The Site of Elevated Vascular Resistance in Early Paraquat Lungs: A Morphometric Study of Pulmonary Arteries

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SAWAI, T., FUJIYAMA, J., TAKAHASHI, M. and TAKAHASHI, T. The Site of Elevated Vascular Resistance in Early Paraquat Lungs: A Morphometric Study of Pulmonary Arteries. Tohoku J. Exp. Med., 1994, 174 (2), 129–140 —— In interstitial lung diseases the pulmonary vascular resistance is more or less elevated. Although this usually is attributed to collapse of capillary bed by fibrosis, vasoconstriction of pulmonary arteries may be another important mechanism. Segments of pulmonary arteries supplying fibrotic areas, if subjected to hyperreactivity and overstraining of the wall, are expected to precipitate thickening of muscular media, revealing when and where vasoconstriction takes place. This was examined by morphometry of arteries in autopsy lungs from 21 patients dying in various stages of fibrosis, with five normal lungs as control. In microscopic lung slides, cross-sectioned pulmonary arteries were submitted to the measurement of $D_m$, the medial thickness, and $R$, the radius, standardizing variously shrunken vessels at a circularly stretched elastic membrane. In each case, ten to thirty arteries were measured so as to cover a wide range of $R$ from 50 to 1,000 $\mu$m. In all cases, there was a linear log-log correlation between $R$ and $D_m$. In paraquat lungs, $D_m$ began to rise as early as the 8th day, i.e., almost simultaneously with beginning deposition of fibrogenic matrix on alveolar wall, suggesting that the medial hypertrophy is the result of hypoxic vasoconstriction due to alveolar-capillary block. Medial thickening was the strongest in small arteries of acinar level. Hypoxic vasoconstriction of pulmonary arteries is likely to occur in an early stage of fibrotic lung disease and contribute to elevated vascular resistance. The intra-acinar small arteries are most liable to respond. —— paraquat lung; pulmonary artery; morphometry; media; vasoconstriction

In fibrosing lung diseases, the pulmonary vascular resistance tends to be elevated, creating pulmonary hypertension and reducing the flow of blood (Heath and Smith 1988). As yet, however, the mechanism of increased resistance is not fully understood. The impediment to blood flow has widely been attributed to fibrotic changes which cause collapse of capillaries and significant narrowing of...
the pulmonary vascular bed. Yet, there are other factors involved, each possibly playing some role in generating pulmonary hypertension.

At an autopsy of paraquat intoxication, fibrosis of the lung is a constant finding if the patient survived longer than 5 days (Bullivant 1966; Smith et al. 1974; Rebello and Mason 1978). Recently, we had an opportunity to study a series of autopsy lungs from patients dying a variety of days after ingestion of paraquat. Fibrosis progresses so far that finally most alveoli are obliterated with dense collagen, leaving only alveolar ducts and sacs. Comparative analysis of pulmonary vessels, if undertaken among these cases, will be helpful in understanding what is responsible for elevating the vascular resistance in fibrotic lungs.

In this study, we focussed our attention on the behavior of medial smooth muscles of pulmonary arteries. This is because we assume that the major resistance-elevating factor in earlier stages of interstitial lung disease may be vasoconstriction. As is well known, segments of the pulmonary artery respond by constricting when the area of lung they supply is subjected to hypoxia due to parenchymal disease or airway obstruction (Hounge 1970). When constriction is sustained in a segment of pulmonary artery, the overstrained medial coat is expected to thicken, as does that of systemic arteries in essential hypertension (Suwa and Takahashi 1971). In fact, this appears to occur in the present series of cases (Figs. 1A and B). In view of this, morphometry of arterial media was

![Fig. 1. A: Small pulmonary artery about 100μm in radius, from a patient dying 13 days after paraquat ingestion. B: Pulmonary artery of about the same dimension from a control case. Note a remarkable thickening of media in the former. Elastica-Goldner stain.](image-url)
undertaken with the intention of visualizing in what stage of disease medial hypertrophy begins and what level of pulmonary arteries are involved in it. This will provide information on the way vascular resistance is elevated with the development of fibrotic lung disease.

MATERIALS AND METHODS

The basic material comprised autopsy lungs from 21 patients dying of paraquat intoxication (Table 1). The age of the patients ranged from 24 to 68 years, with a mean of 37 years. Paraquat was ingested either accidentally or with suicidal intent. The period after ingestion of poison until death was various, ranging from 21 hr to 102 days. Besides, normal lungs from five patients were selected from autopsy material; with no apparent abnormalities being found in the lung and heart, these were added as control (Table 2). In each case, several specimens were taken from lungs which were non-infusion fixed in 10% formaldehyde; they were embedded in ordinary paraffin, sectioned 3 μm thick and stained with Elastica-Goldner stain. The total area of lung sections used for analysis was various from case to case, but amounted to about 25 cm² in average. In each case,

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Time until death</th>
<th>Dₐ(100) (μm)</th>
<th>Sₗ (%)</th>
<th>Histological features of lung</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>F</td>
<td>21 hr</td>
<td>7.0</td>
<td>1</td>
<td>Diffuse edema</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>F</td>
<td>31 hr</td>
<td>8.7</td>
<td>1</td>
<td>Edema</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>F</td>
<td>69 hr</td>
<td>6.8</td>
<td>1</td>
<td>Edema</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>M</td>
<td>2.5 days</td>
<td>8.2</td>
<td>2</td>
<td>Hemorrhage, atelectasis</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>M</td>
<td>3 days</td>
<td>9.1</td>
<td>2</td>
<td>Edema, diffuse hemorrhage</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>M</td>
<td>5 days</td>
<td>7.4</td>
<td>31</td>
<td>Edema, focal hemorrhage</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>M</td>
<td>6 days</td>
<td>9.2</td>
<td>14</td>
<td>Hemorrhage, proliferation</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>M</td>
<td>8 days</td>
<td>10.1</td>
<td>2</td>
<td>Hemorrhage, proliferation</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>M</td>
<td>9 days</td>
<td>8.1</td>
<td>19</td>
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</tr>
<tr>
<td>10</td>
<td>28</td>
<td>M</td>
<td>10 days</td>
<td>10.8</td>
<td>6</td>
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</tr>
<tr>
<td>11</td>
<td>25</td>
<td>M</td>
<td>12 days</td>
<td>13.3</td>
<td>2</td>
<td>Proliferation, fibrosis</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>M</td>
<td>13 days</td>
<td>12.0</td>
<td>6</td>
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<td>13</td>
<td>36</td>
<td>F</td>
<td>18 days</td>
<td>13.8</td>
<td>23</td>
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<td>31</td>
<td>F</td>
<td>19 days</td>
<td>13.9</td>
<td>3</td>
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</tr>
<tr>
<td>15</td>
<td>38</td>
<td>F</td>
<td>20 days</td>
<td>14.9</td>
<td>4</td>
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</tr>
<tr>
<td>16</td>
<td>27</td>
<td>M</td>
<td>23 days</td>
<td>17.4</td>
<td>27</td>
<td>Fibrosis, honeycombing</td>
</tr>
<tr>
<td>17</td>
<td>28</td>
<td>F</td>
<td>24 days</td>
<td>14.2</td>
<td>20</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>18</td>
<td>51</td>
<td>M</td>
<td>39 days</td>
<td>16.6</td>
<td>42</td>
<td>Honeycombing, fibrosis</td>
</tr>
<tr>
<td>19</td>
<td>44</td>
<td>M</td>
<td>42 days</td>
<td>13.1</td>
<td>21</td>
<td>Fibrosis, honeycombing</td>
</tr>
<tr>
<td>20</td>
<td>68</td>
<td>M</td>
<td>90 days</td>
<td>16.9</td>
<td>47</td>
<td>Honeycombing, cancer</td>
</tr>
<tr>
<td>21</td>
<td>32</td>
<td>M</td>
<td>102 days</td>
<td>18.0</td>
<td>16</td>
<td>Fibrosis, honeycombing</td>
</tr>
</tbody>
</table>
10 to 30 cross-sectioned pulmonary arteries were sampled in microscopic slides so as to cover a range from larger arteries of about 1.5 mm in diameter to small arteries of 0.1 mm or less.

The principle of morphometry is shown in Fig. 2. In an autopsy lung, arteries are usually fixed in variously constricted states, where the apparent medial thickness of an artery depends on the grade of constriction: the more constricted, seemingly the thicker the media. To correct this, morphometry was designed according to the method of Suwa and Takahashi (1971), which allows us to define the radius of an artery and its medial thickness at a standardized state. In a cross-sectioned artery, the perimeter length $L$ of the inner elastic membrane and the sectional area $S_M$ of the media was measured. Then the corrected radius $R$ and medial thickness $D_M$ were defined in a state in which the artery was stretched into a circle without changing $L$ or $S_M$ (Fig. 2). Accordingly,
\[ S_m = 2\pi RD_m \]

and
\[ L = 2\pi \left( R - \frac{D_m}{2} \right) . \]

By solving these, we obtain
\[ R = \frac{S_m}{\sqrt{L^2 + 4\pi S_m} - L} \]

and
\[ D_m = \frac{\sqrt{L^2 + 4\pi S_m} - L}{2\pi} \]

The measurements of \( L \) and \( S_m \) were performed with the aid of a desktop computer (Hewlett-Packard, model 310): A microphotograph of an artery was placed on a digitizer, where the contours of the outer and the inner elastic membranes were inputted by tracing with a cursor. The software for the measurement and calculation was written by one of us (T.T.).

Since we had the impression that not only the media but also the intimal layer of pulmonary arteries thickens with the progression of lung fibrosis, we added another morphometry to evaluate what percent of the luminal area is occupied by intima. On a cross-sectioned artery, the sectional area \( S_i \) of intima was measured; the percentage stenosis of lumina by thickened intima was calculated upon a model of concentric circles in which the intima of \( S_i \) in area was forming a circular belt of uniform thickness, adjacent to the internal elastic membrane (Fig. 2).

**Results**

Prior to showing the result of morphometry, a brief comment should be made on the way fibrosis progresses in the lungs examined. The earliest changes, found on about the 3rd day, include diffuse pulmonary edema with accumulation of proteinaceous material in air spaces. This is followed by swelling and desquamation of alveolar epithelia, and focal or diffuse alveolar hemorrhage. Fibrosis starts with deposition upon the alveolar surface of dense matrix in which mesenchymal cells begin to proliferate (Smith et al. 1974); this matrix layer, without capillaries growing inside, forms as a diffusion barrier which blocks alveolar-capillary gas exchange. It was shown in another morphometric study by one of us (Takahashi et al. 1994) that the barrier emerges on day 6 and thickens thereafter (Fig. 8). By about day 20, the alveoli are destroyed with their spaces totally filled with the matrix, in which a large amount of collagen has been deposited. In pulmonary arteries, smooth muscle cells of the media showed no degenerative changes even in the early phase of intoxication in which alveolar tissues are involved in severe toxic damages.
Fig. 3 demonstrates the result of morphometry in a 48-year-old female of the control group (Case 26), shown on bilogarithmic scale. A close linear regression is apparent. $D_M(100)$, the value of $D_M$ at 100 $\mu$m $R$, was calculated at 6.10 $\mu$m from the regression equation.

Fig. 3 demonstrates the result of morphometry in a 48-year-old female of the control group (Case 26) dying of breast carcinoma, where the measurements of pulmonary arteries are shown on log-log coordinates. There is a close linear correlation between $D_M$, the medial thickness, and $R$, the arterial radius. Similar correlation proved to exist in all cases examined, not only in the control but in the paraquat group. Since among the five control cases there was no significant difference in the regression equation, the whole data from a total of 132 arteries from these cases were pooled as in Fig. 4. Thus, we obtained a common regression

Fig. 4. Measurement data from 132 arteries from the five control cases were pooled and a common regression equation was obtained.
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\[
\log D_m = 0.628 \log R - 0.178,
\]

which served as a normal standard.

Fig. 5 is the result obtained from a patient of paraquat poisoning, a male aged 25 years and dying on the 13th day (Case 12). If in this figure one compares the measurement values with the regression line from the pooled data of control lungs, it is clear that in this case of paraquat intoxication, the media of pulmonary arteries is hypertrophic in the range of \( R \) smaller than about 500 \( \mu \)m, as shown by the elevated values of \( D_m \). The difference in the regression equation between this paraquat case and the normal standard proved to be significant \( (p < 0.05) \). Apparently, the thickening of media becomes stronger toward the terminal muscularized arterioles. This suggests that it is at the periphery of the pulmonary artery that the most active vascular response takes place. At the level of 100 \( \mu \)m in radius, the expected \( D_m \) is calculated at 12.0 \( \mu \)m from the regression line, that is about 1.5 times thicker than in the control case in which it was 7.7 \( \mu \)m.

In the same way, the degree of medial thickening was compared among the 21 cases of paraquat intoxication by calculating \( D_m(100) \), the expected value of \( D_m \) at 100 \( \mu \)m in \( R \) (Table 1). As shown in Fig. 6, the media remains unthickened until about day 7, maintaining the normal level of \( D_m(100) \); then it begins to rise and about one month after the ingestion of paraquat, a plateau is reached, where \( D_m(100) \) stays at a level of approximately 17 \( \mu \)m, more than double the normal thickness. Fig. 7 visualizes the stenosing effect of thickened intima, where the
percent luminal narrowing is plotted against the days after ingestion. Each point represents the mean percent narrowing measured in one case. Though the data are widely dispersed, there is a broad tendency for the grade of stenosis to advance over time.

In addition, we had an impression that in paraquat lungs, the medial smooth muscles emerge and develop in the terminal region of pulmonary arterioles where, normally, muscle cells are not found (Compare Figs. 4 and 5). This medial extension toward the periphery is a change commonly found in pulmonary
hypertension (Wagenvoort and Wagenvoort 1977).

**DISCUSSION**

The above morphometry of medial thickness disclosed how the pulmonary arteries behave in lungs with advancing fibrosis. Medial smooth muscles were shown to thicken over time. For the reason discussed below, we think that this is due to vasoconstriction of pulmonary arteries, which overstrains the medial muscles and at the same time elevates the resistance to pulmonary blood flow. The medial thickening cannot be explained from direct toxic effect of paraquat on smooth muscle cells, not only because there were no signs of muscle cell degeneration even in the acute phase of intoxication but because the media continued to thicken and kept an elevated thickness in the late stages in which the ingested poison had already been excreted. Hypertrophy of vascular muscles can also occur secondarily as a response to elevated blood pressure as an adaptive phenomenon to increased wall tension (Suwa and Takahashi 1971). However, muscular hypertrophy in this very early stage of paraquat intoxication excludes such a possibility. That in paraquat intoxication the pulmonary arteries undergo medial hypertrophy was described by Smith and Heath (1974), but the present morphometry contributes to better understanding of its functional significance.

There are two important aspects in the results obtained. The first striking finding is that the hypertrophy of muscular layer begins as early as the 7th day after ingestion. The grade of remodeling of alveolar structure in paraquat lung was expressed with $D_a$, the mean thickness of alveolar septa, which was determined by morphometry on 15 lungs of patients dying various days after ingestion. Thickening of alveolar septa by fibrosis is shown to begin already within one week. Reproduced from *Human Pathol.*, 25(7), p. 705, 1994.
after the ingestion of paraquat, a stage in which the amount of collagen has not yet significantly increased in lung tissue (Yamaguchi et al. 1986). Instead, one finds in this stage of disease a deposition of plasma-like matrix emerging on the alveolar surface and growing thereafter, in which macrophages and fibroblasts begin to proliferate. Fig. 8 shows the result of previous morphometry by one of us; here the mean thickness of alveolar wall $D_A$ was quantified including the superficial matrix layers, applying a geometric model and relying on principles of stereology (Takahashi et al. 1994). One can see in the figure that the alveolar wall begins to thicken on about the 6th day, and the thickening accelerates thereafter, following an exponential curve. It appears quite significant that the thickening of arterial media starts simultaneously with the beginning of this alveolar alteration or shortly thereafter.

The coincidence strongly suggests the possibility that the medial hypertrophy is the result of hypoxic arterial constriction triggered by the formation of matrix layer. The matrix forms so as to diffusely cover the alveolar surface as a growing deposit, blocking the gas exchange between the capillary blood and the alveolar air in the form of alveolar-capillary block (Amdt et al. 1970). Focal hemorrhage and edema, usually complicating the lung lesion in paraquat intoxication, may also contribute to creating local hypoxia and vasoconstriction. In an artery supplying a blocked area and overstrained in sustained hypoxic constriction, medial hypertrophy is expected to precipitate. The mechanism of the hypoxic constriction of pulmonary arteries has been related with several factors, including stimuli from the central nervous system (Stroud and Conn 1954) or from sympathetic nerves (Parsons 1972). Histamin has also been considered to mediate this type of vasoconstriction (Fishman 1976). A direct response of pulmonary arteries to hypoxia not mediated by neural or humoral agents, has also been considered (Zakheim et al. 1975). Urbanner et al. (1973) demonstrated that the media of pulmonary arteries becomes hypertrophic when animals are exposed to hypoxia, in an experiment in which rats were kept for one to two weeks in a low-oxygen atmosphere. Also it is well known that dwellers of high altitudes have pulmonary arteries with media significantly thicker than those of low-altitude dwellers, a phenomenon attributable to sustained response to hypoxia in the former (Wagenvoort and Wagenvoort 1973). So far, the increased vascular resistance in interstitial lung diseases has been attributed mainly to obliteration of alveolar capillaries and small pulmonary arteries by fibrosis, but this appears to be too simplified an explanation.

Another significant result of the present analysis is that in the paraquat lungs, the maximum hypertrophy of smooth muscles proved to occur in the terminal region of pulmonary arteries. Thus, if hypoxic vasoconstriction is responsible for this hypertrophy, one can assume the response to be the strongest at the terminal region of muscularized arteries. In the pulmonary arterial tree, the segments from 50 to 100 $\mu$m in $R$ where $D_M$ proved to maximally enlarge, correspond to
those running parallel with the respiratory bronchioles, i.e., to the small arteries
around the neck of pulmonary acini. That this level of arteriole is especially
prone to reactive constriction is suggested by other observations. The site coin-
cides with the segments where, in patients with advanced pulmonary hyperten-
sion, plexiform lesions develop (Yaginuma et al. 1990), and these have also been
regarded as a result of arterial injury caused by vasoconstriction (Wagenvoort and
Wagenvoort 1977). In an experiment where paraquat was given to hamsters,
Kokubo et al. (1984) observed that in earlier stages of intoxication there were
severe hemorrhages in the lungs but these developed as conglomerates of essen-
tially focal lesions, with a single hemorrhagic focus approximately corresponding
to an acinus. This observation led them to assume that the hemorrhage is
attributable to constriction of the acinus-supplying arteries. From every view-
point, the terminal region of pulmonary arteries is likely to be the site of the
highest vascular reactivity. It is no wonder that also in hypoxia the region is
especially susceptible to vasoconstriction, causing the most pronounced medial
hypertrophy.

With regard to this, the recent study of Ebina et al. (1990) is worth attention.
In a morphometric study of smooth muscles of airways, they showed that in
normal lungs, the comparative thickness of the muscular layer increases toward
the periphery of the airway tree. In the terminal bronchioles, muscles are
developed far more than required to antagonize the recoil pressure exerted upon
the airway wall by the elastic system of the alveolar tissues. This led to the
conclusion that the peripheral bronchioles are equipped with excessive muscles
with which to actively constrict and regulate acinar ventilation. If the site of
regulation for ventilation and that for alveolar blood flow closely co-exist at the
neck of acinus, it may be quite significant from a lung pathophysiology point of
view.

References
Perfusion ratios in patients with the clinical syndrome of alveolar-capillary block. J.
Clin. Invest., 49, 408-422.
Distribution of smooth muscles along the bronchial tree. Am. Rev. Respir. Dis., 141,
1322-1326.
Circ. Res., 38, 221-231.
the Lung, edited by W.H. Thurlbeck, Thieme Medical Publishers, Stuttgart, pp. 687-
750.
6) Hounge, A. (1970) The pulmonary vasoconstriction response to acute hypoxia:
7) Kokubo, T., Takahashi, M., Furukawa, F., Nagano, K., Hayashi, Y. & Takahashi, T.


