: (0.002 ± 0.0001) s⁻¹; after 48 h: (0.001 ± 0.0001) s⁻¹]. At 6 and 24 hours a high contrast between the infarcted and remote myocardium could be detected. By using MALDI imaging we detected a decreasing amount of Gadofluorine M over time [infarct after 6 h: (0.012 ± 0.0009) mM; after 24 h: (0.007 ± 0.0006) mM; after 48 h: (0.002 ± 0.0008) mM; healthy myocardium after 6 h: (0.003 ± 0.0008) mM; after 24 h: (0.002 ± 0.0002) mM; after 48 h: (0.0003 ± 0.0002) mM]. Comparing the MRI and MALDI imaging data there was a good agreement between the data of the two imaging techniques (Figure 2B).

Currently, several gadolinium(III)-based contrast agents are available for clinical applications. More and more, targeted molecular contrast agents are being evaluated to study specific cellular and molecular processes in vivo both in health and disease. As illustrated in Figure 3, three clinically approved, as well as one experimental paramagnetic MRI contrast agents, were evaluated for possible detection by MALDI imaging. Gadopentetate dimeglumine (Gd-DTPA, Bayer Healthcare, Berlin, Germany) is the most widely applied contrast agent in clinical practice. After intravenous administration Gd-DTPA quickly extravasates from the vasculature by diffusion and accumulates within the extracellular space, from which it is eliminated at a half-life of approximately 90 minutes. Gadobutrol (Gadovist, Bayer Healthcare, Berlin, Germany) is a macrocyclic complex with increased thermodynamic stability as well as increased r1 and r2 relaxivity compared to Gd-DTPA. Gadobenate dimeglumine (Gd-BOPTA, Bracco Imaging, Milan, Italy) is based on a linear structure and has a high affinity towards albumin after the agent is administered into the circulation. The identification of the contrast agent signals was again verified by the m/z signal itself as well as by comparison of the isotope signals with the calculated isotope pattern using isotope pattern software (Bruker Daltonik, Bremen, Germany). All four contrast agents demonstrated specific signals and good ionization properties (Figure 3). These results gave evidence, that gadolinium(III)-based contrast agents in general could...
easily be analyzed by MALDI imaging mass spectrometry. Its high sensitivity makes MALDI imaging an ideal tool for tissue-region-specific semiquantitative analysis of gadolinium-(III)-based contrast agents and therefore could be of great interest in the context of contrast agent development as it is already of interest in drug development.\textsuperscript{[5,14]} Quantification of gadolinium in tissue can also be performed using conventional mass spectrometry,\textsuperscript{[15]} but this leads to disruption of the tissue architecture and therefore it is impossible to generate information on distinct tissue regions. Also the mere quantification of gadolinium ions, for example, by ICP-MS may be misleading because the biological environment may lead to partial metal decomplexation. Autoradiography is a traditional method for quantitative detection of compounds, but the disadvantage of that method is that it requires radiolabeling of the compound of interest. Tissue-bound gadolinium can also be visualized on a microscopic scale using transmission electron microscopy,\textsuperscript{[16]} but this approach is not quantitative. Besides MALDI imaging mass spectrometry there are also new ambient ionization techniques which were introduced as the first mass spectrometry imaging method that provides highly specific molecular information (high resolution and accuracy in mass) at cellular dimensions (high resolution in space) and thus could be also very promising for analyzing contrast agents.\textsuperscript{[17,18]} A further aspect for a combined analytic approach of MRI and MALDI imaging data is pointed out in a recently published standard operating procedure for performing three-dimensional MALDI imaging-based semiquantification of contrast agents in tissue. This approach holds great promise for the in situ evaluation of various imaging agents administered in vivo.

Received: October 29, 2014
Published online: \textsuperscript{[19]}

Keywords: gadolinium · imaging agents · kinetics · mass spectrometry · biochemistry


\begin{figure}
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\includegraphics[width=\textwidth]{figure3}
\caption{Overview of analyzed gadolinium(III)-based contrast agents. Structural formula of analyzed compounds and resulting mass spectra of ex vivo treated tissue samples. The molecular ion signals are indicated by asterisks.}
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Contrast Agents: As a consequence of nonlinear correlation between the contrast agent concentration in tissue and the MRI signal obtained in vivo, quantification of certain biological or pathophysiological processes by MRI remains a challenge. MALDI imaging is a valuable tool for corroborating the in vivo imaging MRI signals. This method enabled in situ quantification of the contrast agent directly in tissue with high spatial resolution.