Dose Optimization for Using the Contrast Agent Gadofosveset in Magnetic Resonance Imaging (MRI) of Domestic Pig Brain

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Summary

Pigs are useful models in stroke research, and Magnetic Resonance Imaging (MRI) is a useful tool for measurements of brain pathophysiology. Perfusion Weighed Imaging (PWI) with standard Gd-based chelates (i.e. gadobutrol) provides crucial information about breakdown of the Blood-Brain-Barrier (BBB) in patients. Gadofosveset is also a Gd-based contrast agent, but with a higher binding to serum albumin. The prolonged plasma-half life of gadofosveset allows the acquisition of steady state angiographies, which may increase the sensitivity for detection of BBB leakage. We hypothesize that the contrast dosage with gadofosveset can be optimized for PWI and subsequent steady-state Magnetic Resonance Angiography (MRA) in pigs. Anesthetized domestic pigs (females; N=6) were MRI scanned four times in one day: they were initially imaged during a standard gadobutrol bolus injection (0.1 mmol/kg). Then they received three successive gadofosveset bolus injections of varying dosages (0.015-0.09 mmol/kg). Based on projection from our data, we suggest that a bolus injection of 0.0916 mmol/kg gadofosveset would yield contrast similar to that of a standard dose of 0.1 mmol/kg gadobutrol in dynamic susceptibility contrast MRI at 3 T. In conclusion, our results demonstrate the feasibility of gadofosveset based PWI in pig brain research. The relaxation and plasma half-life properties allow detailed steady-state MRA angiographies and may prove useful in detecting subtle BBB disruption of significance in stroke models and human patients.

Introduction

Pigs are widely used in brain research (*Lind et al., 2007*). Several porcine models of human brain diseases are under development, such as the transgenic model of Alzheimer´s disease. Other models already exist, such as the MPTP model of Parkinson disease (*Mikkelsen et al., 1999*) and the middle cerebral artery occlusion model of stroke (*Sakoh and Gjedde, 2003; Sakoh et al., 2000; 2001; 2003; Watanabe et al., 2007*). The imaging techniques positron emission tomography (PET), computerized tomography (CT) and magnetic resonance imaging (MRI) provide useful tools for non-invasive measurements of the brain pathophysiology in these models (*Alstrup & Winterdahl, 2009)*. Perfusion Weighed Imaging (PWI) provides crucial diagnostic and prognostic information in a patient presenting with symptoms of acute stroke and may therefore also be relevant for evaluation of the stroke models. Current PWI protocols utilize standard Gd-based chelates with rapid vascular clearance to the extracellular fluid (ESF) space. While this property is ideally suited for subsequent visualization of tissue with severe Blood-Brain-Barrier (BBB) breakdown, it has been speculated that rapidly declining blood-tissue Gd-chelate concentration gradients may preclude the

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detection of more subtle BBB leakage. In acute stroke, recent findings indeed suggest that early BBB leakage may herald subsequent hemorrhagic transformation (*Hjort et al., 2008*). These findings are however inconsistent across studies, suggesting that it is crucial to further explore the prognostic potential of BBB leakage (*Hjort et al., 2008*). The use of contrast agents with a long plasma half-life at high field for PWI and subsequent post contrast T1 weighted imaging is a particular promising strategy towards this goal. Meanwhile, prolonged blood-pool contrast allows steady-state Magnetic Resonance Angiography (steady-state MRA), with high spatial resolution (*Bluemke et al., 2001; Grist et al., 1998; Knight et al., 1998*). In principle, steady-state MRA with a blood-pool contrast agent allow rapid identification of intracranial vascular occlusion or carotid stenosis to establish indications for acute thrombolytic therapy or subsequent endarterectomy, without additional contrast agent administration. Gadofosvest trisodium is a Gd-based contrast agent with high binding to serum albumin (*Caravan et al., 2002*). The prolonged plasma-half life allows the acquisition of steady state angiographies for up to an hour after injection. Although the use of gadofosveset for dynamic susceptibility contrast (DSC) MRI has not been reported in the literature, we hypothesize that contrast dosage can be optimized for PWI and subsequent steady-state MRA. In this analysis we simultaneously optimize gadofosveset dose to yield signal loss in T2* weighted DSC similar to that of a standard dose of Gd-chelate and provide sufficient Contrast-to-Noise ratio (CNR) of contrast enhanced MRA in domestic pigs.

Materials and Methods

Laboratory animals

We examined six Danish Landrace/Yorkshire pigs (female, body weight 36 to 41 kg) from the university animal facility. The pigs were fed with a restricted pellet diet and iron supplement, and were group-housed for 1 week prior to the study. The animals were fasted 16 hours prior to the study but had free access to tap water. All experimentation was done in accordance with the Danish National Committee for Animal Experiments.

Animal anesthesia and preparation

Sedation was initiated by i.m. injection of 0.25 mL/kg midazolam (5 mg/ml, Dormicum, Roche, Denmark) mixed with 0.25 mL/kg ketamine (25 mg/ml, S-ketamin, Pfizer, Denmark). Venous access was established in an ear vein, and an additional bolus of 0.25 mL/kg midazolam and 0.125 mL/kg ketamine was administered. The animals were then placed in dorsal recumbency and wrapped with thermostatically controlled heating blankets to maintain normal body temperatures. In order to achieve optimal MR-headcoil placement, orotracheal intubation was discarded in favour of tracheotomy using a 7.0 mm tube. Artificial ventilation with 2% isoflorane (Forene, Abbott, Solna, Sweden), 0.5 L/min O_2 and 1.2 L/min atmospheric air was initiated and maintained by use of a respirator. Urinary catheterization was performed. Surgical exposure of femoral vessels and insertion of catheters (Cordis Avanti 7 F and 6 F, Johnson and Johnson, Miami, FL) enabled blood pressure measurements and blood sampling from the femoral artery. The femoral vein provided the route for repeated bolus injections of contrast agent. Upon completion of the experiment, anesthetized pigs were euthanized with at least 20 mL (0.5 mL/kg) i.v. pentobarbital (200 mg/mL).

Monitoring

Palpebral and interdigital reflexes were checked thoroughly before any surgical intervention and during experimentation to assure sufficient anesthesia. Blood pressure and heart rate were continuously monitored (PM-9000 Vet, Mindray. Shenzhen, China). Arterial blood was sampled at regular intervals yielding blood gas-, oximetry- and acid-base values (ABL520, Radiometer. Brønshøj, Denmark). Urine production was also monitored.

Contrast agent

Gadofosveset trisodium (Vasovist®, Bayer Schering Pharma. Berlin, Germany) is a gadolinium-

based, intravascular contrast agent registered for angiography. Being highly (80-96%) and reversibly bound to the most ubiquitous plasma protein, albumin, it exhibits a very long terminal plasma half-life of 18.5 ± 3.0 h.

For dose optimization, gadofosveset was compared with a typical extracellular agent, gadobutrol (Gadovist 1.0®, Bayer Schering Pharma. Berlin, Germany) with a plasma half-life of roughly 1.9 h (Bayer I. Product Monograph. Gadovist ©. 1.0).

Gadofosveset has high relaxivity (*Rohrer et al., 2005*), with a recommended dose for gadofosveset enhanced MRA of 0.03 mmol/kg, compared with 0.1 mmol/kg recommended for gadobutrol. Gadofosveset longitudinal (R_1) and transverse (R_2) relaxivities in plasma at 3T at 37°C are 9.9 $L \cdot mmol^{-1} \cdot s^{-1}$ and 60 $L \cdot mmol^{-1} \cdot s^{-1}$, respectively (*Rohrer et al., 2005*), compared with 5.0 $L \cdot mmol^{-1} \cdot s^{-1}$ and 7.1 $L \cdot mmol^{-1} \cdot s^{-1}$ for gadobutrol (*Rohrer et al., 2005*).

MR protocol

Animals were scanned using a 3.0 T GE Signa HDx scanner (General Electric Medical Systems, Milwaukee, WI, USA). Contrast agents were injected at a rate of 5 mL/s followed by 30 mL saline flush using an automated MR-compatible power injector (Spectris Solaris, Medrad, Pittsburgh, USA). The pigs were initially imaged during a standard (0.1 mmol/kg) gadobutrol bolus injection, followed by a break of 42 min or more to allow partial renal contrast agent clearance (gadobutrol half-life is 114 minutes). Pigs then received three successive gadofosveset bolus injections of varying dosages (0.015-0.09 mmol/kg). Injections were separated by at least 15 minutes.

T2 imaging parameters*

DSC T2* MRI was performed by gradient recalled echo (GRE) single shot (TR/TE 1500/30 ms, 60° flip angle) echo-planar imaging (EPI) with 14 slices (slice thickness of 4 mm), and a 128 x 128 acquisition matrix, covering a 24 x 24 cm axial field of view (voxel volume 4 x 1.875 x 1.875 mm^3). Imaging was started 20 seconds prior to bolus arrival.

T1 imaging parameters

Steady state MRA was performed using a 3D sequence (TR/TE 6.624/2.86 ms, 14° flip angle) with 60 slices (slice thickness of 1 mm), and a 256 x 256 acquisition matrix, covering a 24 x 24 cm axial field of view (voxel volume of 1 x 0.9375 x 0.9375 mm^3). T1-weighted images were acquired immediately after each bolus injection to provide CNRs for a range of contrast agent concentrations.

Image analysis

Movements across serial examination were corrected by co-alignment and all images were then co-registered to an in-house landrace brain atlas using linear transformation with 6 to 12 freedom angles and scaling. White- and grey-matter VOIs (volumes of interest) were created from predefined atlas masks (frontal white matter and frontal cortex) with a cut-off confidence level of 70%. A combined white- and grey-matter VOI was also created where both masks had a level of confidence above 90%. This "shrinkage" of predefined masks was constructed to avoid possible errors originating from co-registration imprecision.

T2 Dynamic susceptibility contrast analysis*

For the DSC imaging, we assumed a linear relationship between injected contrast agent dosage, plasma concentration, and change in transverse relaxation ΔR_2*:

$$d \propto C \propto \Delta R_2 * \qquad (1)$$

where d is the injected contrast dosage, C is the plasma concentration. The relationship between C and ΔR_2 is given by:

$$C(t) = k \cdot \Delta R_2 * = -k \cdot \ln \frac{S(t)}{S_0} \cdot \frac{1}{TE} \qquad (2)$$

k is a constant of proportionality, S_0 is the signal intensity at baseline while S(t) is the intensity at a given time point. TE is Time of Echo.

Signal-time curves from the VOIs described above (grey-, white- and both combined) were extracted

from all T2* images and normalized to the mean value of the ten pre-bolus signals. See Figure 1.

Results

Examples of steady-state MRA and comparison with digital subtraction angiography (DSA), along with hemodynamic maps are shown in Figure 2.

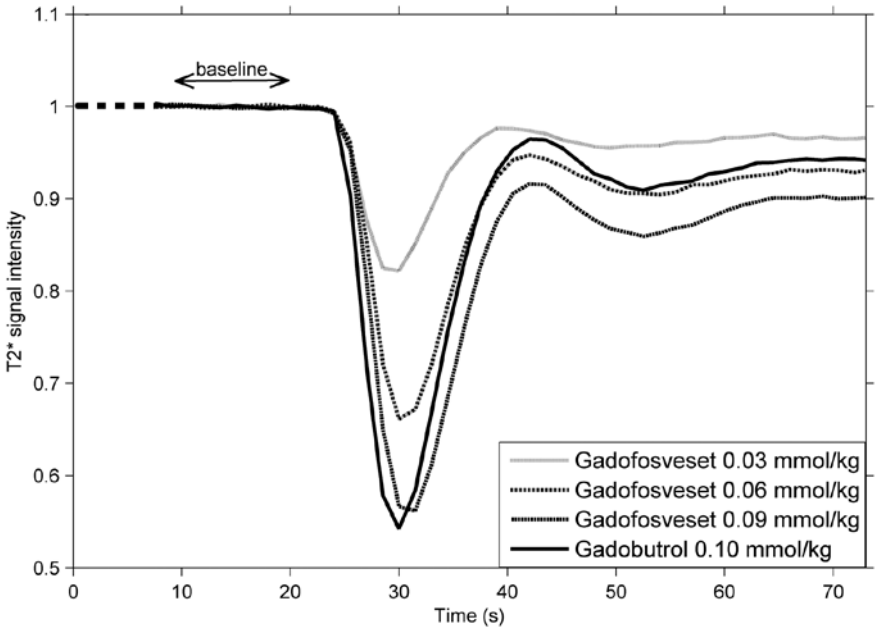

Figure 1. T2* signal intensity drop during bolus passage in pig no. 1. Notice the similarity between 0.1 mmol/kg gadobutrol and 0.09 mmol/kg gadofosveset.

T1 steady-state MRA Contrast-Noise Analysis

Contrast-to-noise ratio was determined for vessels

$$CNR = \frac{S_{vessel} - S_{white-matter}}{\sigma_0} \qquad (3)$$

relative to white matter signal intensity:

S_{vessel} is the maximum observed vessel signal intensity, $S_{white\text{-}matter}$ is the mean white matter intensity and σ_0 is the noise standard deviation of a ROI (region of interest) in artifact free air flanking the pig. Maximum values within the vessel-mask was used in order to avoid partial volume effects.

T2 Dynamic Susceptibility Contrast*

Figure 3 shows maximal R_2* change corresponding to different gadofosveset dosages in grey and white matter, respectively, with corresponding linear regression lines. The horizontal lines correspond to the average R_2* change in grey and white matter, respectively, for gadobutrol. Notice the interception at approximately 0.09 mmol/kg. Figure 4 shows R_2* change in the combined grey- and white-matter VOI with linear regression and 95% CI. Initial 0.1 mmol/kg gadobutrol injection in each pig (n=6) produced a mean R_2* change of 5.926 arbitary (arb.) units/mmol/kg (95% CI ±0.845). This result is illustrated as the horizontal line on the figure. Interception of the horizontal and linear regression line reveals the

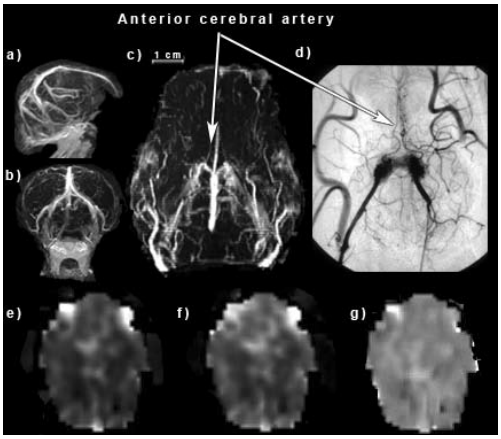

Figure 2. a,b,c) Gadofosveset steady-state MRA MIP. Sagittal, coronal, and basal view, respectively. (TR/TE 6.624/2.86 ms, 14° flip angle. 24 x 24 cm axial field of view, voxel volume 1 x 0.9375 x 0.9375 mm^3). d) Example of DSA. e,f,g) Basal view of Gadofosveset based perfusion maps. CBV, sCBF and sMTT (left to right). 3x3 uniform smoothing kernel used.

estimated gadofosveset dosage needed to generate the same R_2*-change as would 0.1 mmol/kg gadobutrol . This value is 0.0916 mmol gadobutrol/kg. Note that there is an overlap of two data points in Figure 4 at 0.03 mmol/kg, for the R_2*-value of 0.19 arb. units.

T1 steady-state MRA Contrast-Noise-Ratio

Figure 5 shows CNR values for all six pigs as a function of accumulated gadofosveset dose, describing vessel conspicuity relative to white matter. The curve initially increases as accumulated dose increases, and then levels off. After the plateau, no clear drop is seen for the measured dosages. The vertical dashed line marks the optimal gadofosveset dose found for T2* as described above. The value at this point also seems to be optimal for CNR for steady-state MRA, indicating a minimal optimal dose of 0.0916 mmol/kg.

Discussion

We propose a bolus injection of 0.0916 mmol/kg gadofosveset yields contrast similar to that of a standard dose of 0.1 mmol/kg gadobutrol in DSC MRI at 3 T. Based on overlapping confidence intervals in our R_2*/dose results (table 1) we can not discard the null-hypothesis that gadofosveset and gadobutrol display identical R_2*-value.

The ideal condition for bolus tracking (T2*) would have been to wait for total clearance (approx. 5 x 18.5 hours). As this was not possible, we chose to wait for at least 42 minutes after gadobutrol injection and 15 minutes after each gadofosveset injection. The interest is in the signal drop seen as each bolus passes the brain, e.g. the change from the each pre-injection baseline. As long as saturation is not reached, this drop in signal will be relatively large compared to the baseline. However, the signal drops (Figure 1) are clearly dependent on the concentration even for the last injection of high concentration. Therefore, we assume that saturation was not reached.

The ideal condition would be to give only one injection of gadofosveset with the total dose. However, since the half-life is long (18.5 hours) we found it reasonable to use the accumulated dosages, as the steady-state signal enhancement on T1 weighted images would in theory be hardly affected by splitting up the administration of the contrast agent into three successive time points.

Studies using 1.5 T have reported a peak in CNR or best reader diagnosis accuracy at the recommended MRA dose of 0.03 mmol/kg. We found CNR to peak around 0.09 mmol/kg at 3 T. Rohrer *et al.* have reported the value for 3 T plasma R_1 for gadofosveset to be half of the value at 1.5 T (*Rohrer et al., 2005*). This explains the higher dosage needed for CNR to peak at 3 T.

While time-of-flight (TOF) and first-pass contrast enhanced MRA remain predominant approaches to detect steno-occlusive disease, we speculate that steady-state MRA may represent a convenient means of diagnosing extracranial internal carotid stenoses, believed to account for 10-20% of all

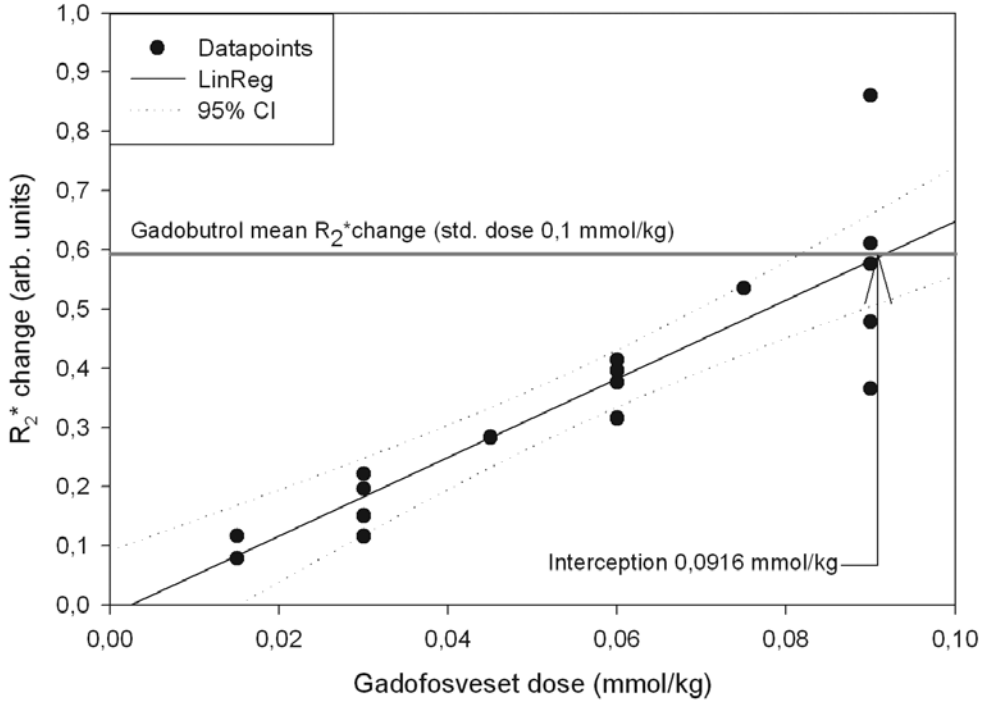

Figure 4. R_2* change as a function of gadofosveset trisodium dose**.** Linear regression line also shown y = -0.016 + 6.645x (r^2 = 0.79). Dotted lines are 95% CI. In comparison, the horizontal dashed line at y = 0.592 (95% CI ± 0.085) marks mean R_2* change from six injections of a standard 0.1 mmol/kg gadobutrol dose. Line interception is at x = 0.0916 mmol gadofosveset/kg. Note that there is an overlap of two data point at 0.03 mmol/kg, for the R_2*-value of 0.19 arb. units.

ischemic strokes (*Sacco et al., 1989*). In particular, the rapid imaging of multiple vascular segments following a single contrast bolus injection may prove important for stroke management: atherosclerotic plaques in the aortic arch are significant predictors of recurrent ischemic stroke and other vascular events (*Amarenco et al., 1996*).

Steady-state MRA, however, has the disadvantage of venous enhancement, rendering visual distinction between arteries and veins difficult, especially on 2D Maximum Intensity Projection (MIP) images where spatial information is lost. Three-dimensional MIP rendering and computerized vessel tracking or segmentation may serve to circumvent this problem.

Another speculated advantage of gadofosveset is improved prediction of hemorrhagic transformation (HT) after ischemic stroke. In ischemic stroke, prolonged, local hypoperfusion is thought to cause blood-brain-barrier (BBB) disruption (*del Zoppo et al., 1998*). Evidence of contrast agent extravasation through a disrupted BBB can be demonstrated by MRI. These manifestations are known as parenchymal enhancement (PE, on T1-weighted imaging) and hyperacute reperfusion marker (HARM, on T2 FLAIR) and correlate with increased risk of HT and consequently poor clinical outcome. We hypothesize that conspicuity of these predictive manifestations will increase with utilization of gadofosveset. Better knowledge of whether or not BBB-disruption has occurred is crucial information when appraising

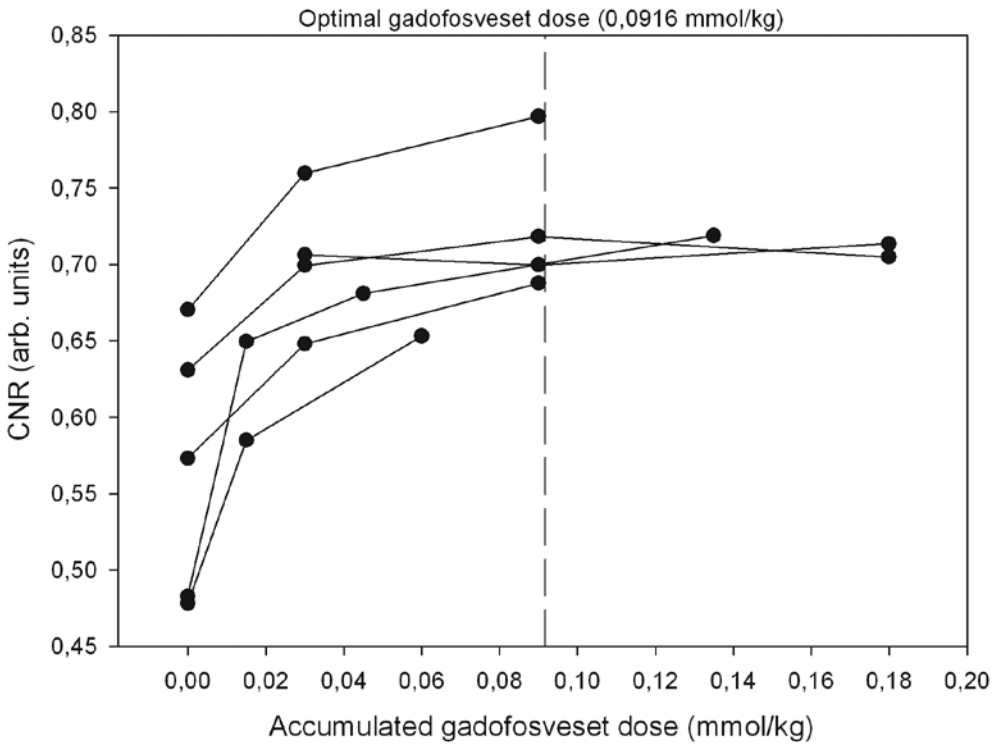

Figure 5. T1 CNR line graphs for all six pigs. Dashed line marks optimal gadofosveset dose found for T2*. See text for details.

Table 1. R_n* changes at different contrast dosages.

Contrast agent	Gadobutrol	Gadofosveset					
Dose (mmol/kg)	0.1 (n=6)	0.015 (n=2)	0.03 (n=5)	0.045 (n=1)	0.06 (n=4)	0.075 (n=1)	0.09 (n=5)
Mean ΔR_2*	0.593 (±0.085)	0.098 (±0.026)	0.176 (±0.033)	0.283 (N/A)	0.375 (±0.036)	0.534 (N/A)	0.578 (±0.145)
Mean ΔR_2*/dose	5.926 (±0.845)	6.520 (±4.495)	5.878 (±1.106)	6.295 (N/A)	6.252 (±0.604)	7.123 (N/A)	6.427 (±1.607)

95% CI in parentheses. Mean ΔR_2*/dose for all gadofosveset measurements = 6.277 (±0.609) and gadobutrol = 5.926 (±0.845)

if stroke patients will benefit from thrombolytic or neuroprotective intervention.

The European Medicines Agency and others have investigated the adverse effects of gadofosveset

(*Perreault et al., 2003*). Generally the agent is well tolerated, with the most frequent treatment-related adverse effects being pruritus, paresthesia, burning sensation, vasodilation, headache and nausea. The adverse effects are dosage-dependent but usually mild, with rapid onset and short duration (European Medicines A. Vasovist: Scientific discussion. 2008). No maximum allowed dosage is provided by the manufacturer or the European Medicines Agency. Even though no cases of nephrogenic systemic fibrosis (NSF) have been documented, caution should be taken before administering gadofosveset to renally impaired patients. We observed no side effects in the six tested pigs.

There are several limitations to the study. The number of experimental animals subjects (n=6) is relatively small. However, we believe that the sample size was large enough to conclude that gadofosveset is useful for pig studies. Due to the long half-life of gadofosveset (18.5 h) we did not correct for the time interval between scans.

Gadofosveset may not only be used in laboratory pigs, but also in human patients with acute symptoms of stroke. Although the circulation and renal function of pigs is believed to be similar to that of humans, optimization of gadofosveset dose must be performed for humans at relevant MRI field strengths.

In conclusion, our results have demonstrated the feasibility of gadofosveset based PWI. The relaxation and plasma half-life properties allow detailed steady-state MRA angiographies and may prove useful in detecting subtle BBB disruption of significance in, for example, acute ischemic stroke. Demonstration of the potential advantages of early identification of BBB disruption awaits preclinical and clinical trials. We believe that the pig is a good model for such studies. We suggest a bolus dose of 0.0916 mmol/kg for acquiring PWIs equal to those generated with 0.1 mmol/kg gadobutrol and steady-state MRA with good CNR.

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