Branching Architecture of Canine Pulmonary Arteries : A Quantitative Cast Study

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KOIKE, K., OHNUKI, T., OHKUDA, K., NITTA, S. and NAKADA, T. *Branching Architecture of Canine Pulmonary Arteries : A Quantitative Cast Study*. Tohoku J. exp. Med., 1986, **149** (3), 293-305 — The purpose of this investigation is to analyze the branching pattern of pulmonary artery. The excised right lower lobes of six mongrel dogs were fixed by perfusion with 1.2% glutaraldehyde solution. Pressure of the pulmonary artery, vein and airway were controlled during perfusion. After fixation, resin was injected manually into the artery. When resin was completely polymerized, the lung was placed in 15 N NaOH solution to remove lung tissue. The diameters and lengths of branches were measured by stereomicroscopy. The results obtained were as follows: (1) The basically dichotomous pulmonary arteries showed not only fairly regular dichotomies but monopodies, in the latter of which the diameter of parent branch was almost the same as that of its larger daughter branch. (2) The diameter of monopodial branch was less than 500 μm , even when the parent branch was a large central segment. In arteries of over 1,000 μm in diameter, 75% or more of branchings were monopodies, whereas in small arteries of 200-300 μm in diameter, half the bifurcations were monopodies. (3) At a bifurcation, the average daughter-to-parent diameter ratio was 0.8-0.9 for the larger daughter branch and 0.5 for the smaller one. (4) The average length of branches was in proportion to their average diameter. ——— pulmonary artery; anatomy; microcirculation; branching pattern

Studies on lung structure have mostly focussed on the airways. Elucidation of airway branching patterns by Weibel (1963) has benefited various studies in the field of lung physiology (Pedley et al. 1970). But regarding the pulmonary arteries, little has been studied on the circulatory behavior based on their branching pattern (Cumming et al. 1969; Overholser et al. 1982), because complete analysis that could be used for designing a pulmonary arterial model has not been achieved due to the lack of morphological knowledge on the architecture of pulmonary arteries. Moreover, an asymmetry of the pulmonary arterial system was pointed out (Singhal et al. 1973). Therefore, we should analyze morphological factors such as the diameter and length of vessels which appear closely related with the pulmonary vascular resistance (Bayliss 1962).

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These circumstances urged us to analyze the pulmonary arterial branching architecture as a fundamental factor of a pulmonary arterial model that is to be established for the future simulation of pulmonary hemodynamics.

MATERIALS AND METHODS

Pulmonary vascular cast

Six mongrel dogs weighing 9–10 kg were anesthetized with sodium pentobarbital (30 mg/kg) and heparinized (500 IU/kg) intravenously. Ventilation was controlled under intratracheal intubation and the right lower lobes were excised. A vinyl tube (10 mm I.D.) was inserted into each excised lung bronchus and the airway pressure was kept at 10 cm H₂O. After siliconized tubes (5 mm I.D.) had been inserted into the pulmonary artery and vein, intravascular blood was washed out with about 100 ml of heparinized saline solution (4 IU/ml). Using the perfusion system of pressure control shown in Fig. 1, the excised lobes were fixed by vascular perfusion at a constant pressure level with 1.2% glutaraldehyde (pH 7.4 phosphate buffer) solution. Fixation was carried out under three different conditions of perfusion pressures (P_{PA} : pulmonary arterial perfusion pressure, P_{PV} : pulmonary venous perfusion pressure) with a zero point at the hilum; *Group A*, P_{PA} 40 cmH₂O and P_{PV} 7 cmH₂O (2 dogs), *Group B*, P_{PA} 70 cmH₂O and P_{PV} 7 cmH₂O (2 dogs) and *Group C*, P_{PA} 70 cmH₂O and P_{PV} 47 cmH₂O (2 dogs). After fixation for one hour acrylic resin (Mercocryl, Dainihon Ink Co.) was injected manually from the arterial side at a pressure same as the perfusion. After the injection that had been continued until the congelation of resin, the lobes were kept at room temperature for 12 hr to allow its complete polymerization.

These lobes were immersed in 15 N NaOH to remove lung tissues. NaOH solution was changed four to five times. After most of the lung tissues had been removed, the casts with remaining tissue debris were washed in running water.

Fig. 1. The excised right lower lobe (RLL) was fixed by perfusion with 1.2% glutaraldehyde solution at constant pulmonary arterial pressure (P_{PA}), pulmonary venous pressure (P_{PV}) and airway pressure (P_{ALV}). After fixation, resin was injected manually at the same pressure as that of perfusion.

Measurement of pulmonary arterial casts

The portions of capillary-networks, veins and branches that were not recognized as either arterial or venous were removed using forceps and needles. In this way, the pulmonary arterial branches were completely exposed. We measured the branches that were clearly identified as arteries. The methods of measurements are shown in Fig. 2.

Diameter of branch. At each bifurcation, the diameters of parent branch (d_n) and two daughter branches (d_{n+1} , d'_{n+1} ; $d_{n+1} \geq d'_{n+1}$) were measured. When the section of a branch was elliptical, the diameter was obtained as an arithmetical mean of the major and minor axes (Cumming et al. 1969).

Length of branch. By definition of Weibel (1963), the monopody branches are the small daughter branches at the bifurcation at which the parents were equal in diameter to their large daughter branches. When we measure a length of branch (l_n), there are three considerable pattern; the lengths between two non-monopodial bifurcations, between non-monopodial and monopodial, and between two monopodial bifurcations. In the first case, the length was defined as the distance between the midpoints of the triangles at bifurcations. In the second, the length was defined as the distance between the midpoint of the triangle at non-monopodial bifurcation and the central axis of monopody branch. In the last case, the length was defined as the distance between the central axes of two monopody branches.

To clearly the branching pattern, we divided the bifurcations into two groups; monopodial and non-monopodial branchings. In monopodial bifurcation, we regarded the

Fig. 2. Measurements of diameters and lengths of pulmonary arterial branches were carried out in two ways. In the left figure, measurement was carried out at each bifurcation for diameter (d_n) and length (l_n) of parent branches and diameters of large (d_{n+1}) and small daughter branches (d'_{n+1}). In the right figure, if the branches from the same stem were found equal in diameter, they were regarded as one major branch whose diameter (D_n) and length (L_n) corresponded to the diameters of large (D_{n+1}) and small daughter branches (D'_{n+1}). The diameters of monopody branches (D_{mon}) arising from the stem of the major branch were also measured.

parent and the large daughter branch as one major branch because their diameters were equal. In these major branchings, we measured the diameter (D_n) and length (L_n) of parent and diameters of large (D_{n+1}) and small (D'_{n+1}) daughter branches which were non-monopodial bifurcation. The diameters of monopody branches (D_{mon}) branching from the stem of major branches were also measured.

These measurements were carried out by stereomicroscopy and rounded off at a unit of $100\ \mu\text{m}$. We divided branches of the diameters above $1,100\ \mu\text{m}$ into two groups; $1,100$ – $1,500\ \mu\text{m}$ and over $1,600\ \mu\text{m}$. The number of branches measured for diameter totalled to 2614 ± 578 (mean $\pm$ s.d., $n=6$) for each lower lobe. Half of them were below $500\ \mu\text{m}$ in diameter. For those above $600\ \mu\text{m}$ in diameter, the number of branches counted for each diameter class was 50–150. The number of branches counted for various diameters were nearly the same for the three different perfusion conditions. The number of branches measured for length was 1499 ± 483 ($n=6$).

RESULTS

Change in diameter at bifurcations

Fig. 3 shows the distribution of the diameters of large daughter branches.

Fig. 3. Diameters of large daughter branches arising from parent branches (left vertical column) are plotted horizontally and their frequencies (f) vertically. Branches over $1,000\ \mu\text{m}$ in diameter are divided into two groups; over $1,600\ \mu\text{m}$ under $1,500\ \mu\text{m}$. These groups are plotted horizontally according to the branching ratios (d_{n+1}/d_n). The large daughter branches are nearly the same in diameter as their parent branches.

Where that parents were over $1,100\ \mu\text{m}$ in diameter, 75% or more of the large daughters had equal diameters to their parent branches. On the other hand, the smaller the parents, the lower the frequency of large daughters having the same diameter as their parent branches,

Fig. 4 shows the distribution of the diameters of small daughter branches. In 75% or more of the bifurcations from the parents above $1,100\ \mu\text{m}$ in diameter, the diameter of small daughters were less than 0.3 in ratio to the diameters of their parent branches. This tendency was also confirmed in branches of $600\ \mu\text{m}$ in diameter. Small daughter branches were predominated by the diameter of $100\ \mu\text{m}$ and majority had a diameter below $300\ \mu\text{m}$. For small daughter branches under $500\ \mu\text{m}$ in diameter, the tendency was practically the same with smaller branches increased and large one decreased in frequency.

The bifurcations at which the large daughter branches were smaller than the parents are shown in Fig. 5. For parent branches with a diameter of $700\ \mu\text{m}$ or less, most of the large daughters had diameters smaller than those of their parents only by $100\ \mu\text{m}$. On the other hand, large daughters that branches from the

Fig. 4. Diameters of small daughter branches arising from parent branches (left vertical column) are plotted horizontally and their frequencies (f) vertically. Branches over $1,100\ \mu\text{m}$ in diameter are divided into two groups; over $1,600\ \mu\text{m}$ and under $1,500\ \mu\text{m}$. These groups are plotted horizontally according to the branching ratios ($d'n+1/dn$). The majority of the small daughter branches are under $500\ \mu\text{m}$ in diameter.

parents above this diametric level were smaller than their parents by 100–200 μm . In case of parents over 1,100 μm in diameter, the diameter ratio of parents to the large daughter branches was 0.7–1.0.

The bifurcations at which the large daughters were smaller than their parent branches were selected. In these bifurcations, the relationship between parent and small daughter branches is presented in Fig. 6. The distribution was nearly symmetrical for all the diameters of the parent branches. In 50–70% of the small daughter branches, the diameter was about half that of the parent branch.

Monopody branches were selected and shown in Fig. 7. Eighty percent or more of these monopody branches having parent branches of over 1,100 μm in diameter had a diameter ratio (ratio of a monopody branch to its parent branch) below 0.3 with a peak at the ratio of 0.1. The same tendency was found for the parent branches having diameters between 1,000 and 500 μm . In both cases, the monopody branches were under 500 μm in diameter, and the majority of these were under 300 μm . But as for the parent branches below 500 μm in diameter, the monopodies of 100 μm in diameter were predominant.

Fig. 5. In branching patterns excluding monopodies, diameters of large daughter branches arising from parent branches (left vertical column) are plotted horizontally and their frequencies (f) vertically. Branches over 1,100 μm in diameter are divided into two groups; between 1,100 and 1,599 μm in diameter and over 1600 μm , and their branching ratios (D_{n+1}/D_n) are plotted horizontally. The majority of large daughter branches are 100 μm smaller than their parent branches.

Fig. 6. In branching patterns excluding monopodies, diameter of small daughter branches arising from parent branches (left vertical column) are plotted horizontally and their frequencies (f) vertically. Branches over $1,100\text{ }\mu\text{m}$ in diameter are divided into two groups; between $1,100$ and $1,500\text{ }\mu\text{m}$, and their branching ratios ($D'n+1/Dn$) are plotted horizontally. In 50% of small daughter branches, the diameters were about half those of their parent branches.

Diameter and length

The curves obtained by plotting the lengths of branches against their diameters are shown in Fig. 8. The branches of $200\text{ }\mu\text{m}$ in diameter had lengths of about $500\text{ }\mu\text{m}$, those $600\text{ }\mu\text{m}$ in diameter lengths of about $700\text{ }\mu\text{m}$ and those $1,000\text{ }\mu\text{m}$ in diameter lengths of about $900\text{ }\mu\text{m}$. Nostatistical differences were found in the length-diameter relationship among the three groups by Student *t*-test.

Branches excluding monopodies were found to be one major branches. Fig. 9 shows the relationship between the diameters and lengths of these branches. Branches of $200\text{ }\mu\text{m}$ in diameter were approximately $900\text{ }\mu\text{m}$ in length, those of $600\text{ }\mu\text{m}$ in diameter approximately $2,200\text{ }\mu\text{m}$ in length and those of $1,000\text{ }\mu\text{m}$ in diameter approximately $4,500\text{ }\mu\text{m}$ in length. Compared with the $\ln/dn$ ratio in Fig. 8, the $\ln/dn$ ratio was much higher. Statistically, there were no differences among the three experimental groups by Student *t*-test.

Fig. 7. Diameters of monopody branches arising from parent branches (left vertical column) are plotted horizontally and their frequencies (f) vertically. Branches over $1,100\ \mu\text{m}$ in diameter are divided into two groups; between $1,100$ and $1,500\ \mu\text{m}$, and over $1,600\ \mu\text{m}$, and their branching ratios ($D_{\text{mon}}/D_{\text{n}}$) are plotted horizontally. The diameters of monopody branches are below $500\ \mu\text{m}$ ($D_{\text{mon}}/D_{\text{n}} < 0.5$).

Frequency of monopody

On the data of Fig. 3, there are two branching patterns. One is the monopodial pattern, and the other is the non-monopodial pattern in which the parents were larger than daughter branches. Based on these two different patterns, the frequency of monopody was calculated and shown in Fig. 10. Approximately 75% or more of the bifurcations were monopodies if the parent branches were larger than $1,000\ \mu\text{m}$. As the diameter of branches decreased, the frequency of monopody diminished. Half the bifurcations from the parent branches with diameters of $200\text{--}300\ \mu\text{m}$ were monopodies.

The frequency was also calculated in Fig. 11 using the data of the lengths of branches shown in Figs. 8 and 9.

$$f = (L_{\text{n}} - l_{\text{n}}) / L_{\text{n}} \times 100$$

Where f is the frequency of monopody and l_{n} and L_{n} are described in Fig. 2. Using this method of calculation, the result showed almost the same frequency patterns as indicated in Fig. 10. These two results were identical in spite of the

Fig. 8. The lengths of branches (mean $\pm$ s.d.) are plotted against their diameters in three experimental conditions. The lengths of branches are proportional to their diameters.

Fig. 9. The lengths of branches without monopody (mean $\pm$ s.d.) are plotted against their diameters in three experimental conditions. The lengths of branches are proportional to their diameters.

Fig. 10. Frequencies of monopodies at bifurcations at which the diameters of large daughter branches were the same as those of their parent branches. Seventy-five percent or more of the bifurcations were monopodies for which the parent branches had diameters of over $500\ \mu\text{m}$.

Fig. 11. Frequencies of monopodies in three experimental conditions. Calculations were based on the average length of branches (l_n) and the average length of branches excluding monopodies (L_n). The curves of frequencies are nearly the same as the curves shown in Fig. 10.

difference in the methods used. Perfusion pressure did not influence these results.

DISCUSSION

The frequency of monopodies in pulmonary arteries differed depending on the diameters of the monopody-branching parent branches; approximately 50–80% of the branchings were found to be monopodies. The large daughter branches were nearly the same as or only 10–20% smaller than their parents in diameter. This means that the large daughter branches gradually reduce their diameters, although little change in diameter was found at bifurcations. On the other hand, the diameter of the smaller daughter at monopodies was different from that at monopodial dichotomies. Although small daughter branches in monopodies (monopody branches) even from a large central branch measured generally below 500 μm in diameter, small daughter branches produced in non-monopodial dichotomies had about half the diameter of their parent vessels.

The irregularity of the pulmonary arterial system in autoptic human lungs has been pointed out (Weibel 1963; Reid and Heard 1963), though this system is in general considered to be parallel to the airway characterized by regular dichotomy (Pump 1961). But recently an irregularity of the bronchial tree was indicated (Horsfield et al. 1982). This irregularity has also been indicated by the analysis of the pulmonary arterial branching pattern using the opaque injection method (Elliot and Reid 1965). Other studies using casts have reported that the branching pattern of human pulmonary arteries is characterized by monopodies in the range of parent branches larger than 800 μm (Cumming et al. 1969; Singhal et al. 1973).

According to the results of the present study, especially in Figs. 3 and 4, we can conclude that the pulmonary arterial system represents a combination of two different branching patterns and there are no differences among individuals. One is the pattern of monopody that occurs in 50–80% of all bifurcations. Especially in case of large parent branches with a diameter over 1,000 μm , 75% or more of the bifurcations are monopodies. But the frequency of monopodies becomes lower with decreasing diameter of parent branches. The other is the pattern of more or less regular dichotomy where the larger daughter branch has 70–80% diameter of its parent branch while the smaller one is of 50% diameter. These branching patterns occur in 20–50% of all bifurcations.

The lengths of branches became longer in proportion to their diameters for both $d_n/\ln$ and D_n/L_n . However, the variance of length on diameters was large. As there is a diversity of length in the lung airway system (Weibel 1963), there is naturally a considerable irregularity in the lengths of arterial branches because pulmonary arteries basically run parallel with the bronchi (Pump 1961) and are characterized by many monopodies. The irregularity of geometrical structures is not characterized only in lungs but also in other systemic organs (Suwa et al. 1963).

The vascular cast preparation in this experiment involved resin injection after perfusion-fixation with low-concentration glutaraldehyde solution at a constant pressure level, and this was considered appropriate with minimum artifacts. Previous morphological studies using casts of lung have been mainly focused on the bronchial tree and have used a method of resin injection after dry fixation (Ross 1957), formalin fixation (Horsfield and Cumming 1968) or formalin steam fixation (Weibel 1963). Also, in earlier studies of the pulmonary vascular system using casts, resin was injected after perfusion fixation with formalin solution (Hyland et al. 1963; Cumming et al. 1969). However, the tissue fixed with formalin is more prone to constriction than with glutaraldehyde solution. Casting materials such as plastic (Ross 1957) or methacrylate (Murakami 1971) pose problems due to higher viscosities and stronger constriction when polymerized. We chose three different experimental conditions in order to determine the influence of the pressure of resin injection. The branching patterns demonstrated no difference in relation to these experimental conditions.

In this study, the branching pattern of large and small pulmonary arteries ranging from 100 μm in diameter up to central branches were measured and analyzed by stereomicroscopy. In our previous studies, hypoxic vasoconstriction has been observed in branches of pulmonary arteries below 400 μm in diameter, and concept of functional vascular unit has been proposed (Nitta et al. 1969). In view of the result that the frequency of monopodies was strikingly low among peripheral branches of 400 μm or less, one may regard these branches as corresponding to a functional unit comparable to the physiological significance of small pulmonary arteries.

References

- 1) Bayliss, L.E. (1962) The rheology of blood. In: *Handbook of Physiology. Circulation Volume I*, edited by W.F. Hamilton, Waverly, Baltimore, pp. 108-126.
- 2) Cumming, G., Henderson, R., Horsfield, K. & Singhal, S.S. (1969) The functional morphology of the pulmonary circulation. In: *The Pulmonary Circulation and Interstitial Space*, edited by A.P. Fishman & H.H. Hecht, University Chicago Press, Chicago, pp. 327-340.
- 3) Elliot, F.M. & Reid, L. (1965) Some new facts about the pulmonary artery and its branching pattern. *Clin. Radiol.*, **16**, 193-198.
- 4) Horsfield, K. & Cumming, G. (1968) Morphology of the bronchial tree in man. *J. appl. Physiol.*, **24**, 373-383.
- 5) Horsfield, K., Kemp, W. & Phillips, S. (1982) An asymmetrical model of the airways in the dog lung. *J. appl. Physiol.*, **52**, 21-26.
- 6) Hyland, J.W., Smith, G.T., McGuire, L.B., Harrison, D.C., Haynes, F.W. & Dexter, L. (1963) Effect of selective embolization of various sized pulmonary arteries in dogs. *Amer. J. Physiol.*, **204**, 619-625.
- 7) Murakami, T. (1971) Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. *Arch. Histol. Jap.*, **32**, 445-454.
- 8) Nitta, S., Arakaki, Y., Okaniwa, G. & Nakada, T. (1969) Vascular unit and its significance in pulmonary circulation. *Sci. Rep. Res. Inst. Tohoku Univ., Ser. C*, **16**, 58-67.

- 9) Overholser, K.A., Bhattacharya, J. & Staub, N.C. (1982) Microvascular pressures in the isolated, perfused dog lung: Comparison between theory and measurement. *Microvasc. Res.*, **23**, 67-76.
 - 10) Pedley, T.J., Schroter, R.C. & Sudlow, M.F. (1970) The prediction of pressure drop and variation of resistance within human bronchial airways. *Resp. Physiol.*, **9**, 387-405.
 - 11) Pump, K.K. (1961) The circulation of the primary lobule of the lung. *Dis. Chest*, **39**, 614-621.
 - 12) Reid, J.A. & Heard, B.E. (1963) The capillary network of normal and emphysematous lungs studied by injections of Indian ink. *Thorax*, **18**, 201-210.
 - 13) Ross, B.B. (1957) Influence of bronchial tree structure on ventilation in the dogs lung at inferred from measurements of plastic cast. *J. appl. Physiol.*, **10**, 1-14.
 - 14) Singhal, S., Henderson, R., Horsfield, K., Harding, K. & Cumming, G. (1973) Morphometry of the human pulmonary arterial tree. *Circulat. Res.*, **33**, 190-197.
 - 15) Suwa, N., Niwa, T., Fukasawa, H. & Sasaki, Y. (1963) Estimation of intravascular blood pressure gradient by mathematical analysis of arterial cast. *Tohoku J. exp. Med.*, **79**, 168-198.
 - 16) Weibel, E.R. (1963) *Morphometry of the Human Lung*, Springer-Verlag, Berlin.
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