Hyperpolarized $^{129}$Xe MR Imaging with Balanced Steady-state Free Precession in Spontaneously Breathing Mouse Lungs

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Purpose: We investigated the characteristics of hyperpolarized (HP) $^{129}$Xe magnetic resonance (MR) imaging obtained from balanced steady-state free precession (SSFP) measurement of mouse lungs, especially under spontaneous breathing, and compared the results with those obtained using traditional spoiled gradient echo (SPGR) method, focusing on improved signal-to-noise ratio (SNR) and reduced total acquisition time.

Methods: We calculated magnetization response of the HP$^{129}$Xe gas for the balanced SSFP sequence under spontaneous breathing to derive optimal conditions for the imaging experiment. We then placed an anesthetized mouse in the magnet (9.4T) supplied with oxygen gas and a mixture of HP$^{129}$Xe gas supplied from a continuous-flow hyperpolarizing system. We obtained an axial plane image of the lung through balanced SSFP and SPGR sequences, changing the various magnetic resonance (MR) imaging parameters, and measured the SNR of these images.

Results: We demonstrated the clear dependence of image intensity on flip angle and number of shots. The SNR was higher in balanced SSFP than in SPGR and 2.3-fold higher compared at each maximum. In contrast, total acquisition time in balanced SSFP was shortened to about one-eighth that of SPGR using a one-shot acquisition mode.

Conclusion: In HP$^{129}$Xe MR imaging of the lung of a spontaneously breathing mouse, balanced SSFP sequence with multi-shot and centric order acquisition provides higher SNR in a shorter acquisition time than SPGR.

Keywords: balanced steady-state free precession, hyperpolarized $^{129}$Xe, MR imaging, mouse lungs, spontaneous breathing

Introduction

Some difficulties are recognized in applying $^1$H magnetic resonance (MR) imaging to lungs because proton density is very low in the lung and $T_2^*$ becomes very short as a result of the large difference in magnetic susceptibility from complex alveolar structures. However, since 1994, when Albert's group first reported MR imaging of the lungs using hyperpolarized (HP) $^{129}$Xe MR imaging, which allows direct imaging of the void space, such imaging has been expected to become a powerful tool for morphological diagnosis and functional evaluation of lungs. Among the noble gases used for HP gas MR imaging, $^3$He has a large gyromagnetic ratio with low solubility in tissue, such that HP $^3$He MR imaging is suited for morphological analysis of lung air space, and various studies have been performed based on $^3$He imaging of lungs. On the other hand, $^{129}$Xe is highly soluble in tissue and exhibits a large range of distribution in chemical shifts in dissolved states. Using these features, HP $^{129}$Xe nuclear magnetic resonance (NMR) can be used to investigate the gas transport mechanism and the structure in the lung parenchyma as well as to obtain information about other organs, such as the brain, that can be reached by HP $^{129}$Xe gas through the blood circulation. Therefore, HP $^{129}$Xe NMR can provide much information on organs to which Xe can be transported. However, HP MR imaging using $^{129}$Xe suffers from lower signal...
intensity than that with $^3$He, and sensitivity enhancement is very important for HP $^{129}$Xe MR imaging, even if it is already enhanced by the HP technique.

For widespread clinical application, the performance of both gas- and dissolved-phase studies using the same element will be most effective. For example, HP gas and transmitter/receiver coils must be prepared for the 2 types of nucleus when gas-phase studies are performed using $^3$He and dissolved-phase studies are performed using $^{129}$Xe. Furthermore, $^3$He is a very rare isotope available mainly as a byproduct of tritium ($^3$H) decay. Taking these factors into account, signal enhancement of gas-phase study of $^{129}$Xe would be very effective for widespread use of HP gas.

HP $^{129}$Xe MR imaging has mostly been measured using sequences that acquire a gradient echo signal, such as fast low angle shot (FLASH). In recent years, improved gradient hardware techniques have increased use of the balanced steady-state free precession (SSFP) sequence, which can offer images with high signal-to-noise ratio (SNR) within a very short acquisition time. In HP MR imaging, $^3$He and $^{13}$C measurements using balanced SSFP have been reported, and higher SNR was revealed using this sequence than spoiled gradient echo (SPGR), which is used conventionally in HP MR imaging. In HP $^{129}$Xe MR imaging, the superiority of balanced SSFP over SPGR was estimated by simulation only.

We applied balanced SSFP to HP $^{129}$Xe gas-phase MR imaging in mouse lungs and estimated its effectiveness in signal enhancement and shortening of acquisition time. Prior to the experiments, we simulated magnetization response of HP $^{129}$Xe for balanced SSFP sequence in spontaneously breathing lungs and estimated the effect of experimental conditions on the SNR. We then performed balanced SSFP experiments in mouse lungs to examine the optimized conditions of measurement, compared results with those from SPGR, and identified SNR improvement and shortening of acquisition time.

## Materials and Methods

### Theoretical background

Figure 1a provides a schematic diagram of the balanced SSFP sequence, in which the excitation train comprises an initial $\alpha/2$ preparation pulse followed by a train of alternating $\pm \alpha$ excitation pulses. The time interval between the $\alpha/2$ pulse and the first $-\alpha$ pulse is repetition time $(TR)/2$, and $\pm \alpha$ pulses are separated by the $TR$. Each applied gradient pulse is compensated by a gradient pulse with opposite polarity within the $TR$. The evolution of the magnetization in the SSFP sequence was simulated using a matrix product method for thermal equilibrium magnetization, as described previously. Here, we assumed no offset frequency, so the magnetization right after $n$-th $\pm \alpha$ pulse, $M_n = (M_{x,n}, M_{y,n}, M_{z,n})$, is described as:

![Fig. 1. (a) Illustration of balanced steady-state free precession (SSFP) sequence. $G_{ax}$ is the gradient pulse in slice-selection direction; $G_{ro}$ in read-out direction; and $G_{pe}$ in phase-encoding direction. After $\alpha/2$ preparation pulse followed by repetition time $(TR)/2$ time interval, the alternating $\pm \alpha$ excitation pulses are applied with the time interval of the $TR$. This illustration shows a single segment. Each segment is separated by an inactive time (IT) period and repeated until all phase-encoded data are collected. The applied gradient pulses are compensated by the gradient pulses with opposite polarity within the $TR$ for all 3 gradient axes. (b) Illustration of spoiled gradient echo (SPGR) sequence. Spoiler gradient pulse is applied to dephase transverse magnetization after acquisition of gradient echo.](image-url)
$\mathbf{M}_1 = P_1 R (TR/2) P_{\text{prep}} \mathbf{M}(0) + M_0 \{1 - \sqrt{E_{1app}}\} \mathbf{P}_1 \hat{k}$

$$= \begin{pmatrix} M_0 & 0 & 0 \\ 0 & 0 & 0 \\ \exp(-t/T_2) & 0 & 0 \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ M_0 \end{pmatrix}$$

$\mathbf{R}(t) = \begin{pmatrix} \cos(\alpha/2) & \sin(\alpha/2) \\ -\sin(\alpha/2) & \cos(\alpha/2) \\ 0 & 0 \end{pmatrix}$

$P_{\text{app}} = \begin{pmatrix} 0 & \cos(\alpha) & 0 \\ \cos(\alpha) & 0 & 0 \\ 0 & -\sin(\alpha) & \cos(\alpha) \end{pmatrix}$

$\mathbf{M}(0) = \mathbf{P}_n \mathbf{R}(TR) \mathbf{M}_{n-1} + M_0 (1 - E_{1app}) \mathbf{P}_n \hat{k}$

$$= \begin{pmatrix} M_{x,n-1} E_{2app}^* \\ M_{y,n-1} E_{2app}^* \\ M_{z,n-1} E_{2app}^* \end{pmatrix} \cos \alpha + (-1)^n \{M_{z,n-1} E_{1app} + M_0 (1 - E_{1app})\} \sin \alpha$$

$\mathbf{M}_1 = \mathbf{P}_1 R (TR/2) \mathbf{P}_{\text{prep}} \mathbf{M}(0) + M_0 \{1 - \sqrt{E_{1app}}\} \mathbf{P}_1 \hat{k}$

$$= \begin{pmatrix} M_0 \sqrt{E_{2app}} \sin(\alpha/2) \cos \alpha - M_0 \{1 - \sqrt{E_{1app}} \{1 - \cos(\alpha/2)\}\} \sin \alpha \\ M_0 \sqrt{E_{2app}} \sin(\alpha/2) \sin \alpha + M_0 \{1 - \sqrt{E_{1app}} \{1 - \cos(\alpha/2)\}\} \cos \alpha \end{pmatrix} \quad (n = 1)$$

$\mathbf{M}_n = \mathbf{P}_n R (TR) \mathbf{M}_{n-1} + M_0 (1 - E_{1app}) \mathbf{P}_n \hat{k}$

$$= \begin{pmatrix} M_{x,n-1} E_{2app} \\ M_{y,n-1} E_{2app} \\ M_{z,n-1} E_{2app} \end{pmatrix} \cos \alpha + (-1)^n \{M_{z,n-1} E_{1app} + M_0 (1 - E_{1app})\} \sin \alpha$$

$\mathbf{M}_2 = \mathbf{P}_2 R (TR) \mathbf{P}_{\text{prep}} \mathbf{M}(0) + M_0 \{1 - \sqrt{E_{1app}}\} \mathbf{P}_2 \hat{k}$

$$= \begin{pmatrix} M_0 \sqrt{E_{2app}} \sin(\alpha/2) \cos \alpha - M_0 \{1 - \sqrt{E_{1app}} \{1 - \cos(\alpha/2)\}\} \sin \alpha \\ M_0 \sqrt{E_{2app}} \sin(\alpha/2) \sin \alpha + M_0 \{1 - \sqrt{E_{1app}} \{1 - \cos(\alpha/2)\}\} \cos \alpha \end{pmatrix} \quad (n \geq 2)$$

where $E_1 \equiv \exp(-TR/T_1)$; $E_2^* \equiv \exp(-TR/T_2^*)$; and $\hat{k}$ is the unit vector in the $z$ direction (direction of the static magnetic field). Here,
In SPGR sequence (Fig. 1b), the RF pulse of small flip angle $\alpha$ is applied followed by spoiler gradient to dephase transverse magnetization. The transverse magnetization induced by the $n$-th RF pulse, $M_{y,n}$, depends on longitudinal magnetization just before the application of the RF pulse, $M_{z,n}$.

$$M_{z,n} = M_{z,n-1} \sin \alpha.$$  

From the above equations, $M_{y,n}$ is described as:

$$M_{y,n} = \left[ M_{y,n-1} \frac{\cos \alpha}{\sin \alpha} E_{1\text{app}} + M_0 (1 - E_{1\text{app}}) \right] \sin \alpha \quad (n \geq 2)$$

$$M_{y,1} = M_0 \sin \alpha \quad (n = 1) \quad [3]$$

Figure 2a illustrates the transverse magnetization evolved versus the RF pulse number simulated by Eqs. [2] and [3]. Measurement parameters are $TR = 3.6 \text{ ms}$ and $\alpha = 25^\circ$, $50^\circ$, $100^\circ$, and $160^\circ$ in the balanced SSFP and $TR = 300 \text{ ms}$ and $\alpha = 15^\circ$, $30^\circ$, and $45^\circ$ in the SPGR. Relaxation parameters are commonly set to $T_{1\text{app}} = 1000 \text{ ms}$, $T_{2\text{app}} = 3 \text{ ms}$ in the 2 sequences. These values reflect those reported by the present authors for mouse lungs under spontaneous breathing. In the balanced SSFP, the decay of magnetization is very fast. It is also estimated from another simulation (Fig. 2b) that when $TR$ becomes shorter, this decay of magnetization becomes more gradual near this $TR$ value (a few ms) in balanced SSFP. However, hardware restriction prevented further shortening of the $TR$. Therefore, enhancement of signal intensity in the balanced SSFP will benefit from the acquisition of echoes in a segment mode of phase encoding and insertion of a proper waiting time (inactive time, IT, in Fig. 1b) after acquisition of a definite number of echoes for sufficient replenishment of HP $^{129}\text{Xe}$ magnetization in the slice by ventilation. In addition, acquisition in the centric order of phase-encoding will be useful instead of the conventional acquisition of sequential order.

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**Fig. 2.** (a) Simulation of transverse magnetization evolution in balanced steady-state free precession (SSFP) (left) and spoiled gradient echo (SPGR) (right). Repetition time ($TR$) = 3.6 ms in balanced SSFP, whereas $TR = 300 \text{ ms}$ in SPGR and $T_{1\text{app}} = 1000 \text{ ms}$, $T_{2\text{app}} = 3 \text{ ms}$. The decay of transverse magnetization is faster in balanced SSFP than in SPGR. (b) Transverse magnetization evolution in balanced SSFP when the $TR$ is varied. The longer the $TR$, the faster the decay of transverse magnetization.
Continuous-flow hyperpolarizing system

We used a xenon gas mixture (Japan Air Gases, Tokyo, Japan) consisting of 3% natural abundance xenon ($^{129}$Xe: 26.4%), 12% nitrogen, and 85% helium as a source of HP gas for $^{129}$Xe MR imaging measurements. HP $^{129}$Xe gas was produced by spin exchange optical pumping method using a home-built continuous-flow polarizing apparatus. We placed a cylindrical polarizing cell (Pyrex glass, 6 cm diameter, 30 cm long) in the fringe field, at approximately 12 mT, of the superconducting NMR magnet (9.4T). A droplet of rubidium (about 0.2 g) was deposited into the polarizing cell, which was maintained at an approximate atmospheric pressure and 110°C using a hot-air blower (LEISTER Hotwind S, Sarnen, Switzerland). Circularly polarized light was irradiated at 795 nm into the cell for optical pumping, using 90 W diode laser arrays (FAP-system, 30 W, and DUO FAP-system, 60 W, Coherent Inc., Santa Clara, CA, USA). The xenon gas mixture was passed through a vessel containing Na–K alloy to dry it before reaching the polarizing cell. The xenon gas mixture was steadily supplied at the xenon gas mixture just before entering the mask at approximately 20 mL/min and mixed with the mask at approximately 20 mL/min and mixed with the xenon gas mixture just before entering the mask.

Animal preparation

All animal procedures conformed to the requirements of the Institutional Animal Care and Use Committee of the Division of Health Sciences, Graduate School of Medicine, Osaka University. We used 3 male ddY mice (body weight 35–45 g, Japan SLC, Inc., Shizuoka) for this study. Each animal was anesthetized with intraperitoneal injection of pentobarbital (40 to 50 mg/kg), had a mask attached for spontaneous breathing of the xenon gas mixture, and was then fixed in a ϕ30 acrylic cylinder for insertion into the vertical magnet at 9.4T.

HP $^{129}$Xe imaging

We performed all measurements on an NMR spectrometer (Varian Unity INOVA-400WB) equipped with a vertical 9.4T superconducting magnet and a gradient unit for imaging and used a dual tunable (1H and $^{129}$Xe) imaging probe (bore diameter: 32 mm, Doty Scientific Inc., Columbia, SC, USA).

Prior to HP $^{129}$Xe imaging, we observed 1H MR imaging in coronal, axial, and sagittal planes to confirm the position of the mouse lungs. These measurements were performed using a gradient echo sequence with $TR$/echo time ($TE$) = 50/2 ms; matrix = $128 \times 128$; field of view (FOV) = 40 × 40 mm$^2$ (axial) and 50 × 50 mm$^2$ (coronal, sagittal); slice thickness = 5 mm; and number of averaging (NA) = 2. To examine the effect of the IT on signal intensity in the balanced SSFP sequence, we performed HP $^{129}$Xe MR imaging measurements with variable IT, such as 0.25, 0.5, 1.0, 1.5, and 2.0 s. In the HP $^{129}$Xe balanced SSFP imaging, acquisition parameters were: $TR/TE = 3.6/1.8$ ms; matrix = $32 \times 32$ (zero filled to 64 × 64); FOV = 40 × 40 mm$^2$; slice thickness = 5 mm; NA = 16; number of shots (number of segments of phase encoding) = 4; and flip angle = 140°, and we observed the axial plane image with centric order of phase encoding. We then examined the dependence of HP $^{129}$Xe balanced SSFP image SNRs on the flip angle and number of shots, where we tested flip angles of 25°, 50°, 75°, 100°, 120°, 140°, 160°, and 180° and number of shots of one, 2, 4, and 8, maintaining other parameters at the same values mentioned. Total acquisition times were 2 min 18 s for the 8-shot images, one minute 10 s for the 4-shot images, 36 s for the 2-shot images, and 19 s for the one-shot images. For comparison, $^{129}$Xe SPGR imaging was undertaken in the axial plane under the conditions of $TR/TE = 300/1.5$ ms; matrix = $32 \times 32$ (zero filled to 64 × 64); FOV = 40 × 40 mm$^2$; slice thickness = 5 mm; NA = 16; and flip angle = 15°, 30°, and 45° in a sequential order of phase-encoding. Total acquisition time was 2 min 33 s.

SNR evaluation

In this study, we evaluated SNR directly from the pixel intensity in the HP $^{129}$Xe images and calculated SNR as the ratio of the mean signal intensity in the whole lungs, $S_{\text{signal}}$, to that in noise regions of 10 × 10 mm$^2$, $S_{\text{noise}}$, which were set at 4 corners of the FOV, namely, $\text{SNR} = S_{\text{signal}}/S_{\text{noise}}$.

We averaged the SNR data from 3 mice and normalized the SNR values for each mouse’s datum by the value from the balanced SSFP image measured with 25° excitation pulses in one shot.

Results

Figure 3a shows HP $^{129}$Xe images obtained at various ITs; Fig. 3b shows the SNR measured from each image; and Fig. 3c shows the SNR divided by each total acquisition time taking account of total acquisition time. From the balance of SNR and total acquisition time, we concluded that sufficient signal intensity was obtained at IT = 1.0 s. Using this parameter, we then performed further acquisitions changing such variables as the number of
Fig. 3. (a) Hyperpolarized (HP) $^{129}$Xe images at various inactive time (IT) values in the balanced steady-state free precession (SSFP). IT = 0.25, 0.5, 1.0, 1.5, and 2.0 s from left to right. (b) The signal-to noise ratios (SNRs) measured from the images in Fig. 3a are plotted against the IT. (c) The values of the SNR divided by total acquisition time are plotted against the IT.

Fig. 4. (a) Balanced steady-state free precession (SSFP) images obtained when the number of shots and flip angle are changed. The number of shots was changed to 8, 4, 2, and one from the top to bottom, and the flip angle was changed to $25^\circ$, $50^\circ$, $75^\circ$, $100^\circ$, $120^\circ$, $140^\circ$, $160^\circ$, and $180^\circ$ from left to right. (b) Hyperpolarized (HP) $^{129}$Xe spoiled gradient echo (SPGR) images observed at flip angles of $15^\circ$ (left), $30^\circ$ (middle), and $45^\circ$ (right). (c) Proton reference image obtained in axial plane.

shots (= 1, 2, 4, and 8) and the flip angle (= $25^\circ$, $50^\circ$, $75^\circ$, $100^\circ$, $120^\circ$, $140^\circ$, $160^\circ$, and $180^\circ$) (Fig. 4a). Details of images obtained with 8 shots were clear, but with fewer shots, images were blurred and signal intensity was reduced. In contrast, high signal intensity was obtained at flip angles of $140^\circ$ to $160^\circ$. We also observed SPGR images at flip angles of $15^\circ$, $30^\circ$, and $45^\circ$ (Fig. 4b) and the $^1$H image...
in the axial plane for reference (Fig. 4c) and measured the SNRs of these HP $^{129}$Xe images (Fig. 5). In relatively small flip angles, the SNRs of balanced SSFP images and SPGR were similar, but in larger flip angles, SNRs of the balanced SSFP were remarkably high, having a maximum value at 140° to 160°. The SNR of balanced SSFP (8 shots, 140°) was 2.3 times larger than that of SPGR (45°) when compared at the maximum values of SNR attained in each sequence. Furthermore, the SNR was seen to increase as the number of shots increased in the balanced SSFP.

The superiority of balanced SSFP over SPGR was also shown with regard to total acquisition time. It was clear that the acquisition time of balanced SSFP was shorter than that of SPGR (see Materials and Methods), and one-shot image of the balanced SSFP was taken in one-eighth the total time of the SPGR.

**Discussion**

We estimated that signal decay will become very fast in HP $^{129}$Xe MR imaging balanced SSFP measurement, as shown in Fig. 2. With a shorter TR, the decay would be more gradual because it is influenced more by $T_{2\text{app}}$. However, we could not set a shorter TR because of hardware restrictions. To reduce the influence of this fast decay on image intensity, we performed the acquisition in a centric order with multi-shot mode. Thus, we observed greater signal intensity in the balanced SSFP within shorter acquisition time than in the results of SPGR. As well, in each maximum SNR datum, the balanced SSFP image provided 2.3-times larger SNR than the SPGR image. Because the Ernst angle is about 45° from our acquisition parameters, the SPGR image with flip angle of 45° is considered to have been acquired in the condition causing the largest signal intensity. In balanced SSFP measurement, reducing the number of shots remarkably shortens total acquisition time, but the accompanying decrease in SNR and blurring effect will become serious in the resulting image. This is caused by acquiring more decayed signals for high frequency elements and reducing the number of first echoes that are most intense in the image components, when the number of shots is reduced.

Several studies have reported the superior utility of balanced SSFP compared with SPGR: $^1$H images in mouse brain are improved by a factor of 3.4 in SNR; $^3$He images in human lungs are improved by a factor of 3 to 4 in SNR; and $^{129}$Xe images of human lungs are simulated to be improved by a factor of 3.2 in SNR. We observed similar improvement for the HP $^{129}$Xe images in mouse lungs under spontaneous breathing. However, the factor of SNR improvement was 2.3, which was a little smaller than the result of simulation in human lungs. In the case of mouse lungs, we observed that $T_{2\text{app}}$ was 2 to 3 ms, whereas $T_{2\text{app}}$ was set to 20 ms in the above-mentioned simulation calculation. Therefore, such a difference in $T_{2\text{app}}$ is considered to have induced a little difference in the SNR values. $T_{2\text{app}}$ is influenced by not only the external magnetic field, but also by the respiration condition of controlled or spontaneous breathing and the diffusion dephasing under the imaging gradient.

Gas-phase signal as well as dissolved-phase signal is frequently measured for investigations into the lung gas transport mechanism or the structure of the lung parenchyma as well as for HP $^{129}$Xe gas-phase investigations. Sensitivity enhancement of HP $^{129}$Xe gas-phase MR imaging is very useful for these measurements.

**Conclusion**

In the balanced SSFP sequence during spontaneous breathing, the effect of remarkably fast decay in magnetization will be reduced by adopting the centric order with multi-shot. Using such a mode of measurement and optimizing the flip angle and number of shots establishes the balanced SSFP sequence to provide more than twice the SNR values of SPGR in spontaneously breathing mouse lungs at 9.4T. On the other hand, reducing the number of shots makes acquisition much shorter for a balanced SSFP image than an SPGR image.

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