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ANALYSIS

CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING OF LUNG CELL CULTURES

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Abstract: Lung cancer is one of the most common types of cancer diagnosed, and the development of methods to image diseased lung tissue by MRI is of utmost importance. Contrast-Enhanced Magnetic Resonance Imaging (CE-MRI) was used to noninvasively evaluate spin-spin relaxation time, T_1, of lung cell cultures infused with various clinical gadolinium-based contrast media for imaging. In this study we used a clinical 1.5 Tesla scanner and the contrast agents: Omniscan, MultiHance, Gadovist, and ProHance. A significant five-fold reduction of T_1 relaxation time was obtained.

Keywords: contrast media, magnetic resonance imaging, gadolinium, lung cancer, cell cultures

Due to the fact that lung cancer has a very poor prognosis, new diagnostic techniques to image early stages of lung tumors may improve therapeutic outcomes as the current lung tumor survival rate is very low (1). The diagnosis of lung tumors often requires invasive removal of tissues for histological analyses. From 1972, Magnetic Resonance Imaging (MRI) has been extremely successful in diagnosing disease in soft tissue (2). MRI can be an extremely useful non-invasive clinical tool for monitoring the therapeutic effect of anti-cancer treatments. However, MRI of lung tumors is very difficult due to the low spin density of hydrogen nuclei in the lungs. This situation creates the susceptibility effect of lung tissue-air boundaries (3). Recently, the use of laser hyper-polarized (HP) noble gas isotopes such as ^{3}He and ^{129}Xe has been of increasing interest for use in a variety of MRI applications including imaging lung tissue. Conventional MRI sequences are usually based on the fact that the polarization state is initially in equilibrium and will recover after RF pulsing. Since HP gas is not an equilibrium state, imaging procedures must be adapted accordingly, and the expense of the required laser optical pumping facility limits the potential of HP MRI in clinical medicine applications.

CE-MRI is based on a procedure to shorten spin-lattice relaxation time (T_1) and thereby increase the repetition rate (TR). A shorter T_2 can result in lower sensitivity. Contrast agents have found multiple applications in diagnostic medicine because of their ability to improve the quality of contrast. Due to the accumulation of the contrast medium in tissue and the pulse sequence performed, we can obtain two types of images: hyperintensive (T_1 dependent images) and hypointensive (T_2 dependent images). In Poland, there are only a few MR research sites and only a fraction of these sites is studying methods in quantitative MRI as in clinical practice, the quantitative approach is not generally utilized. However, medical MRI and experimental MRI are both based on quantitative measurements. The main reasons for this are time constraints and possession of special knowledge for measurements. The use of quantitative measurements is extremely important in achieving a better contrast between healthy and pathological different tissues in clinical diagnosis. The main action of contrast agents is to reduce T_1 relaxation time in tissue. As a result, tissues that have absorbed the contrast agent give a stronger signal in the images (4, 5). On the pharmacy market, gadolinium-based contrast agents come in two forms: ionic and

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non-ionic. It should be noted that contrast agents used in CE-MRI are much less likely to cause allergic reactions. In this work, we imaged lung cell culture *in vitro* with clinical contrast agents to evaluate T_1 relaxation time. In this manner, we would like to present results that suggest that CE-MRI can be useful in the imaging of *in vitro* lung cell culture.

EXPERIMENTAL

Materials

The A549 cell line (ATCC® CCL-185 ™) was from the ATCC (American Type Culture Collection (ATCC®) MANASSAS, VA, USA) and was purchased through LGC (Łomianki, Poland). Culture medium Dulbecco's Modified Eagle's Medium, Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham Penicillin-Streptomycin-Neomycin Solution Stabilized were purchased from Sigma-Aldrich (Sigma Aldrich, MO, USA). Fetal Bovine Serum was purchased from Biochrom, Germany. Accutase Cell Detachment Solution was from Corning (Corning, NY, USA) and tissue culture flasks from ThermoFisher Scientific (ThermoFisher Scientific, MA, USA). Contrast agents such as Omniscan was purchased from General Electric Healthcare (Oslo, Norway), MultiHance was purchased from Bracco Imaging (Bracco Imaging Polska Sp. z o.o. Warsaw, Poland), Gadovist was purchased from Bayer HealthCare Pharmaceuticals (Berlin, Germany).

Cell cultures

The A549 cell line (ATCC® CCL-185™) were cultured under standard conditions: temperature 37°C, 5% CO_2 and 95% humidity. The culture medium consisted of Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, MO, USA), Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham (Sigma-Aldrich, MO, USA), Fetal Bovine Serum (Biochrom, Germany) and Penicillin-Streptomycin-Neomycin Solution Stabilized (Sigma-Aldrich, MO, USA). The culture of lung cancer cells was passaged in 3rd day with the use of Accutase Cell Detachment Solution (Corning, NY, USA) into 70 mL tissue culture flasks (ThermoFisher Scientific, MA, USA). This study used lung cancer cells A549 prepared in 4 separate samples indicated as Cells 1, Cells 2, Cells 3, and Cells 4. Each sample was centrifuged and the final density was approximately 10^{10} cells/mL. The contrast media used consisted of 50 µL Omniscan in 1 mL water (25 mmol/L), 50 µL MultiHance in 1 mL water (25 mmol/L), 50 µL Gadovist in 1 mL water (50 mmol/L) and 50 µL ProHance in 1 mL water (25 mmol/L). These are gadolinium-based agents used in clinical diagnostics.

Available pharmaceutical contrast agents are:

Omniscan (Fig. 1), also called Gadodiamidum, is produced by General Electric Healthcare (Oslo, Norway). The active substance of Omniscan is gadodiamide (GdDTPA-BMA) available at a concentration of 287 mg/mL (0.5 mmol/mL) and the other ingredients are sodium hydroxide or hydrochloric acid for pH adjustment.

MultiHance (Fig. 2), also called Dimeglumini gadobenas, is produced by Bracco. The active substance of MultiHance is gadobenic acid, 1 mL solution for injection contains gadobenic acid at a concentration of 334 mg (0.5 mmol/mL) in the form of gadobenate dimeglumine (529 mg/mL).

Gadovist (Fig. 3), also called as Gadobutrolum, is produced by Bayer. The active substance of Gadovist is gadobutrol at a concentration of 529 mg/mL (0.5 mmol/mL), and other ingredients are calculobutrol sodium trometamol and 3.6% hydrochloric acid in water for injections.

ProHance (Fig. 4), also called Gadoteridolum, comes from Bracco. The active substance is gadoteridol at concentrations of 279.3 mg/mL (0.5 mmol/mL). In addition, ProHance contains Calteridol calcium salt, tromethamine, hydrochloric acid or sodium hydroxide for pH adjustment, and water for injections.

Magnetic Resonance Imaging

All MRI scans were performed with an Optima MR360 magnetic resonance spectrometer from General Electric Healthcare (Milwaukee, Wisconsin, USA). The camera was supported by the SV23 software version. Lung cancer cells in samples A-D were placed in an MR tunnel and subjected to a series of measurements to determine relaxation times T_1 and T_2. The lung cancer cells in the vials were placed on the FLEX Small transceiver. To perform the measurements, the Fast Spin Echo (FSE) sequence was used with the following parameters: FOV field of view = 10 × 10 cm; Matrix = 320 × 224; NEX = 2.0; Slice Thickness = 1.0 mm; spacing = 0.5. TR time varied in the range of 48 ÷ 15000 ms (in the following steps: 48, 50, 100, 150, 200, 300, 500, 1000, 1500, 2000, 3000, 5000, 10000, 15000) and TE was 3 ms. To determine T_1 relaxation time of cancer cells with the contrast agent, measure-

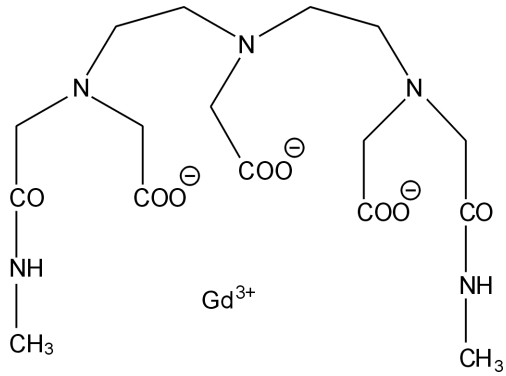

Figure 1. Omniscan.

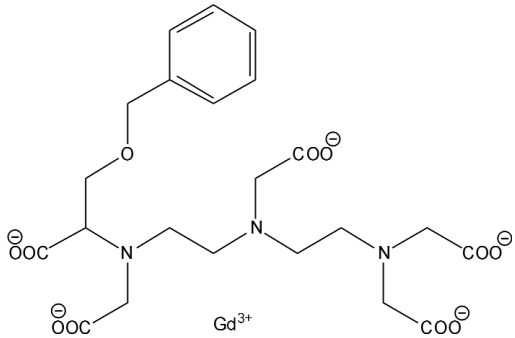

Figure 2. MultiHance.

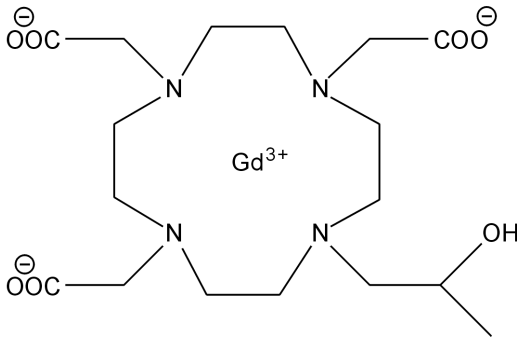

Figure 3. Gadovist.

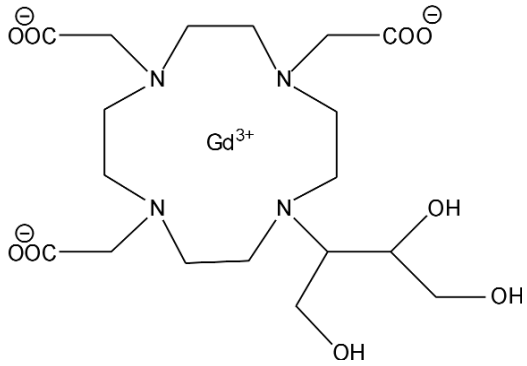

Figure 4. ProHance.

Table 1. Concentration of contrast agents for steps 1-5 in mmol/L.

Contrast Agent	Step 1 (mmol/L)	Step 2 (mmol/L)	Step 3 (mmol/L)	Step 4 (mmol/L)	Step 5 (mmol/L)
Omniscan	25	25	23.8	22.7	20
MultiHance	25	25	23.8	22.7	20
Gadovist	50	50	47.6	45.5	40
ProHance	25	25	23.8	22.7	20

ments were performed in 5 contrast-dilution steps for Omnicsan, MultiHance, Gadovist, and ProHance, respectively. All prepared lung cell culture samples were imaged and T_1 relaxation time was determined before contrast agents were added. The addition of contrast agents to lung cells culture was performed as follow: **Step 1-** 50 µL of the contrast in 1 mL of H_2O; **Step 2-** after 24, the measurements of step 1 was repeated; **Step 3-** 50 µL of H_2O was added to step 2 sample; **Step 4-** 100 µL of H_2O was added to step 2 sample and **Step 5-** 250 µL of H_2O was added to step 2. The contrast agent concentrations for steps 1-5 are listed in Table 1 in mmol/L.

Statistical analysis

Results are presented as the mean ± standard deviation (SD) of six independent experiments.

RESULTS AND DISCUSSION

The relaxation time of lung cancer cells: cells 1, cells 2, cells 3 and cells 4 were 1017± 11 ms, 1485 ± 14 ms, 1092 ± 11 ms and 1378 ± 13 ms, respectively (Fig. 5A). Then, samples cells 1, cells 2, cells 3 and cells 4 were treated with 50 µL of Omniscan, MultiHance, Gadovist and ProHance in 1 mL water, respectively (Figure 5B). The samples were measured again after 24 h. In the next step, the same samples were tested with 100 µL of H_2O added (Fig. 5 C).

Step 1: 50 µL of the contrast agents Omniscan, MultiHance, Gadovist, and ProHance was dissolved in 1 mL of H_2O and added to the cells. T_1 relaxation times of pure contrast agents Omniscan, Multi-

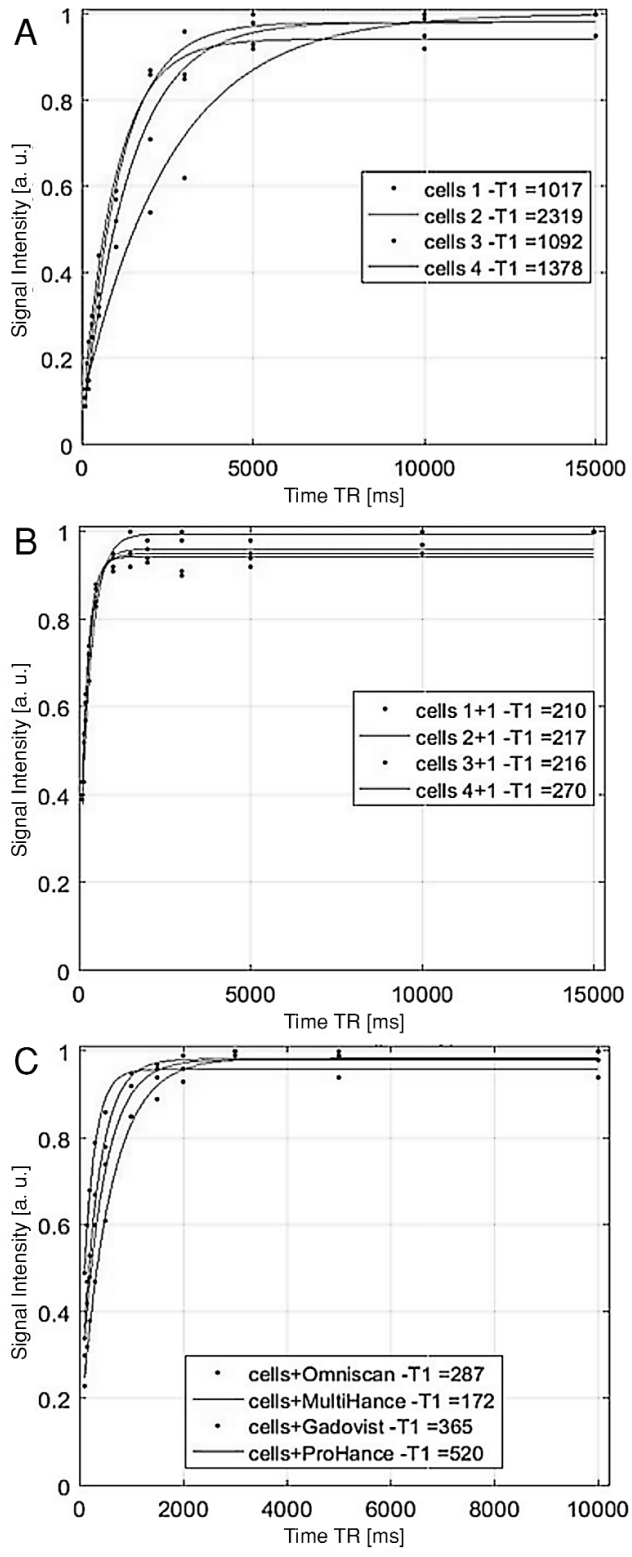

Figure 5A-C. Signal intensity (a.u.) as a function of time (ms) for 5A) Cell cultures 1-4 without contrast agent, 5B) Cell cultures 1-4 treated with 1 mL of water containing 50 µL of Omniscan, MultiHance, Gadovist and ProHance, 5C) Cell cultures 1-4 treated with 1 mL of water containing 50 µL of Omniscan, MultiHance, Gadovist, and ProHance plus 100 µL of H_2O added to each sample.

Hance, Gadovist and ProHance were 210 ± 4, 217 ± 4, 216 ± 4 and 270 ± 5 ms, respectively. The relaxation times show that the maximum discrepancy was between the Omniscan and ProHance contrast agents equal to 60 ± 2 ms. The presented data are mean values ± standard deviation of six independent experiments.

Step 2: The measurement was repeated after 24 h. For the cells treated with Omniscan contrast agent, the difference is a 77 ± 5 ms increase, for Gadovist the relaxation time T_1 increased by 55 ± 5 ms, and for ProHance it increased by 220 ± 5 ms. In the case of MultiHance, the result dropped by 45 ms, when compared to step 1.

Step 3: A 50 µL of water was added to each sample and the T_1 relaxation time was again determined from six independent experiments.

For three contrast agents, a decrease in the T_1 time was observed. In the case of Omniscan, the value of T_1 decreased by another 25 ± 5 ms, for Gadovist 74 ± 5 ms, for ProHance it decreased by 245 ± 5 ms. In the case of MultiHance, the value increased by 18 ms.

Step 4: A further 100 µL of water was added to the same samples. In the case of Omniscan, the observed difference in T_1 relaxation time was a 17 ms increase. However, in the case of the other three contrast agents, the values increased for MultiHance by 10 ± 5 ms, for Gadovist by 101 ± 5 ms, and for ProHance by 121 ± 5 ms.

Step 5: A 250 µL of water was added, an increase in the T_1 relaxation time value was observed for all four contrast agents. For Omniscan it increased by 56 ± 5 ms, for MultiHance by 32 ± 5 ms, for Gadovist by 107 ± 5 ms, and for ProHance the highest increase of 214 ± 5 ms was observed.

Figure 6 below shows the changes in T_1 relaxation times for the prepared samples after 5 steps included in Table 2. The various changes in T_1 measurements are presented in Figure 6.

In a review by Dietrich et al., T_1 relaxation time in the lung parenchyma in healthy people in the 1.5 Tesla field was described. It was determined that the average T_1 relaxation time for healthy lung tissue at 1.5 Tesla was approximately 1200 ms with a standard deviation of 150 ms (6). Paramagnetic contrast substances are well-soluble in water, in addition, they have the ability to completely absorb from the digestive system and circulation into the intercellular spaces. In addition, no drug interactions have been reported so far. Iron-based shading agents are most often used to determine focal lesions in the liver and other gastrointestinal pathologies. For liver pathology, manganese-based contrasts are also used, but unlike iron-based contrasts, the goal is to make healthy tissues visible. In contrast, agents based on

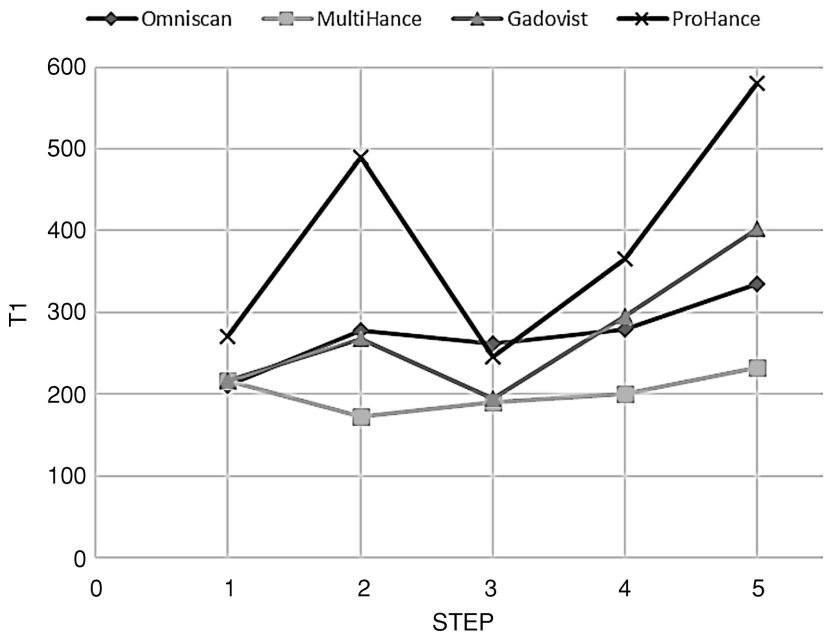

Figure 6. T_1 relaxation time corresponding to steps 1-6.

dysprosium are used in imaging of heart and brain diseases. Generally, positive contrast agents in medical diagnostics are used to diagnose diseases of the central nervous system, heart, digestive system, with particular emphasis on the liver and kidneys as well as the musculoskeletal system. In addition, the whole group of positive contrast agents can be divided into extracellular contrast agents, mainly used for angiographic studies (showing stenosis or aneurysms) and intracellular, which are absorbed into the cells by binding to proteins. Intracellular shaders are invaluable for imaging small vessels. They are most often used in imaging of tumors, due to the fact that with their use, the doctor can realistically assess the size of the tumor and its location. In addition, they are able to visualize all kinds of inflammatory changes and ischemia. The second group of shading agents are negative contrast agents that reduce the intensity of the signal in the tissue (reduce the T_2 relaxation time). Unlike positive contrast agents, the MR image is darker. One of the most commonly used agents is magnetite, it is administered orally or intravenously, usually for liver tests. Numerous scientific studies present the use of contrast agents in scientific research using methods of determining relaxation times. The longitudinal and transverse relaxation times T_1 and T_2 are the times after which they reach a certain defined value. For T_1 it will be about 63% of the total magnetization value, while for T_2 it will be 37%. By browsing PubMed's scientific database, we can find papers describing the use of contrast media for research on cell cultures (7-10). Table 3 presents MRI study with contrast agents used in clinical diagnostics. Contrast agents used in MRI have found wide application due to the properties of improving

Table 2. Results of T_1 relaxation times for lung cancer cell cultures cells 1-4. The presented data are mean values ± standard deviation of six independent experiments.

	Omniscan	MultiHance	Gadovist	ProHance
	cells 1	cells 2	cells 3	cells 4
	T_1 (ms)	T_1(ms)	T_1(ms)	T_1(ms)
	1017 ± 7	1485 ± 9	1092 ± 6	1378 ± 7
Step 1	210 ± 4	217 ± 4	216 ± 4	270 ± 5
Step 2	287 ± 1	172 ± 3	268 ± 2	490 ± 2
Step 3	262 ± 2	190 ± 2	194 ± 3	245 ± 3
Step 4	279 ± 2	200 ± 2	295 ± 2	366 ± 1
Step 5	335 ± 2	232 ± 2	402 ± 1	580 ± 2

Table 3. A review of CE-MRI studies.

Authors/year	Ref	Experiment	Result
Sobhani T. et al. 2019	(11)	Manganese-zinc ferrite nanoparticles and L929 cell lines	Longitudinal relaxivity 85.5 mM^{-1} s^{-1}
Haedicke I.E. et al. 2019	(12)	Manganese porphyrin contrast agent	Four-fold lower T_1 relaxation time.
Huang Q. et al. 2019	(13)	Iron-doped carbon quantum dots for 3.92 $mM^{-1}\cdot s^{-1}$	Longitudinal relaxivity
Li X. et al. 2019	(14)	Silane-polyethylene glycol modified HS-Fe (HS-Fe-PEG) NPs	T_2-weighted MR imaging
Wang J. et al. 2019	(15)	Glucose and levofloxacin	Longitudinal relaxivity 5.79 $mM^{-1}\cdot s^{-1}$
Yang Y. et al. 2014	(16)	Gd-DOTA	Longitudinal relaxivity 7.55 mM-1 s^{-1}
Yuan Z. et al. 2007	(17)	Gd-DTPA	Gd-DTPA enhanced MRI
Crisci R. et al. 1997	(18)	Gd-DTPA	Gd-DTPA enhanced MRI.
Carney C.E. et al. 2015	(19)	Gd-DTPA	Gd-DTPA enhanced MRI

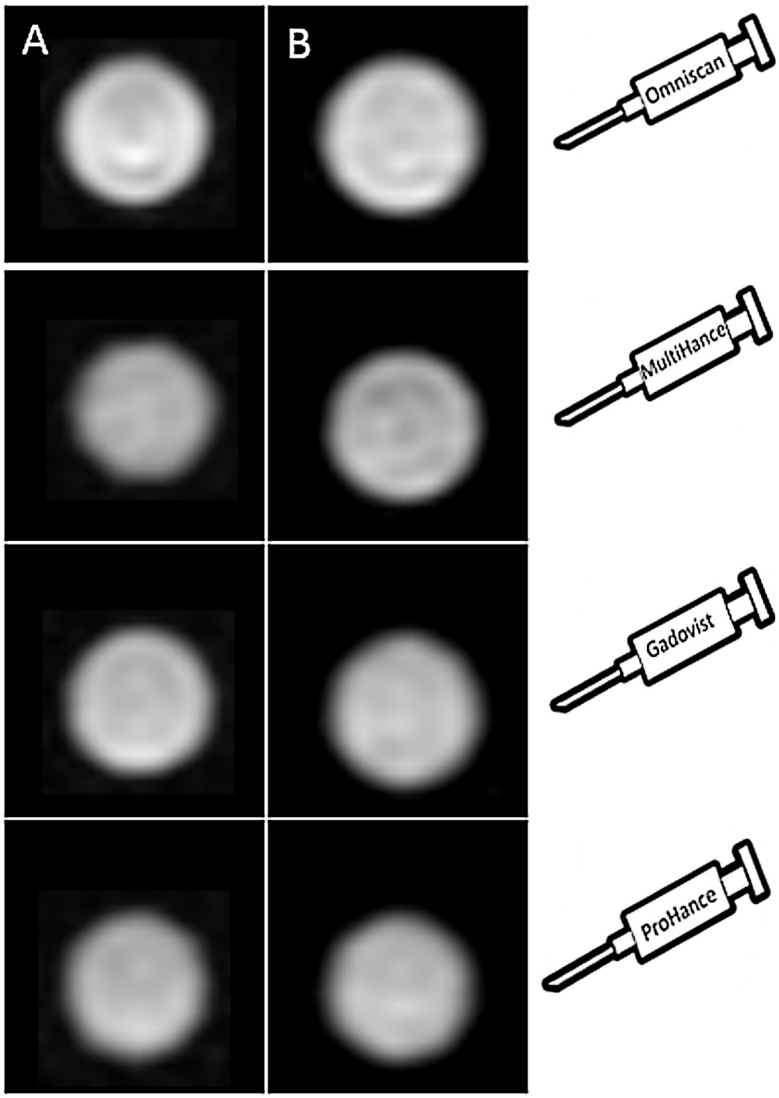

Figure 7. MRI images of lung cancer cell culture A) before treatment and B) after treatment with contrast agents Omniscan, MultiHance, Gadovist, and ProHance.

the quality of diagnostic images. In diagnostics, it is important to obtain a satisfactory contrast. One of the most commonly used contrast agents in clinical practice is gadolinium, which is based on one of the metals from the so-called "rare earth" group, from the lanthanide group. These are metals with paramagnetic properties, they have magnetic properties in the external magnetic field. The main principle of gadolinium is to reduce the T_1 relaxation time.

Currently, new and easier ways of identifying drug forms are being sought. In our view, MRI techniques are needed to provide more efficient early-stage diagnosis, predict disease course, and develop a more effective therapy. We propose a qualitative and quantitative study of important agents such as contrast agents (Fig. 7) to increase contrast agent specificity. We want to develop MRI methodology to test new contrast agents before they are used in the clinic. However, we want to establish our technique by using available and known contrast agents. We hope that our pre-clinical methodology will be helpful for clinical studies and pharmaceutical practices. One of the initial assumptions of this project is that we will use MRI measurements to model drug formulations. These proposed measurements will allow us to assess the physic-chemical properties of already existing drugs for the benefit of improving medicine. Qualitative and quantitative analysis of

drugs using MRI will enable to study molecular properties.

The interdisciplinary aspect of this project will allow for simultaneous spectroscopic and imaging studies. At each stage, optimization of conditions and parameters of imaging, spectroscopic and analytical measurements will be performed. Estimation of change in drug form and stability of the drug substance can be analyzed by MRI as a function of pH, temperature and light exposure. One of the goals of this project was to use the 1.5 Tesla MRI scanner for molecular research on relaxation measurements. We hope that clinicians working in the field of in vitro contrast-enhanced MRI for lung cancer diagnostics will be able to use the generated data to further improve diagnostic methods. In addition, we hope that this study of contrast agents in cell culture will inspire other researchers to develop MRI methodology for future clinic studies.

CONCLUSIONS

With MRI, we are able to provide non-invasive ways to visualize molecular events during controlled-release dosage. Using MRI, we also have a tool that is helpful in understanding the processes that occur in drug metabolism. This may have a significant impact on the development of a new generation of pharmaceuticals such as contrast agents.

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Conflicts of interest

The authors declare no conflict of interest.

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