Pulmonary Atherosclerosis and Pulmonary Arterial Pressure in Cholesterol-Fed New Zealand White Rabbits

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ABSTRACT. The lung produces many vasoactive substances originating from its vascular endothelium and plays an important part in various pathoses. The present study was carried out to clarify pulmonary atherosclerosis and pulmonary arterial pressure, and to elucidate a part of the pulmonary pathosis in cholesterol-fed rabbits. Atherosclerosis was induced by feeding the animals a cholesterol-rich diet. When the rabbits were fed the cholesterol-enriched diets for 15 weeks, the grade of the atherosclerosis was severer than in 8W-feeding rabbits. The lesions of 8W-feeding rabbits were mainly composed of foam cells and fibrous components, whereas in 15W-feeding rabbits, the aggregation of foam cells beneath the endothelium of the vessel was infiltrating the media and severe stenose of the lumen was observed. In the entire pulmonary arterial system, the severe obstructive vascular lesions were localized and not diffused. The pulmonary arterial pressures of the rabbits increased slightly with time and the mean pressures were 11.3 ± 0.9 (control group), 11.8 ± 1.0 (8W group) and 13.7 ± 1.5 mmHg (15W group) respectively. A significant difference existed in the mean pressure between the control group and 15W-feeding group, but there were no significant differences in the systolic and diastolic pressures among the three groups. In conclusion, we could induce pulmonary atherosclerosis in rabbits by feeding them a hyper-cholesterol diet but not overt pulmonary hypertension.

KEY WORDS: cholesterol-fed rabbit, pulmonary atherosclerosis, pulmonary hypertension.

The lung produces many vasoactive substances, such as endothelin [9, 24], nitric oxide [3, 15, 19] and adrenomedullin [21, 35], that originate in the vascular endothelial cells and has receptors [17, 26]. So it is thought that the lung plays an important role in hemodynamic regulation and in various pathoses. Many researchers are performing investigations on the lung of refractory diseases such as primary pulmonary hypertension. Cholesterol-fed rabbits are widely used for research on atherosclerosis [5–7, 12, 20], fat metabolism [2, 4, 7], and vascular remodeling [11, 29, 30, 32] et cetera. Previous structural studies in cholesterol-fed rabbits observed high-grade vascular occlusive lesions in the coronary artery [4, 20, 22, 25] and aorta [2, 6, 12, 16, 22, 25, 31, 36], but little is known about the pathosis of the lung. Incidentally, perfusion pressure of the lung is lower, as is that of the heart, than that of the aorta. Based on the above observation, we hypothesized that pulmonary atherosclerosis and vascular obstructive lesion-induced pulmonary hypertensive-state could be observed in cholesterol-fed rabbits. This study was conducted to clarify the progression of pulmonary atherosclerosis and the pulmonary arterial pressure in cholesterol-fed rabbits and to investigate whether the rabbits would be suitable as a pulmonary hypertension model animal or not.

MATERIALS AND METHODS

The present study was approved by the committee of Animal Experimentation, Faculty of Medicine, Kagoshima University.

Animals: Twenty conventional male New Zealand White rabbits weighing 3 kg (2.8–3.2 kg) were purchased from Kuroda Laboratory Animal Center Co. Ltd. (Kumamoto Japan). The rabbits were caged individually in an automatically air conditioned room (temperature: 22 ± 2°C, Humidity: 55 ± 10%, Air exchange: 10 times/h, 12 hr light/dark cycle). Water was provided ad libitum. After acclimating to the animal facilities for a week, the rabbits were used in these studies.

Control group: Rabbits of this group (n=6) were fed a standard rabbit chow (Nihon Nosan Kogyo K. K., Yokohama, Japan) for 8 weeks at a daily amount of 90 g per head.

Experimental groups: Atherosclerosis was induced by feeding the animals a cholesterol-rich diet supplemented with 0.5% cholesterol and 0.25% cholic acid. The cholesterol diet was prepared by mixing it with a standard rabbit chow (Nihon Nosan Kogyo K. K., Yokohama, Japan). The rabbits of experimental group 1 (n=7) were fed the cholesterol enriched rabbit chow (90 g/head/day) for 8 weeks and the other rabbits were fed the diet (90 g/head/day) for 15 weeks (experimental group 2: n=7).

Measurements of pulmonary arterial pressure: After the feeding period of the diet, rabbits were anesthetized with medetomidine (0.1 mg/kg i.m.) plus ketamine (30 mg/kg i.m.). A 5-French-sized Swan Ganz catheter (American Edwards Laboratories, California, U.S.A.) was introduced into the pulmonary arterial trunk via the left jugular vein to measure the pulmonary arterial pressure under fluoroscopy and a catheter for monitoring the arterial blood pressure was introduced into the left carotid artery. Then blood samples were taken through the Swan Ganz catheter to measure serum cholesterol levels of the rabbits.
Histopathological analysis: After measurement both of the systemic and the pulmonary arterial pressure, the rabbit were euthanatized with sodium pentobarbital and then the lung was excised immediately. Fixation of the lung was performed by immersion in 10% buffered formalin. The pulmonary sections were embedded in paraffin by standard histological techniques, and 5-µm sections were cut. Tissue sections were stained with hematoxylin and eosin or Van Gieson stain and were examined microscopically in detail. The extent of atherosclerosis in the lung was examined in the pulmonary arteries from the trunk to the peripheral artery histopathologically. As the pulmonary arteries branch in a manner very similar to that of the bronchial tree and generally run parallel to the bronchi and bronchioles, we should be able to guess the degree of atheromatous development from the anatomical character of the bronchus. For example, the tracheal cartilage is absent in lobular bronchioles as is the peribronchial smooth muscle in terminal bronchioles.

In evaluation of the pulmonary vascular disease, the stenotic rate of the vessel was determined by estimating the percentage of intimal area involved with atherosclerotic lesions using computer program software (NIH image V.1.56) after taking a photograph of the vessel. To evaluate the severity of pulmonary vascular disease in the entire pulmonary arterial system, the index of pulmonary vascular disease (IPVD) advocated by Yamaki et al. [33] was also used (Fig. 1). In all rabbits of the three groups, fifty voluntary points of the pulmonary arteries were histologically examined and values of the index were calculated. This index varies in theory from 1.0 to 4.0. The index defined in this way made possible evaluation of PVD in the whole pulmonary arterial system of the individual cases.

Statistical analysis: All values are expressed as the mean ± SD and statistical analysis was performed by factorial ANOVA between groups (StatView-J 4.02, Abacus Concepts, Inc). Significance was assumed at p<0.05.

RESULTS

Serum cholesterol level: Following the completion of these diets, the serum cholesterol levels in the rabbits increased from approximately 29-fold (experimental group 1: 1230 ± 310 mg/dl) to 35-fold (experimental group 2: 1490 ± 410 mg/dl) the control values (42 ± 10 mg/dl) (Fig. 2). A significant difference in the serum concentration of cholesterol was seen between the control group and the other groups.

Systemic and pulmonary arterial pressure: These parameters were measured using six rabbits from each of the three groups. Concerning systemic blood pressure, there was no significant difference among the groups: the values were 110 ± 14 mmHg (systolic pressure) and 82 ± 12 mmHg (diastolic pressure) (Fig. 3-a). The pulmonary arterial pressures in rabbits of the control group were 14.8 ± 2.2 mmHg (systolic), 11.3 ± 0.9 mmHg (mean), and 9.5 ± 0.6 mmHg (diastolic) respectively. The values in the rabbits of the experimental groups were 15.0 ± 1.4, 11.8 ± 1.0, 10.3 ± 1.0

![Diagram](A) (A = point 1 : No intimal reaction)

![Diagram](B) (B = point 2 : Intimal thickening with cellular proliferation)

![Diagram](C) (C = point 3 : Fibroelastic proliferation of intima)

![Diagram](D) (D = point 4 : Partial or total destruction of media)

An index of pulmonary vascular disease (IPVD)

\[ IPVD = \frac{(1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4)}{N_1 + N_2 + N_3 + N_4} \]

Fig. 1. The index of pulmonary vascular disease (IPVD). N1, N2, N3 and N4 are the numbers of the pulmonary arterial sections bearing the respective scores. (From reference 33)
PULMONARY PATHOSIS IN CHOLESTEROL-FED RABBIT

mmHg in group 1, and 17.5 ± 1.7, 13.7 ± 1.5, 11.8 ± 1.7 mmHg in group 2, respectively (Fig. 3-b). Regarding the mean pressure of the pulmonary artery, there was a significant difference between the control group and experimental group 2, but there were no significant differences among the three groups in systolic and diastolic pressures.

**Histopathological findings of the pulmonary artery and IPVD:** Figures 4 and 5 show the histopathological findings in the pulmonary arteries of the experimental groups. **Control group:** No atheromatous lesion was recognized in the pulmonary artery of any rabbit. In normal rabbits, the wall of the pulmonary artery was thicker than that of mice and rats and was characterized by its thickness of tunica media [1]. **Experimental group 1** (Fig. 4): Atheromatous lesions were mainly observed in the elastic pulmonary artery and the large-sized muscular pulmonary arteries which run parallel with the segmental bronchi and the interlobular bronchiole. The lesions, mainly composed of foam cells, were isolated and showed intimal proliferation but the grade of atherosclerosis was slight. The index of pulmonary vascular disease (IPVD) in this group ranged from 1.06 to 1.33. **Experimental group 2** (Fig. 5): Atherosclerotic lesions were more severe and they were mainly composed of foam cells. The aggregation of foam cells beneath the endothelium of the vessel was infiltrating the media and severe stenosis of the lumen was observed. Atheromatous lesions were recognized in the medium-sized muscular pulmonary arteries running parallel with the lobular bronchioles and in peripheral pulmonary arteries partly, too. Medium to high-grade atheroscleromatous lesions were seen in rabbits of experimental group 2. The index of PVD in this group varied from 1.18 to 1.46.

**DISCUSSION**

Until now, there has been only one report by Komuro and Rosenzweig [23] on both pulmonary atherosclerosis and pulmonary hypertension in cholesterol-fed rabbits. Komuro and Rosenzweig had tried to induce pulmonary atherosclerosis and hypertension by feeding a high-fat diet to New Zealand White rabbits, but this diet alone produced neither atherosclerosis nor hypertension. Only pneumonectomy plus a high-fat diet induced persistent pulmonary hypertension and progressive pulmonary atherosclerosis consisting of discrete atheromata in large-sized pulmonary arteries. One reason why an atherosclerotic rabbit was not obtained with the diet alone was found to be the low serum cholesterol concentration in the rabbits. As the atherogenic substance in the diets was not pure cholesterol but an esterized fatty acid polymer, the serum cholesterol level of the rabbits with this diet protocol reached 500 mg/dl after 12 weeks of feeding and was considerably lower than that of our rabbits. We could make atherosclerotic rabbits by feeding a hypercholesterol diet only because the serum cholesterol levels increased to about 1,200 mg/dl at 8 weeks and 1,500 mg/dl at 15 weeks with our diet protocol. It was thought that the difference in the serum cholesterol concentration in the rabbits of the two studies (Komuro and ours) influenced the development of atherosclerosis. From the histopathological findings, high-grade atheromatous lesions (severe stenosis of the lumen, etc.) were identified in rabbits of our experimental groups but not in Komuro’s rabbits. Ruan et al. [28] reported on the immunohistological changes of the macrophage-derived foam cells in cholesterol-fed Japanese White rabbits. The atherosclerotic lesions of the rabbits were very similar to those in our rabbits but Dr. Ruan did not make mention of the pulmonary arterial pressure. Because of the severe obstructive vascular changes in cholesterol-fed rabbits with our diet protocol, we supposed that the pulmonary arterial pressure had increased and the lung had been in a hypertensive state. Though the pulmonary arterial pressure in these experimental rabbits was not so high, a significant difference was evident between experimental group 2 and the control group in the mean pulmonary arterial pressure (p=0.046). The values of pulmonary arterial pressure in these cholesterol-fed rabbits were considerably lower than those reported by Komuro and Rosenzweig [23]. However, Ohar et al. [27] and Deuchar et al. [8] reported that the mean pulmonary arterial pressures in normal rabbits were 12 ± 1 and 11.5 ± 0.8 mmHg, respectively. In pulmonary hypertension, severe thromboembolic lesions in the pulmonary arteries can cause an increase in the pulmonary arterial pressure. In this study, since we observed luminal obstructive lesions in large to medium sized pulmonary arteries but no substantial increase in the pulmonary arterial pressure, we speculate that these lesions did not develop throughout the entire lung.

From the index of pulmonary vascular disease (IPVD) too, the mean of the index indicated a value of 1.35 or less (control group: 1.02, experimental group 2: 1.18, experimental group 3: 1.35). The index values suggest that many of the vascular arterial diseases were low-grade lesions composed of slight intimal thickening with foam cells and...
fibrous tissue. An index of 2.2 can be regarded as a critical point of pulmonary vascular disease, therefore, the IPVD of rabbits in this study indicated that the grades of the pulmonary arterial lesions were mild. Also, no other characteristic signs of pulmonary hypertension, such as right ventricular hypertrophy [13, 34] or a proliferation of the media with smooth muscle cells in the muscular pulmonary arteries [10, 14, 18], were recognized. The result of the present study, however, that the pulmonary arterial pressure in rabbits of the experimental groups had slightly increased with time and that a significant difference in the mean pressure existed between the control group and experimental group 2, is notable. As a rise of the mean pulmonary arterial pressure suggests an increase of pulmonary vascular resistance, we believe the results of this study to be significant in the pulmonary pathosis of cholesterol-fed rabbits. To our knowl-
edge, the present study is the first to report changes both in atherosclerotic lesions and pressure in the pulmonary arteries of cholesterol-fed rabbits with the atherogenic process. In conclusion, we could produce pulmonary atherosclerosis but not induce a recognizable pulmonary hypertensive state in New Zealand White rabbits by feeding with a hypercholesterol diet.

Fig. 4. Experimental group 1 (8 week-feeding of the diets: a, b, c). An accumulation of foam cells and slight proliferation of fibrous components in the subendothelial layer of a pulmonary arterial trunk (a) and middle-sized muscular pulmonary arteries running parallel to the interlobular bronchiole (b, c). The luminal stenosis was slight and stenosal rates of the vessels were 34.3% (b) and 30.5% (c), respectively. (Bar: a=200 µm, b and c=500 µm)
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Fig. 5. Experimental group 2 (15 week-feeding of the diets: d, e, f, g). The lesion composed mainly of foam cells and fibrous components was infiltrating the media (d, f) and severe stenosis of the vessels was recognizable. Rates of luminal stenosis are 59.8% (e-1), 61.9% (e-2), 86.3% (f) and 90.8% (g) respectively. (Bar: d and g=200 µm, e and f=500 µm)
REFERENCES


