Right Ventricular Hypertrophy Following Chronic Pulmonary Embolism Induced by Repeated Administration of Sephadex Particles

TAKEHIRO MITSUHASHI, ROKURO ARAI* and HISASHI SAWA*

Department of Pathology and *Department of Radiology, Osaka City University Medical School, Osaka 545

Twenty-four rabbits were divided into three groups. Ten and eight of these animals were injected with Sephadex G 100 particle suspension [particle size 40 to 120 µm; 72,000 particles in 0.5 ml phosphate buffered saline (PBS)] and with Sephadex G 50-coarse particle suspension [particle size 150 to 300 µm; 1,800 particles in 0.5 ml PBS], respectively, twice a week intravenously, and the others were injected with PBS alone as control in the same way. Fifteen or 60 min before sacrifice these animals were treated with injections of 10 to 20 µCi of thallium-201 in 0.2 ml saline. In the Sephadex G 100 embolic animals, the mean weight ratio of the right ventricular free wall (RV) to the left ventricular free wall (LV) plus septum (S) was 36.7% at 4 to 6 weeks, 41.4% at 7 to 9 weeks and 33.4% at 10 to 13 weeks. The mean ratio in the total Sephadex G 100 embolic animals was 36.8±1.9 (s.d.) %, which was approximately 1.30 times as high as control values (control 27.3±0.9%, p<0.005). In the Sephadex G 50-coarse embolic animals, the weight ratio was 45.5% at 6 weeks, and the mean ratio was 35.3% at 7 to 9 weeks and 31.0% at 10 to 13 weeks. In the total Sephadex G 50-coarse embolic animals it was 34.4±2.6%, which was also significantly different from control value at p<0.05. These data suggest that repeated pulmonary embolization can produce right ventricular hypertrophy. In the distribution of thallium-201, the mean activity ratio of RV to LV+S was 16.4±3.9% in control animals. In the Sephadex G 100 embolic animals it was 31.4% at 4 to 6 weeks, 23.4% at 7 to 9 weeks and 21.1% at 10 to 13 weeks. The mean activity ratio in the total Sephadex G 100 embolic animals was 24.0±2.7%. In the Sephadex G 50-coarse embolic animals the ratio was 49.0% at 6 weeks. The mean ratio was 19.4% at 7 to 9 weeks, and 12.5% at 10 to 13 weeks. In the total Sephadex G 50 embolic animals it was 21.0±5.1%. These data suggest that the RV weight in experimental right ventricular hypertrophy induced by repeated pulmonary embolization was not always proportional to the increased uptake of the tracer in the myocardium.

Pulmonary embolism, by virtue of a reduction in the functional pulmonary vascular bed, is known to increase the right ventricular afterload and to alter its performance. On the other hand, it is well known that the degree of cardiac hypertrophy is most likely related to the long course of gradually increasing
severity of workload during the normal growth period. Therefore, repeated showers of small pulmonary emboli would be able to produce right ventricular hypertrophy (Harrison 1951).

Recently it has been reported that thallium–201 is concentrated in the myocardium, and myocardial thallium–201 imaging has been widely used to assess left ventricular perfusion in patients with ischemic heart disease. Cohen et al. (1976) and Khaja et al. (1979) demonstrated that this technique is also useful for the detection of patients with right ventricular hypertrophy or enlargement secondary to pulmonary hypertension or other causes, although the right ventricle is usually not well-visualized during thallium–201 myocardial perfusion. Not much is known, however, about the correlation of the distribution of tracer in the myocardium to right ventricular afterload induced by chronic pulmonary embolization.

The purpose of this paper is to describe a new animal model of right ventricular hypertrophy, following chronic pulmonary embolism induced by repeated injections of Sephadex particle suspension into rabbits intravenously, and to report the distribution of thallium–201 in the myocardium of the animals as mentioned above.

**MATERIALS AND METHODS**

**Animals**

White male and female rabbits weighing 1.55 to 3.1 kg were used in this study. The animals were sacrificed by exanguination. The lungs were immediately inflated to normal size via the trachea with buffered neutral formalin. In all cases the heart and lungs were removed en bloc. After fixation in buffered neutral formalin, the auricles of the heart were removed from the ventricles and the latter were separated into right and left in accordance with the method of Fulton et al. (1952). The routine histologic processing was carried out.

**Embolization**

Sephadex G 100 (particle size; 40–120 µm) and Sephadex G 50-coarse (particle size; 100–300 µm) were obtained from Pharmacia, Uppsala, Sweden. Particle sizes from 150 to 300 µm of Sephadex G 50 were obtained by straining Sephadex G 50-coarse through a stainless steel mesh (150 x 150 µm). One g of Sephadex particles was suspended in 100 ml of Dulbecco’s phosphate buffered saline (PBS). The particle numbers of Sephadex G 100 (40–120 µm) and Sephadex G 50 (150–300 µm) per 0.5 ml of the PBS suspension were about 72,000 and 1,800, respectively.

Microembolism was produced by the injection of Sephadex particle suspension into the lateral ear vein. Pilot experiments had indicated that 0.8 ml of Sephadex particle suspension corresponded approximately to an LD₅₀; hence for the study of repeated pulmonary embolization, we injected 0.4 ml each of the suspension twice weekly for the first two weeks and 0.5 ml each twice a week thereafter. Control rabbits received similar injections of PBS.

**Distribution of thallium–201 in the myocardium**

The animals received injections of 10 to 20 µCi of thallium–201 (thallium chloride) in 0.2 ml saline at 15 or 60 min before exanguination. The concentration of tracer in the heart was determined in a scintillation well counter.

In a series of 10 rabbits, weighing 1.3 to 2.7 kg, the tissue distribution of thallium was determined as a function of time. The rabbits were sacrificed by exanguination
Right Ventricular Hypertrophy

at 15, 60 or 120 min after intravenous administration of 15 μCi of thallium in 0.2 ml saline. The concentrations of tracer in the heart, lungs, liver, kidneys and spleen were determined in a scintillation well counter.

RESULTS

Body weight. Both control and embolic rabbits gained weight at a steady rate and the rate was almost the same; 0.076 kg/week in control, 0.079 kg/week in the embolic with Sephadex G 100, and 0.079 kg/week in the embolic with Sephadex G 50.

Heart weight. There was no evidence of left ventricular hypertrophy in experimental animals. The left ventricle plus septum to body weight ratios of control and embolic animals were similar; 0.16±0.01 (s.D.)% in control, 0.16±0.01% in the embolic with Sephadex G 100, and 0.15±0.004% in the embolic with Sephadex G 50. In normal animals the weights of right ventricular free wall (RV) and left ventricular free wall (LV) plus septum (S) were correlated with the body weight. Therefore, for the assessment of right ventricular hypertrophy it was more practical to compare the weight ratios, RV/(LV+S), in PBS control and experimental animals than to compare the RV weights.

The mean ratio of RV/(LV+S) in the control animals was 27.3±0.9 (s.D.)% (Table 3). In the Sephadex G 100 embolic animals it increased to 36.7% at 4 to 6 weeks and to 41.4% at 7 to 9 weeks, and then decreased to 33.4% at 10 to 13 weeks. The mean ratio in the total Sephadex G 100 embolic animals was 36.8±1.9% (Table 1), which was significantly increased from the control values (p<0.005). In the Sephadex G 50 embolic animals, the ratio was 45.5% at 6 weeks, and the mean ratio was 35.3% at 7 to 9 weeks and 31.0% at 10 to 13 weeks. The mean ratio

<table>
<thead>
<tr>
<th>Duration of embolization (weeks)</th>
<th>Number of animals</th>
<th>Initial body weight (kg)</th>
<th>Final body weight (kg)</th>
<th>RV weight (g)</th>
<th>LV+S weight (g)</th>
<th>Weight ratio; RV/(LV+S) (%)</th>
<th>Thallium activity ratio; RV/(LV+S)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>11</td>
<td>2.0</td>
<td>2.35</td>
<td>1.9</td>
<td>4.5</td>
<td>42.2</td>
<td>35.0</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>1.9</td>
<td>2.65</td>
<td>1.6</td>
<td>4.2</td>
<td>38.1</td>
<td>19.8</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>2.2</td>
<td>2.5</td>
<td>1.2</td>
<td>4.02</td>
<td>29.9</td>
<td>39.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.7</td>
<td>31.4</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>2.0</td>
<td>2.9</td>
<td>2.35</td>
<td>5.05</td>
<td>50.0</td>
<td>32.3</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>2.0</td>
<td>2.5</td>
<td>1.55</td>
<td>4.25</td>
<td>36.5</td>
<td>21.1</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>1.9</td>
<td>2.35</td>
<td>1.25</td>
<td>3.3</td>
<td>37.9</td>
<td>16.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41.4</td>
<td>23.4</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>1.9</td>
<td>2.7</td>
<td>1.4</td>
<td>4.05</td>
<td>34.6</td>
<td>17.8</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>2.2</td>
<td>2.8</td>
<td>1.46</td>
<td>4.67</td>
<td>31.3</td>
<td>26.5</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>2.2</td>
<td>3.0</td>
<td>1.62</td>
<td>4.25</td>
<td>38.1</td>
<td>22.0</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>2.7</td>
<td>3.6</td>
<td>1.49</td>
<td>5.01</td>
<td>29.7</td>
<td>17.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33.4</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Mean ratio in total Sephadex G 100 embolic animals 36.8±1.9 24.0±2.7

LV, left ventricle; RV, right ventricle; S, septum.
in the total Sephadex G 50 embolic animals was 34.4±2.6% (Table 2), which also significantly increased from the control values (p<0.05). These results led us to a conclusion that the repeated pulmonary embolization produces right ventricular hypertrophy.

**Histopathological findings.** There was little evidence of hemorrhagic infarction in both control and embolic animals. In the lungs of the embolic animals

### Table 2. Measurements of Sephadex G 50-coarse (particle size 150 to 300 μm) embolic animals

<table>
<thead>
<tr>
<th>Duration of embolization (weeks)</th>
<th>Number of animals</th>
<th>Initial body weight (kg)</th>
<th>Final body weight (kg)</th>
<th>RV weight (g)</th>
<th>LV+S weight (g)</th>
<th>Weight ratio; RV/(LV+S) (%)</th>
<th>Thallium activity ratio; RV/(LV+S) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>18</td>
<td>2.4</td>
<td>3.0</td>
<td>2.0</td>
<td>4.4</td>
<td>45.5</td>
<td>49.0</td>
</tr>
<tr>
<td>7</td>
<td>57</td>
<td>2.65</td>
<td>3.15</td>
<td>1.84</td>
<td>4.92</td>
<td>37.4</td>
<td>27.8</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>2.65</td>
<td>3.25</td>
<td>1.84</td>
<td>4.92</td>
<td>37.4</td>
<td>27.8</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>2.3</td>
<td>2.8</td>
<td>1.75</td>
<td>4.05</td>
<td>40.4</td>
<td>17.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35.3</td>
<td>19.4</td>
</tr>
<tr>
<td>10</td>
<td>59</td>
<td>2.6</td>
<td>3.0</td>
<td>1.77</td>
<td>5.12</td>
<td>34.6</td>
<td>10.4</td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>1.7</td>
<td>2.9</td>
<td>1.28</td>
<td>4.16</td>
<td>30.0</td>
<td>10.6</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>1.8</td>
<td>2.8</td>
<td>1.11</td>
<td>4.02</td>
<td>27.6</td>
<td>12.3</td>
</tr>
<tr>
<td>11</td>
<td>38</td>
<td>1.55</td>
<td>2.5</td>
<td>1.56</td>
<td>4.29</td>
<td>51.7</td>
<td>16.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31.0</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Mean ratio in total Sephadex G 50-coarse embolic animals 34.4±2.6 21.0±5.1

### Table 3. Measurements of control animals

<table>
<thead>
<tr>
<th>Duration of PBS injections (weeks)</th>
<th>Number of animals</th>
<th>Initial body weight (kg)</th>
<th>Final body weight (kg)</th>
<th>RV weight (g)</th>
<th>LV+S weight (g)</th>
<th>Weight ratio; RV/(LV+S) (%)</th>
<th>Thallium activity ratio; RV/(LV+S) (%)</th>
<th>Distribution of thallium-201 (% dose/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>24</td>
<td>2.6</td>
<td>2.9</td>
<td>1.60</td>
<td>5.23</td>
<td>30.6</td>
<td>12.4</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>3.1</td>
<td>3.3</td>
<td>1.32</td>
<td>5.42</td>
<td>24.4</td>
<td>9.6</td>
<td>2.45</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>2.7</td>
<td>3.2</td>
<td>1.39</td>
<td>4.80</td>
<td>29.0</td>
<td>9.0</td>
<td>2.4</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>2.75</td>
<td>3.35</td>
<td>1.28</td>
<td>4.84</td>
<td>26.4</td>
<td>10.5</td>
<td>3.3</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>2.0</td>
<td>2.4</td>
<td>1.60</td>
<td>3.61</td>
<td>27.7</td>
<td>29.8</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>2.2</td>
<td>2.6</td>
<td>1.28</td>
<td>4.99</td>
<td>25.7</td>
<td>27.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Mean ratio in total PBS control animals 27.3±0.9 16.4±3.9 2.6±0.2

### Table 4. Distribution data of thallium-201 (mean % dose/organ) over time

<table>
<thead>
<tr>
<th>Starting time of exanguination for sacrifice (min)</th>
<th>15 (n=3)</th>
<th>60 (n=3)</th>
<th>120 (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td>1.7</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>LV+S</td>
<td>2.9</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Liver</td>
<td>2.8</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.7</td>
<td>3.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Kidneys</td>
<td>11.8</td>
<td>10.6</td>
<td>8.7</td>
</tr>
</tbody>
</table>
there were various stages of granulation tissue around the emboli in the branches of the pulmonary arteries. Nothing in particular was found in the bronchial arteries.

**Distribution of thallium-201.** Data on the tracer distribution in normal animals are summarized in Table 4. The myocardial distribution in normal rabbits in relation to the body weight is shown in Fig. 1. These data suggested that the percent ratio of RV activity to LV activity could hardly be affected by either time or body weight factors.

The mean ratio of RV activity to LV+S activity in control animals was 16.4±3.9 (s.d.) % (Table 3). The myocardial uptake by both the ventricles of these rabbits was 2.6±0.2% dose/organ. In the Sephadex G 100 embolic animals the mean ratio went up to 31.4% at 4 to 6 weeks, and down to 23.4% at 7 to 9 weeks and to 21.1% at 10 to 13 weeks. The mean ratio in the total Sephadex G 100 embolic animals was 24.0±2.7% (Table 1). In the Sephadex G 50 embolic animals, the ratio was 49.0% at 6 weeks. The mean ratio decreased to 19.4% at 7 to 9 weeks and to 12.5% at 10 to 13 weeks. The mean ratio in the total Sephadex G 50 embolic animals was 21.0±5.1% (Table 2).

**DISCUSSION**

Experimental models of right ventricular hypertrophy previously reported are several in number. They had been induced by administration of monocrotaline pyrrole and its derivatives (Hayashi and Lalich 1967; Meyrick and Reid 1979), feeding under hypoxic circumstances (Meyrick and Reid 1978; Rabinovitch et al. 1979), banding of the main pulmonary artery (Bishop and Melsen 1976; Welham et al. 1978), and repeated pulmonary embolization with fibrin clot (Harrison 1951). On the other hand, many experimental models of pulmonary embolism have been reported. They were induced by administration of a wide variety of embolic materials, including seeds of various types (Dalen et al. 1967). Penrose tubing,
barium sulfate, Lycopodium spores, lead shot, polystyrene beads, plastic micro-
spheres (Cuénoud et al. 1978), starch granules, glass beads and blood clots. How-
ever, there was no report of pulmonary embolism induced by Sephadex particles.

The technique used in this study to investigate right ventricular hypertrophy
offers several advantages: (1) The Sephadex particles used as emboli have little
toxicity and well defined particle sizes, and are insoluble in water. (2) The various
particle sizes are available, and they are obtained easily. (3) In rabbits as experi-
mental animals, it is easy to make pulmonary embolism by repeated injections
into the lateral ear veins, resulting in the long-term right ventricular afterload. In
experimental right ventricular hypertrophy induced by chronic pulmonary embo-
lism with blood clots, it is difficult to assess the size and volume of the emboli
and the degree of resultant pulmonary arterial obstruction. Although blood clot
is native as an embolus, Hyland et al. (1963) reported that, in the assessment of
the number of emboli required for producing incipient pulmonary hypertension,
the animals given glass beads and polystyrene spheres gave the results which were
identical with animals given blood clots.

The mean weight ratio of RV to LV+S in the total Sephadex G 100 embolic
animals was 36.8±1.9%, which was 1.30 (range; 1.09–1.83) times as much as that
in the total PBS control animals. We compared the RV weights in the experimental
and the normal animals. In ten normal rabbits at our Laboratory a correlation of
the RV weight to body weight was seen (r=0.87, RV=0.21±0.40 BW) (Fig. 1).
The mean deviation of the RV weight from this line was −5% in the total
PBS control animals and +23% in the total Sephadex G 100 embolic animals.
Therefore, the mean deviation of the RV weight of the embolic animals increased
1.30 times as high as that of the PBS control. Also the mean weight ratio of RV
to LV+S in the total Sephadex G 50 embolic animals was 34.4±2.6%, which was
1.26 (range; 1.01–1.67) times as high as the control value. Experimental cardiac
hypertrophy seldom resulted in an increase in right ventricular mass of more than
100%. Most studies reported a 10–60% increase in weight. According to Har-
rison (1951) who produced right ventricular hypertrophy by repeated showers of
small pulmonary emboli consisting of blood clots, the weight ratio of the right to
the left ventricular free wall increased 1.35 (range; 1.02–1.67) times as high as
that in his control.

Hyland et al. (1963) demonstrated that a close relationship existed between
the number of vessels of a given size and the number of these vessels which had to
be occluded before incipient pulmonary hypertension occurred; and that this was
ture for all vessel sizes examined ranging from 0.17 mm to 5–6 mm in diameter.
The number of emboli (polystyrene spheres, particle size; 0.18±0.03 and 0.30±
0.05 mm) required for producing incipient pulmonary hypertension in their dogs
were 89,000±4,000 and 28,000±10,000, respectively. In our experiments
Sephadex G 50 (particle size; 0.15–0.30 mm) embolic animals at 4 to 13 weeks
were administered with 13,000 to 45,000 particles. However, it is difficult to
interpret the findings that in the Sephadex G 100 embolic animals the mean weight
Right Ventricular Hypertrophy

ratio of RV to LV+S showed a peak of increase at 7th to 9th week and then decreased and that in the Sephadex G 50 embolic animals the mean weight ratio was also decreased after the peak at 6th week.

There is general agreement that infarction as a sequel of experimental pulmonary embolism rarely occurs in healthy animals. Roach and Laufman (1955) demonstrated that infarction was induced by embolization of dogs with autologous blood clot only in areas of the lungs where atelectasis or pneumonia pre-existed. In our experiments there was little evidence of hemorrhagic infarction in either embolic or control animals.

Thallium–201 is concentrated in the myocardium under circumstances of normal flow. Thallium requires a short but finite time to clear it from the blood and entire tissue. Strauss et al. (1975) demonstrated that its concentration in the dog myocardium was 2.08% dose/organ at 10 min after administration and 1.34% at 20 min. Rabinovitch et al. (1979) also described the concentration in the heart of rats was 3% at 10 min after administration. Myocardial uptake of it in our PBS control rabbits was 2.6% dose/organ, similar to the results of Rabinovitch et al.

Rabinovitch et al. (1979) showed that, in their experimental right ventricular hypertrophy of hypoxic rats, the increase in right ventricular mass was proportional to the increase in thallium–201 uptake. However, in our experiments, increase in right ventricular weight of the embolic rabbits was not always proportional to increase in the uptake of thallium in it. We should discuss here about pulmonary embolism and its effects on coronary blood flow. Scherf and Schönbrunner (1935) had found electrocardiographic changes in 2 of 10 dogs with experimental pulmonary embolism. They attributed the changes to reflex contraction of coronary arteries with resultant decreased coronary flow. Horn et al. (1939) studied the records of 42 cases of pulmonary embolism and found structural myocardial changes in 8. They attributed the changes in cases of pulmonary embolism to myocardial ischemia induced by coronary insufficiency as a result of interplay of three probable factors: (1) shock, (2) asphyxia, and (3) exaggerated vagal reflex resulting from obstruction of the pulmonary embolism.

However, Guzman et al. (1964) showed a statistically significant increase in blood flow in all the region of the coronary circulation after pulmonary embolization (starch granules as emboli). They suggested the increase in coronary blood flow was due to a widespread coronary vasodilatation. Dalen et al. (1967) also supported this interpretation. In experimental right ventricular hypertrophy induced by pulmonary embolization, the increased right ventricular weight may not always be proportional to the increased uptake of thallium–201 in it.

References


