Aerosol Delivery in Ventilated Newborn Pigs: An MRI Evaluation

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ABSTRACT: Pulmonary deposition of inhaled drugs in ventilated neonates has not been studied *in vivo*. The objective of this study was to evaluate pulmonary delivery of gadopentetate dimeglumine (Gd-DTPA) following nebulization in ventilated piglets using magnetic resonance imaging. Seven ventilated piglets (5 ± 2 d old, weight 1.8 ± 0.5 kg) were scanned in the Bruker/Siemens 4T magnetic resonance scanner using T1 weighted spin-echo sequence. Aerosols of Gd-DTPA were generated continuously using the MiniHeart jet nebulizer. Breath-hold coronal images were obtained before and every 10 min during aerosolized Gd-DTPA for 90 min. Signal intensity (SI) changes over the lungs, kidneys, liver, skeletal muscle, and heart were evaluated. A significant increase in SI was observed in the lungs, kidney, and liver at 10, 20, and 40 min respectively after start of aerosol. At the end of 90 min, the SI increased by 95%, 101%, and 426% over the right lung, left lung, and kidney, respectively. A much smaller increase in SI was observed over the liver. In conclusion, we have demonstrated effective pulmonary aerosol delivery within 10 min of contrast nebulization in ventilated piglets. Contrast visualization in the kidneys within 20 min of aerosol initiation reflects alveolar absorption, glomerular filtration and renal concentration. (*Pediatr Res* 64: 159–164, 2008)

Neonatal hypoxemic respiratory failure (NHRF) is associated with potentially reversible pulmonary hypertension that causes right-to-left shunting and profound hypoxemia. Inhaled nitric oxide, a selective pulmonary vasodilator, has revolutionized the treatment of NHRF (1). However, lack of sustained improvement in 30–46% of infants and the need for specialized delivery systems make the treatment expensive and limit availability. Several investigators have explored the use of aerosolized prostaglandin E_1 (PGE_1) as a selective pulmonary vasodilator in patients with respiratory failure to improve oxygenation because of its selective action not only on the pulmonary circulation, but also on well-ventilated lung units (2–5). We have previously described the safety and feasibility of inhaled PGE_1 (IPGE₁) in NHRF in a phase I/II unblinded clinical trial (5). We have also reported the stability and emitted dose of IPGE₁ following jet nebulization in a neonatal ventilator circuit and predicted primarily alveolar

deposition based on the aerosol particle size distribution (6). Alveolar deposition is important for optimal drug effect. Although it is recognized that aerosolized drug delivery is more efficient in spontaneously breathing subjects compared with those undergoing assisted ventilation and in adults compared with children and neonates, the deposition pattern of inhaled drugs in ventilated neonates remains largely unknown because of ethical concerns in this age group (7,8). Several investigators have reported the feasibility of performing magnetic resonance (MR) ventilation imaging in animal models and human adults with aerosolized Gadopentetate dimeglumine (Gd-DTPA) as a contrast agent (9–17). The present study was designed to assess aerosol delivery in neonatal pigs undergoing mechanical ventilation using functional MR imaging (MRI) with aerosolized gadopentetate dimeglumine (Gd-DTPA) as a surrogate.

MATERIALS AND METHODS

Subjects. The study was performed on nine healthy domestic piglets (2–6 d old) from a specific pathogen free litter after approval by the Institutional Animal Care and Use Committee. All animals received care in compliance with the National Institutes of Health guidelines.

Animal preparation. Animals were intubated oro-tracheally after intramuscular premedication with midazolam and ketamine. The cephalic vein was catheterized percutaneously for vascular access. Anesthesia was maintained by i.v. infusion of ketamine, midazolam, and fentanyl titrated to the level of anesthesia as assessed by monitoring heart rate, blood pressure, and response to painful stimuli. Maintenance fluids consisting of dextrose-saline were administered. Body temperature was maintained at 38–39°C. Baseline measurements of blood gas parameters were obtained followed by MRI.

Mechanical ventilation. Positive pressure ventilation was provided manually at a rate of 15 bpm and peak inspiratory pressure of 18 cm H₂O using room air by trained research staff during the MRI protocol. The ventilator circuit used is similar to the one used for hospitalized neonates undergoing mechanical ventilation in the MRI suite. Oxygen saturations were monitored using a pulse oximeter. The gas delivered into the circuit was neither heated nor humidified. All experiments were carried out at room temperature.

Administration of continuous aerosol. The low flow MiniHeart jet nebulizer (Westmed Inc., Lakewood, Co) was used to generate continuous aerosols as described previously (5). The nebulizer was placed in the inspiratory limb of the ventilator circuit close to the ETT. The oxygen flow through the jet nebulizer was set at 2 LPM. Because oxygen is known to be a paramagnetic contrast agent, the nebulizer was placed in the nebulizer circuit from the beginning of the experiment to assess baseline conditions. At the start of aerosol therapy, the nebulizer chamber was primed with 4 mL of Gd-DTPA (0.5 mol/L) followed by continuous delivery into the nebulizer chamber at a rate of 8 mL/h. The MiniHeart nebulizer generates aerosols with a mean particle size of 2–3 μ m.

Abbreviations: Gd-DTPA, gadopentetate dimeglumine; MR, magnetic resonance; NHRF, neonatal hypoxemic respiratory failure; PGE_1 , prostaglandin E_1 ; SI, signal intensity

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MR imaging. Anesthetized term newborn piglets undergoing manual positive pressure ventilation were scanned in the Bruker 4T MR scanner in the supine position using T1 weighted spin-echo sequence with a head coil used for excitation and reception. The first scan was performed before the administration of aerosolized Gd-DTPA to assess baseline conditions. Next, Gd-DTPA was infused into the nebulizer chamber without moving the animal from its original position inside the tomograph. Serial images at the same anatomic level were obtained for 90 min during ventilation with aerosolized Gd-DTPA. To reduce motion artifacts from breathing, respiratory gating was accomplished by data acquisition in the inspiratory phase (18 s) during a breath hold. Respiratory-gated coronal images were obtained before and every 10 min during aerosolized Gd. The MRI parameters included: TE of 12 ms, TR of 200 ms, one NEX (number of excitations), field of view of 250×125 mm², slice thickness of 5 mm with nine continuous slices acquired interleaved, base resolution 256, and phase resolution of 96 then interpolated to 512×256 , phase partial Fourier 6/8. The reception bandwidth was 150 Hz. Signal intensity changes over corresponding regions of the lungs, liver, kidneys, skeletal muscle, and heart were monitored over time to assess Gd-DTPA deposition.

MR images were analyzed using our internally developed MR signal processing in NMR (SPIN) software that is written in Visual C++ for the Microsoft Windows platform (18). Signal intensities were measured in defined regions of interest before and at each time point after administration of aerosolized agent (0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min). Regions of interest were defined in lungs, liver, heart, kidney, and skeletal muscle. Regions were chosen to contain a minimum of 100 pixels. The percent enhancement of signal intensity (SI) over baseline was calculated by means of the following formula: $([SI_{\text{postcontrast}} - SI_{\text{precontrast}}] / SI_{\text{precontrast}}) \times 100$. Subtraction images were generated as the difference between the Gd-DTPA-enhanced images and baseline images.

Tissue concentration of Gd was extracted from the ratio of the signal intensity as a function of time, $s(t)$, to the signal intensity at baseline, s_0 . Signal intensity as a function of time is proportional to $[1 - \exp(-TR/T_1(t))]$. The ratio $s(t)/s_0$ can thus be calculated directly. To convert this ratio to concentration requires a knowledge of $T_1(0)$ in the equation $1/T_1(t) = 1/T_1(0) + \alpha \times c(t)$, where α is approximately 5 (mM sec)^{-1} , $c(t)$ is the tissue concentration of Gd as a function of time, and $T_1(t)$ is calculated by $T_1(t) = -TR / \ln\{1 - [1 - \exp(-TR/T_1(0))] s(t)/s_0\}$ for spin echo. Because $T_1(0)$ is not well characterized at 4T, a range of $T_1(0)$ values for the lung (0.8, 1.2, and 1.6 s) and kidney (1.4, 1.6, and 1.8 s) were used to estimate a range of concentrations.

Euthanasia. At the end of the experimental protocol, the piglets were euthanized by i.v. overdose of pentobarbital (90 mg/kg) and bilateral pneumothoraces.

Statistical analyses. Descriptive statistics were used to summarize sample characteristics. Sample means and standard errors of the detected signal intensities were calculated for lungs, kidney, liver, heart, and skeletal muscle at each of the 10 time points before the administration of the contrast agent till the end of the experiment. The mean signal intensities (standard errors) were plotted against time to delineate time trends. Linear mixed models were used to assess the increases in detected signal intensities over time (19). Although the detected signal intensities for the left lungs appeared more variable than those from the right lungs, the difference was not statistically significant. Data from the two lungs were thus combined in analysis. Similarly, average data for the kidneys was used in the analysis. Statistical significance was defined as a p value of less than 0.05. Statistical analyses were performed using the SPSS statistical package, version 15.0.1 (SPSS Inc., Chicago, IL) and SAS/STAT software, Version [9.1.3] (SAS Institute Inc., Cary, NC).

RESULTS

Nine intubated term newborn piglets underwent MR scans. Two pigs died during transport before contrast administration (tension pneumothorax = 1; massive pulmonary hemorrhage = 1). The remaining seven pigs received aerosolized Gd. Only data for the seven pigs that received aerosolized Gd-DTPA are presented. The mean $\pm$ SE age and weight of the piglets was 5 ± 2 d and 1.8 ± 0.5 kg, respectively.

On visual inspection of the MR scans, enhancement of the collecting system of the kidneys was dramatic and observed in all animals at 30 min (Fig. 1). Figure 2 shows marked enhancement of the left lung at the end of 90 min that is better

Figure 1. SI changes on T1-weighted images in healthy ventilated piglets after inhalation of Gd-DTPA. (A) At baseline, before contrast administration, both lungs have low SI (21 for left lung and 14 for right lung) and the renal collecting system appears dark (arrowheads). The liver (L) and gastric contents (G) are indicated. (B) 30 min after aerosolized Gd-DTPA inhalation, both lungs enhance homogeneously (SI = 37 for left lung, 26 for right lung) and contrast is visualized in the renal collecting system (arrows). The SI over the liver and gastric contents is unchanged.

visualized on the subtraction image obtained by subtracting the baseline image from the 90 min postcontrast image.

The percent increase in lung SI in healthy ventilated piglets after administration of aerosolized Gd is shown in Figs. 3 (first 20 min) and 4 (over entire duration of study). The baseline SI before administration of aerosolized Gd was assigned a value of 0. After inhalation of aerosolized Gd-DTPA, the detected signal intensity in lungs increased significantly ($p < 0.05$) above baseline as early as 10 min after onset of aerosol for both lungs (mean $\pm$ SE increase in SI for left lung $9 \pm 3\%$, $p < 0.05$; right lung $13.5 \pm 2\%$, $p < 0.01$; average for both lungs $11.3 \pm 2\%$, $p < 0.01$) (Fig. 1). There was a significant increase in SI over both lungs with time on longitudinal data analysis using proc mixed (estimate = 1.4, SE = 0.4, $p < 0.05$) (Fig. 2). At the end of 90 min, the signal intensity in the right and left lung increased by (mean $\pm$ SD) 95 ± 40 and $101 \pm 47\%$, respectively, with an average increase of $98 \pm 38\%$. The change in SI over the lungs as a function of time could be described by the following linear regression equation: Change in SI = $1.2 \times \text{Time (min)} - 10$ ($r^2 = 0.9$).

Significant increase in SI over the kidneys was first detected 20 min after the onset of aerosol therapy ($31 \pm 11\%$, $p < 0.05$) (Fig. 1). There was a significant increase of SI over the kidneys with time (estimate 6.1, SE = 1.0, $p < 0.01$) (Fig. 2); at the end of 90 min, the signal intensity in the collecting

Figure 2. Significant increase in SI over left lung (outlined) at 90 min (B) compared with baseline (A). This enhancement is better visualized on the subtraction image (C). Change in signal intensity over the right lung is not as marked in these scans.

Figure 3. Changes in SI over lungs (□) and kidneys (■) during first 20 min of aerosol administration.

Figure 4. Changes in SI over lungs (□) and kidneys (■) during aerosol administration over 90 min.

system of the kidneys was $426 \pm 60\%$ above baseline. The change in SI over the kidneys as a function of time could be described by the following linear regression equation: Change in SI = $5.1 \times \text{Time (min)} - 29$ ($r^2 = 0.98$).

Although there was a significant increase in signal intensity over the liver and skeletal muscle with duration of aerosol therapy (estimate 0.1, SE = 0.05, $p < 0.05$), the absolute

Figure 5. Tissue concentration of Gd in kidney (solid lines) and lung (interrupted lines) over time as calculated from the changes in signal intensity. T1(0) values used were 0.8 s (◇), 1.2 s (□), and 1.6 s (△) for lung; and 1.4 s (◆), 1.6 s (■), and 1.8 s (▲) for kidney.

increase in signal intensity was much lower than over the kidneys and lungs and was first observed 40 min after start of aerosol therapy. At the end of 90 min, the increase in SI was over the liver and skeletal muscle was $14 \pm 2\%$ and $16 \pm 6\%$ above baseline, respectively. The change in SI over the heart was insignificant over the course of the experiment.

Tissue concentrations of Gd in the lung and kidney for different values of T1(0) are shown in Fig. 5. The lung concentrations were higher than the kidney concentrations in the first 20 min. The kidney concentration at 90 min (0.69–1.13 mM) was much higher than the lung Gd concentration at 90 min (0.15–0.42 mM). There was a significant positive correlation between the tissue concentration of Gd in the lungs and kidneys (correlation coefficient = 0.91, Fig. 6).

DISCUSSION

We have successfully demonstrated effective pulmonary aerosol deposition in mechanically ventilated neonatal pigs using MRI with aerosolized Gd-DTPA. Although aerosolized Gd-DTPA has been used to define regional lung ventilation in healthy animals, experimental disease models, and human

Figure 6. Correlation of Gd concentrations in lung and kidney over time. A $T1(0)$ of 1.2 s was used for lung and 1.6 s for kidney. The correlation coefficient is 0.91.

adults, this is the first report of the use of aerosolized Gd-DTPA to evaluate pulmonary drug delivery in a neonatal animal model undergoing mechanical ventilation (9–17). Neonatal pigs undergoing mechanical ventilation were chosen, as it is known that aerosol drug delivery is decreased in ventilated subjects as well as in infants and children. Pulmonary deposition was demonstrated as early as 10 min following onset of aerosol therapy indicating successful delivery further supported by the detection of increased SI over the kidneys 20 min after aerosol initiation. MR images were obtained every 10 min for 90 min allowing assessment of SI changes over time. Comparison of SI changes over different organs permitted assessment of drug disposition over time. Tissue Gd concentration in lungs and kidneys was quantified from the signal intensity data.

Pulmonary aerosol deposition has been evaluated by theoretical *in vitro* models, pharmacokinetic studies and pulmonary imaging in animals and humans (7,8,20–25). Theoretical models and pharmacokinetic studies cannot directly demonstrate the site of aerosol deposition; in the latter, it is inferred that plasma and urine levels reflect lower respiratory tract deposition (8). Functional imaging of the lung may allow determination of site of aerosol delivery. Gamma scintigraphy, the gold standard for functional imaging studies, is limited by poor spatial and temporal resolution and radiation exposure (22). Single photon emission computed tomography and positron emission tomography, three-dimensional lung imaging techniques that allow detailed assessment of regional deposition have their limitations. Single photon emission computed tomography data has to be transformed to conceptual lung models that need to be validated. Positron emission tomography is not widely used because of the short half-life of the unstable positron-emitting radioisotopes necessitating an on-site accelerator for production. In contrast, MRI does not rely on ionizing radiation and permits ventilation imaging of the lungs with high spatial and temporal resolution using paramagnetic contrast agents. However, MRI of the lung parenchyma is problematic because of relatively low number of hydrogen protons, cardiac and respiratory motion, and

susceptibility effects induced by multiple air and tissue interfaces (10,26,27). These problems can be overcome by the use of pulse sequences with a very short echo time (TE), breath hold imaging, and cardiac gating. Several investigators have reported the feasibility of performing MR ventilation imaging in animal models and human adults with aerosolized gadolinium (Gd)-DTPA as a contrast agent (9–17). Because of imaging with this $T1$ -shortening contrast agent, a high SI can be generated within the pulmonary air spaces. The highly soluble droplets dissolve rapidly and are absorbed into the blood by rapid diffusion across pulmonary epithelium and capillary endothelium. Once the contrast agent is absorbed from the lung into the plasma, the pharmacokinetics are similar to those of a compound administered intravenously. Calculated half-life for pulmonary clearance is approximately 2 h, with completion of urinary elimination by 30 h. Additional advantages of Gd-DTPA aerosol include its wide availability, comparatively low cost, ability to use with existing MR systems without modification, and safety. The present study was designed to assess aerosol delivery in neonatal pigs undergoing mechanical ventilation using functional MRI with aerosolized Gd-DTPA as a surrogate.

We chose the neonatal pig as a model for this *in vivo* study as the functional thoracic anatomy, lung morphology, pulmonary vasculature, and postnatal development of the pig is close to that of the human being (28,29). In this study, there was a significant increase in the SI over the lungs within 10 min of aerosol administration reflecting early effective aerosol delivery to the lungs. There was progressive increase in SI over time. These results are comparable to those reported in ventilated animal and human adults in whom variable increases in SI over the lungs have been reported at 10–30 min (ranging from 22 to 216%) (12–17,30). The greater variability in SI changes over the left lung compared with the right reflects difficulty in accurately measuring SI because a large portion of the left hemithorax is occupied by the heart. To determine whether the increase in SI over the lungs reflected presence of contrast agent in the interstitial fluid or blood vessels, we determined the change in SI over vascular organs like the liver, skeletal muscle, and heart. Although there was a significant increase in SI over the liver and skeletal muscle over time, the SI increase was delayed and smaller in magnitude compared with the lungs suggesting a low concentration in circulating plasma. This implies that SI changes in the lungs reflect the presence of contrast in the interstitial compartment. Montgomery *et al.* (31) have previously shown that aerosolized Gd-DTPA selectively enhances the extravascular—extracellular water spaces of the lung. Significant increase in SI over the kidneys was evident 10 min after the increase in lungs reflecting extensive alveolar absorption; thereafter, the SI increased rapidly over the kidneys, surpassing the SI increase over the lungs. This can be explained by a combination of rapid clearance from the lung, prompt elimination and concentration by the kidneys, and better visualization of a solid organ by MRI. Tissue concentrations of Gd in the lung and kidney were characterized by higher values for the lungs in the first 20 min followed by higher kidney concentrations subsequently. There was a significant positive correlation

between tissue Gd concentration in lungs and kidneys. This is to be expected if the contrast agent is successfully getting into the blood from the alveoli and is being filtered and concentrated in the kidneys.

This study describes for the first time effective pulmonary drug delivery and distribution following jet nebulization in an *in vivo* neonatal animal model using functional MRI. However, despite the important findings of this study, there are limitations. First, we used a surrogate agent to determine pulmonary deposition. Aerosol particle size depends upon the physicochemical properties of the agent, nebulizer, and patient related factors. Both PGE₁ and Gd-DTPA are water-soluble compounds. We have previously reported the physicochemical properties of PGE₁ and the aerosol particle size distribution (6). In this report, we have used aerosolized Gd-DTPA as a paramagnetic contrast agent. Physicochemical properties and aerosol particle size have been previously characterized for Gd-DTPA (11,12). The aerosol particle size for both PGE₁ and Gd-DTPA favors alveolar deposition; therefore, these results predict pulmonary drug deposition for PGE₁ following jet nebulization in a neonatal ventilator circuit. Second, inhaled molecular oxygen has been shown to be an effective MR contrast agent in MR ventilation imaging because of its paramagnetic properties (27,32,33). Although animals in this study received the same flow rate of oxygen during baseline and aerosolized Gd-DTPA administration to avoid the confounding effect of oxygen on SI changes, it is possible that enhancement of signal intensity after administration of aerosolized Gd-DTPA may be attenuated. To address this confounding effect, scans acquired before inhalation of Gd-DTPA were subtracted from those obtained after inhalation of Gd-DTPA (30). Third, although we have demonstrated effective pulmonary deposition, we have not quantified the actual percent of aerosol delivered. Future mass balance studies involving a closed system with collection of exhaled gas and urinary output and complex mathematical models will permit quantification of aerosolized drug delivery. Fourth, in the present study, the animals were manually ventilated without positive end-expiratory pressure using a modified ventilator circuit with extremely long inspiratory tubing and nebulizer close to the ETT. Despite the fact that these factors would have decreased aerosol delivery to the animal, we were able to demonstrate effective pulmonary aerosol delivery. Although precautions were taken to be consistent in delivering breaths of similar duration and inspiratory pressure, an MR compatible mechanical ventilator may have allowed greater consistency in ventilation thus decreasing regional SI differences (12). The subjects of this study were heavily sedated to avoid motion artifacts. Once the animal was placed in the scanner, no changes were made in the position to avoid degradation of image quality during serial scans. However, other factors may have contributed to degradation of image quality including misregistration between serial images because of relatively long time intervals between imaging and cardiac motion. This may account for the relatively large variation in the lung enhancement observed. The low echo time of 12 ms used in these experiments along with the maximum concentrations of 1–2 mM limits the T2 effect to less than 5–10% in the kidney.

For concentrations on the order of 0.1–0.2 mM, this effect reduces to less than 1%.

In conclusion, our study shows the feasibility of serial MRI assessment of pulmonary drug delivery following administration of aerosolized medications. We have demonstrated effective pulmonary aerosol delivery within 10 min of contrast nebulization in ventilated piglets. Contrast visualization in the kidneys within 20 min of aerosol initiation reflects extensive alveolar absorption and renal concentration. Future studies are needed to quantify efficiency of aerosolized drug delivery in an *in vivo* closed system.

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