An Experimental Study of Atherosclerosis as a Sequela of Coronary Arteritis

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In order to examine the effect of corticosteroids on coronary atherogenesis in collagen diseases, an experimental study of serum sickness was performed. Forty-two rabbits were divided into four groups (Groups A–D). Group B, C and D rabbits received four intravenous injections of bovine serum albumin (250 mg/Kg) at 16-day intervals. Groups A, C and D rabbits were fed ad libitum cholesterol supplemented diet (1%) 16 days after the last injection. Group D rabbits received subdermal injections of prednisolone (1 mg/Kg) three times per week in the same period. After 124 days, all rabbits were sacrificed. Serum cholesterol and phospholipid increased in Group A, C and D rabbits. Group A rabbits showed intimal foam cell proliferation. Group B rabbits showed slight fibrous intimal thickening. The coronary arteries of Group C rabbits showed fatty-proliferative intimal thickening and an increase in the incidence of vascular lesions (13.9% of the coronary arteries as compared with 11.7% for Group A and 8.4% for Group B). The coronary lesions of Group D showed the same pattern as those of Group C, but the incidence of lesions was 6.0%. It was concluded that prednisolone did not augment immunologically induced atherosclerosis.

IN the past, vasculitis was the major lesion in coronary arteries of collagen disease, but recently atherosclerosis is overtaking this.¹ Bulkley and Roberts stated that long-term administration of corticosteroids was the cause of coronary atherosclerosis.² In Japan, however, neither the "Vascular Lesion of Collagen Disease Research Committee"³ or our group⁴ could find any significant relationship between atherosclerosis and the administration of corticosteroids. We postulated that the intimal change was a preceding factor of coronary atherosclerosis.⁴ Thus, we performed the following experiment concerning serum sickness^{5,6} to

examine this hypothesis.

MATERIALS AND METHODS

Forty-two male white rabbits weighing about 2 Kg were divided into four groups (Groups A–D). Group B, C and D rabbits received four 250 mg/Kg-intravenous-injections of bovine serum albumin (BSA) at 16-day intervals. Small dose desensitizing injections of BSA were given 36 hours and 12 hours prior to the 2nd, 3rd and 4th booster injections of BSA. From the 1st to 63rd day, rabbits were fed on ad commercial rabbit ration. From the 64th day, Group A, C and D rabbits were fed a libitum cholesterol supplemented diet containing 1% cholesterol by weight for 60 days prior to sacrifice. During the same period, Group D rabbits received subdermal injections of prednisolone at a dosage of 1 mg/

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TABLE I AVERAGE VALUES OF SERUM LIPIDS BEFORE AND AFTER EXPERIMENT

	Total cholesterol		HDL-cholesterol		Phospholipid	
	Before	After	Before	After	Before	After
Group A Fed cholesterol-supplemented diet	45 ± 32.6	2084 ± 695	24.0 ± 11.0	12.0 ± 0.0	76.6 ± 27.8	622 ± 214
Group B Injected with BSA	52 ± 36.1	45 ± 1.0	28.7 ± 5.0	26.7 ± 5.0	90.0 ± 25.5	76 ± 19.9
Group C Injected with BSA followed by cholesterol-supplemented diet	65 ± 31.9	2088 ± 616	21.2 ± 6.7	9.6 ± 5.4	95.4 ± 25.2	525 ± 327
Group D Injected with BSA followed by cholesterol-supplemented diet and steroid	55 ± 47.7	2597 ± 685	24.0 ± 7.2	34.0 ± 3.5	74.0 ± 16.4	422 ± 277

(mg/dl) (mean ± standard deviation)

TABLE II INCIDENCE AND HISTOLOGIC TYPES OF THE CORONARY ARTERIES

Group and regimen	No. of arteries examined per rabbit	Incidence of lesions per rabbit	Histologic types of lesions percent of coronary arteries examined*		
			Fatty	Proliferative	Fatty-proliferative
Group A Fed cholesterol-supplemented diet	538.8 ± 150.06	11.7 ± 4.0%	10.4 ± 4.04%	0.3 ± 0.24%	1.0 ± 0.42%
Group B Injected with BSA	748.7 ± 186.9	8.4 ± 3.4%	0%	8.4 ± 3.40%	0%
Group C Injected with BSA followed by cholesterol-supplemented diet	621.8 ± 192.89	13.9 ± 3.2%	2.9 ± 1.07%	1.2 ± 0.61%	9.8 ± 2.23%
Group D Injected with BSA followed by cholesterol-supplemented diet and steroid	917.3 ± 273.37	6.0 ± 2.4%	1.4 ± 1.10%	0.8 ± 0.21%	3.8 ± 1.75%

(mean ± standard deviation)

*The incidences were expressed as percent to all coronary arteries examined.

Kg three times per week.

Levels of serum lipid were measured periodically from the marginal vein.

After 124 days, rabbits were sacrificed under ether anesthesia and necropsied. Hearts were fixed by 10% neutral formalin. The base of the heart was cut sagittally into 6 to 8 blocks and the ventricles were cut horizontally into 2 to 3 blocks. Tissue specimens were embedded in paraffin, thin-sectioned by the routine method

and stained with hematoxyline and eosin, azan, elastica- van Gieson stain and PAS method. The sections were microscopically scanned under a magnification of 4 (objective) × 10 (eyepiece) using Olympus BH-2.

The arteries were categorized according to the diameter of the internal elastic layer, 30–100 microns as small size, 101–250 microns as medium size and over 250 microns as large size. The arterial lesions with intimal thickening were

TABLE III DISTRIBUTION OF CORONARY LESIONS ACCORDING TO ARTERIAL SIZE.

	Percent of lesions in each size group*		
	Large	Medium	Small
Group A Fed cholesterol-supplemented diet	4.9 ± 1.64%	18.6 ± 5.57%	11.2 ± 4.30%
Group B Injected with BSA	15.3 ± 9.2%	17.7 ± 12.92%	7.5 ± 2.96%
Group C Injected with BSA followed by cholesterol-supplemented diet	28.0 ± 12.3%	25.3 ± 5.00%	11.7 ± 3.51%
Group D Injected with BSA followed by cholesterol-supplemented diet and steroid	11.8 ± 3.36%	22.0 ± 2.51%	4.8 ± 1.85%

(mean ± standard deviation)

*The figures were expressed as percent to the number of each sized arteries.

categorized as to histologic type according to Hardin et al.⁷ i.e., fatty, proliferative or fatty-proliferative.

RESULTS

Twenty-six rabbits out of 42 died from anaphylactic shock or sepsis induced by cutaneous ulcer of injection sites. In those 26, various degrees of active vasculitis, endo- and epicarditis and myocardial fibrosis were observed. The number of rabbits surviving per group was five in Group A, three in Group B, five in Group C and three in Group D.

The levels of serum total cholesterol, HDL-cholesterol and phospholipid prior to the examination and on the 124th day are shown on Table I. The average values of serum total cholesterol and phospholipid were elevated in Groups A, C and D. HDL-cholesterol decreased in Groups A and C, and was elevated in Group D.

The number of coronary arteries examined was 301–1219, mean 675.1 ± 224.7 (standard deviation) per rabbit. More than 80% of coronary arteries were small, and less than 5% were large. The histological change in Group A was mainly a fatty change. In Group B only proliferative change was observed. In Groups C and D all three changes were observed, but fatty-proliferative change was more prominent. In all groups, the majority of lesions were found in the small sized coronary artery. In the BSA injected

groups, Groups B, C and D, the incidence of medium and large size arterial lesions tended to increase (Table II, III).

DISCUSSION

Although the number of rabbits in the experiment reported here is small, two principal findings were confirmed.

Frequent injections of BSA will induce necrotizing vasculitis and proliferative vasculitis, which will lead to intimal fibroelastosis later. Group C, which received frequent injections of large doses of BSA followed by a cholesterol-supplemented diet, had a higher incidence of proliferative intimal lesions compared with Group A that were only fed a cholesterol-supplemented diet. This finding suggests that immunologically injured intima will accumulate lipids preferentially, causing fatty-proliferative change, and that in man injured intima or fibrocellular thickened intima has a tendency to develop into mature atherosclerosis.

The grade and incidence of immunologically induced atherosclerosis was not accelerated by the administration of prednisolone. In our experiment, prednisolone was administered 16 days after the last booster injection of BSA, when the coronary lesion would have been in the healing stage. There is little possibility that prednisolone reacted as an anti-inflammatory agent to active vasculitis and reduced the incidence of

the arterial lesions. This result does not support Bulkley and Roberts' theory of the atherogenicity of steroid hormones in systemic lupus erythematosus. Therefore, fibrocellular intimal thickening is considered to be a preceding lesion of coronary atherosclerosis and 1 mg/kg of prednisolone does not have atherogenicity.

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