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MICROVASCULAR CASTING OF THE LUNG: BRONCHIAL VERSUS PULMONARY ARTERY FILLING

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Abstract

To determine the importance of the pulmonary or bronchial arteries as the site of injection to cast the lung vasculature of rats, we retrospectively compared a group of normal Sprague-Dawley rats that were cast through either the caudal vena cava and right ventricle leading to the pulmonary arteries or through the aorta and bronchial arteries. There was no difference in the appearance of the whole casts or cast-to-lung weight ratios between the two groups, although an incompletely cast lung that was injected through the aorta showed preferential filling of the pleural and peribronchial regions. Nuclear impressions, a sign of good filling, were more frequent and the grade of the images was higher in the group filled through the vena cava. The microvascular density index was not different between the two groups, and a multivariate analysis of variance showed the injection site was not important in determining vascular density. The pulmonary vessels fill nearly as well when injected from the bronchial circulation indicating that the bronchial circulation can immediately perfuse the lung if the pulmonary arteries are obstructed, and casting can be carried out from either side with nearly the same results.

Introduction

The lung has two arterial blood supplies-the pulmonary and bronchial. The pulmonary circulation is designed for gas exchange and the bronchial circulation for nourishment of the central structures. The bronchial circulation can perfuse the pulmonary vasculature if the pulmonary circulation is obstructed (Virchow, 1847; Liebow et al., 1950). When this occurs extensive new bronchial vessels are formed, although the time required to develop normal flow is unknown (Weibel, 1960). The interconnections between these two systems can be studied by casting. Pulmonary vessels are cast by injecting resin into the pulmonary artery, right ventricle, or vena cava. The bronchial circulation is cast by injecting resin into the aorta and bronchial arteries, but it has long been known that the two systems are interconnected (Miller, 1947; von Hayek, 1960). Study of their connections and interactions gives important insight into pulmonary physiology (Charan et al., 1984; Deffebach et al., 1987; Schraufnagel, 1987). Investigators have found that material injected into the bronchial arteries is found in the pulmonary veins (Magno and Fishman, 1982), but attempts to cast the lung by aortic filling have not been reported.

In a casting experiment, the superior vena cava was damaged, so the resin was injected through the aorta. The lung vasculature filled well. We repeated this procedure on several occasions, but until now did not make any comparison of the two injection sites. In this study the casts of the lungs filled through the vena cava and pulmonary artery were compared with the casts filled through the aorta and bronchial arteries.

Materials and Methods

This study is a retrospective analysis of animals that were used in two previously reported studies on casting technique (Schraufnagel and Schmid 1988a, 1988b) and in pilot studies not reported. Fifty-five, female, Sprague-Dawley rats that weighed about 250 g were deeply anesthetized with pentobarbital. The abdomen was opened and the caudal vena cava and abdominal aorta were cannulated at the level of the renal arteries. Forty-four animals received warmed, heparinized saline, fixative, and partially polymerized

methylmethacrylate (Mercox, Ladd, Burlington, VT) through the vena cava to cast the lung vessels. In the other 11 animals the vena cava was ruptured during the time the aorta and caudal vena cava were being separated or it was punctured by the cannula as it was threaded up the caudal vena cava. In these 11 animals the injection of the methylmethacrylate was done retrograde through the aorta. Precasting fixation and rinsing varied with the protocol. Perfusion fixation was done with glutaraldehyde, formaldehyde, or nothing (Schraufnagel and Schmid, 1988b). Rinsing was done until the aortic effluent was clear or with 50 ml of fixative (Schraufnagel and Schmid 1988a). Vasodilators were not used. The infusion pressure of the rinsing and fixation solutions was about 5 cm of water. The capillary pressure during casting was not measured. After casting, the lungs of both groups were treated the same. The animals were placed in a 45°C bath for an hour to complete the polymerization. The lungs were removed, weighed, and placed in 10 N sodium hydroxide until the tissue was digested. The specimens were rinsed in detergent, water, and alcohol and reweighed. The quality of casts was judged and measurements were made without knowledge of the injection site.

Sections about 1 mm thick were then cut with razor blades. The specimens were fastened to aluminum studs with double-sided tape,sputter-coated with a layer of palladium-gold about 20 nm thick and viewed with a JEOL JSM-35C scanning electron microscope. The final aperture was 200 µm; the accelerating voltage was l5 kV; the tilt angle was 0°; the working distance was l5 mm. Images for density measurements were taken at 100 X magnification and simultaneously transmitted to a DEC Vax computer by Deltapro (Computer Design and Applications, Waltham, MA) image analysis system. Up to 4 studs were made per animal and all the area on the studs was photographed. Based on a past study (Schraufnagel and Schmid 1988c), the gray level of the images was preset to give a black-white picture where regions out of the plane of focus appeared black and regions in focus appeared white. The ratio of the white area to the total area was defined as the vascular density index.

In addition to density measurements the images were graded. Excellent micrographs were scored 1; good were scored 2; satisfactory were scored 3; below average were scored 4; and poor were given a 5. Extravasation of resin was assessed by counting the images that had alveolar and lymphatic plastic. Signs of poor filling such as lack of nuclear impressions and the presence of non-connecting capillary segments (dead ends) were also tabulated.

The quality of the casts was compared by the rank sum test (SAS, 1985); two by two tables were analyzed by Fisher's exact test (Fisher, 1922). The average cast-to-lung weight and the vascular density index were compared by the t test. The relative importance of the injection site compared to the other variables studied, namely the fixation and rinsing procedures, was measured by analysis of variance (SAS, 1985).

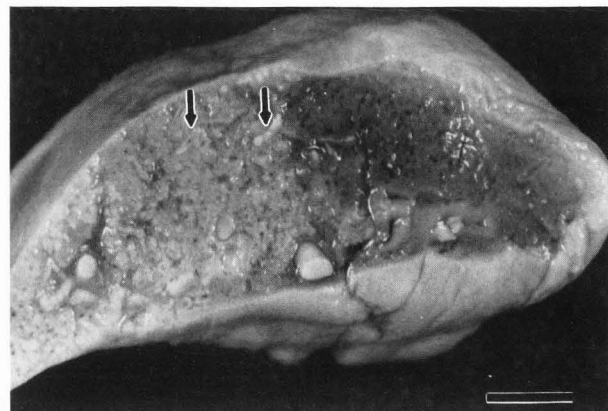As a second (prospective) study, I cast the lungs of 3 rats after clamping the heart to prevent the

Figure 1. This rat lung was injected through the aorta and did not fill completely. The pleural surface is well cast; peribronchial areas (arrows) are better filled than surrounding lung parenchyma. Bar = 4 mm.

methylmethacrylate from entering the lung retrograde through the heart.

Results

Table 1 shows the assessment of the filling of the casts was not different between the injection groups, although a specimen injected from the aorta that did not fill completely showed preferential casting of the peribronchial and pleural regions (figure 1). The cast to organ weight ratio was 0.27 (SD 0.26) for the aortic group and 0.26 (SD 0.21) for the vena caval group, which was not significantly different. Although there was no noticeable difference in the whole casts or images between the two groups (figures 2-3), the vena caval group had a better average grade of 1.6 (SD 0.76) compared to the aortic group which had a grade of 2.1 (SD 0.78)(p < 0.01). All 140 micrographs of the vena caval group had nuclear impressions on the casts, but only 85% of the 53 micrographs of the aortic group had them (p < 0.01). The percentage of images with dead ends was not different between the vena caval (32%) and the aortic groups (28%). Alveolar and lymphatic filling, which indicates leakage of resin, occurred more frequently in the aortic group (42%) than in the vena caval group (19%) (p < 0.01). The leakage occurred primarily in the non-fixed lungs. In a lung injected through the aorta the extravasated plastic was trapped beneath the pleura giving a replica of the inner pleural surface (figure 4).

On the pleural surface the vascular density index was 0.48 (SD 0.08) for the vena caval group and 0.50 (SD 0.08) for the aortic group. On the alveolar surface the density index was 0.39 (SD 0.09) for the vena caval group and 0.37 (0.13) for the aortic group. These differences were not significant. The analysis of variance showed that the injection did not account for any significant variation in the density index (table 2).

The heart clamping experiment showed that the lungs filled with methylmethcrylate even though the heart was obstructed.
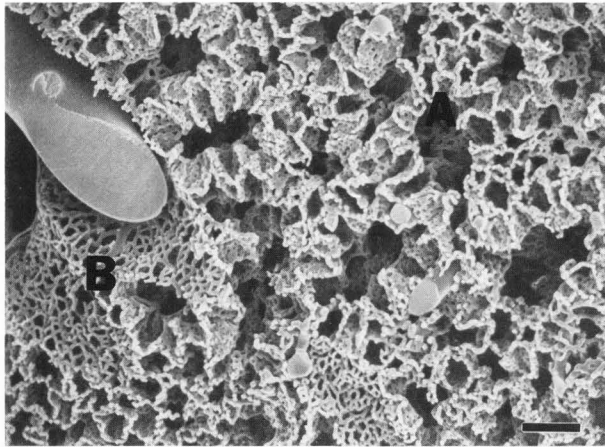
Figure 2. Normal lung cast from vena cava. The alveolar capillary baskets (A) and peribronchial capillaries (B) appear normal. Bar.= 100 µm.
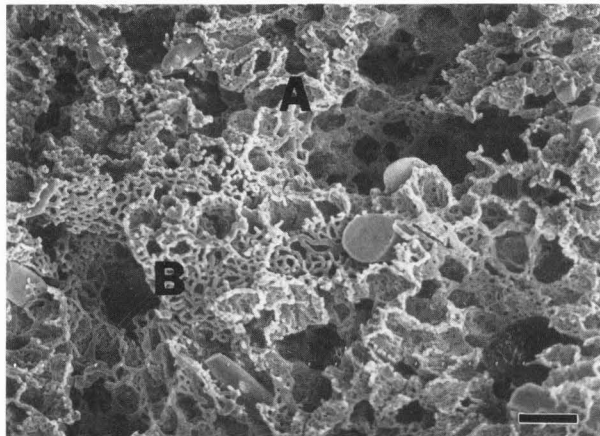

Figure 3. Normal lung cast through the aorta. The alveolar capillary baskets (A) and peribronchial capillaries (B) appear normal. Bar = 100 µm.

Table 1. Assessment of filling by viewing the whole cast with a dissecting microscope

| | | Filling From | | |
		Vena Cava	Aorta	Total
Excellent cast produced	No	20	5	25
	Yes	24	6	30
Total		44	11	55

The percentage of excellent casts produced by aortic filling was 6/11 or 54.5%. The percentage of excellent casts produced by vena caval filling was 24/44 or also 54.5%.
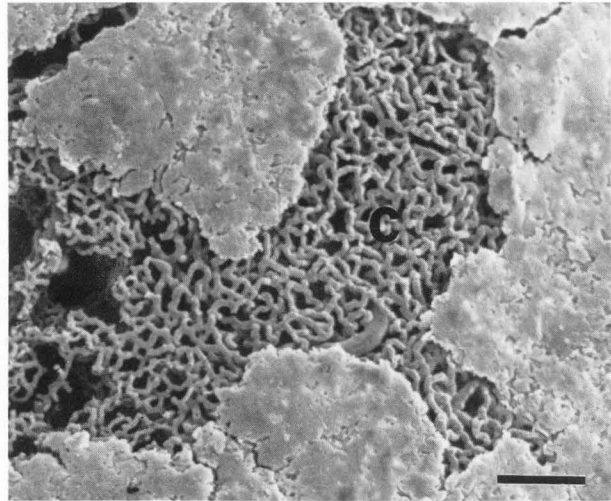

Figure 4. In a lung cast from the aorta, extravasated resin was trapped beneath the pleura giving a replica of the inner pleural surface. Part of the sheet was removed to show the capillaries (C) beneath it. Bar = 100 µm.

Table 2. An analysis of variance table comparing the effects of different preparations on the vascular density index of 28 animals and 233 images. The only variable that was not important was whether the lungs were injected through the vena cava or aorta.

Variable	F value	P <
Fixation Method[1]	30.22	0.0001
Thorough rinsing[2]	61.81	0.0001
Injection Site[3]	0.92	0.34
Animal[4]	43.79	0.0001
Surface[5]	254.76	0.0001

1. Fixation method could be with 2.5 or 1.2% glutaraldehyde, 10% formalin, or nothing.
2. The rinsing was until the effluent was clear or with only 50 ml of fixative.
3. The injection site was caudal vena cava or thoracic aorta.
4. This variable accounted for the difference between animals.
5. The surface was either the pleural or cut (alveolar) surface.

Discussion

Methylmethacrylate fills all vessels including capillaries well and is ideal to study connections between the pulmonary and bronchial circulations (Schraufnagel et al., 1986; Schraufnagel, 1987). It is known that with obstruction of a major pulmonary artery, the bronchial circulation eventually takes over without necrosis of the lung, but it was surprising that nearly perfect casts were produced with injection through the aorta. This indicates that the

communications are extensive and that the bronchial circulation can immediately supply the pulmonary circulation when a pulmonary artery is obstructed.

The second point is that casting can be carried out from either the right or left side of the heart with nearly equal results. Although the grade of the images and nuclear impressions were better in the vena cava filled group, no difference was detectable by observation alone. These experiments had different fixation and rinsing procedures, but had the same aim to produce a good quality cast. The fixation and rinsing were clearly more important that the injection site. The difference in nuclear impressions and grading may indicate less complete filling with aortic injection, but the difference was not enough to significantly affect the vascular density index. Vessels may not be completely distended even though capillaries are well filled.

Acknowledgements

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Discussion with Reviewers

S. A. Dilly: Does methyl methacrylate come out of the bronchial arteries in the vena caval group, and does it reach the main pulmonary arteries and veins in the aortic group? I would expect this if the casts are really identical.
Author: When the rats receive 20 ml of methylmethacrylate it usually appears in the aortic outflow. In these studies we did not specifically look at the main pulmonary arteries in those animals cast through the aorta or at the bronchial arteries in those animals cast through the pulmonary arteries.

S. A. Dilly: It is generally taught that pulmonary infarction due to pulmonary arterial obstruction only occurs in previously damaged lungs since in normal lungs the bronchial circulation is sufficient to maintain the tissue. You have investigated pathological conditions, such as emphysema and fibrosis. Have you noted any difference in ability of the aorta to supply pulmonary tissue with pulmonary arterial insufficiency?
Author: If previous lung damage is important in the pathogenesis of pulmonary infarction, it is likely to be related to the adequacy of the collateral circulation (both pulmonary and bronchial). Infarction is more likely to occur if many small emboli are lodged in the pulmonary arteries. Multiple emboli and involvement of more lobes are more important than the presence of emphysema or fibrosis. Chronicity is a factor because patients who have been sick longer also are more likely to have infarction when compared to patients with pulmonary embolism without infarction. (Tsao et al., 1982, Schraufnagel et al., 1985). Hyperplasia of the bronchial circulation occurs within a week or two after pulmonary artery obstruction, so that the blood flow to the lung nears normal (Weibel, 1960). This study shows that there is enough connection between the two circulations to cast the pulmonary circulation after injection of the bronchial arteries. Presumably this is enough to prevent necrosis.

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