Mixing oxygen with hyperpolarized $^3$He for small-animal lung studies

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ABSTRACT: Hyperpolarized helium (HP $^3$He) is useful for direct MR imaging of the gas spaces of small animal lungs. Previously, breaths of 100% HP $^3$He were alternated with breaths of air to maximize helium signal in the lungs and to minimize the depolarizing effects of O$_2$. However, for high-resolution imaging requiring many HP $^3$He breaths (hundreds) and for pulmonary disease studies, a method was needed to simultaneously deliver O$_2$ and HP $^3$He with each breath without significant loss of polarization. We modified our existing computer-controlled ventilator by adding a plastic valve, additional relays and a controller. O$_2$ and HP $^3$He are mixed at the beginning of each breath within the body of a breathing valve, which is attached directly to the endotracheal tube. With this mixing method, we found that $T_1$ relaxation of HP $^3$He in the guinea pig lung was about 20 s compared to 30 s with alternate air/HP $^3$He breathing. Because imaging times during each breath are short (about 500 ms), the HP $^3$He signal loss from O$_2$ contact is calculated to be less than 5%. We concluded that the advantages of mixing HP $^3$He with O$_2$, such as shorter imaging times (reduced $T_1$ losses in reservoir) and improved physiologic stability, outweigh the small signal loss from the depolarizing effects of oxygen on HP $^3$He. Copyright © 2000 John Wiley & Sons, Ltd.

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INTRODUCTION

Ideally, for hyperpolarized (HP) $^3$He studies of lungs, HP $^3$He should be delivered with normal levels of O$_2$ to maintain normal blood oxygen levels. However, this is difficult to do because contact with paramagnetic oxygen has a strong depolarizing effect on HP $^3$He. An additional problem for small animal lung microscopy is that multiple breaths of HP $^3$He may be required to accumulate sufficient imaging data. In the past, our solution to these problems has been to use breaths of 100% HP $^3$He alternated with breaths of air. For the short imaging times generally used in these early studies of normal animals, this procedure was effective for imaging and physiologically appropriate; but for studies of pulmonary disease models, such as emphysema and asthma, and for the longer imaging times needed for high-resolution three-dimensional acquisitions, simultaneously delivering O$_2$ with HP $^3$He becomes a necessity. Thus, the purpose of this study was: (1) to develop a method for controlled delivery of mixtures of HP $^3$He and O$_2$ while minimizing the loss of polarization from contact with oxygen; and (2) to assess the depolarizing effects of the mixing method by measuring the $T_1$ of HP $^3$He in the lungs.

METHODS

For mixing of O$_2$ and $^3$He in the same breath, we modified our existing computer-controlled ventilator (see Fig. 1). These modifications allow O$_2$ and $^3$He to be mixed within the body of the breathing valve at a point just before the gases enter the endotracheal tube. Mixing occurs only at the beginning of each inspiration, thus minimizing the contact time between the two gases. The MRI-compatible breathing valve attaches directly to the animal’s endotracheal tube (see Figs 1 and 2, insert). This breathing valve has individual ports for controlling inspiration (INSPIR) and expiration (EXPIR) gas flows and ports to control the supply of normal breathing air and HP $^3$He (Fig. 3). These valve ports are controlled pneumatically by electromechanical valves, which in turn are controlled by DC pulses from a digital I/O board (TIO-10, National Instruments, Austin, TX) in a
computer (Macintosh Power PC, Apple Computer Inc, Cupertino, CA) using LabVIEW software (National Instruments). Our modification consisted of adding the components shown as dashed rectangles and lines in Fig. 1. These additional components are also shown in Figs 2–4. Primary control for the added mixing function is provided by a remote microcontroller (PIC, peripheral interface controller, BASIC Stamp II, Parallax Inc, Rocklin, CA) that is interposed between the digital board outputs and the electropneumatic valves. This microcontroller (PIC) has 16 I/O ports and is programmed on a PC using a modification of BASIC (PBASIC). Programs are then downloaded to the microcontroller and stored in permanent memory. Modifying the ventilator LabVIEW program to perform gas mixing was not possible because of an insufficient number of digital I/O ports and relays. The PIC accepts outputs from the TIO-10 board and then generates new outputs to control the switching valve.

Figure 1. Block diagram of MRI-compatible, computer-controlled ventilator. Dashed-line components represent modifications to existing ventilator (solid rectangles) that allow mixing of oxygen and helium with each breath. The shaded area contains components that are positioned within 1 m of the magnet bore. LCD, liquid crystal display; PR, pressure regulator; PVR, pressure/vacuum regulators; FR, flow restrictor.
Figure 3. Schematic of the pneumatic control of the MRI-compatible breathing valve and switching valve for mixing oxygen and helium. The large rectangle represents the body of the breathing valve made of machined Lexan, four valve ports, and a pressure sensor (PS). Arrowed lines indicate machined channels for gas flow. A connector at the output arrow to the lungs attaches directly to the endotracheal tube (see Fig. 2 insert). Each valve port is controlled by pulses of pressure/vacuum (P/V) from electro-pneumatic valves (DC) controlled by the digital I/O board and PIC microcontroller. The $^3$He reservoir, positioned about 1 m from the magnet bore, is a Plexiglas cylinder containing a Tedlar collapsible bag filled with $^3$He. The cylinder is pressurized with $^4$He. The $^3$He source line is connected to the helium valve port on the breathing valve. A pneumatic diode placed in this line prevents contamination of the $^4$He reservoir by blocking back flow of gas in the event of a pressure imbalance in the breathing valve body. A similar diode is in the O$_2$/air line. During mixing, a pneumatically controlled switching valve also changes the gas source from the normal air/isoflurane to the special mixture of oxygen, and simultaneously both AIR and HELIUM valve ports are opened. Thus, mixing occurs in the body of the breathing valve where the two source gas channels merge before the inspiration valve port. V, anesthetic vaporizer; R, pressure regulator.

Figure 4. Pneumatic control interface box containing the PIC microcontroller, relay boards, and associated dials and toggle switches for operation (right section of panel). The LCD is connected directly to the PIC controller. The upper panel (A) shows the screen on initial start-up of the PIC and lower (B) shows the microcontroller set for 640 breaths of mixed gas breathing. During operation, an actual breath count is shown next to the present mix breath number. In this case, the suspended breath count is zero. See Methods for details.
Through a series of added relays (SC 2062 relay board, National Instruments), it re-routes other TIO-10 outputs to provide modified control of the valve ports on the breathing valve (see below). On the Pneumatic Control Interface box (see Figs 1 and 3) is a toggle switch that is used to change from normal breathing mode to gas mixing mode. Dials on the box are used for pre-setting the number of mixed breaths in an imaging series. The PIC also controls the passage of the output trigger from the ventilator to the scanner to trigger imaging acquisitions only on $^3$He/O$_2$ breaths. A liquid crystal display (LCD) connected to the PIC indicates the status of PIC control and shows the number of pre-set breaths and also keeps a current count of the number of mixed breaths actually delivered. Other features of the microcontroller are described below.

Gas mixing occurs by two simultaneous actions: (1) the pneumatically controlled accessory valve (switching valve) in the breathing air supply line switches from normal breathing supply (21% O$_2$/isoflurane) to a source of pre-mixed O$_2$ and N$_2$ (usually 50:50) and isoflurane; and (2) both the $^3$He and air supply valve ports on the breathing valve are kept open. Proportional mixing of $^3$He (20–79%) and O$_2$/N$_2$ is achieved by maintaining both these sources at the same pressure and adjusting the flow of O$_2$/N$_2$ using plastic flow restrictors (FR, AirLogic, Racine, WI). Flow is restricted to achieve the desired oxygen level measured with an oxygen meter (Hudson Respiratory Care Inc, Temecula, CA). Typically, we use about 25% O$_2$/25% N$_2$ with the balance being $^3$He. For rat and guinea pig studies, tidal volumes are generally about 3 cm$^3$ and were set by adjusting the source gas pressures and durations of inspiration.

The PIC microcontroller can also be programmed to deliver a pre-set number of mixed breaths and then to suspend breathing at full inspiration for a pre-set ‘breath’ count. Thus, the gas content of the lungs can be equilibrated with the $^3$He/O$_2$ mixture. Then, with a full tidal volume of gas in the lungs, data can be collected over periods up to 30 s. We used this method to evaluate the effect of our mixing method on the $T_1$ of $^3$He in the lung. HP $^3$He was generated using previously described optical pumping methods. MR data acquisition utilized a 2 T, 30 cm-bore magnet controlled by a Signa console (GE Medical Systems, Milwaukee, WI) and a 7-cm-diameter birdcage coil. For measurements of $^3$He $T_1$, we used anesthetized guinea pigs and a progressive saturation technique (hard pulses of 132 μs duration) during 24 s suspensions of breathing. Under such conditions, the hyperpolarized gas signal intensity measured after the $n$th RF excitation is given by

$$S(n) = S_0 \exp\left[-(n-1)T_R/T_1\right] \cos^{n-1} \alpha \sin \alpha.$$  \hspace{1cm} (1)

In the first series of experiments, the animal received alternate air/isoflurane and HP $^3$He breaths at a rate of 60 breaths/min for 24 s to build up a steady concentration of HP gas in the lungs. Non-localized free induction decays (FID, $n = 16–64$) were then collected during a breath hold after full inspiration of noble gas. After a 60 s recovery with air/isoflurane breaths to allow normalization of exhaled CO$_2$, the complete procedure was repeated three to five times with different settings for $\alpha$ ($5–10^\circ$) and $T_R$ (0.375–1.5 s). The O$_2$ fraction of the air breaths, FiO$_2$, was then changed (FiO$_2$ between 19 and 98%) using an oxygen blender (Bird, Palm Springs, CA) and the complete protocol was repeated for the new gas compositions. Waiting periods of at least 5 min ensured the establishment of a new steady-state gas composition in the lungs after switching to the new air mixture.

In a second series of experiments, gas was delivered to the animal using mixing of O$_2$ and $^3$He (60 breaths/min) as described above. The progressive-saturation sequence was started after 15 breaths of the mixture ($n = 12–24$, $\alpha = 5–10^\circ$, $T_R = 1–2$ s) and repeated with similar recovery and waiting periods as in the first series. FiO$_2$ was varied between 21 and 82% (with $^3$He filling the balance) using calibrated gas flow restrictors (see above).

**RESULTS AND DISCUSSION**

Pulmonary $^3$He relaxation times measured in the lung of live guinea pigs under conditions of alternate breathing and mixing of O$_2$ and $^3$He are plotted in Fig. 5. The observed $T_1$ decays for both curves with increasing FiO$_2$ agree well with previous in vitro results with $^3$He/O$_2$ mixtures, indicating that $^3$He spin-lattice relaxation in the lung gas space is dominated by dipolar interaction with paramagnetic oxygen. Compared with alternate breathing, $T_1$ is shortened from about 30 to 20 s after mixing with 21% O$_2$. For imaging times of up to 500 ms/breath, the signal loss due solely to $T_1$ decay from oxygen mixing and residence in the lungs is less than 5%. Furthermore, arterial gases with this method of

![Figure 5. $T_1$ relaxation of HP $^3$He in the guinea pig lung under condition of alternate breathing of 100% $^3$He and air compared to every breath being a mixture of $^3$He and O$_2$. See text for details](image)
mixing are not different from those using normal air on every breath (mix vs air: \(pO_2\) mmHg, 101 vs 90; \(pCO_2\) mmHg, 21 vs 25; pH, 7.59 vs 7.56). Different inspiratory levels of oxygen could also be used, but polarization losses would also differ (Fig. 5). The method is sufficiently flexible to allow changing the volume of \(^3\)He in each breath by adjusting the percentage of \(N_2\) in the breathing mixture while leaving the tidal volume and \(FiO_2\) the same.

We conclude that the advantages of mixing HP \(^3\)He with \(O_2\), such as shorter imaging times (hence reduced \(T_1\) losses in reservoir) and improved physiologic stability, far outweigh the insignificant losses due to the depolarizing effects of oxygen on HP \(^3\)He. This imaging strategy has been used for \textit{in vivo} studies of an elastase model of emphysema\(^8,9\) and is currently being used in a high-resolution study of the distal gas spaces of the small-animal lung, as shown in Fig. 6.

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**REFERENCES**