Adaptive response to pneumonectomy in puppies

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ABSTRACT   When left pneumonectomy was performed on 9-week-old puppies, the right lung increased in weight, volume, surface area, and number of alveoli so that at age 20 weeks these variables were the same as those of both lungs of control animals and significantly larger than those of the right lung of control animals. The adaptive response of the right lung after pneumonectomy was greater in the lower lobe than in the middle or cardiac lobes. The number of alveoli per ml and the average interalveolar wall distance, hence the surface to volume ratio, was the same in both lungs of control animals and the same in the cardiac, middle, and lower lobes of their right lungs.

After pneumonectomy in experimental animals, the contralateral lung increases in volume, weight, collagen content, protein and deoxyribonucleic acid (DNA) to approximate to the values in both lungs of control animals.[1-12] There are conflicting views on the effect of pneumonectomy on alveolar multiplication,[3 4 6 8 10 11 13-19] and these have been reviewed elsewhere.[19] In part, the reason for the controversy stems from older and less rigorous methods of counting alveoli, but also results from inherent practical and theoretical difficulties in counting alveoli and calculating alveoli per unit volume.[19 20] In general, alveolar surface area increases directly with lung volume after pneumonectomy rather than to the 2/3 power which would be expected by simple overinflation, suggesting increased complexity of alveolar surface. This could be brought about either by lengthening of alveolar septa[18 19] or increased alveolar number.[19] We found that, after pneumonectomy in 10-week-old rabbits, the contralateral lung increased in volume, surface area, and alveolar number to the levels of these variables in both lungs of controls four weeks later.[10] In rats, the adaptive response was less complete; when pneumonectomy was performed at 12 weeks of age, the relative response to the contralateral lung was less than when pneumonectomy was performed at 4 to 8 weeks of age.[19] Statistically significant alveolar multiplication did not occur when pneumonectomy was performed at 12 weeks of age.

The only previous study in dogs showed that the average alveolar diameter was increased in the contralateral lung of two adult dogs after pneumonec-

tomy but not when pneumonectomy was performed on three puppies.[14] These data suggest that alveolar multiplication occurs after pneumonectomy in puppies but not in adult dogs. Dogs are highly suitable animals for the study of lung mechanics and gas exchange and we wondered what the adaptive response would be to pneumonectomy performed in puppies. In this paper we describe the morphological findings.

Methods

Left pneumonectomy was performed on four male and four female mongrel puppies, 9 weeks old. Three male and four female mongrel puppies of the same age, litter mates of the experimental animals, underwent similar anaesthesia and a chest wall incision without entering the pleural cavity. It has been shown that collapse of the lung will induce cellular multiplication and weight increase in the contralateral lung.[7 21 22] All animals were of approximately the same weight at the time of operation. At the age of 20 weeks, the animals underwent tests of pulmonary mechanics and gas exchange. While still anaesthetised, the animals were exsanguinated. The postmortem mechanical properties of the lungs were then studied. Four pneumonectomy specimens, at age 9 weeks, all the contralateral lungs of the pneumonectomised animals and three left and seven right lungs of the controls at 20 weeks were excised and weighed. The right upper lobe was removed, distended at a transpulmonary pressure of 25 cm of saline and its volume measured by water displacement. We intended to make biochemical measurements from this lobe, but there was a technical mishap. The rest of the right lung and the left lungs were

fixed for at least two days with intra-bronchial formalin at a transpulmonary pressure of 25 cm of formalin. Lung volumes were measured by water displacement and the lobes cut into sagittal slices. The volume proportions of parenchyma (tissue less than 1 mm in diameter) and non-parenchyma (tissue greater than 1 mm in diameter) were then determined by point counting the slices of lung. The measurements listed below were made in parenchyma and corrected to the whole lung. Three random blocks were taken from the mid-sagittal slice of each lobe of the left lung. Sections were cut at 7 μm thickness and stained with haematoxylin and eosin. Shrinkage was determined and all values refer to dimensions of fixed lung volume. The slides were randomised and coded. The following measurements were made on the lungs using standard techniques or minor modifications.[19]

Inter-alveolar wall distance (L_m), volume proportion of alveolar air, volume proportion of alveolar duct air (the "core" of air internal to alveoli) and the number of alveoli per unit area. Alveolar surface area[23] and the total number of alveoli and average alveolar volume were calculated using standard methods with the assumption that the alveolar shape factor was 1·55[24] and that the distribution of characteristic linear dimensions of alveoli was normal. No correction was made for thickness of histological slides. Total alveolar number was projected to the fixed lung volume plus the saline filled right upper lobe.

Student's *t* test was used for statistical analysis.

Results

The findings in the right lung of the "pneumonectomy" animals were compared with the right lung and with both lungs of control animals (figure). This shows that the right lungs of the pneumonectomy animals had significantly larger volumes, alveolar surface area, and total number of alveoli than the right lungs of the controls and that there was a trend for alveolar duct proportion to be increased in the pneumonectomy animals. Alveolar dimensions were not different between the two groups and alveolar surface area increased directly with lung volumes in the pneumonectomy animals. There were no statistically significant differences between the right lungs of pneumonectomy animals and both lungs of the controls. There was a trend for the two lungs of the control animals to have more alveoli than the single contralateral lung in the pneumonectomy animals.

Table 1 shows a comparison of the dimensions of the structures in the right and left lungs of controls and shows that there are no differences. The right lung was 63·6% larger than the left lung and therefore the total surface area and total number of alveoli were correspondingly higher in the right lung. We have also recalculated our data assuming that the four left lungs of the control animals not subjected to morphometry had the same number of alveoli per unit volume and L_m as their right lungs. The results are exactly the same as when the comparison was made between pneumonectomy animals and both
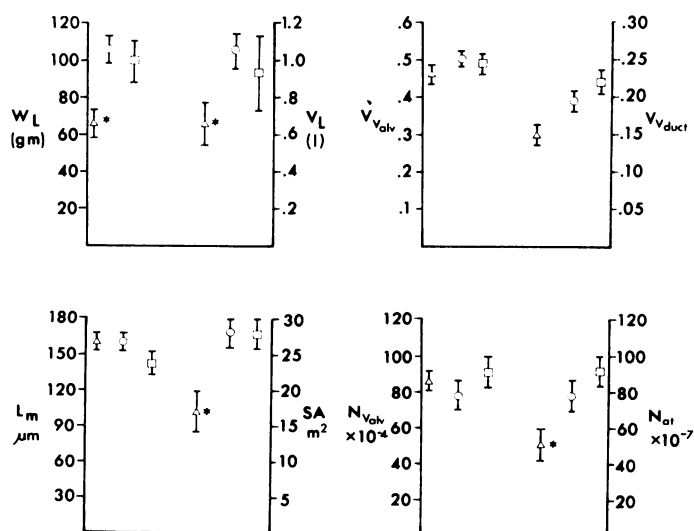

Figure   *Dimensions of the right lung of animals ($\triangle$), the right lung of animals undergoing pneumonectomy ($\bigcirc$,) and both lungs of control animals ($\square$) are compared. Bars represent $\pm$ 1 SE. The variables are: lung weight (W_L), lung volume distended at a transpulmonary pressure of 25 cm formalin (V_L), volume proportion of alveoli ($V_{v\ alv}$), volume proportion of "alveolar ducts" $V_{v\ duct}$, average interalveolar wall distance (L_m), alveolar surface area (SA) number of alveoli per ml ($N_{v\ alv}$), and total number of alveoli (N_{at}).*
**Asterisk indicates a difference, significant at p 0·05 or less.*

Table 1 *Morphometric variables in control lungs in 20-week-old animals and in the left lung excised at 9 weeks of age (mean ± SEM)*

	Right lung (controls) 20 Weeks n=7	Left lung (controls) 20 Weeks n=3	Left lung pneumonectomy 9 Weeks n=4
Lung volume (ml)	627±84	383±63	113±15
Interalveolar wall distance (μm)	158±7	136±11	141±5
Volume proportion of alveoli	0·46±0·02	0·50±0·04	0·47±0·01
Volume proportion of ducts	0·15±0·03	0·20±0·03	0·28±0·01
Alveoli per ml (× 10⁻³)	876±60	931±29	1100±76
Volume proportion of parenchyma	0·76±0·03	0·82±0·05	0·85±0·1
Volume proportion of non-parenchyma	0·24±0·03	0·18±0·05	0·15±0·1

Table 2 *Lung volumes and lobar comparisons of number of alveoli per ml and average interalveolar wall distance in the right lung*

Right lung volume (ml)		Number of alveoli per ml (× 10⁻³)			Average interalveolar wall distance (μm)	
Control n=7	Pneumonectomy n=8	Control n=7		Pneumonectomy n=8	Control n=7	Pneumonectomy n=8
627±84	1062±97	Cardiac 962·9±129·7		623·7±84·8*	159±13	166±12†
		Middle 748·5±61·5		682·4±80·1†	157±11	155±11
		Lower 903·8±48·8		902·8±55·3	151±7	145±7

* Different from control p < 0·05, different from lower lobe pneumonectomy p < 0·01.
† Different from lower lobe pneumonectomy, p < 0·05.

lungs of the control animals as was illustrated in the figure. Table 1 also compares the dimensions of structures in left lungs at pneumonectomy in 9-week-old puppies with left lungs in the 20-week-old controls. Once again the dimensions of structures are the same but lung volume increased by 339%, surface area by 362%, and total alveolar number by 288%.

Table 2 gives a comparison of the dimensions in the lobes of the right lung. There are no differences between the lobes in the controls. In pneumonectomy animals there were fewer alveoli per ml in middle (p<0·05) and cardiac (p<0·01) lobes compared with the lower lobe, and the inter-alveolar wall distance was greater in the cardiac lobe (p<0·05) than in the lower lobe suggesting a greater adaptive response in the lower lobe. The cardiac lobe in pneumonectomy animals had fewer alveoli per unit volume than the controls (p<0·05) suggesting that the adaptive response was less complete in this lobe.

Discussion

Our data show that in the dog, as in the rat and rabbit, after pneumonectomy the contralateral right lung increases in lung weight, lung volume, surface area, and total alveolar number. These values closely approximate to those of both lungs of the control animals, and these variables were also significantly greater than in the single left lung of control animals.

The adaptive responses were almost as complete as in the rabbit,[10] in that the total number of alveoli in the contralateral lung was less than in both lungs of the controls, but not significantly so. The argument about the significance of alveolar number persists[18] [19] and has been discussed previously.[19] Surface area increased directly with lung volume and we interpret this to mean increased alveolar surface complexity brought about by formation of new alveoli. Alternatively, alveolar walls may become more complex[18] but we could find no evidence for this. If alveolar walls elongated to produce increased surface area, then the volume proportion of alveolar air would increase and the volume proportion of alveolar duct air, as we have defined it, would decrease. This did not occur in our experiment although we have had evidence for such an adaptive change in rats subjected to pneumonectomy at 12 weeks of age.[19] We have assumed that the upper lobe, which was intended for biochemical assessment of lung growth, had undergone the same change as the lung as a whole. This may not have been the case, so that the response in terms of alveolar number and surface area may not have been as complete as it appears. This may be offset by our projection of mean number of alveoli per unit volume derived from three lobes to the whole lung. As indicated below, the lower lobe (which in any event is the largest lobe) may have expanded most and thus total alveolar number may be underestimated. However, it is clear that an

extensive response occurs in the dog which is more complete than in the rat where a similar assumption was made.[19] The lower lobe appears to respond more completely in that it had significantly more alveoli per unit volume than either the middle or the cardiac lobe, and the average inter-alveolar wall distance and the mean alveolar transection length (L_m times alveolar air proportion) were smaller. Further, the cardiac lobe had significantly fewer alveoli per unit volume than the cardiac lobe of control animals suggesting an incomplete response. It has been suggested that stretch is the primary stimulating factor to the adaptive response since the response can be reduced or abolished by plombage of the pleural cavity on the side of the pneumonectomy.[2 9 12] Although we have no data concerning individual total volumes, it may be that different mechanical stresses occur on the lobes of the lung after pneumonectomy and thus it may be that the lower lobe is stretched the most as it herniates through the incomplete mediastinum of the dog. It therefore undergoes a more complete adaptive response. It seems unlikely that increased blood flow, increased ventilation or the recently described post-pneumonectomy serum factor[25] would produce an uneven lobar response. Variation of response in the lobes were noted in the rat[19] but not in the rabbit.[10] Since active lung growth and alveolar multiplication occurred between nine and 20 weeks of age, the adaptive response appears to be a speeding up of this process.

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