Determination of a preclinical protocol for quantitative measurements of perfusion and permeability in the rat lung using dynamic contrast enhanced-MRI

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ABSTRACT

Quantitative values of pulmonary perfusion and permeability are useful in the diagnostics of common pulmonary diseases. Furthermore, they can be employed to quantify changes after therapeutic intervention in preclinical drug research. These parameters can be measured by dynamic contrast enhanced MRI using an intravenous injection of a paramagnetic contrast agent. MR imaging of rodents include extra requirements such as better spatial and temporal resolution. Therefore, protocols for DCE-MRI measurements of perfusion and permeability in the rat lung were developed in this work. The contrast agent concentration was optimized for the perfusion protocol. Since the contrast agent Gd-DTPA has only a linear signal to contrast agent concentration relationship for a specific range of concentrations, this range was determined for the current situation. The range was first investigated in vitro and after that linked to the in vivo situation by a combined experiment placing in vitro samples in FOV during a DCE-MRI measurement in rat. Unexpected factors affected the combined experiment which consequently motivated a direct in vivo measurement. The results from the in vivo measurement were largely affected by inflow and 3D scanning should therefore be used in order to reduce these effects. However, it was not possible to sample as rapid 3D sequences as needed for the perfusion measurements with the present technical limitations. Even though it was not feasible to perform perfusion measurements in this report the major pitfalls in performing pulmonary perfusion were investigated. Permeability is a slow process having less demand of good temporal resolution. 3D scanning was therefore used for the permeability measurements. After the optimization of the acquisition method, contrast agent concentration and flip angle, the resulting protocol included an untriggered 3D sequence with flip angle of 20 ° and contrast agent concentration of 0.4 mmol/ml. A pilot study investigating changes in pulmonary permeability between healthy rats and rats with lung inflammation was performed using the resulting permeability protocol. It showed that the protocol was successfully implemented with regards to acquisition method and relative enhancement. However, no changes in permeability could be seen in the pilot study and consequently the method and analyses require further evaluation.
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1 INTRODUCTION

Functional pulmonary imaging is important for the diagnostics of many pulmonary diseases. There are three feasible techniques for functional pulmonary imaging today; nuclear medicine, computed tomography (CT) and magnetic resonance imaging (MRI). The primarily advantages of MRI compared to nuclear medicine are the absence of ionizing radiation and a considerably better spatial and temporal resolution (Ohno et al., 2004). CT is the standard for lung morphology in clinical imaging but functional CT imaging for clinical applications is not as developed. Techniques as multiple detector computed tomography (MDCT) and the use of contrast agents have thus enabled functional CT imaging. However, the radiation dose becomes considerably high in dynamic CT scanning and is therefore a limiting factor.

MRI includes no ionizing radiation and has both morphologic and functional possibilities, which in the future would make it possible to perform one combined scan giving both morphologic and functional information (Kauczor et al., 2000). Furthermore, many lung diseases are chronic and require multiple scans for follow-up over time, which makes the lack of ionizing radiation in MRI even more important (van Beek, 2008). Hence, there is a large interest of the development and research of functional MR imaging in the lung.

The gas exchange of the lung can be determined by functional MRI methods. It is important to have a match between the perfusion of blood and the ventilation of air in order to have an effective and properly working gas exchange (Mistry et al., 2008). There is often a mismatch between these parameters in many pulmonary diseases. Measurements of perfusion and ventilation can therefore be used for the diagnostics and follow up of pulmonary diseases such as chronic obstructive pulmonary disease (COPD), cystic fibrosis and pulmonary hypertension (Kauczor et al., 2009).

Measurements of pulmonary perfusion with dynamic contrast enhanced MRI have been investigated in clinical settings both in 2D (Hatabu et al., 1999) and 3D (Ohno et al., 2004). However, quantitative perfusion MRI is not very common in clinical diagnostics and preclinical quantitative assessments are even more uncommon. Quantitative measurements of pulmonary perfusion with rodents have only been done in one study (Mistry et al., 2008). MRI of rodents involves difficulties such as requirements for better spatial resolution due to smaller vessels and organs. The body weight of rat is around 300 times less compared to man. Additionally, better temporal resolution is needed because of the cardiac rate in rats is about 5 times faster compared to man (Mistry et al., 2008).

Permeability is another important parameter that can be investigated by functional MRI methods. A change in permeability can indicate a region of inflammation in the lung. Methods and models for permeability measurements have mostly been developed for applications in cancer (Naish et al., 2009). However, they are also applicable in functional lung imaging.
Consequently measurements of pulmonary perfusion and permeability are useful tools in clinical diagnostics. In addition, they can be applied in preclinical research, e.g. in the pharmaceutical industries, where these parameters can be used in lung disease models during the development of novel drugs.

The aim of this project is to develop protocols suitable for quantitative measurements of perfusion and permeability in the rat lung using dynamic contrast enhanced MRI. Additionally, the developed protocol will be used in a pilot study including healthy animals and animals with lung inflammation.
2 THEORY

2.1 Respiratory system

The main function for the respiratory system is to oxygenate the blood by a gas exchange between oxygen, O₂ and carbon dioxide, CO₂. The breathing air flows through the trachea, which divides and continues as two main bronchi in the two lungs. Further, the main bronchi get more and more narrow and become bronchioles, which end in a small lumen surrounded by a capillary net, the alveoli (fig. 1). The air in the alveoli oxygenates the blood flowing through the capillary net and a gas exchange occurs (Sand, et al., 2007).

![Respiratory system diagram](http://www.southtynesidepic.nhs.uk/master_code/childrenscentre/philsinl/117.gif)

**Figure 1.** Respiratory system of man. The lung contains millions of alveoli, which is the place of the gas exchange between oxygen and carbon dioxide.

(http://arditobook.pbworks.com/f/1180404291/Alveoli_diagram.png)

2.2 Perfusion and permeability in the lung

Perfusion is the capillary blood flow and is measured in units of ml min⁻¹ 100g⁻¹, indicating the blood volume, that perfuse through a mass of tissue, per unit time. It is important that the ventilation of air match the capillary perfusion of blood in order to have a properly working gas exchange. A change in the perfusion and/or the ventilation can therefore be an indication of a pulmonary disease. Consequently, measurements of these parameters can be used as a diagnostic tool (Mistry et al., 2008).

Permeability is the possibility for particles and fluid to penetrate a barrier. An increased permeability can be an indicator of inflammation, which is the response of the immune
system to an infection (Wilhelm, 1973). Blood plasma containing immune cells and proteins are gathered in the tissue by an increased vascular permeability, resulting in an oedema.

2.3 MRI of the lung

MRI is a well developed image modality for many parts of the body. However, MRI of the lung is challenging and has been limited to research applications. It has even been stated that among all organs imaged by MRI, the lung is very likely the most difficult (Kauczor et al., 2009). However, the development of new MRI techniques have made it possible to perform pulmonary MRI, which today is an area of increased interest with many potential applications.

2.3.1 Physical principles of MRI

The most common molecule in our body is water. Every proton within the water molecule has a rotational motion called spin and is positively charged. According to the laws of electromagnetism a small magnetic moment can be detected from each proton. If an external magnetic field is applied to the water molecule, the protons within it will align from being in random directions. Larmor’s equation gives the frequency of the precessional motion called larmor frequency

\[ f = \frac{\gamma}{2\pi} \cdot B_0 \]

Eq. 1

where f is the frequency [Hz], \( \gamma \) is the gyro magnetic ratio [MHz T\(^{-1}\)] and \( B_0 \) [T] is the static external magnetic field. This relation is also called the resonance condition and has to be satisfied in order to transfer energy to the system.

All protons in a system, exposed to the same magnetic field will resonate with the same frequency in the same direction. Together they will create a net magnetic moment along the direction of the external field. The amplitude of the net magnetization vector in equilibrium is called \( M_0 \) and depends on the strength of the static magnetic field (\( B_0 \)) and on the proton density.

The small net magnetic moment coming from the protons in tissue is only detectable if it is flipped to an angle, \( \alpha \) to the external field \( B_0 \). This is done by energy from a radio frequency (RF) pulse, which has to satisfy the resonance condition to be able to transfer energy. The net magnetization is flipped and the result is two components of the initial vector; one longitudinal (\( M_z \)), which is parallel to the static field \( B_0 \) and one transverse (\( M_{xy} \)), which is perpendicular to \( B_0 \).

After the disturbance of the RF pulse, the spins return back to equilibrium by two different processes, \( T_1 \) and \( T_2 \) relaxation (2.3.2). The MR signal is created from the transverse net magnetization, \( M_{xy} \) which rotates in the transverse plane and gives rise to an oscillating magnetic field. This oscillation induces a current in a receiver coil, which becomes the MR signal, called free induction decay (FID).
For image formation, different sequences of RF pulses and magnetic field gradients are applied. The pulse sequences create echoes in the form of a spin echo (SE) or gradient echo (GE).

2.3.2 $T_1$ and $T_2$ relaxation theory

A proton in a strong static magnetic field behaves approximately like a small needle magnet; it will align to the direction of the static field. The alignment happens due to a relaxation mechanism called $T_1$ or spin-lattice relaxation. The same relaxation mechanism will have an effect after a disturbing RF pulse.

There are two types of relaxation mechanisms after a disturbing RF pulse, $T_1$ and $T_2$ relaxation. They happen simultaneously but $T_2$ is always shorter than $T_1$.

$T_1$ relaxation occurs when the longitudinal component of the magnetic moment returns back to its initial value $M_0$ after an RF pulse. The energy that the spins absorbed from the RF pulse is transferred to the surrounding lattice by dipole-dipole interactions. The magnetic dipole moment from the surrounding lattice has to fluctuate at the larmor frequency and satisfy the resonance condition in order to enable energy transfer. The fluctuation of the magnetic dipole moment arises from the random molecule motion and is dependent on the physical state and structure of the tissue. Only a number of spins will oscillate at the larmor frequency and consequently different amounts of spins will contribute to $T_1$ relaxation in different types of tissue.

Immediately after the RF pulse, all spins will resonate in phase at the larmor frequency and the transversal net magnetization, $M_{xy}$ has its maximum value. The transverse relaxation, $T_2$, occurs as the phase coherence is lost between the spins and $M_{xy}$ decays back to zero. The dephasing is caused by small magnetic field inhomogeneities. Either internal caused by the random molecule motion ($T_2^*$) or external caused by the external field ($T_2^*$).

The efficiency of $T_1$, $T_2$ and $T_2^*$ relaxation is dependent on the structure of tissue, which makes relaxation time to a characteristic property for different types of tissue (McRobbie et al., 2007). If MRI is done with a suitable pulse sequence, contrast between different tissues can be achieved weighted on the $T_1$, $T_2$ or proton density (McRobbie et al., 2007).

2.3.3 Difficulties with MRI of the lung

MRI of the lung is difficult due to a number of reasons. First, the lung region is disturbed by respiratory and cardiac motions, which can contribute to motion artifacts. Secondly, the structure of the lung is not optimal for MRI. It has a low proton density creating low signal and it contains many interfaces between air and tissue arising from the large number of air filled lumen, alveoli. The interfaces results in susceptibility differences, which creates magnetic field inhomogeneities in the tissue. Consequently $T_2^*$ relaxation time is short in the lung (Kauczor et al., 2009).
2.3.4 Dynamic contrast enhanced MRI

Dynamic contrast enhanced MRI (DCE-MRI) is a functional MRI method, which is based on a T₁ weighted dynamic MRI sequence, measured during an intravenous injection of contrast agent (CA). The effect of CA in blood is a shortening of T₁ leading to an increased signal intensity on T₁ weighted images (2.3.6). The signal intensity for each image is plotted, resulting in an enhancement curve (fig. 2). In ideal cases the enhancement curve should display the first passage of CA. However, very good temporal resolution is needed in order to track the first passage of the CA.

![Enhancement curve](image)

**Figure 2.** A typical enhancement curve from a DCE-MRI measurement.

The identification of changes of perfusion and permeability values requires different parts of the CA enhancement curve and has therefore different demands on temporal resolution. The first part of the curve is needed for calculation of perfusion values and consequently, perfusion imaging demands very good temporal resolution as the CA perfuse out in tissue with the heart rate. The last part can be used for the calculation of permeability. If there is increased vessel permeability in the lung, CA will leak out in the parenchyma and build up a more or less constant concentration in tissue. CA concentration in normal tissue decays more continuously. A permeability change can therefore be seen as a change of the after-peak-slope in the CA enhancement curve. Permeability is therefore a rather slow process requiring less temporal resolution.

Finding quantitative values of perfusion and permeability includes curve fitting of different mathematical models. Dependent on the mathematical model used, an arterial input function (AIF) can be required. It shows the concentration of CA at each time point in the blood supply of the tissue of interest. How well the AIF can be determined, has often a large effect on the accuracy of the resulting quantitative values of perfusion and permeability. However, AIF determination is challenging as it requires good temporal resolution (Yankeelov, 2007).
2.3.5 FLASH

FLASH (Fast Low Angle SHot) is a spoiled gradient echo (GRE) sequence which belongs to the group of fast GRE sequences. This group of sequences decreases the scan time by having flip angles ≤ 90° and shorter repetition times (TR). When TR is shorter than the $T_2$ relaxation time, there is a nonzero transverse magnetization left at the time of the next repetition. After a few regular TRs the remaining transversal net magnetization will come to a constant value at each TR and a nonzero steady state will be reached. The transversal steady state creates several echoes parts from the gradient echo coming from the FID. Spoiled GRE sequences like FLASH remove the transverse net magnetization before each TR (McRobbie et al., 2007).

FLASH is a useful pulse sequence for DCE-MRI. Strongly T1 weighted images can be produced using short repetition times and small flip angles. The advantage of using GRE-sequences compared to SE-sequences in DCE-MRI is mainly the short achievable acquisition time.

The signal equation for FLASH can be written as (McRobbie et al., 2007)

$$ S = \rho \left( 1 - e^{-TR \cdot R_1} \right) \cdot \sin \alpha \cdot e^{-TE \cdot R_2^*} ,$$

Eq. 2

where $S$ is the signal, $\rho$ is the proton density, $R_1 = \frac{1}{T_1}$ and $R_2^* = \frac{1}{T_2^*}$ are the relaxation rates [s$^{-1}$] and $\alpha$ is the flip angle [rad].

In DCE-MRI a CA is distributed in tissue. The effect on the relaxation rate of a CA can be written as

$$ R' = R + r \cdot C ,$$

Eq. 3

where $R, R'$ [s$^{-1}$] are the relaxation rate before and after the administration of contrast agent, $r$ is the specific relaxivity [mmol$^{-1}$·ms$^{-1}$] demonstrating the effect of $T_1$ and $T_2$ from CA and $C$ is the CA concentration [mmol·ml$^{-1}$].

The signal from FLASH after administration of such a CA can be written as a combination of Eq. 2 and Eq. 3

$$ S' = \rho \left( 1 - e^{-TR \cdot (R_1 + r_1 \cdot C)} \right) \cdot \sin \alpha \cdot e^{-TE \cdot (R_2^* + r_2^* \cdot C)} ,$$

Eq. 4

where $S'$ is the signal after administration of CA.
The flip angle, \( \alpha \) giving the highest SNR from the GRE pulse sequence is the so called Ernst angle, which is dependent on TR and \( T_1 \) (McRobbie et al, 2007)

\[
\alpha_{ernst} = \cos^{-1}(e^{-TR/T_1})
\]

Eq. 5

2.3.6 Contrast agent theory

Contrast agents affect the MR signal by shortening of the relaxation times and can consequently increase the contrast between tissues. There are a lot of complexes used for this purpose and more are on the research level. As mentioned earlier (2.3.5) the specific relaxivity of the contrast agent specifies how effective the CA can reduce \( T_1, T_2 \) which is characteristic for different kinds of CA.

The most common contrast agents in MRI today are Gadolinium (Gd) – DTPA complexes. On the market it can be found as, for example Magnevist® (Gd – DTPA) or Omniscan® (Gd-DTPA-BMA), two solutions having the same CA properties.

Gd – DTPA is a contrast agent consisting of two parts, the pure Gadolinium and the chelate DTPA. DTPA is required for shielding since Gadolinium is toxic in its normal state (McRobbie et al., 2007).

Gadolinium (7+) is a paramagnetic compound meaning that it has one or more unpaired electrons in its atomic structure. The magnetic moment of an unpaired electron is much larger than from a proton, which results in stronger dipole-dipole interactions when CA is administrated in tissue. Stronger dipole-dipole interactions make the relaxation more efficient and consequently \( T_1 \) is shortened. Furthermore, CA can shorten \( T_2 \) by varying the correlation time (Wood, Hardy 1993).

Gd-DTPA belongs to the group of positive enhancers, which mainly affect \( T_1, T_2 \) and \( T_2^* \) are thus also affected but with only to a smaller extent. However, at larger concentrations of CA, the susceptibility differences between the paramagnetic CA and tissue will be so extensive that \( T_2^* \) gets even more pronounced. When \( T_2^* \) dominates over \( T_1 \) the result is a suppressed signal on \( T_1 \)-weighted images. Consequently, there is a nonlinear signal concentration curve for higher concentrations of CA (fig. 3). The effect from CA and hence the linear range of concentrations are dependent on the pulse sequence and its timing parameters (Shahbazi-Gahrouei et al., 2001).
Figure 3. Example of a signal CA concentration curve showing that the signal from a distributed range of CA has a non linear relationship due to $T_2^*$ effects.
3 MATERIAL AND METHODS

3.1 General

3.1.1 Animals and animal preparation

The experiments including animals were all performed with permission from the ethical committee of University of Göteborg, according to ethical approval no. 400-2008 and 40-2008.

All animals in the study were male Wistar rats, which had body weights in a range from 239 g - 412 g. During the experiments the rats were anesthetised with a mixture of air, pure oxygen and 2-3 % isoflurane. Their temperature was kept constant by surrounding tubes with a temperature of 36° C. Their breathing rate was kept constant at 50-60 breath/minute by adjusting the settings of the anesthesia.

Injections of CA were performed during the study. These injections were all performed intravenously in the tail. An extra long injection line made it possible to perform more than one injection in the same rat, without removing it from the scanner.

A disease model was included in one of the studies. It was a lipopolysaccharide (LPS), which is an antigen creating inflammation. The administration of LPS was done intratracheally.

3.1.2 Contrast agent injection

The contrast agents used in this study were Magnevist® (C = 0.5 mmol/ml Gadolinium-DTPA, Bayer-Schering, Berlin, Germany) and Omniscan® (C = 0.5 mmol/ml Gadolinium-DTPA-BMA, NYCOMED IMAGING AS Oslo, Norway). Magnevist® was used in all experiments apart from the pilot study (3.3.2) where Omniscan® was used due to the lack of Magnevist®. Dilution was done with saline in order to get the concentration and volume of interest. The volume of CA solution used in all experiments was 0.4 ml, which was injected manually during 10 seconds.

All notations of contrast agent concentration in the report are noted in mmol of Gd-DTPA/ Gd-DTPA-BMA per ml of the total injected volume.

3.1.3 MR scanner

The MR scanner used for all MR imaging was a 4.7 T Bruker Biospec (Bruker, Ettlingen, Germany) 47/40 at Astra Zeneca in Mölndal, Sweden. A quadrature proton coil with a diameter of 72 mm and a Gradient insert S112 with max gradient 400 mT/m was used during the experiments. Image acquisition and reconstruction of the MR data was done with Paravision 5.0 (Bruker, Ettlingen, Germany).
3.1.4 Respiratory triggering

A triggering module for respiratory triggering (SA instruments, Inc, Stony Brook, NY, USA) was connected to the scanner. Respiratory triggering reduces artifacts from motion by a trigger pulse that activates the scanner at a constant position of the diaphragm. The sensor, a small pad, is fastened on the chest of the rat giving a signal when it is compressed by the rising diaphragm (fig. 4).

![Diagram of respiratory triggering](Position of the diaphragm)

**Figure 4.** Schematic overview of the principle of triggering where the trig pulse is set to activate the scanner at a constant position of the diaphragm.

3.1.5 Imaging method

The method used for the measurements of perfusion and permeability was DCE-MRI, which was based on a T1-weighted FLASH sequence optimized for a short echo time (TE) (Månsson, 2008).

DCE-MRI measurements were all performed according to a standard scheme presented here. The rat being imaged was lying supine with the head first in the scanner. The breathing rate was assessed by a connected triggering module. Before the image acquisition could start, the rat was positioned in the center of the scanner with assistance of scout images and a suitable slice containing as much lung as possible was chosen.

A 2D FLASH acquisition sequence was used for the perfusion measurements with TE/TR 0.6/2 ms, slice thickness 4 mm and the number of excitations (NEX) was 4. The temporal resolution for this sequence was, because of the triggering, dependent on the breathing rate, but had an average of about 1 second. It was the highest temporal resolution achievable with the current equipment.

In the permeability experiments a 3D FLASH sequence was used with TE/TR 0.6/5 ms, which had a temporal resolution of 1 minute per 3D slab, containing 20 slices.

The CA injection started 30 seconds after the start of 2D FLASH and 2 minutes after 3D FLASH in order to both have pre-contrast baseline images and images of the CA enhancement. The different delays of injection between 2D and 3D were due to the temporal resolution of each sequence.

If two injections were performed in the same rat, they were separated by 20 min in order to have a sufficient wash-out (Weinmann et al., 1984). A complete wash out is important in order to return back to same baseline signal after each injection and avoid build up of the signal.
3.2 Perfusion measurement

3.2.1 The signal concentration curve

The signal concentration curve of the CA is not linear (2.3.6). At higher concentrations T2* effects will contribute, which result in a suppressed signal. A suitable CA concentration for a perfusion measurement has no suppressed signal and lies consequently in the linear range. The aim of this section was therefore to determine the linear signal concentration range for a DCE-MRI perfusion measurement in the lung.

3.2.1.1 In vitro experiment

The purpose of this experiment was to determine the relationship between the MR signal and the CA concentration in vitro with the same FLASH sequence used as in the DCE-MRI measurements. This was done in order to find the interesting range of CA concentrations for further investigation.

The phantom used was built from a plastic tube holding smaller glass tubes (3.7 ml) filled with different concentrations of contrast agent (fig. 5). They were all surrounded by water. The investigated concentrations of CA ranged from 0.005 mmol/ml to 0.2 mmol/ml and were divided on two scans according to Table 1. A 2D FLASH (matrix 96 x 128; FOV 6.47 x 6.65 cm²; respiratory triggering off) according to section 3.1.5 was used as acquisition sequence.

![Image](image.png)

**Figure 5.** The left image a) shows a schematic setup of the phantom for the in vitro experiment and the right image b) shows the resulting axial MR image.
Table 1. The investigated CA concentrations in the in vitro experiment were divided in two scans with five samples each.

<table>
<thead>
<tr>
<th>Scan 1</th>
<th>Scan 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0.005 mmol/ml</td>
<td>1 0.05 mmol/ml</td>
</tr>
<tr>
<td>2 0.01 mmol/ml</td>
<td>2 0.06 mmol/ml</td>
</tr>
<tr>
<td>3 0.05 mmol/ml</td>
<td>3 0.07 mmol/ml</td>
</tr>
<tr>
<td>4 0.1 mmol/ml</td>
<td>4 0.08 mmol/ml</td>
</tr>
<tr>
<td>5 0.2 mmol/ml</td>
<td>5 0.1 mmol/ml</td>
</tr>
</tbody>
</table>

3.2.1.2 Combined in vitro/in vivo experiment

The in vitro and in vivo relation of the signal concentration curve is not comparable and consequently a linking experiment was needed in order to find the signal CA concentration curve in vivo. The next step was therefore to use in vitro samples with concentrations from the linear range, determined in the original in vitro experiment (3.2.1.1), as a reference of linearity to in vivo DCE-MRI measurements with different concentrations of CA. However, the in vitro signal can only be compared with the in vivo signal if they are done in the same scan. Therefore, the principle of this experiment was to combine an in vitro and an in vivo experiment by placing in vitro tubes in the FOV during a DCE-MRI measurement in rat. The relationship of the signal concentration curves was investigated to determine whether the resulting concentrations in vivo in the lung were in the linear range or not.

A standard DCE-MRI measurement as described in section 3.1.5, 2DFLASH (flip angle 20°; matrix 96 x 128; FOV 7-7.5 x 5-5.8 cm² (suited for individual animal size); respiratory triggering on) was carried out for two injections of CA, 0.1 mmol/ml and 0.2 mmol/ml. Additionally, in vitro samples made of 2 ml glass tubes with CA concentrations 0.01, 0.02, 0.04 mmol/ml were placed next to the neck of the rat (fig. 6). The samples were heated to 36° before the measurement started to preserve the same temperature for all reference in vitro samples.

The reference in vitro samples were measured two by two during injection 1 and 2, having one sample in common as a reference. The receiver gain that controls the MR signal was set to a constant value, which allowed for a comparison between the two scans as the experiment was repeated in two animals.
3.2.1.3 Investigation of potential confounders of the combined in vitro/in vivo experiment

The samples from the original experiment and the ones used in the combined in vitro/in vivo experiment differed in parameters like respiratory triggering on/off, tube size and surrounding media. These three parameters were investigated in order to see if some of them affected the in vitro signal concentration curve.

**Respiratory triggering** was used during all injections in the combined in vitro/in vivo experiment but not in the original in vitro experiment. To see if triggering affected the signal from the in vitro samples next to the rat, one precontrast scan was performed with the same sequence as in the combined in vitro/in vivo experiment but with respiratory triggering off.

The **tube size** of the original in vitro experiment was 3.7 ml compared to 2 ml in the combined in vitro in vivo experiment. The signal from three different CA concentrations, 0.01 mmol/ml, 0.02 mmol/ml and 0.04 mmol/ml in 3.7 ml tubes was compared with the signal from the same concentrations in 2 ml tubes to see if the geometry of the samples had an effect. The two sets of samples were measured with the exact same pulse sequence as in the in vitro experiment (3.2.1.1), both surrounded by water.

The **surrounding media** of the original in vitro experiment was water but in the combined in vitro/in vivo experiment it was air. The dependence of different surrounding media was therefore investigated by scanning three CA concentrations 0.01 mmol/ml, 0.02 mmol/ml and 0.04 mmol/ml with the same tube size (2 ml) surrounded by two different media, air and water. The same acquisition sequence as in the in vitro experiment (3.2.1.1, flip angle 20º) was used with a slightly different FOV (6.97 x 6.65 cm²).
3.2.2 In vivo experiment
The combined in vitro/in vivo experiment resulted in unexpected parameters dependencies (4.3.1.2), which consequently motivated a measurement of the signal concentration relation directly in vivo. Four CA concentrations 0.05 mmol/ml, 0.1 mmol/ml, 0.2 mmol/ml and 0.3 mmol/ml were injected and measured by standard DCE-MRI (3.1.5) with 2DFLASH (flip angle 20°; matrix 96 x 128; FOV 5.0 x 5.0 cm²; respiratory triggering on) in the same rat. The experiment was carried out in four animals with similar body weight. The minimal delay time between two measurements for sufficient wash out was 20 min (Weinmann et al., 1984). This was important to consider since the baseline signal for all measurements in the same rat should be constant. Measurements including delay times in all four rats required a distribution of the measurements over two days according to the injection scheme in figure 7.

![Figure 7. The scheme of injections for the in vivo experiment in one rat.](image)

3.2.3 Partial volume effect
The objective of this experiment was to determine if partial volume effects were a potential problem in DCE-MRI measurements in rat. Partial volume effects arise if more than one type of tissue contributes to the signal in one voxel. If the voxels gets smaller e.g. the slice thickness is reduced, the potential effect should be decreased.
Two measurements with the same contrast agent concentration, 0.05 mmol/ml but with different slice thicknesses, 2 mm and 4 mm, were performed in the same rat. The position and FOV was kept constant when changing the slice thickness. The measurements were performed by standard DCE-MRI (3.1.5) with 2DFLASH (flip angle 20°; matrix 96 x 128; FOV 5.0 x 5.0 cm²; respiratory triggering on).

3.2.4 Inflow effect
The inflow effect may appear when vessels with significant blood flow are in an imaged slice. Since the RF pulse only affects the blood that is in the slice at the moment, fully relaxed blood can enter the slice of interest between RF pulses and give high signal in GRE
sequences (McRobbie et al., 2007). This is a problem when searching for small signal changes due to different concentrations of CA, which can be saturated by the inflow signal. 3D scanning can reduce inflow artifacts because the RF pulse will then be applied not only in one slice but over a larger volume. More blood gets excited and inflow artifacts are reduced.

The aim of this experiment was to investigate if inflow effects caused problems when analyzing the 2D FLASH signal from enhanced vessels. 2D scans from the perfusion section 3.2.2 was compared with 3D scans from the permeability section 3.3.1.3, which had the same CA concentrations, 0.1 mmol/ml and 0.2 mmol/ml. For details of the measurements see each section.
3.3 Permeability measurements

Pulmonary permeability was the second aim of the project. The latter part of the enhancement curve is mainly affected by permeability, which is a slow process and consequently less temporal resolution is needed. The saved time can therefore be used in 3D scanning. 3D scans have several advantages over 2D like the reduction of inflow effects and the possibility of image reconstruction in any plane of interest (McRobbie et al., 2007).

3.3.1 Optimization of the imaging method

All permeability measurements were done with a 3D FLASH sequence. To make it suitable for DCE-MRI permeability measurements, optimization of imaging method, flip angle and CA concentration were done.

3.3.1.1 In vivo 3D scanning

The 3D sequence was first studied in vivo to have a starting point for the optimization. According to the standard injection scheme (3.1.5), DCE-MRI measurements using a 3D FLASH sequence (flip angle 20°; matrix 192 x 192 x 20; FOV 5 x 5 x 4 cm$^3$; NEX 4; respiratory triggering on) with CA concentration 0.5 mmol/ml were carried out in one rat.

3.3.1.2 Optimization of the acquisition scheme

One scan was done with respiratory triggering on and one was done with respiratory triggering off in order to see if triggering in a combination with the 3D FLASH sequence caused artifacts. Besides triggering, the two scans were identical standard DCE-MRI measurements (3.1.5) with 3D FLASH (flip angle 20°; matrix 192 x 192 x 20; FOV 5 x 6.5 x 6.5 cm$^3$; NEX 4). A plastic immovable QA phantom was used in this experiment (fig. 8).

A dynamic series should consist of at least 8 images to be able to show if the occurring signal fluctuations were a coincidence. However, for the untriggered 3D FLASH sequence the number of images that could be sampled consecutively was limited by the gradients of the scanner, which were overloaded after four repetitions and automatically stopped the sequence. A dynamic series of 8 images was therefore sampled by setting the acquisition sequence to stop after each completed 3D image set and start over again with the next one during 8 repetitions. The gradient system could then recover between each image. This way of sampling is henceforward called repeated 3D FLASH.
Figure 8. An MR image of the static, plastic QA phantom used for the trigger optimization. The marked region is the region of interest (ROI) used for the analyses.

3.3.1.3 In vivo optimization of repeated3DFLASH
In this experiment the repeated3DFLASH sequence was studied in vivo. Two rats were measured for the in vivo optimization of the flip angle and the CA concentration. Two CA concentrations, 0.1 mmol/ml and 0.2 mmol/ml were therefore investigated with two different flip angles, 20° and 30° using the standard DCE-MRI (3.1.5) with the repeated3DFLASH (matrix 192 x 192 x 20; FOV 5 x 6.5 x 6 cm³; NEX 8; respiratory triggering off) according to Table 2. NEX was increased from 4 to 8 in order to compensate for the fact that no triggering was used in the repeated3DFLASH sequence.

Table 2. The injection scheme for the in vivo optimization of repeated 3DFLASH.

<table>
<thead>
<tr>
<th>Animal 1</th>
<th>Animal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>20° 0.1 mmol/ml</td>
<td>0.2 mmol/ml</td>
</tr>
<tr>
<td>30° 0.1 mmol/ml</td>
<td>0.2 mmol/ml</td>
</tr>
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</table>

3.3.2 Pilot study
After the optimization of the protocol, a pilot study was performed to investigate if it was possible to find a significant difference in pulmonary permeability in healthy rat lungs compared to rat lungs with inflammation. The inflammation was created by 0.2 ml (C = 1 mg/ml) solution of LPS per animal. The time between administration of LPS and measurement was 24 h. The same standard DCE-MRI measurement (3.1.5) with the repeated3DFLASH (flip angle 20°; matrix 96 x 96 x 20; FOV 5 x 6.5 x 6 cm³; NEX 8; respiratory triggering off) was performed with CA dose 0.4 mmol/ml before and after administration of LPS (day 0 and day 1). Anatomical landmarks were used to ensure the same position of the FOV on both days.
3.4 Image analysis

The image analysis of the MR data was performed using ImageJ (Rasband WS. ImageJ, Bethesda, USA; Abramoff MD et al. 2004). When enhancement curves was considered in the analyses, a region of interest (ROI) was defined by hand in order to find representative curves for different types of tissue. If the ROI consisted of more than one pixel, an average was done over all pixels within it. The ROI selection in the pulmonary artery was done by choosing a homogenous region in the pulmonary bifurcation and in the lung it was done by taking a large homogenous region avoiding clearly enhanced blood vessels (fig. 9).

![Figure 9](image)

Figure 9. Examples of ROI selections of the pulmonary artery (upper ROI) and the lung (lower ROI).

3.4.1 Perfusion measurements

The measurement of the samples in the original in vitro experiment (3.2.1.1) was done in two scans. One of the samples (CA concentration 0.05 mmol/ml) was used in both scans for normalization in order to show a signal concentration curve over the whole range.

DCE-MRI measurements generated enhancement curves of the passage of CA. All enhancement curves for the perfusion measurements were normalized to the signal of the baseline of each curve, defined as the second to the last precontrast image.

The in vivo signal CA concentration curve was investigated in the in vivo experiment (3.2.2). The signal value for each concentration was calculated using a signal average of five images after the maximum concentration had passed (image 50 – 55). In addition, the resulting signal average was averaged over the four rats in order to have one signal value for each concentration. Image 50 – 55 were chosen instead of the maximum concentration images because the magnitude of the peak was dependent on the time of injection.
3.4.2 Permeability measurements

All enhancement curves for the permeability measurements were normalized to an average of their own baseline signal consisting of two precontrast images.
4 RESULTS

4.1 Contrast agent enhancement

All DCE-MRI measurement in rat resulted in an enhancement of the blood vessels as in figure 10 where image a) demonstrates a precontrast image and the following images different times after administration of CA. Image c) demonstrates the maximal enhancement of the lung and image d) demonstrates the enhancement of the liver.

Figure 10. Enhancement of a rat lung from DCE-MRI measurements with 0.2 mmol/ml CA injected. Image a=1 second, b = 29 seconds, c = 33 seconds, d = 59 seconds and e = 360 seconds after the start of the MRI acquisition sequence.
4.2 The optimal flip angle

$\alpha_{\text{ernst}}$ was simulated for different $T_1$ values (fig. 11), showing that $\alpha_{\text{ernst}}$ is larger for decreasing values of $T_1$. $T_1$ in the lung at 4.7 T is about 1500 ms (Abdlamer, 2007), which results in $\alpha_{\text{ernst}}$ of $\approx 3^\circ$. However, when choosing the flip angle for the FLASH sequence in DCE-MRI measurements, more parameters have to be considered. First, the distribution of CA will shorten $T_1$, which will result in a variable but larger $\alpha_{\text{ernst}}$ (fig. 11). Secondly, stronger $T_1$-weighting can be achieved with larger flip angles, which is desirable in DCE-MRI. A simulation of the signal after administration of CA (fig. 12) shows the degree of linearity. The third parameter that affects the choice of $\alpha$ is the fact that inflow effects can be reduced with larger flip angles. Therefore, considering these three parameters the flip angle used in the FLASH sequence for DCE-MRI measurements was $20^\circ$.

**Figure 11.** Ernst angle for different values of $T_1$, showing an increased $\alpha_{\text{ernst}}$ for decreasing values of $T_1$.

**Figure 12.** Calculations of the signal concentration curve for different flip angles (TE/TR 0.6/2ms; $T_1$ 1500ms; $T_2$ 50 ms; $r_2$ 0.005 mmol−1ms−1), showing that the linearity decreases with a decreased flip angle.
4.3 Perfusion measurements

4.3.1 Signal concentration curve

4.3.1.1 In vitro experiment
Measurements of in vitro samples with different concentrations of CA surrounded by water resulted in a maximum signal at a CA concentration of 0.06 mmol/ml (fig. 13). From this experiment a linear signal concentration range (0 – 0.06 mmol/ml) was defined. This range was used as the starting point for the combined in vitro/in vivo experiment.

![Signal concentration curve in vitro](image)

**Figure 13.** The signal concentration curve from the in vitro experiment. The maximum signal was seen at a CA concentration of 0.06 mmol/ml.

4.3.1.2 Combined in vitro/in vivo experiment
Image analysis of the combined in vitro/in vivo experiment showed that the signal-concentration curve from the reference in vitro samples placed next to the rat (fig. 14) did not show the same linearity as the signal concentration curve from the samples in the original in vitro experiment (fig. 13). The same effect was seen in the analysis of the two animals.

Some parameters differed in the set-up of the combined in vitro/in vivo experiment compared to the in vitro experiment, which were studied in order to find the contributing factor to the difference of the in vitro signal CA concentration relationship.
Figure 14. The resulting signal concentration curve from the in vitro samples in the combined in vitro/in vivo experiment. The signal from all three CA concentrations showed almost the same signal level.

When the effect from triggering was investigated, it showed that the signal variations from the in vitro samples were about the same for the triggered and the untriggered sequence (fig.15), indicating that triggering had no effect.

The next factor to be studied was the dependence from the sample geometry. The result showed that the larger samples of 3.7 ml had a slightly more linear curve than the samples of 2 ml (fig. 16).

The last parameter investigated was the surrounding media. The result showed a difference of the relationship for samples surrounded by air and by water (fig. 17). The difference could primarily be seen in the third and largest CA concentration, i.e. different surrounding media had the strongest effect at the highest CA concentration.

Figure 15. Signal from a triggered and an un triggered 2DFLASH sequence. The signal variations were about the same indicating no effect from triggering.
The signal concentration curves for different surrounding media. The signal concentration relation differed for air and water, indicating that the surrounding media had an impact on the signal concentration curve in vitro.

Figure 16. The signal concentration curves for different sample geometry showing that the relation is slightly more linear for the larger samples than for the smaller samples.

Figure 17. The signal concentration curves for different surrounding media. The signal concentration relation differed for air and water, indicating that the surrounding media had an impact on the signal concentration curve in vitro.
4.3.1.3 In vivo experiment
The four DCE-MRI measurements in the four rats were all completed and could all be analyzed. The results showed different signal concentration curves from a ROI of the lung (fig. 18a) compared to a ROI of the pulmonary artery (fig. 18b). The four concentrations in the lung gave almost a linear signal relation whereas all four concentrations had about the same signal in the pulmonary artery. The same result could be seen when plotting relative enhancement curves of the pulmonary artery and the lung in the same diagram for one rat (fig. 19) (see Appendix A1 for all four rats). This result indicated a suppressed signal in the pulmonary artery and an unsuppressed signal in the lung.

![Figure 18](image.png)

**Figure 18.** The signal concentration curves from the in vivo experiment taking a ROI in a) the lung and b) in the pulmonary artery. The signal is an average of image 50-55 in the dynamic series. The error bars represent the standard deviation of the average.
Figure 19. Enhancement curves of a ROI in the lung and a ROI in the pulmonary artery for concentrations 0.05, 0.1, 0.2, 0.3 mmol/ml in the same rat, indicating a suppressed signal in the pulmonary artery.

4.3.2 Partial volume effect

The results from the investigation of different slice thicknesses and thus a potential partial volume effect showed that the relative enhancement for slice thickness 4 mm were higher compared to 2 mm, for both the lung and the pulmonary artery (fig. 20). However, the relation between the relative enhancement curves of the pulmonary artery and the lung did not change by a thinner slice thickness, indicating that the suppressed signal was not caused by a partial volume effect.
Figure 20. The results from the investigation of the partial volume effect for different slice thickness. The relative enhancement curves for 4 mm slice thickness in the left figure and 2 mm slice thickness in the right figure. No change of the relative enhancement could be seen in the pulmonary artery.

4.3.3 Inflow effect

The effect from inflow was investigated in order to find what contributed to the suppressed signal in the pulmonary artery. Since 3D scans have the ability to reduce inflow, the investigation included a comparison between a 2D and a 3D scan for two different CA concentrations 0.1 mmol/ml and 0.2 mmol/ml.

The difference of the relative enhancement between the lung and the pulmonary artery was different in the 3D scan compared to the 2D scan (fig. 21). The 3D scan showed a higher relative signal in the pulmonary artery compared to lung. In addition, the signal from the pulmonary artery increased for an increased CA concentration, whereas the 2D scan did not. This result indicated that inflow contributed to the suppressed signal in the pulmonary artery.
Figure 21. Comparison between 2D and 3D scans for two CA concentrations, 0.1 mmol/ml and 0.2 mmol/ml, showing that the relation between the relative enhancement curves of the pulmonary artery and the lung were changed.
4.4 Permeability measurements

4.4.1 Optimization of the imaging method

4.4.1.1 In vivo 3D scanning

3D scanning was first investigated in vivo with the purpose to define parameters that required optimization. The resulting enhancement curves for the pulmonary artery, lung, muscle and image background (noise) are shown in the same plot (fig. 22). The result showed oscillations of the signal both in the lung and in the pulmonary artery which could indicate a problem with triggering or low SNR. The relative enhancement of around 3 was also considered as too low. Consequently, triggering and CA concentrations were two issues to be solved in the optimization process.

Figure 22. The result of the first in vivo 3D FLASH scan, showing enhancement curves from a ROI in the pulmonary artery, lung, muscle and noise. Abnormal signal fluctuations could be seen for both the pulmonary artery and the lung. The relative enhancement in the lung and the pulmonary artery had a maximum around 3.

4.4.1.2 Optimization of the acquisition scheme

The investigations of different acquisition schemes in vitro showed that a triggered 3D FLASH sequence (fig. 23, green line) gave fluctuations whereas an untriggered 3D FLASH sequence did not (fig. 23, blue line). The best alternative for the permeability measurements were the untriggered adapted sequence, repeated3DFLASH (fig. 23, red line) because there was a problem with the gradients. However, the repeated3DFLASH had more fluctuating signal than the untriggered 3D FLASH sequence but less variation compared to the triggered one.
Figure 23. The result from an in vitro measurement using different acquisition methods. The triggered 3DFLASH sequence (green line) gave abnormal signal variation compared to the untriggered 3DFLASH sequence (blue line). The repeated 3DFLASH sequence was used because of the limitation of the gradients.

4.4.1.3 In vivo optimization of repeated 3DFLASH

NEX was increased (doubled from 4 to 8) in order to compensate for the fact that no triggering was used during the repeated 3DFLASH sequence. The in vivo measurement with this sequence (fig. 24) gave reduced signal fluctuations compared to the measurements with the triggered 3DFLASH sequence (fig. 22). The combination of no triggering and increased NEX was therefore considered to be successful.

Considering the different flip angles, 20º gave a larger relative enhancement compared to 30º for both CA concentrations. However, the effect was even clearer for the largest concentration 0.2 mmol/ml (fig. 24).

The relative enhancement was almost doubled, when doubling the CA concentration from 0.2 mmol/ml to 0.4 mmol/ml at 20º flip angle.
Figure 24. Enhancement curves from measurements with repeated 3DFLASH. The relative enhancement at flip angle 20° is higher than 30°.

4.4.2 Pilot study

The purpose of the pilot study was to demonstrate the feasibility of the optimized pulse sequence in order to detect changes in permeability in the rat lung.
All DCE-MRI measurements were successfully conducted and all four rats were in good condition for the measurement day 1. The resulting enhancement curves from a ROI in the lung were smooth indicating that the acquisition method repeated 3DFLASH worked and that the relative enhancement was around 10, which was sufficient.

The resulting images day 0 and day 1 was inspected and compared visually but no distinct change in contrast could be seen in any of the four rats.
Furthermore, enhancement curves from a ROI in the lung day 0 and day 1 were studied. No differences of the slope after the peak were seen in any of the four rats (fig. 25).
Figure 25. Resulting enhancement curves from the four animals in the pilot study. No changes in the after peak slope can be seen.
5 DISCUSSION

The method for measurements of pulmonary perfusion and permeability with dynamic contrast enhanced MRI in rat was investigated in this thesis. One pilot study was performed, which investigated the permeability in the rat lung using a LPS model.

5.1 The optimal flip angle

The flip angle used in the FLASH sequence was set to 20º, being a tradeoff between high SNR, the reduction of inflow effects and T₁ weighting. The selection was done after simulation of the FLASH sequence.

More T₁-weighting can be achieved with larger flip angles since the relative difference of the net magnetization vector (M₀) in tissues with different T₁ gets larger for an increased flip angle. A larger relative difference can hence be seen as better T₁ weighted contrast.

Theoretically, the relaxivity $r₂^*$ should be used in Eq. 4 giving the effect of $T₂^*$ from CA. However, in the simulation of the postcontrast signal, the value of $r₂^*$ was approximated by the value of $r₂$. This was done because the effect of CA on $T₂^*$ is very complex involving diffusion among other factors. Finding a more accurate value of $r₂^*$ would include advanced additional measurements, which was not within the scope of this work. Furthermore, using a more accurate value of $r₂^*$ should not affect the conclusions done. Therefore, $r₂$ was considered to be a reasonable approximation in order to show the linearity of the signal concentration curve for different flip angles.

The second reason to the choice of a flip angle as high as 20º was the capacity of larger flip angles to reduce inflow effects. This is a consequence of the required time to reach the steady state for different flip angles. The steady state is reached immediately at flip angle 90º while it takes some time for flip angles less than 90º. If there is fast flowing blood through an imaged slice and the approach of steady state is slow, the blood will hence be out of the slice before steady state is reached. Consequently fully relaxed blood will enter and give inflow effects. Larger flip angles can thus be used to reduce such effects (Roberts, 2005).

Even though the largest SNR is achieved at $α_{\text{emst}}$, much higher flip angles were preferable in the FLASH equation for DCE-MRI. Since good SNR was not the primary objective, it was more important to have strong T1-weightening and to reduce inflow effects. Furthermore, SNR could be increased in other ways such as increased slice thickness, NEX or CA concentration (lying within the linear range).
5.2 Perfusion measurement

The primary objective was to develop a protocol for perfusion measurements in a preclinical situation. In order to enable a reliable perfusion measurement, the linear range of the signal-CA concentration curve was determined.

Many investigators using contrast agent Gd-DTPA assume that the relationship between signal and the CA concentration used is linear. However, the linearity is dependent on pulse sequence parameters and CA concentration (Shahbazi-Gahrouei et al., 2001). Therefore, finding the linear range of the signal concentration curve in vivo is an important step in the process finding accurate values of pulmonary perfusion.

An in vitro experiment was first done in order to determine the linear range of the signal CA concentration curve in vitro. From this experiment, three CA concentrations, which worked as in vitro references were placed in FOV during DCE-MRI measurements in rat. It was essential to perform such a linking experiment in order to find out the in vivo relation, which differed considerably from the one in vitro.

Another consideration to do this kind of experiment was difficulties in calculating the dilution of the injected CA. The CA was diluted from the tail vein to the pulmonary artery. To which degree was difficult to calculate since it is dependent on blood volumes, injection times and pharmacokinetics of the CA.

However, the same CA concentrations showed different signal CA concentration relationships in the original in vitro experiment and the combined in vitro/in vivo experiment. Additional experiments were performed in order to determine the factors contributing to the deviation between the original in vitro and the combined experiment. Investigating merely three samples were probably too few but it could nevertheless be used as an indicator of the situation. The investigation showed that the surrounding media and the geometry of the samples had an effect on the signal from the in vitro tubes.

The surrounding media dependence can be explained by the fact that different surrounding media have different susceptibilities. There are larger susceptibility gradients in the border between air and the CA sample, than between water and the CA sample. Larger susceptibility gradients contribute to a more inhomogeneous magnetic field profile over the sample. Taking the same ROI in a sample surrounded by air and by water can therefore give a different MR signal, caused by the differences in the magnetic fields. Consequently, the size of the sample also has an effect of how homogenous the magnetic field is in the ROI.

Another potential parameter that affected the signal in the in vitro samples could be the temperature. According to Reichenbach, 1997 there is a temperature dependence of the relaxivity of Gd-DTPA. The temperature of the original sample tubes were not controlled during the experiments but the reference samples in the combined experiment were measured at 36°C, which may explain the difference in signal.

Inhomogenities in the static magnetic field have also to be considered since the samples in the two experiments had different placing and orientations towards $B_0$. The samples in the original experiment were placed horizontal but in the combined experiment they were placed vertical.
The fact that the signal concentration curve most probably is dependent on parameters as for example the surrounding medium resulted in the decision to end the combined in vitro/in vivo experiment without further analyses. Even if all contributing factors could be identified, it was hard to estimate the size of the effect, which would lead to an uncertain in vitro/in vivo comparison.

Measurements of different CA concentrations directly in rat are presumably the most straightforward way to find the signal concentration curve in vivo. The in vivo experiment was carried out measuring four CA concentrations in four rats. The analyses of the relative enhancement curve in the lung and the in pulmonary artery indicated different signal CA concentration relationships. The signal differences for different CA concentrations were much smaller in a ROI in the pulmonary artery compared to a ROI in the lung. This indicated a suppressed signal in the artery.

Theoretically, if the injected CA concentration in rat was in the linear range, the relative signal enhancement in the pulmonary artery should be higher compared to the lung, as there is more blood containing more CA in an arterial voxel (100%) than in the lung (relative blood volume in the lung tissue). The result of the in vivo experiment did not show that difference, which indicated a suppressed signal in the pulmonary artery. Partial volume, inflow and T2* effects were potential causes. The partial volume effect was investigated by varying the slice thickness and the inflow effect was investigated by reflecting the differences between a 2D and a 3D scan. Even though the partial volume effect did not show any impact, the investigation of this effect was even more important to investigate in rat compared to man as the pulmonary artery in rat is considerably smaller. This increase the risk of partial volume effects despite the smaller voxel sizes in animal scanners compared to human scanners. The investigation was only done in the slice direction. However, partial volume effects in plane could also be a potential contributing effect. The investigation of inflow was a comparison between a 2D and a 3D scan. Since 3D scans have the capacity to reduce effects of inflow and this experiment showed a reduction, inflow was very likely a reason to the suppressed signal in the pulmonary artery.

However, it cannot be assumed that the inflow effect is the only contributing factor to the suppressed signal in the pulmonary artery. In addition, there could be effects from T2*, giving a suppressed signal from the higher CA concentrations just as in the original in vitro experiment for CA concentrations higher than 0.06 mmol/ml. The risk of T2* effects should also be larger in the pulmonary artery where less dilution occurs. However, if effects from T2* were the dominating effect, even the lowest CA concentration 0.05 mmol/ml would be a too large concentration lying outside the linear range. This is rather unlikely considering a reasonable degree of dilution that must occur during the passage of the blood vessel from the injection site to the pulmonary artery. The inflow effect is therefore most likely the dominating factor of the suppressed signal.
Another reason that makes the performing of the in vivo experiment difficult is choosing a reasonable CA concentration range without knowing the degree of dilution. One way to avoid the problem could be to do the injection of CA in the jugular vein. It would allow more control of the concentration in the pulmonary artery since the dilution is less from the jugular vein, than from the tail vein. However, this is a complicated method to perform in practice, among other things because the difficulty to find the jugular veins for an injection.

If the temporal resolution of around 1 second is sufficient since the cardiac rate in rat is around 360-500 beats/ min (Wekstein, 1965). During the time between two images in a perfusion measurement with temporal resolution of 1 sec, 6 - 8 heart beats will pass, which means that the enhancement of the lung happens from one image to another. Theoretically it is thus possible to lose important information if the maximum concentration occurs between two time points. However, the area under a theoretical signal peak occurring between two time points is so small compared to the total area of the curve that it would probably not make any difference (fig. 26). If there was extra time it should rather be invested in 3D scanning.

![Potential peak between two time points](image)

**Figure 26.** The red point displays a theoretical peak between two measuring points. Note the small change of the total area under the curve.

The most feasible solution to perform perfusion measurements would be to use rapid 3D scanning. It would reduce effect from inflow but still have a good temporal resolution in order to catch the rapid perfusion process. 3D scanning are also preferred if the method should be used in diseased lungs. The whole lung parenchyma is rarely homogenously afflicted with disease and a 2D slice will therefore not be that helpful (Risse, 2009). Technical limitations of the scanner and settings made it impossible to sample rapid 3D sequences as needed for measurement of pulmonary perfusion.
Further studies could use methods that reduce the scan time of each slice order to increase the temporal resolution. Reduced spatial resolution, timesaving sampling strategies and parallel imaging could be some alternatives (McRobbie et al., 2007).

Important error sources that were limiting for perfusion measurement were identified in the perfusion section in this work. This was an important step in the process finding a method suitable for DCE-MRI measurements of pulmonary perfusion.
5.3 Permeability measurement

Permeability measurements could be done in 3D because of its little requirements in terms of temporal resolution. Optimization of the 3DFLASH sequence was done for DCE-MRI measurements in the rat lung. The optimization process resulted in a CA concentration injection of 0.4 mmol/ml, which was measured with an untriggered acquisition strategy called repeated3DFLASH and flip angle 20°. In addition, NEX was set to 8 in order to reduce artifacts from motion since no triggering was used. The optimized protocol was used in a pilot study investigating changes in pulmonary permeability after administration of LPS. However, no changes were found between images day 0 and day 1 after visual inspection of images and analysis of enhancement curves.

The CA concentration of 0.4 mmol/ml was chosen after considerations of the relative enhancement of signal from measurements with CA concentration of 0.1 mmol/ml and 0.2 mmol/ml. A concentration that high could be chosen since the latter part of the enhancement curve is used for permeability calculations. At latter time points in the dynamic series, the dilution in the lung is large resulting in a smaller concentration of CA and hence less risk for \( T_2^* \) effects from CA.

The strategy of acquisition with 3DFLASH was investigated using a static plastic phantom. Theoretically a signal from a static phantom should be constant, independent of if the acquisition scheme is triggered or not. However, the investigation of the acquisition method indicated that triggering of the 3DFLASH sequence gave an oscillation of the signal. Potential explanations could be the fact that the time between two trigger pulses was too short to be able to sample a complete 3D slab, which for example can cause acquisition and steady state problems.

The untriggered repeated3DFLASH acquisition method was used because the gradients limited a subsequent scan to 4 repetitions. NEX was increased since no triggering was used in the repeated3DFLASH sequence. NEX can reduce motion artifacts by averaging scans affected by respiratory motion, which commonly is quite steady. Hence, a larger number of averages will reduce the artifacts.

The optimized protocol was used in a pilot study including four animals measured before and after the administration of the inflammatory antigen LPS. Since it was expected to easily see changes from the distributed dose of LPS, no verification of inflammation was done. However, visual comparison of the images from the pilot study day 0 and day 1 did not show any findings and hence it could not be stated whether the inflammation was not visible on the MR images or did not exist at all. The comparison of the enhancement curves from a ROI in the lung day 0 and day 1 showed neither any difference.

If LPS is correctly administrated it will create an inflammation in some part of the lung. However, the inflammation will not cover the whole lung and therefore it can be hard to define the affected region if no visible change is seen. In the analyses it is important to be able to select a ROI that is healthy day 0 and inflammed day 1 in order to detect a change in
permeability. This becomes difficult and also more important when the inflammatory region
not covers the whole lung.
Therefore, further studies should use verification methods as inflammatory cell count or
morphologic MRI in order to define a region of inflammation for further analyses. T2-
weighted SE imaging could be useful if edema as a consequence of the inflammation already
was present (takes often ≥ 48 h). Inflammatory cell counting was not possible to perform in
this study due to technical limitations.
CONCLUSION

Protocols for quantitative measurements of pulmonary perfusion and permeability using a preclinical MR scanner were successfully developed. However, there were difficulties involved that limit the application of the perfusion protocol in practice. Finding an appropriate CA concentration from the linear signal CA concentration range was hard using 2D scanning due to inflow effects. To avoid inflow effects, further experiments should investigate the capacity of rapid 3D scanning for the determination of the signal CA concentration range but also for the protocol of quantitative pulmonary perfusion measurements.

A protocol for measurements of pulmonary permeability was successfully developed in this report. During a pilot study investigating permeability changes after an administration of LPS, no change could be found by visual inspection of the resulting images. However, this was due to difficulties to define a region of inflammation since the entire lung was not affected of inflammation. Therefore, further pilot studies have to use verification methods of inflammation e.g. cell counts in order to have a reliable starting point in the identification of changes in pulmonary permeability.
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APPENDIX

A1. Enhancement curves from the in vivo experiment in the perfusion section (4.3.1.3) for all four rats (A, B, C, D)