Magnetic resonance imaging study of diamagnetic and paramagnetic agents for optical clearing of tumor-specific fluorescent signal in vivo

Alexei A. Bogdanov, Natalia I. Kazachkina, Victoria V. Zherdeva, Irina G. Meerovich, Daria K. Tuchina, Ilya D. Solovyev, Alexander P. Savitsky, Valery V. Tuchin

Published online on: 09 Feb 2022

How to cite: Alexei A. Bogdanov, Natalia I. Kazachkina, Victoria V. Zherdeva, Irina G. Meerovich, Daria K. Tuchina, Ilya D. Solovyev, Alexander P. Savitsky, Valery V. Tuchin. 09 Feb 2022, Magnetic resonance imaging study of diamagnetic and paramagnetic agents for optical clearing of...
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Introduction

The task of efficient delivery of light into deep subcutaneous layers of biological tissue has been long recognized as a complicated task due to the presence of multiple light scattering layers and inclusions [1]. However, there is a multitude of in vivo diagnostic applications that could greatly benefit from improvements in the efficacy of light delivery (reviewed in [2–4]). They range from Raman spectroscopy-enhanced diagnostics [5] to photodynamic therapy [6]. The anatomy and structure of skin and underlying tissues such as subcutaneous fat and connective tissue such as fascia and muscle fibers create multiple barriers to light penetration deeper into the body due to strong scattering and absorption (reviewed in [7]). It is well established that the stratum corneum and epidermis contain scatterers that are particularly strong, while deeper layers of the skin, such as the dermis and hypoderm, have very large scattering coefficients which exceed the absorption coefficients of these tissues by 10–100 times [8, 9]. Therefore, optical clearing directed at better light delivery into the tissue is expected to be critical for improving in vivo imaging techniques due to its ability to decrease scattering of light in multimodal settings [10, 11]. Optical clearing of skin surface is known as one of effective approaches for increasing depth penetration and improving quality of optical diffusion tomography and multiphoton tomography. For example, it has been shown recently that the use of an X-ray contrast agent (Omnipaque®) shows promise as optical clearing agent for fluorescence diffusion tomography due to its ability to decrease background fluorescence of the upper layers of the skin [12].

It is believed that OC compositions work because of three main possible mechanisms that are acting in concert with each other in vitro, which primarily includes clearing during the immersion in OC solutions. The same factors may potentially be at work in vivo. The first of those mechanisms is based on equalizing the difference between the values of refractive indices between various components of biological tissues and tissue fluids due to the penetration of OC into the tissues [13]. The second effect is due to the dehydration of tissues caused by hyperosmotic effects of the components of OC compositions [14, 15]. The third hypothetic mechanism is based on reversible dissociation of collagen fibers due to OC components interfering with hydrogen bonds (which keep the collagen structure together), leading to lowering of light scattering of collagen by reducing size of scattering particles [16, 17]. Out of all available optical clearing compositions tested so far, those containing glycerol and/or DMSO are used in
Handbook of Tissue Optical Clearing

Materials and methods

Optical clearing solutions

We used topical applications of a diamagnetic mixture of glycerol/DMSO/water (70% glycerol, 5% DMSO, 25% water, ACS for analysis grade or similar), which was prepared by mixing, equilibrated for 1 h and used within 24 h for experiments in vitro and in vivo. Gadovist® (gadobutrol, 1.0 mmol/ml, Bayer HealthCare Pharmaceuticals, Germany) was used nondiluted as a paramagnetic OC solution.

Animal model

Animal studies were approved by the Ethics Committee of the Saratov State Medical University (Protocol No 8, April 10, 2018). Optical measurements were performed using athymic nu/nu mice (Research Center of Biotechnology of the RAS, Moscow, Russia). An animal model of cancer was obtained using a group of mice (n = 7, male) that were inoculated subcutaneously with epithelioid human carcinoma (HeP2 or A549) tumor cell suspension (10⁶ cells in 100 µl of DPBS) in the right flank. Cells were expressing a far-red cell marker protein Tag RFP [21, 22].

Optical imaging

For low resolution imaging, UVP iBox studio (Analytik Jena AG) was used for locating subcutaneous fluorescent tumor nodules. Fluorescence measurements were performed in anesthetized animals using an inverted microscope (Eclipse TE 2000 U, Nikon) equipped with DCS-120 Confocal Scanning System (Becker & Hickl GmbH, Berlin, Germany) setup, the WL-SC-480-6 supercontinuum laser, acousto–optic tunable filter AOTF-V1-D-FDS-SM (FIANIUM, UK), and HPM-100-40 hybrid detector (Becker & Hickl GmbH, Berlin, Germany). The signal was acquired using DCS-120 system with a beam splitter quartz plate and longpass HQ 550LP Chroma and bandpass 580BP40 Omega filters. TagRFP fluorescence was excited in vivo at 540 nm. To study the OC effect, the OC mixtures were applied on the skin surface above the tumors for 15 min. Thereafter, the excess of the mixture was carefully absorbed using a cotton swab and the images were recorded.

Magnetic resonance imaging

MRI was performed at IT (M3, Aspect Imaging, Shoham, Israel) with animals maintained at 37°C under gas anesthesia (1.5–2% isoflurane in oxygen) using body RF coil. Initially, pre-contrast images were obtained (T2w FSE pulse sequence: TR/TE = 4000/42, FOV 40x40, 128x240 matrix, ETL 8, NEX 4) selecting 4–6 1 mm-thick tomographic slices on scout images. Anesthesia-mediated breath frequency was kept at 30–40 b/min, and built-in respiratory triggering was used during the acquisitions to minimize motion artifacts. In some T2w FSE experiments, an inversion prepulse TI=120 ms was used for fat suppression. Diffusion-weighted pulse sequences (DWI) were applied in some experiments: TR/TE=1200/52.5 ms, b=133.8, 407.1, 760.3 and 975.6 s/mm², δ = 2 ms. Scanning time was approximately 5 min. 3D Gradient-echo (GRE) T1-weighted sequences (TR/TE=10/2.9 ms, FA 40, or TR/TE=60/2.9 ms, FA 20, NEX 7, FOV 40 × 40 mm, 256 × 256 imaging matrix) were used in experiments involving gadobutrol topical application. Scanning times were 6 min (short TR) and 24 min (long TR), respectively. The application of diamagnetic OC composition (n = 5) and gadobutrol (n =3) was performed in separate groups of animals using the same conditions as described above. The cradle with the coil was placed back in the bore of
Magnetic resonance imaging study and optical clearing study

The magnet and series of T2w FSE or 3D GRE images were acquired. Signal intensity changes were determined by 16-bit TIFF image analysis using Fiji/ImageJ. ADC values were calculated by fitting \( \ln(S/S_0) \) dependence vs. \( b \) factor, where \( S/S_0 \) is a ratio of diffusion-weighted to non-diffusion-weighted MR signals.

**Statistical analysis**

The differences in fluorescence intensity before and after OC were considered statistically significant (\( p < 0.05 \)) according to one-tailed nonparametric Mann–Whitney–Wilcoxon (MWW) t-test paired observation. MRI intensity data were analyzed using one-tailed nonparametric (unpaired) MWW t-test, \( n = 6–12 \) data points per group.

**Results and discussion**

**Fluorescence and MRI measurements.**

Optical clearing of skin using a diamagnetic glycerol and DMSO containing mixture

Subcutaneous tumors expressing red fluorescent TagRFP with an emission maximum of 584 nm [23] were used as a source of fluorescence in animal experiments. Cells expressing TagRFP can be imaged in vivo at 2–3 weeks after tumor inoculation into the flank of the animals [24]. The location of tumors was first identified by using a CCD camera with imaging at low resolution to verify the location, and then the animals were placed in a special cassette for macroscopic imaging of tumor nodules in more detail using the setup involving excitation with a tunable laser source and data collection using a time-correlated single photon counting detector. The animals were imaged to measure fluorescence intensity changes before and after the topical application and after the removal of the excess of glycerol/DMSO/water OC compositions. As shown in Figure 25.1 fluorescence intensity measurements provide semiquantitative assessment of OC efficacy; a comparison of the images of two closely located tumor nodules before and after OC indicate that fluorescence of both nodules increased after the application. These differences become especially apparent after the removal of the excess of OC composition (Figure 25.1 A, B, and C) and the observed differences could be compared quantitatively by measuring fluorescence intensity change along the trace shown in Figure 25.1 C. The representative trace plot shows that the tumor nodule that appeared only weakly fluorescent before OC showed a strong increase in fluorescence intensity (Figure 25.1).

Fluorescence intensity measurements allowed the enhancement of the background vs. enhancement of true sources to be compared, i.e. small areas occupied by red fluorescent cells within the area they occupy under the skin of mice. To assess the effectiveness of measurements performed over the skin surface of tumors in mice, the measurements were performed over time to determine fluorescence intensity of TagRFP before and after the optical clearing during tumor progressions. We determined that in those areas, the mean photon counts were increased by a factor that varied between 1 and 3 after a 15-minute optical clearing using a mixture containing glycerol.

**FIGURE 25.1** TagRFP red fluorescence imaging of two closely located subcutaneous Hep2 tumor nodules before (A) and after OC application (B) and after the removal of the excess of OC (C). The representative combination of fluorescence intensity profile plots corresponding to images A–C is shown in Figure 1D with two distinct areas (1 and 2) of regional fluorescence enhancement clearly visible. Nodules 1 and 2 and corresponding intensity peaks are numbered on the images accordingly. Pseudo color scale is shown in panel A.
and DMSO. The calculated ratios of maximum fluorescence intensity show that an optical clearing effect ($F_i/F_o$) was observed in 19 regions of interest (ROI) over a total of 22 total cases in three animals (Figure 25.2). The OC effect showed a tendency to increase over the course of tumor growth. This observed dependence on the time post-cell implantation may be a consequence of the increase in the density of fluorescent cells within the field of view and their relative proximity to the surface of the skin.

A variety of contrast-enhanced [25, 26] and noncontrast MRI techniques are available for measurements of small blood volume changes in the tissue [27–29]. As one of the potential options compatible with diamagnetic OC solutions low field proton ($^1$H) magnetic resonance imaging (MRI at 1T) T2-weighted pulse sequences were chosen as means to investigate the potential changes of skin/subcutaneous tissue properties before and after OC composition was applied. For MRI in mouse models of cancer, glycerol/DMSO OC composition was used and fast spin echo (FSE) pulse sequences with T2* relaxation properties of different tissues become more apparent. MR images acquired over time using T2w fast spin-echo (FSE) MRI pulse sequences with approximately 10–15 min delays between the acquisitions in the same animals shortly after optical imaging (Figure 25.3 A–D) showed significant quantitative differences between normalized MR signal intensities of axial peripheral tissue/skin slices before and after OC mixture applications in five animals with implanted ectopic subcutaneous tumor (Figure 25.3).

The application of OC composition onto the skin resulted in peripheral low-level “darkening” T2-weighted MR signal of the subcutaneous space below the treated skin, i.e. at the tumor periphery (Figure 25.3 A–D). Even though the differences in signal intensity caused by OC application were difficult to appreciate visually, the changes in MR signal were measurable by using local region-of-interest analysis. The differences in MR signal allowed the measurement of signal-to-noise (SNR) ratios (where $SNR = SI_{\text{mean}}/SD_{\text{noise}}$), an analysis that reflected normalized MR signal change differences which were negative in value after OC treatment. This indicated, as expected, the increase of the ROI-specific mean T2w MR signal. The obtained results obtained in a group of five animals with two different tumor types expressing TagRFP are summarized in Figure 25.3E. In all animals, SNR decreased after OC and in three out of five of the peripheral, subcutaneous tumors, the measured changes in SNR were statistically significant.

The measured change of SNR on T2-weighted MR images could potentially be a direct consequence of OC dilution in the skin, in subcutaneous tissue layers, and in the lymph of the subdermal extracellular space. To investigate whether dilution is causing the change in SNR, we conducted phantom experiments. After diluting OC and applying the same T2w FSE pulse sequences, we observed no statistically significant differences in normalized T2w MR signal in the case of signal intensities measured in 100% OC, in OC mixtures at various dilutions, and in pure water. Thus, this control MRI experiment showed that the observed differences in T2w MR signal intensity were not due to OC dilution when glycerol or DMSO concentrations decrease after penetrating the tissue.

Diffusion-sensitive MR pulse sequences may be applied to monitor $H_2O$ diffusion changes in tumor tissue vs. normal tissue before and after application of OC composition. Diffusion-weighted MR signal changes should be derived from the images obtained at $b > 100$ s/mm$^2$ in soft tissues [34]. The obtained apparent diffusion coefficients (ADC) were eliminated by using a 180° pulse of spin-echo sequence [33]. In T2 imaging strength of the MR signal is judged by hypointensity of images, since the signal intensity (SI)

$$SI = k[H(1 - e^{-TR/T1})]e^{-TE/T2},$$

where $H$ is the proton density and SI is determined by the factor $e^{-TE/T2}$ if $TR > T1$.

To apply FSE with MR signal change occurring primarily due to T2 relaxation, one should wait for most of the longitudinal magnetization to recover, and then apply a series of 180-degree radiofrequency pulses to collect series of echoes that emphasize T2 decay of magnetization (nuclear spin dephasing). With longer echo times, the differences in T2 relaxation properties of different tissues become more apparent. MR images acquired over time using T2w fast spin-echo (FSE) MRI pulse sequences with approximately 10–15 min delays between the acquisitions in the same animals shortly after optical imaging (Figure 25.3 A–D) showed significant quantitative differences between normalized MR signal intensities of axial peripheral tissue/skin slices before and after OC mixture applications in five animals with implanted ectopic subcutaneous tumor (Figure 25.3).

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**FIGURE 25.2** TagRFP fluorescence intensity change post implantation, showing the dependence of fluorescence intensity ratio changes (expressed as $F_i/F_o$ where $F_i$ is the initial maximum fluorescence intensity of given ROI before OC). In 19 ROI out of 22 total (n = 3/animals) a fluorescence intensity increase (i.e. $F_i/F_o$ >1) was detected indicating optical clearing effect. Individual observations for each ROI (black circles) and mean ± SD values are shown. The values plotted above the dashed line $F_i/F_o$ = 1 indicate the OC effect.
Magnetic resonance imaging study and optical clearing study compared by assuming monoexponential dependence on high $b$ factor values and calculated using the fitting the data using standard equation: $SI = S_0 e^{-bD}$, where $SI$ is the signal intensity with DW; $S_0$ is the signal intensity without DW; $b$ is the attenuation coefficient (s/mm$^2$); and $D$ (ADC) is the diffusion rate constant for the given voxel (mm$^2$/s).

Overall, the ADC values were larger in the tumors than in the normal muscle (Figure 25.4). This observation was expected since H$_2$O diffusion in cancer tissue as a rule is faster than in the normal muscle [35]. Also as expected, most of the skin/subdermal ROI showed an increase of ADC after OC, both in tumors as well as normal muscle (Figure 25.4). In the case of tumors, those changes were in most cases statistically significant. The penetration of OC components into the skin and possibly subdermal layer should induce the gradients of water diffusion because of water structuring and creation of differentially mobile water pools in the tissue.

Therefore, based on the results obtained without and with diffusion sensitization, the following explanation of observed MR signal differences could be put forward (Figure 25.5). Glycerol and DMSO are known to alter skin water content transiently due to the penetration into the skin and sequestration of water in dermis/epidermis [14, 36]. According to molecular dynamic simulations, higher glycerol content results in more extensive hydrogen-bond networks since the hydrogen bonding lifetime shows a tendency to increase as glycerol concentration increases while water molecules show faster reorientational motions than glycerol [37]. The consequence is shorter proton relaxation times of tissue/glycerol structured water and hydroxyls of glycerol molecules. One cannot exclude a potentially more complex interaction of both DMSO and glycerol with the tissue resulting in transient water depletion of the deeper subdermal layers and relative increase in blood volume (capillary density) in the peripheral tissue. The consequence of that would be an increase in deoxyhemoglobin content, and this effect may contribute to a paramagnetic effect and resultant shortening of water proton relaxation times. The same effect, i.e., relative increase of blood volume and local tissue concentration of paramagnetic deoxyhemoglobin, may
be caused by also by mild local hyperthermia due to OC interfering with normal heat exchange which may be followed by vasodilation.

**The use of MR contrast agents for OC**

The development of various cross-overs of imaging modalities providing anatomical and functional data (with the notable exception of radioisotope-based SPECT and PET combined with CT and MRI, such as SPECT/CT, PET/CT, and PET/MRI), is still at relatively early stages of conceptualization and preclinical translation [38–40]. However, such new multimodality hybrids may have important advantages over traditional in vivo imaging. One important and early recognized advantage of optical imaging combined with MRI is its safety due to the lack of exposure to ionizing radiation [41].

The progress towards acquisitions of simultaneous or back-to-back MRI (for anatomy and functional imaging) and optical (functional and molecular) images and data sets has so far been limited. It was recognized early on that MRI is capable of providing both structural and metabolic information, while optical tomography delivers specific and quantitative data reflecting anatomy and metabolism in experimental oncology [42, 43]. Several previous reports suggested that multimodality imaging integrating, for example, described multispectral frequency domain diffuse optical tomography with MRI, by which MRI-generated maps may be used to instruct the algorithms involved in optical reconstructions. This approach afforded an estimation absorption coefficient of an inclusion in a mouse-size phantom with 11% error [44]. Furthermore, the application of fluorescence lifetime imaging mode enabled improvements in MRI-assisted tumor identification in the liver of experimental animals [45]. These achievements, though incremental in nature, also spurred activity in multimodality optical/MRI contrast agent development [46, 47]. Nevertheless, the limitations in efficiency of light delivery to deeper tissues and resultant complexity of imaging data interpretation are major obstacles on the road to comprehensive integration of optical imaging with other modalities. The use of optical clearing compositions with paramagnetic properties that can be detected by MRI may be viewed as a measure directed at improving the prospects of optical and MR image integration.

Paramagnetic clinical MR contrast agents (MR CA) are highly soluble and thermodynamically stable low molecular weight chelates of Gd(III), which have been in clinical use for contrast agent-assisted MRI since the 1980s due to their ability to shorten relaxation rates of water protons (relaxivity) [48–50]. For in vivo IV use, the high-relaxivity Gd(III)-based MR CA are supplied formulated at high concentrations (at 0.5M or 1M). These MR CA are hyperosmolar (1300–1600 mOsm) and usually viscous (2–5 Pa s) solutions that do not absorb light in the visible/near infrared ranges of the spectrum [51]. In this regard, Gd(III)-based compounds have advantages as potential OC agents over experimental iron (III)- and manganese (II)-containing low-molecular-weight experimental paramagnetic contrast agents [52, 53] because the majority of chelated iron (III) and manganese (II), i.e. high relaxivity complexes absorb light in the visible range of the spectrum. The main rationale behind the idea to assess the OC properties of clinical MR CA was in enabling multimodal imaging in vivo. If in similarity with X-ray contrast agents, local application of paramagnetic gadolinium-based contrast agents leads to optical clearing of the skin, it could be also feasible to perform contrast-enhanced MR imaging to determine the time course of MRI contrast agent penetration into the skin. It would be also feasible to collect information contained in MR images that reflects local anatomy and is useful for correcting optical imaging results to account for the influence of fine structure of peripheral layers of normal subcutaneous tissue, or a peripheral lesion.

The initial optical clearing feasibility work using MR CA was conducted by optimization of contrast agent use with the following tests in vitro involving mouse skin samples: 1) measurements of skin thickness with OCT, and 2) measurements of a gain in transmittance. These experiments showed, in the
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case of gadobutrol (Gadovist®), a 12-fold increase in transmittance of light in a broad spectral range during the period of 10 min after application of MR CA on the skin ex vivo [19]. The OCT images showed an increase of light beam probing depth with a better contrast of the skin sample images as a result of MR contrast agent mediated optical clearing. In addition, optical coherence tomography revealed improved images reflecting skin microarchitecture, which were obtained after gadobutrol application (Figure 25.6A). After immersion of the skin in MR CAs, the OCT scans showed less scattering. Overall, results obtained by using gadobutrol indicated that this highly concentrated CA was superior to other CAs tested, if compared by collimated transmittance increase after application onto the skin samples. For example, gadobutrol mediated transmittance (Figure 25.6B) was five times higher than that of GdDTPA (Magnevist®) or GdDOTA (Dotarem®) over a period of 1 h post treatment [19].

Reliable MR imaging of various phase and tissue boundaries such as skin–air interfaces may be complicated, and the resultant images are generally prone to boundary artefacts [54, 55]. In addition, high concentrations of gadolinium-based MR CA generally cause strong magnetic susceptibility artefacts [56]. Due to the nature of these artefacts, the detection of locally applied concentrated MR CA is uncomplicated. However, gathering quantitative useful information reflecting the volume and penetration of MR CA into the tissue by measuring MR signal intensity changes over time strongly depends on chosen pulse sequence. This is why the experiments were initially conducted by following Gd-based contrast resulting from the influence of high Gd(III) magnetic susceptibility (\(\Delta \chi\)) on SNR.

Fast MR image acquisition mode using GRE pulse sequence with high T1-weighting (short TR and minimal TE) resulted in local image distortion by gadobutrol (at 1 M gadolinium) due to water proton relaxation signal affected by local susceptibility with low SNR due to high image noise (Figure 25.7). T1-weighted GRE with higher TR/TE ratio resulted in lower MRI acquisition, high T1 weighting, less distortion, higher image contrast, and higher SNR due to lower noise (Figure 25.7). During the course of gradient echo (GRE) T1 weighted pulse sequences acquisition, it became very apparent that by measuring signal loss, one could follow the fate of the contrast agent on the skin. This can be achieved by using either MRI highly sensitive to local magnetic susceptibility change (\(\Delta \chi\)), or less sensitive which is associated with less signal loss. In the latter case, the overall image distortion due to high \(\Delta \chi\) is less pronounced.

Indeed with the MR signal loss in the case of a solution high magnetic susceptibility causes magnetic field inhomogeneity that results in dephasing of nuclear spins during data acquisition [57]. This leads to a local loss of signal, which is the proportional to voxel size and TE. As any change of MR signal local signal loss can be quantified. Figure 25.7 illustrates the distribution of contrast agent on the surface of the skin and peripheral tissue layers. Skin signal intensity drops after the application of gadobutrol (i.e. the contrast agent showing the highest efficacy of optical clearing in vivo). The observed effect of gadobutrol is still detectable and the signal loss is still present on MR images even at 40 min after CA application.

Signal loss due to 100% gadobutrol application onto the skin surface above ectopic tumors in live mice was measurable with both TR = 10 and TR = 60 ms (Figure 25.8 A and B). It is apparent that GRE acquisitions at shorter TR (Figure 25.8A)
showed a time-dependent decrease of MR signal, reflecting gadobutrol-mediated signal loss in the skin and peripheral tissue (a 2–3 mm thick layer). Over time there was also a small increase in the average SNR observed in the contralateral normal muscle, indicating that the contrast agent may have traversed the skin barrier and was enhancing contralateral skeletal muscle due to the dilution of MR CA in the lymph/extracellular fluid. With GRE acquisitions at longer TR = 60 (Figure 25.8B), the time resolution of imaging was insufficient to track the dynamics of signal change. However, the MR signal intensity loss in the skin (closed circles) is apparent at the endpoint (44 min) after the completion of OC skin treatment, and this change was statistically significant (p < 0.05). The OC amplification apparently resulted in no change of SNR in the muscle (open circles) using this pulse sequence parameter. It is possible that unlike extreme T1-weighting scenario at TR = 60, the enhancement of the contralateral muscle due to transfer the gadobutrol through the skin was either very low, or this effect
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was measurable only at the early time points in the beginning of the experiment because of the short half-life of gadobutrol in mice (about 15–20 min after intravenous administration [58]). In effort to improve the observed OC effect of gadobutrol, an attempt to obtain a system similar to glycerol/DMSO mixture was made by replacing glycerol (the main component of the OC composition) by gadobutrol for the purpose of improving both its transdermal transport and optical clearing. In experimental setup essentially identical to the one used for assessing OC effect of glycerol and DMSO in mouse ectopic tumors (see Figure 25.1), a consistent increase of fluorescence intensity of TagRFP tumor marker was observed over time during the period of 1 h after OC application (Figure 25.9A). Fluorescence intensity quantification showed that OC mixtures containing DMSO resulted in faster clearing, but the overall fluorescence intensity increase effect was similar in terms of red fluorescent protein intensity increase over the period of 1 h. Both CCD measurements and more sophisticated photon counting using a DCS-120 detector showed similar trends in terms of intensity change.

FIGURE 25.9 OC effect of a mixture of gadobutrol/water/DMSO (75:25:5 by volume) after application on the surface of the skin of athymic mice bearing superficial Hep2-TagFRP tumors before, and at 15, 30, and 60 min after application (A). B – Comparative fluorescence intensity measurements of TagRFP emission achieved by using a photon integration mode of DCS-120 detector after OC with 100% gadobutrol (black columns) and gadobutrol/water/DMSO (75:25:5) (gray columns).

FIGURE 25.10 Pseudo-color Z-stack image averages (n = 4, 1 mm slices) obtained using T1-weighted pulse sequence (TR 60/TE 3, FA FOV 40 × 40 mm, 256 × 256 matrix, NEX 5, FA 20°. At 18 min, there is local signal loss in the area above the tumor due to the application of an OC composition consisting of gadobutrol/water/DMSO (70:25:5%).
The effect of OC composition containing gadobutrol and DMSO on MR effect was studied in more detail by applying a 3D gradient echo pulse sequence to measure the signal in the skin and underlying tissue. The local changes of MR signal i.e., its decrease followed by a subsequent increase became more apparent after collecting four consecutive tomographic image slices and generating a z-stack with MR signal averaging (Figure 25.10). The averaged z-stack images allow more precise tracking of the signal change over time in vivo; as expected, the initial decrease in MR signal intensity due to the magnetic susceptibility effect was followed by a signal loss in the interstitium, suggesting that some of the locally applied gadobutrol was absorbing through the skin, potentially aided by DMSO at approximately 40 min after application. After additional 60 min, the increase in MR signal intensity was observed presumably due to dilution in the interstitium, and soon after the CA started to disappear from the tumor due to removal from the imaged tissue volume, and this effect resulted in the loss of MR signal intensity.

Conclusions

In summary, magnetic resonance imaging is complementary to optical imaging of fluorescent protein marker in that it enables tracking the fate of OC compositions on the skin surface and underlying peripheral tissue. Both diamagnetic and paramagnetic solutions with OC properties induce changes in local water proton relaxation properties, and resultant changes in MR signal-to-noise ratios can be followed over time to improve OC protocols. The ability of MRI to resolve kinetics of local changes due to OC-mediated changes in relaxivity of the tissue is strictly pulse sequence–dependent. It is likely that T2w MR hypointensity increases in the case of diamagnetic OC composition tested potentially may be caused by the minute increase of deoxyhemoglobin content due to skin permeability and water immobilization and/or displacement. The clinical MR contrast agent with the highest concentration (gadobutrol) resulted in an OC effect which was readily detectable by optical coherence tomography, collimated transmittance increase, and fluorescence intensity measurements in vivo. MR imaging of topical gadobutrol application suggests transient penetration into the skin, which is enhanced in the presence of DMSO and results in transient changes in tissue signal after applying T1-weighted gradient-echo pulse sequences.

Acknowledgments

This work was supported by the Government of the Russian Federation (grant no. 14.W03.31.0023 to support scientific research projects implemented under the supervision of leading scientists at Russian institutions and Russian institutions of higher education).

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