Respiratory Physiology & Neurobiology 189 (2013) 594–606

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Heterogeneity and matching of ventilation and perfusion within anatomical lung units in rats

Robb W. Glenny[a,*], Christian Bauer[b], Johannes Hofmanninger[c], Wayne J. Lamm[a], Melissa A. Krueger[a], Reinhard R. Beichel[b]

[a] University of Washington, Seattle, USA
[b] University of Iowa, Iowa City, USA
[c] Vienna University of Technology, Vienna, Austria

ARTICLE INFO

Article history:
Accepted 30 July 2013

Keywords:
Regional pulmonary blood flow
Regional ventilation
Ventilation–perfusion matching
Gas exchange
Functional unit of gas exchange
Imaging

ABSTRACT

Prior studies exploring the spatial distributions of ventilation and perfusion have partitioned the lung into discrete regions not constrained by anatomical boundaries and may blur regional differences in perfusion and ventilation. To characterize the anatomical heterogeneity of regional ventilation and perfusion, we administered fluorescent microspheres to mark regional ventilation and perfusion in five Sprague–Dawley rats and then using highly automated computer algorithms, partitioned the lungs into regions defined by anatomical structures identified in the images. The anatomical regions ranged in size from the near-acinar to the lobar level. Ventilation and perfusion were well correlated at the smallest anatomical level. Perfusion and ventilation heterogeneity were relatively less in rats compared to data previously published in larger animals. The more uniform distributions may be due to a smaller gravitational gradient and/or the fewer number of generations in the distribution trees before reaching the level of gas exchange, making regional matching of ventilation and perfusion less extensive in small animals.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The lung efficiently exchanges respiratory gases through the tight matching of regional ventilation ($\dot{V}_A$) and perfusion ($\dot{Q}$) at the level of the alveoli and capillaries. This local matching is remarkable given the heterogeneous spatial distributions of ventilation and perfusion (Glenny and Robertson, 1990; Robertson, 1999). While the mechanisms for the wide variations in regional $\dot{V}_A$ and $\dot{Q}$ have been reasonably well determined and reviewed (Glenny and Robertson, 2011), the mechanisms matching local $\dot{V}_A$ and $\dot{Q}$ require further investigation.

Prior studies exploring the spatial distributions of $\dot{V}_A$ and $\dot{Q}$ have used a number of different schemes to partition the lung into discrete regions. Initial studies used collimated scintillation counters at fixed vertical positions in human subjects (Ball et al., 1962; West, 1962). Reed and Wood (Reed and Wood, 1970) and then Greenfield (Greenleaf et al., 1974) used microspheres to measure blood flow and sampled dog lungs at fixed locations along a Cartesian coordinate system. Most recently, other investigators have used computerized tomography (CT) (Hoffman and Chon, 2005), single photon emission tomography (SPECT) (Petersson et al., 2004), positron emission tomography (PET) (Treppo et al., 1997) and magnetic resonance imaging (MRI) (Hopkins et al., 2007) to visualize the spatial distributions of $\dot{V}_A$ and $\dot{Q}$. All of these studies partition the lungs into voxels within three-dimensional orthogonal coordinate systems and therefore do not respect the anatomical partitions of the lung. Fractal theory (Mandelbrot, 1983) has highlighted that this approach imposes man made geometries onto a biological structure that may have its own natural geometry.

Through embryological development, the lung is partitioned into a hierarchy of anatomical units from right and left lungs, into lobes, segments, sub-segments and acini. Airway and vascular development occur in concert with the parenchyma so that any given lung unit is served by a single airway and vascular pathway. In the final construction, the lung can be partitioned into distinct anatomical units, each supplied by a dedicated airway and vascular pathway.

One problem arising from sampling the lung along a fixed coordinate system is that sampled regions may overlap anatomical units and contain lung from different vascular and airway distributions. Another concern created by sampling from fixed coordinate systems is that lung regions may also contain large airways, vascular structures and parenchyma. Hence, sampling schemes that virtually dissect the lung regardless of anatomical partitions can potentially blur real or create artificial heterogeneity in $\dot{V}_A$ and $\dot{Q}$.

* Corresponding author. Tel.: +1 206 543 4709; fax: +1 206 625 8365.
E-mail addresses: glenny@uw.edu (R.W. Glenny), christian-bauer@uiowa.edu (C. Bauer), jhofmanninger@gmail.com (J. Hofmanninger), wjel@u.washington.edu (W.J. Lamm), krueger@u.washington.edu (M.A. Krueger), reinhard-beichel@uiowa.edu (R.R. Beichel).

1569-9048/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2013.07.027

The observed matching of regional ventilation and perfusion could also be distorted.

Gas exchange in the lung is dependent on both the heterogeneities of $\dot{V}_A$ and $\dot{Q}$ but also the spatial correlation between them (Wilson and Beck, 1992). Prior studies have revealed a range of heterogeneities $\dot{V}_A$ and $\dot{Q}$ depending on the size of the animals studied, methods used to acquire the data and normalization methods. Similarly the local correlation of ventilation and perfusion has varied among different studies. Our prior study using randomly sampled spherical volumes in a rat lung reported relatively poor matching between ventilation and perfusion ($r = 0.49 \pm 0.23$) (Robertson et al., 2010). All of these prior studies have used geometrically defined regions of interest that may not accurately characterize the heterogeneities of ventilation and perfusion.

To investigate the importance of partitioning the lung into anatomically correct units, we measured regional ventilation and perfusion in mechanically ventilated rats using an imaging cryomicrotome and then developed computer algorithms to virtually dissect the lung parenchyma along anatomical boundaries. Regional ventilation and perfusion were mapped using fluorescent aerosols and microsphere, respectively. This allows us to report the heterogeneity for ventilation and perfusion and their matching within anatomical lung regions. We also compare our findings to those obtained through virtual dissection independent of anatomical partitions.

2. Methods

2.1. Animal preparation and imaging

The University of Washington Animal Care Committee approved the experimental protocol. Five male Sprague–Dawley rats weighing between 257 and 290 g were anesthetized by intraperitoneal injection of 80 mg/kg Ketamine and 10 mg/kg Xylazine, sufficient to prevent withdrawal of paw after pinch. A tracheostomy was performed, and an internal jugular vein and carotid artery were cannulated. The animals were mechanically ventilated at a rate of 44 ± 6 breaths/min with a tidal volume of ~3 mL, utilizing a piston pump ventilator. The fluorescent aerosol was generated by an in-line ultrasonic aerosol-generating system (Microstat Ultrasonic Nebulizer, Mountain Medical Equipment, Littleton, CO, USA) that creates 1–2 μm mass median diameter droplets that are then passed through a drying column before entry into the trachea. The inhaled particles were characterized by an aerosol spectrometer (Grimm Aerosol NanoCheck, Germany) to size and count the particles and an aeroTrak 9000 (TSI, Shoreview, MN, USA) that measures the particle lung deposition surface area. Scanning electron microscopy demonstrated that the generated particles were 1–2.5 μm in diameter. The fluorescent microsphere aerosol was generated from 2 mL of a 2.5% suspension of 0.04 μm diameter red fluorescent microspheres (Molecular Probes, Eugene, OR, USA) in distilled water and was administered to the rats for 3–5 min. Regional perfusion to rat lungs was marked during aerosol administration by intravenous injection of 40,000 scarlet fluorescent microspheres 15 μm in diameter. A 0.15 mL arterial blood sample was obtained at the end of the aerosol administration. The rats were then deeply anesthetized with an intraperitoneal injection of Ketamine/Xylazine), their chests widely opened and exsanguinated. The pulmonary artery and left atrium were cannulated. The lungs were removed from the chest, filled via the trachea with Optimal Cutting Temperature media (OCT, Sakura Finetek Inc., Torrance, CA, USA) until they appeared inflated to total lung capacity. To fill the non-capillary vessels, blood was injected. The filled lung was frozen, suspended in a mixture of 99.25% OCT and 0.75% India ink, and frozen in the OCT mixture.

The Imaging CryoMicrotome (Barlow Scientific, Inc., Olympia, WA, USA) is a device that determines the spatial distribution of fluorescence and fluorescent microspheres at the microscopic level. Details of the instrument configuration have been previously reported (Bernard et al., 2000) but the instrument has been improved through a number of modifications. The instrument consists of a Redlake MegaPlus II ES 3200 (San Diego, CA, USA) with a resolution of 2184×1472 pixels, a computer (Dell Computer Corp., Round Rock, TX, USA), metal halide lamp (PE300BF Cermax, Excelitas Technologies, Fremont, CA, USA), excitation filter-changer wheel, emission filter-changer wheel, and a cryostatic-microtome. Fluorescence images are acquired with a 180-mm Micro-Nikkor lens (Nikon, Corp., Tokyo, Japan). The lens is fine-focused for each emission filter with a custom-built stepper motor/gearing system to control for chromatic aberrations. Computer control of the microtome motor, emission and excitation filter wheels, fine-focus, and image capture and display is accomplished through an application written in LabVIEW (8.2, National Instruments Inc., Austin, TX, USA).

The lungs were mounted in the cryomicrotome so that 24 μm thick transverse sections (Z planes) were serially obtained from base to apex. The camera was moved an appropriate distance from the sample block so that the pixel size matched the slice thickness, providing isotropic voxels. Following every cryomicrotome cut, fluorescent emission images of the lung block were acquired for each of the two fluorescent colors (aerosol and microspheres), a full range excitation and emission image to define the lung parenchyma and an autofluorescence pairing (excitation = 485 nm, emission = 530 nm) to highlight the airway walls.

2.2. Image processing and identification of anatomical units

The objective of the image processing is to identify anatomical units at different scales of size. For each spectrum channel acquired, the images from the transverse slices are combined into a single three-dimensional volume. The precise registration of the serial sections in the *x* and *y* directions and the uniform thickness of the transverse sections in the z direction can be demonstrated though coronal reconstructions from the transverse images shown in Fig. 1.

Near-acinar regions: Inspection of the aerosol images reveals structure that suggests anatomical units centered on small airways (Fig. 2). It appears that the aerosol fluorescence has deposited within regions demarcated by walls that have less fluorescence. We interpret this structure as representing the smallest functional unit because they have volumes approximating acinar regions (Mercer and Crapo, 1987; Rodriguez et al., 1987; Yeh et al., 1979). The partitions are automatically segmented using the algorithm depicted in Fig. 3, which is based on the watershed-segmentation algorithm (Vincent and Soille, 1991). The individual processing steps are as follows.

The input image is preprocessed by means of different filter operations. First, a high-pass filter, implemented by subtracting the original image by a Gauss-filtered ($\sigma = 800$ μm) version of the original, is used to reduce the low frequency gray value variations within the lobes (Fig. 4b), which can be caused by in-homogeneous lighting, for example. Second, subsequent median-filtering with a kernel size of $9 \times 9 \times 9$ is utilized to suppress small bright gray-value peaks (Fig. 4c). Third, a Gaussian low-pass filter ($\sigma = 160$ μm) is used to provide smoother transitions between compartments (Fig. 4d) to prevent over segmentation. To ensure the watershed-segmentation will segment along the lobe border, an artificial watershed is added that is derived from the lobe mask (see below). Subsequently, gray values are inverted so that compartments form catchment basins (Fig. 4e), and the watershed transformation is

Download English Version:

https://daneshyari.com/en/article/5926059

Download Persian Version:

https://daneshyari.com/article/5926059

Daneshyari.com