Ventilation inhomogeneity in oleic acid-induced pulmonary edema

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Tsang, John Y. C., Michael J. Emery, and Michael P. Hlastala. Ventilation inhomogeneity in oleic acid-induced pulmonary edema. J. Appl. Physiol. 82(4): 1040-1045, 1997.—Oleic acid causes permeability pulmonary edema in the lung, resulting in impairment of gas-exchange and ventilation-perfusion heterogeneity and mismatch. Previous studies have shown that by using the multiple-breath helium washout (MBHW) technique, ventilation inhomogeneity (VI) can be quantitatively partitioned into two components, i.e., convective-dependent inhomogeneity (cdi) and diffusive-convective-dependent inhomogeneity (dcdi). Changes in VI, as represented by the normalized slope of the phase III alveolar plateau, were studied for 120 min in five anesthetized mongrel dogs that were ventilated under paralysis by a constant-flow linear motor ventilator. These animals received oleic acid (0.1 mg/kg) infusion into the right atrium at t = 0. MBHWs were done in duplicate for 18 breaths every 40 min afterward. Three other dogs that received only normal saline served as controls. The data show that, after oleic acid infusion, dcdi, which represents VI in peripheral airways, is responsible for the increasing total VI as lung water accumulates progressively over time. The cdi, which represents VI between larger conductive airways, remains relatively constant throughout. This observation can be explained by increases in the heterogeneity of tissue compliance in the periphery, distal airway closure, or by decreases in ventilation through collateral channels.

convective-dependent inhomogeneity; diffusive-convective-dependent inhomogeneity; diffusion front; extravascular lung water; multiple-breath helium washout technique; phase III alveolar plateau

PULMONARY EDEMA induced by oleic acid has been well established as an experimental model of adult respiratory distress syndrome (20). After injury, there are physiological changes such as hypoxemia, decrease in pulmonary compliance, and increase in extravascular lung water. Pathological findings include peribronchial cuffing, alveolar flooding, and microvascular thrombosis. Whereas the ventilation-perfusion mismatch has been well studied in this setting, the changes in the distribution of ventilation per se have not been systematically examined. The purpose of the present investigation is to evaluate the changes in the ventilation inhomogeneity after oleic acid-induced pulmonary edema with the use of the multiple-breath helium washout (MBHW) technique.

Ventilation is the transport of a volume of gas into and out of the lung. Its distribution is primarily determined by convection and diffusion (16). Ventilation within the normal lung is quite homogeneous, despite interfering factors such as asymmetrical narrowing and branching of the airways (10) as well as asynchronous ventilation between different gas-exchanging compartments (8, 9). As the bronchi progressively branch out into the periphery, the cross-sectional area of the airways increases exponentially (21). Consequently, the convective flow of the inspired gas steadily decreases as it moves distally until diffusion becomes a larger influence on gas movement than convection (18). The diffusion-convection front for nitrogen, which represents the interface between the inspired gas and the resident gas in the lung, is estimated to be at approximately the 14th to 16th generation of branching or at the level of the terminal bronchiole (6). Because of the greater diffusivity of helium, its diffusion-convection front will be three to four generations mouthward compared with that for nitrogen.

Analysis of expired marker-gas concentration recorded during a multiple-breath washout by the normalized phase III slope (SIII) technique allows for the separation of convective-dependent inhomogeneity (cdi) and diffusive-convective-dependent inhomogeneity (dcdi) components (4). The cdi is due to nonuniform ventilation between larger parallel airways where gases are transported by convection and not mixed by diffusion. On the other hand, dcdi occurs between smaller airway regions where gases are mixed by the interaction of diffusion and convection, in the vicinity of the diffusion-convection front. The present study was performed to investigate the relative changes of these two components of ventilation inhomogeneity induced by permeability pulmonary edema. We analyzed the slope of the phase III alveolar plateau during the MBHW as described by Crawford et al. (4), with modifications by Emery (7).

Our working hypothesis is that after oleic acid-induced pulmonary edema, the total inhomogeneity of ventilation is increased. However, because the edema fluid likely has its effect in the periphery, we expect that the convective-dependent component cdi may be unchanged, whereas the diffusive and convective-dependent component dcdi will be increased.

METHODS

Animals and surgical preparation. Eight mongrel dogs, weighing 20 ± 5 kg, were anesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg), placed in a supine position, and intubated with a cuffed endotracheal tube. In each animal, a 7-Fr Swan-Ganz catheter (Edwards Laboratory) was placed in the pulmonary artery to measure the mean pulmonary arterial pressure (Ppa), mean pulmonary wedge pressure, and cardiac output by the thermodilution technique. For infusion of fluid and medications, a
large-bore intravenous catheter was inserted into the right atrium. A carotid arterial catheter was used to monitor the systemic blood pressure. All pressures were referred to the midchest position. Warming blankets were used to maintain the animal’s body temperature between 36 and 38°C.

Ventilation parameters. All the animals were anesthetized and ventilated with room air under paralysis (pancuronium bromide, 1–3 mg/h iv). A specially designed linear motor ventilator was used so that the inspiratory and expiratory flows during the respiratory cycle were kept equal and constant, i.e., the inspiratory-to-expiratory ratio was 1:1. Positive end-expiratory pressure was set at zero. At the beginning of the experiments, the tidal volume was set between 12 and 15 ml/kg and a respiratory rate at 18 breaths/min to maintain a PCO2 between 36 and 42 Torr. These ventilation parameters were held constant over the entire protocol regardless of the changes in the blood gases afterward, and no further adjustment was made. Specifically, there was no inspiratory hold in the ventilatory cycle and no special maneuver with the ventilatory circuit that may interfere with the end-expiratory lung volume, thus allowing for the natural maneuver with the ventilatory circuit that may interferewith the end-expiratory lung volume, thus allowing for the natural

Physiological measurements. The baseline measurements of Ppa, pulmonary wedge pressure, blood pressure, and hemoglobin as well as the arterial and venous blood gases were obtained at time (t) = 0, which was −50 min after induction of anesthesia. The animals were then randomly divided into two groups. Group 1 (n = 5) received oleic acid (0.1 ml/kg suspended in 5 ml of 0.9% normal saline iv) over 15 min via a large intravenous catheter in the right atrium. Permeability pulmonary edema was allowed to develop in the next 120 min (12). Group 2 (n = 3) served as controls and received only normal saline as the maintenance fluid. These control animals were monitored for the next 2 h without further intervention. All these physiological measurements were repeated at t = 40, 80, and 120 min.

MBHW. Before each washout measurement, 3% helium was added to the inspired gas until an equilibrium was established throughout the respiratory cycle. At the beginning of each run, helium was abruptly discontinued, and its concentration was measured by an on-line mass spectrometer for the subsequent 18 breaths so that the slope of the phase III alveolar plateau in each breath could be obtained. All data recordings were done in duplicate under the same experimental conditions, and they were interfaced with appropriate computer software for subsequent data analysis. The MBHW measurements were performed at t = 0, 40, 80, and 120 min, immediately after the physiological measurements designated at the same time.

Wet-to-dry ratios. At the end of the experiments, the animals were put under deep anesthesia by a large dose of intravenous pentobarbital sodium and killed by injection of a bolus of saturated KCl. The chest of these animals was then opened, and 10 samples from all lobes of the lung were randomly obtained. These samples were placed in pre-weighted empty vials, so that the wet weight of each sample could be measured immediately. All the lung samples were then dried in an oven for a period of 3 wk until a constant weight was reached. After measuring the dry weights, the wet-to-dry ratio of each of these samples was calculated.

SnIII analysis. The nonuniformity of ventilation distribution is quantified in these experiments by measuring the progression of phase III alveolar plateau slopes that occur during a multiple-breath washout of helium. This procedure is the same as that described by Emery (7), which is a modification of the procedure described by Crawford et al. (4).

The profile of helium concentration [He] as a function of expired volume over time is obtained for 0.75–0.95 of the expired tidal volume, designated as VV5 and VV95, respectively; they fall entirely within phase III of the expired gas profile (Fig. 1). The influence of dilution on the expired [He] profiles of successive washout breaths is negated by dividing each slope by the [He] at 0.85 of the expired volume in the same breath. The resulting SnIII of each washout breath is then assumed to be due to only two processes; i.e., cdi and dcdi. The curve-fit method employed for analysis of these results (7) by Emery is described in Fig. 2. Computer-generated best fit values are used for parameters of the biexponential equation that describes SnIII vs. breath number n. SnIII measurements from progressively later washout breaths are given decreased weight in the curve-fit procedure, reflecting the increased uncertainty in the gas measurements with increased helium dilution. The best fit values are used to describe the cdi and dcdi processes that produce total SnIII (SnIIItotal). This method results in an estimate of the cdi and dcdi contributions to the nonuniformity of ventilation from each washout breath, reported for the first washout breath. The influence of systematic and random errors in measurement of SnIII for the first breath is decreased because all washout breaths are used in the best fit estimate of SnIII for breath 1.

Statistics. The hemodynamic and blood gas data before and after the infusion of oleic acid were compared by two-tailed unpaired t-tests. Similarly, the values of SnIII and its partition components at breath 1 were compared within groups at

![Diagram of normalization process of phase III alveolar plateau slope (SnIII) during multiple-breath helium washout (MBHW) method. (He), helium concentration; V, volume of gas expired. Constant expiratory flow allows conversion of SnIII (liters⁻¹) to SnIII (s⁻¹) when tidal volume and respiratory frequency are known. Normally present cardio-genic oscillations have been excluded for clarity.](http://jap.physiology.org/Downloadedfrom)
different times and between groups at the same time during the experiments. The level of significance was considered to be $P < 0.05$.

RESULTS

The hemodynamic data are shown in Fig. 3, A and B. They show that after oleic acid infusion at $t = 0$, the Ppa significantly increased after 80 min in the oleic acid group while the systemic blood pressures were relatively unchanged throughout the experiment. The cardiac output, however, immediately decreased after oleic acid infusion but remained steady afterward. Figure 4 shows the blood gas data. After oleic acid infusion, PO$_2$ decreases immediately while PCO$_2$ remains constant. The results also show that there was no change in hemodynamics or arterial blood gas in the control animals.

The amount of pulmonary edema fluid in the oleic acid group is significantly higher than that in the control group (wet-to-dry ratios are 6.31 ± 1.17 and 4.27 ± 0.37, respectively; $P < 0.05$). Figure 5 shows the plot of $S_{III}$ vs. breath number in one of the experiments before oleic acid-induced injury. Typically, the data show that the $S_{III}$ for cdci ($S_{III\,cdci}$) reaches a steady state at the first few breaths during the MBHW. This observation is consistent with those previously reported in the normal lung (17) and is confirmed by our results in the control animals. Figure 6 shows the changes in $S_{III\,cdci}$ after 120 min of oleic acid-induced injury. $S_{III\,cdci}$ increases significantly after oleic acid infusion.

Figure 7 shows the changes in $S_{III\,t}$. In the oleic acid group at breath 1 over the time course of the experiment. Its components, i.e., $S_{III}$ for cd (S$_{III\,cd}$) and $S_{III\,cdci}$, are also presented. The data show that $S_{III\,t}$ progressively increases as lung water accumulates. As $S_{III\,cd}$ remains relatively constant, the increases in $S_{III\,t}$ can be almost entirely accounted for by the simultaneous increases in $S_{III\,cdci}$.

Figure 8 shows the similar plot for the control group. It shows that $S_{III\,t}$ and its two partition components,
DISCUSSION

Oleic acid-induced lung injury has been established as an experimental model for permeability pulmonary edema. Earlier investigators have reported that after its infusion the amount of extravascular lung water will gradually increase over the course of 120 min and reach a plateau level afterward (12). Pathological findings include alveolar flooding, epithelial damage, and microvascular thrombosis (20). In our present study, we have examined the ventilation inhomogeneity within the lung as lung water gradually accumulates.

The MBHW technique employed in our present investigation is a sensitive experimental method for the study of ventilation inhomogeneity. By measuring the normalized slope of phase III alveolar plateau ($S_{III}$), a characteristic pattern of increasing slope over consecutive breaths can be obtained. An initial rapidly rising phase of $S_{III}$ in the first several breaths gives way to a steady, but less rapid, rise in $S_{III}$ in the later breaths. The rate of the continuous rise in $S_{III}$ measured from the later breaths has been found to be independent of gas diffusivity and is predicted entirely on the basis of ventilation inhomogeneities between lung regions that are too far apart for mixing by diffusion (4). This component of $S_{III}$ has been identified as $S_{III,c,di}$. On the other hand, the rate of rise in the early phase of $S_{III}$ vs. breath number has been found experimentally to be sensitive to gas diffusivity and increasing the value of $S_{III,c,di}$ in the early phase of washouts beyond that produced only by $S_{III,c,di}$. This additional component within $S_{III}$ (18) has been predicted by computer models of gas mixing to be the diffusive-convective-dependent component, i.e., $S_{III,d,cdi}$. The normalized slope of the alveolar plateau during the washout results from $c,di$, $d,cdi$, and gas exchange. With a gas-exchange ratio $<1.0$, the inert-gas concentrations increase during exhalation with ongoing gas exchange. Cormier and Belanger (1) have demon-

![Fig. 5. $S_{III}$, $S_{III,c,di}$, and $S_{III,d,cdi}$ vs. breath no. in one of experiments before lung injury in oleic acid-induced pulmonary edema group.](image)

![Fig. 6. $S_{III}$, $S_{III,c,di}$, and $S_{III,d,cdi}$ vs. breath no. in one of experiments after lung injury at 120 min in oleic acid-induced pulmonary edema group. Data show that after the 5th breath during washout procedure $S_{III,d,cdi}$ also reaches a steady state.](image)

![Fig. 7. $S_{III}$ at breath 1 in oleic acid-induced pulmonary edema group during experiment. Its convective-dependent component, $S_{III,c,di}$, and its diffusive-convective-dependent component, $S_{III,d,cdi}$, are also presented. *Statistically significant difference from baseline value at $t = 0$.](image)

![Fig. 8. $S_{III}$ at breath 1 in control group. Its convective-dependent component, $S_{III,c,di}$, and its diffusive-convective-dependent component, $S_{III,d,cdi}$, are also presented. There are no significant changes in all these parameters over time.](image)
ventilation inhomogeneity in pulmonary edema

...analyzer was calibrated to 
...that gas exchange accounts for only ~10% of
...of the slope under normal conditions. With lung injury, this fraction decreases as heterogeneity (cdi and dcdi) increases in importance. We did not correct for the slight influence of the gas-exchange component on the absolute values of $S_{nIII\text{cdi}}$ and $S_{nIII\text{dcdi}}$ for two reasons: the correction is very small, and the correction for gas exchange would not change the ratio between $S_{nIII\text{cdi}}$ and $S_{nIII\text{dcdi}}$, and thus the conclusions would not be altered.

Our data show that both the $S_{nIII\text{tot}}$ and $S_{nIII\text{dcdi}}$ at breath 1 progressively increase to approximately three times their corresponding baseline values at 120 min after the infusion of oleic acid (Fig. 7). The $S_{nIII}$ results indicate an increasing nonuniformity of ventilation distribution among the airway regions that branch in the vicinity of the diffusion-convection front for He. The front position of $N_2$ has been predicted to be in the vicinity of the terminal bronchioles in humans (6), and airways that divide in this vicinity subtend regions approximately the size of acini (18). The position for helium is a few generations more proximal in the airways, and branch points in this region probably define airway regions of the size that includes groups of acini. Although the dog’s upper airway branching patterns are different from human, distal airway branching geometry is probably comparable between species. The increase in helium $S_{nIII\text{dcdi}}$ after infusion of oleic acid is evidence for a nonuniform injury between airway regions on the scale of groups of acini. $S_{nIII\text{cdi}}$ is not altered by this injury, thus indicating no change in the relative uniformity of ventilation distribution between larger, well-separated regions. This finding is in agreement with the kind of diffuse and patchy injury that is observed anatomically in this model of permeability pulmonary edema (15, 20).

These results suggest that there is a progressively heterogeneous flooding of the acini groups after oleic acid infusion. It is likely that both the volume and the geometry of the distal air spaces have been altered over time. Furthermore, changes in the mechanical properties of the parenchymal tissue, which can occur as a consequence of oleic acid-induced injury, i.e., worsening of the heterogeneity in regional compliance and/or airway resistance, can also explain our observations. Even in the absence of alveolar flooding, interstitial edema will also likely affect the compliance characteristics of the regions involved.

As lung water progressively accumulates after oleic acid-induced injury, it is reasonable to suggest that the lung compliance will decrease over time and the end-expiratory lung volume may also decrease. Previous investigators have shown that both the end-expiratory lung volume (2) and airway closure (3) can affect the ventilatory heterogeneity and alveolar mixing efficiency in the lung periphery. During the experiments, we have minimized the changes in end-expiratory lung volume by ventilating the animals with a high but constant tidal volume (~15 ml/kg) and a short expiratory time (inspiration-to-expiration ratio = 1:1), which may incidentally result in a small intrinsic end-positive expiratory pressure. Room air was used as the inspired gas to avoid absorption atelectasis. However, as permeability pulmonary edema continues to develop, these factors may also contribute to the increasing $S_{nIII\text{dcdi}}$ observed during the experiments. The lung compliance was not measured in either group of animals as we did not perform any inspiratory hold between washouts to avoid changes in lung volume, status of airway closure, distribution of edema fluid in the airway, and $PCO_2$.

Another possible cause of increasingly nonuniform ventilation distribution is the progressive decrease in the gas-mixing mechanism through collateral channels between different lung regions. Electron-microscopic studies have documented the existence of interalveolar pores of Kohn (13) and bronchiole-alveolar channels of Lambert (11) in dogs. However, alterations in gas communication between acini may not be detectable with helium because of the much more proximal position of the diffusion-convection front, beyond which all helium gas is quite uniformly mixed. This consideration significantly diminishes the possibilities that alveolar pores of Kohn and bronchiole-alveolar channels of Lambert may have been altered, leading to changes in $S_{nIII}$ in dogs. On the other hand, interbronchiole channels of Martin (14) have been implicated as being primarily responsible for collateral ventilation in dogs (5) and may be involved in the altered ventilation distribution found in these experiments.

$S_{nIII\text{tot}}$ has been observed to be relatively constant despite the progressive lung injury with its concomitant increase in lung water. There appear to be no major changes in the uniformity of ventilation between the larger compartments, i.e., lung regions subtended by airways proximal to approximately the 10th generation.

The method of determining $S_{nIII}$ and its cdi and dcdi components has been critically evaluated, and modifications to the method have resulted in the current version (7). The current method used to analyze these results utilizes all of the washout breaths to produce a best fit solution to the two exponential equations that describe the contribution of both the cdi and dcdi components of $S_{nIII}$ (Fig. 2). In this way, the dcdi effect that is predicted to continue only to a small degree beyond breath 5 of the washout does not interfere with the best fit of the cdi process. This method weighs the data by a factor of $(1/{\text{breath no.}})$ in the curve fit procedure to give less influence to the later washout breaths. These changes have been designed to improve the ability of the method to find reasonable solutions to noisy data and to take into account the decreasing signal-to-noise ratio that always occurs with measurement of increasingly dilute helium samples during the washout.

In summary, our study described the ventilation inhomogeneity that occurs after oleic acid-induced pulmonary edema measured with a sensitive technique of MBHW. We conclude that the diffusive-convective-dependent component of ventilation inhomogeneity in airways is almost entirely responsible for the increasing total ventilation inhomogeneity as lung water
accumulates progressively over time. These observations can be explained by factors such as changes in acini volume and flooding, worsening heterogeneity of tissue compliance in the regions of small distal airways, peripheral airway closure, and/or decreases in ventilation through some collateral channels. On the other hand, the convective-dependent component of ventilation inhomogeneity, which represents ventilation inhomogeneity subtended by larger conductive airways, remains relatively constant. This finding is compatible with previous pathological observations, which show that the permeability pulmonary edema occurs on a patchy and random basis throughout the lung parenchyma.

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