Application of carbon monoxide diffusing capacity in the mouse lung

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Fallica J, Das S, Horton M, Mitzner W. Application of carbon monoxide diffusing capacity in the mouse lung. J Appl Physiol 110: 1455–1459, 2011. First published February 10, 2011; doi:10.1152/japplphysiol.01347.2010.—In the past decade the mouse has become the primary animal model of a variety of lung diseases. To assess various mechanisms underlying such pathologies, it is essential to make functional measurements that can reflect the developing pathology. In this regard, the diffusing capacity for carbon monoxide is a variable that directly reflects structural changes in the lung. Although measurement of single-breath diffusing capacity of the lung for carbon monoxide (DLCO) has also been previously reported in mice by a number of investigators, a number of technical issues have precluded routine and widespread use of this metric in mouse models. In the present report, we describe a means to quickly and simply measure a dimensionless variable closely related to the DLCO in mice, termed a diffusion factor for carbon monoxide (DFCO). The DFCO procedure involves a 9-s lung inflation with tracer gases in an anesthetized mouse, followed by a 1-min gas analysis time. We have tested the approach with two common models of lung pathology, elastase-induced emphysema and bleomycin-induced fibrosis. Results show a significant 15% reduction in DFCO in emphysema, and a 41% reduction in the fibrosis model. Repeat measurements within a mouse were found to be highly reproducible. This pulmonary function test can thus be used to detect structural changes with these pathological models. The method can also be used to measure changes in pulmonary blood volume, since the uptake of CO is highly dependent on this variable in addition to the gas exchange surface area.

METHODS

DFCO. The measurement of the single-breath DLCO involves inhaling a gas mixture with a small amount of carbon monoxide and an insoluble tracer gas. The inhaled breath is held for a specified time, and then the exhaled gas is sampled during the latter half of the expiration to assess the mean alveolar concentration. The difference between the dilutions of the CO and the tracer gas is a function of how much CO is taken up by the lung. This uptake is a result of both the alveolar surface area and thickness, the blood volume in the alveolar capillaries, and the rate of chemical binding to hemoglobin. Any insult or pathology that affects any of these will lead to a decreased DLCO.

One factor that limits the ability to easily make this measurement in mice is the difficulty of reproducibly sampling the alveolar gas. To get this sample, a system is required to discard the initial part of the expire, which contains unmixed gas in the anatomic dead space. While it is possible to set up stopcocks or T-connectors to enable one to discard the initial expire, and then collect the later alveolar sample, the time it takes to do this with manual switching can be quite variable. And since the time of the breath hold is a key parameter, variability in this time directly reflects in the variability of the measurement. This has often required the method to be done in duplicate or triplicate, but even this can introduce further error, since with each measurement there may be an increasing partial pressure of CO in the blood that lessens the uptake on a subsequent breath hold. An alternative to this manual switching can be had using a mass spectrometer (18), but aside from not being widely available, the method requires a slow inspiration and expiration, which can introduce variability in the measurement of the lung volume. We have chosen to circumvent these switching problems by simply quickly sampling a volume equal to the entire inspired gas. By ignoring the small dead space contamination, the procedure precludes calculation of the true DLCO, but it compensates for this by allowing a more precise control of the time of the breath hold.

The procedure we use employs a gas mixture of approximately 0.5% neon, 0.5% CO, and balance air. The absolute concentration levels of Ne and CO are not critical, since samples from the mice are normalized on a given day to the calibration sample. All protocols were approved by the Johns Hopkins Animal Care and Use Committee. The procedure as used in this study involves anesthetizing mice with ketamine (90 mg/kg) and xylazine (15 mg/kg), performing a tracheostomy, and inserting an 18-gauge stub needle cannula. In a 3-ml syringe, we withdraw 0.8 ml from the gas mixture bag, connect the syringe to the tracheal cannula, and quickly inflate the lung. We wait for a count of 9 s, and then quickly withdraw the 0.8 ml. This 0.8 ml is diluted to 2 ml with room air and injected into a desktop (6 × 10 × 16 in) Gas Chromatograph (Inficon, Micro GC model 3000A). This instrument uses a molecular sieve column with helium as carrier gas.

IN THE PAST DECADE the mouse has become the primary animal model of a variety of lung diseases. To assess various mechanisms underlying such pathologies, it is essential to make functional measurements that can reflect the developing pathology. In this regard, the ability to perform measurements made in human subjects may facilitate the comparison to pathologies. In this regard, the ability to perform measurements can thus be used to detect structural changes with these pathological models. The method can also be used to measure changes in pulmonary blood volume, since the uptake of CO is highly dependent on this variable in addition to the gas exchange surface area.

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In mice, DFCO is thus a dimensionless variable, which varies between 0 and 1, with 1 reflecting complete uptake of all CO, and 0 reflecting no uptake of CO.

Experimental tests in mice. To examine the ability of the DFCO to detect functional and pathological changes, we studied C57BL/6 mice that were subjected to two commonly used pathological models: emphysema caused by instilled elastase, and fibrosis caused by instilled bleomycin. Mice were studied under anesthesia with 100% O2 for 5 min, whereupon the trachea was closed quickly opened and the heart excised. This excision procedure took less than 1 min and was used to drain the lungs of blood. Group C consisted of six mice, anesthetized with isoflurane and given 0.25 U bleomycin (Elastin Products, Owensville, MO) via tracheal aspiration as described elsewhere (9). They were studied 2 wk after this single insult.

Comparison of the control, emphysematous, and fibrotic mice are shown in Fig. 2. Control values of DFCO (means ± SE) were 0.76 ± 0.013. In the emphysematous group the DFCO was significantly reduced to 0.65 ± 0.023, and this was even lower in the bleomycin-treated mice, where DFCO was 0.45 ± 0.043. In the control mice (group A) and emphysematous mice (group C), the second measurements averaged very slightly smaller by 0.007 and 0.012, but these differences were not significant (P > 0.05).

Pulmonary function was done in the control, fibrosis, and emphysematous mice. Mice were anesthetized and tracheostomized as above and then ventilated with a custom ventilator at a rate of 120 breaths/min and a tidal volume of 0.2 ml. One minute after a 5-s deep inspiration to 30 cmH2O, dynamic elastance was measured with an inspiratory occlusion method as previously described (8). After these dynamic measurements were done, the tracheal cannula was obstructed, and the lungs were allowed to degas, whereupon a pressure volume curve was generated, as previously described (3). Total lung capacity was defined as the lung volume at 35 cmH2O.

Comparison between groups was analyzed with a one-way ANOVA and significance level assessed with a Bonferroni correction for multiple comparisons. A P < 0.05 was considered significant.

RESULTS

Mechanical changes caused by the bleomycin and elastase are shown in Fig. 1, A and B. The elastase resulted in a significantly decreased elastance and increased total lung capacity. The bleomycin caused opposite changes, i.e., a significantly increased elastance and decreased total lung capacity.

Comparisons between groups were analyzed with a one-way ANOVA and significance level assessed with a Bonferroni correction for multiple comparisons. A P < 0.05 was considered significant.

Measurement protocols. Measurement of DFCO in groups A, C, and D were made 5 min after being anesthetized, and before being ventilated for the elastance measurement. Within 2 min after the first DFCO measurement sample was taken, a second measurement was made in mice in groups A and C. In group B, measurements were made within 2 min after the heart rate ceased. In both groups B1 and B2, five repeat measurements of DFCO were made over the next 5 min.

Fig. 1. Measurements (means ± SE) of elastance (A) and total lung capacity (B) in control and pathological lungs. All bars are significantly different (P < 0.05).

Fig. 2. Measurements (means ± SE) of diffusing factor of carbon monoxide (DFCO) under different conditions. All bars are significantly different from each other (P < 0.05).

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first four measurements, but a slight significant fall with the fifth measurement. However, in the dead mice that had the heart excised and the pulmonary vasculature drained, the initial value of \( \text{DF}_{\text{CO}} \) was 0.39 ± 0.015, and this was progressively and significantly decreased with each successive measurement, with the value of \( \text{DF}_{\text{CO}} \) after five measurements being 0.07 ± 0.011.

In the degassed mice, we used the initial measurement to calculate the total dead space of the system, which includes air in the tubing and the airway tree. Using the initial and final values of the Ne concentration in the syringe, we calculated an average dead space of 0.16 ± 0.015 ml.

**DISCUSSION**

In this paper we have defined a new simple metric that reflects the gas-exchanging ability of the mouse lung. Although the \( \text{DL}_{\text{CO}} \) is the gold standard for assessing this function in humans and experimental animals (16, 21), the only advantage of making this measurement in mice would be for comparative studies among different species. However, for most potential applications in mouse studies, the objective is to be able to detect a change in gas exchange ability between control and experimental groups. To this end, the \( \text{DF}_{\text{CO}} \) provides a simple reproducible metric, which can detect structural changes in several different lung disease models. Regarding this terminology, we note that Cotes et al. argued for changing the term \( \text{DL}_{\text{CO}} \) to a transfer factor for carbon monoxide, since approximately half of the measurement depends on the chemical reaction rate with the capillary blood (1, 4). Although these same considerations also apply to the mouse, we decided to use the term \( \text{DF}_{\text{CO}} \), since few have paid heed to this recommendation, and nearly all investigators in humans and other species continue to use the \( \text{DL}_{\text{CO}} \) terminology.

Of the several assumptions needed for interpreting the \( \text{DF}_{\text{CO}} \), most are the same as with the more conventional \( \text{DL}_{\text{CO}} \). Approximately half of either measurement results from the rate of chemical composition with hemoglobin in the red blood cells and thus depends on the amount of capillary blood volume. The fact the \( \text{DF}_{\text{CO}} \) in dead mice is much lower than that in living mice must reflect a substantial postmortem loss (almost 50%) of pulmonary capillary blood volume. If the blood were completely drained, then the uptake of CO should be similar to that of Ne, and the \( \text{DF}_{\text{CO}} \) should fall to very low values. In our experiments, however, even after allowing the pulmonary blood to drain freely through severed pulmonary arteries and veins, there was still sufficient blood in the capillaries to keep the first postmortem measurement of \( \text{DF}_{\text{CO}} \) at about 50% of the measurement in a live mouse. This initial value was similar to that measurement with an intact vasculature. However, when the capillary blood was further reduced by successive lung inflations, the mice with severed vessels showed a progressive reduction of \( \text{DF}_{\text{CO}} \) to near zero. With an intact vasculature, any blood expressed from the capillaries during the repeated lung inflations apparently just flowed back into the capillaries, allowing additional CO uptake. The finding that the \( \text{DF}_{\text{CO}} \) was similar with repeat inflations indicates that there is a negligible build up of CO back pressure during the 9-s breath holds we use with our procedure.

Another assumption is that we simply ignore the effect of unmixed inspired gas in the anatomic and equipment dead space. In our system, by not using stopcocks or T-connectors, we have minimized this dead space as much as possible. The dead space calculated from the Ne dilution during the first inflation of the degassed lungs was 0.16 ml. There are few reported measurements of dead space in the mouse (17, 20), but our value is significantly lower than the 0.22 ml reported for this strain by Schulz et al. (20). This may reflect the different method they used (the Fowler method), but 0.22 ml is not consistent with the fact that adequate ventilation can be maintained with a tidal volume of 0.2 ml (12). Nevertheless, during our \( \text{DF}_{\text{CO}} \) procedure, a dead space of 0.16 ml and an inflation volume of 0.8 ml will cause the concentrations of CO and Ne in the mixed alveolar gas recovered to be increased slightly (≈20%) over the true alveolar gas concentrations. Since CO is absorbed from the alveoli, the percentage error will be larger for CO than Ne, and this will decrease the accuracy of the \( \text{DF}_{\text{CO}} \). However, within any experimental protocol with a given mouse strain, the errors will be similar in all groups, so that the ability to detect pathological changes may not be substantially compromised. This ability is clearly shown by the ability to show significant changes with the emphysema and fibrotic models used in the present work.

In both of these models of lung pathology, we found significant decreases in \( \text{DF}_{\text{CO}} \). However, the reasons for these decrease are quite different in the different models. In emphysema, the loss of alveolar surface acts directly to decrease both the surface area for diffusion and the blood volume in those destroyed capillaries. Although we did not attempt any dose-response relations with the elastase, the expectation would be that the more surface area destroyed, the lower would be the \( \text{DF}_{\text{CO}} \). How sensitive the \( \text{DF}_{\text{CO}} \) would be in detecting small
changes remains to be determined. In fibrosis, there is inflammation and a thickening of the diffusion barrier that leads to a reduction in diffusing capacity (14). This increased thickening of the matrix also leads to increased stiffness of the lung. Similar to the elastase model, we used a single dose of bleomycin that we knew to be able to cause a robust injury. This dose resulted in a substantial reduction in the DFCO, but further study will also be required to determine how sensitive this metric will be in detecting small pathological changes.

The procedure we have used here also differs in another important way from what has been used to measure DLCO in mice. In nearly all of these studies in the literature, investigators inflated the lungs to a given pressure. This procedure can lead to different lung volumes during the breath hold. Since in an individual mouse lung surface area must increase with lung volume, the DLCO should also increase with lung volume. For this reason, differences in DLCO often disappear when normalized to lung volume even among different strains (17). In our protocol for the DFCO, however, we use the same volume increment under all conditions, so that any volume dependence will be minimized. The final lung volumes for CO diffusion will only differ to the extent there are differences in functional residual capacity (FRC). Within a strain these are likely to be negligible, but even among strains where FRC may differ by 0.1 ml or more (15), this would only introduce ≈10% variation in the final volume. Furthermore, although there are few data in mouse models about how much FRC changes in different pathologies, even if it doubles in emphysema, it would go from 0.16 to 0.32 ml, which would only be about a 15% change in lung volume (i.e., after the inflation with the CO mixture). By avoiding the necessity to accurately measure and normalize for lung volume differences with an inflation to a specific pressure, our volume inflation procedure both simplifies the method and improves reproducibility. There is, however, one potential issue with using this constant volume inflation that might manifest itself in the fibrosis model. If the lung is made stiffer, then inflation with the same volume will increase the pressure in the lung during the breath hold. This could result in collapse of the capillaries with resultant loss of red blood cells for diffusion. If this were the case, then this would make the DFCO even smaller than just the thickening alone would cause. While this might be a good thing if one were looking for way to detect fibrosis, we did some limited testing of how big this effect might be. In three of the bleomycin-treated mice we measured the DFCO twice, first with an inflation of 0.5 ml and then with the normal inflation of 0.8 ml. In all three mice, the DFCO was slightly larger with the 0.5 ml, and the average increase was 11%. Although this is consistent with there being a small effect of the higher pressure, with the smaller volume, the dead space effect becomes a larger error, and this will also manifest itself as a larger value of the DFCO. The bottom line is that until more is known about the sensitivity of the DFCO measurement in this pathology, there does not seem to be sufficient reason to be concerned about the higher inflation pressure in fibrosis. It is also possible that the increased barrier thickness and stiffness may protect the capillaries from collapse.

Finally, we consider the effect of repeat measurements of the DFCO. Our calculation ignores any possible CO partial pressure with a repeat measurement, the blood will have taken up some CO, and thus any increase in the partial pressure of CO (Pco) could lead to a smaller value of the DFCO. As we reported, however, although there was a very small fall (consistent with this prediction), there was no significant difference between the first and second measurement. A simple calculation supports this result, estimating that the amount of CO taken up in the 9-s breath hold would be unlikely to raise the Pco more than a hundredth of 1 mmHg. With a nominal DlCO of 9 μl·min⁻¹·cmH2O⁻¹ (6, 10, 17), and our test gas’s Pco of 3.8 of mmHg (0.5% CO), the mouse will take up about 6 μl CO in each 9-s test. How much this will raise the blood Pco in a mouse is not known, but we can estimate this by assuming that like other mammals, there is enough Hb to maintain oxygen at ≈20 vol%. Thus, if the mouse has a 2-ml blood volume, fully saturated Hb could hold ≈0.4 ml of oxygen (or carbon monoxide). If the P50 for mouse Hb is similar to that for human Hb, this would make it 0.16 mmHg, which is the partial pressure that would bind with 200 μl CO. With a linear extrapolation to zero, we thus estimate that each 6 μl CO will raise the Pco = 0.005 mmHg. So the gradient for CO uptake on the first measurement is 3.8 mmHg, and on the second measurement it is 3.795 (3.8 – 0.005). This is a trivial difference that supports the reproducibility of the measurement in vivo, and even with no blood circulation.

In conclusion, we have shown how to simply obtain a functional measurement that reflects structural changes in the lung under different pathologies that affect the ability of the lung to exchange gas. The procedure (including gas measurement time) takes less than 90 s. The approach does require the mouse to be anesthetized, but if an intubation procedure were done (13), it would not be necessary to euthanize the mice. The method defines a diffusion factor (DFCO) that is robust, highly reproducible, and sensitive enough to detect structural changes with experimentally induced fibrosis and emphysema.

REFERENCES


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