Production of Experimental Arteriosclerosis in Spontaneously Hypertensive Rats, SHR/NCrj

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(Received 31 October 1983/Accepted 23 March 1984)

ABSTRACT. For the successful production of atherosclerosis, 12-week-old male rats (SHR/NCrj) were fed a high-cholesterol diet for 12 weeks with or without vitamin D₂ (VD) supplement at an initial stage, and clinical and pathological observations of them were carried out. No animals showed clinically abnormal appearance throughout the experimental period. The level of final blood pressure of the rats treated with VD was higher than that of rats untreated with VD or control rats fed normal diet. The levels of total cholesterol and β -lipoprotein of rats fed the diet with or without VD were about three times higher than those of controls. Treatment-related arterial lesions were detected only in rats with VD supplement. In the aorta, intimal fibromuscular plaques and medial sclerosis with calcification were prominent, and marked and irregular proliferation of smooth muscle cells were observed in the intima and media of the mesenteric artery with accumulation of mucopolysaccharides and lipids. Panarteritis nodosa was frequent in some of the same artery.—*Key words:* arteriosclerosis, cholesterol, SHR.

Jpn. J. Vet. Sci. 46(4): 443–451, 1984

INTRODUCTION

The rat has generally been regarded as a species highly resistant to treatment for provoking atheromatous arterial lesions, and studies on successful induction of atheromatous changes in either elastic- or muscular-type arteries of normotensive rats have been relatively few [1–4, 8]. In these, the treatments were so severe that most animals were markedly depressed before ending the experiment. Therefore, using spontaneously hypertensive rats (SHRs), in which only arterial lipidosis has been reported as yet [15], we tried to induce atheromatous lesions without any deression of animals. This paper deals with clinical and pathological findings of the experiment.

MATERIALS AND METHODS

Fifty-two 12-week-old male SHRs (SHRs/

NCrj, Charles River Japan, Inc.) were divided into 3 groups, A, B and C, 16, 18 and 18 animals, respectively. The animals of group A were fed commercial pellets (MF, Oriental Yeast Co., Tokyo), and others of groups B and C were given a high-cholesterol diet, that was the same pellets supplemented with cholesterol (1%), cholic acid (0.25%) and lard (5%) for the first 8 weeks, similar to that of Enomoto and Saito [4], and with cholesterol (3%), cholic acid (1%) and lard (5%) for the following 4 weeks. Animals of group C received an additional oral administration of 40,000 IU of VD (Daiichi Pure Chemicals Co., Tokyo) in 0.2 ml of olive oil (Nakamura Chemical Co., Tokyo) per head per day for the initial 5 days. All animals were housed in a room kept at $24 \pm 1^\circ\text{C}$, with $55 \pm 10\%$ humidity, ventilated 10 times/h and lighting automatically regulated to provide a 12 h-light period and a 12 h-dark period and were

fed the diet and water *ad libitum*. Throughout the experiment, body weight and daily food consumption were measured and systolic blood pressure was recorded by a cuff-sphygmomanometer (DSR-801, Toiden Co., Tokyo) at the 0, 3rd, 7th and 11th week of experiment (*i.e.* at the age of 12, 15, 19 and 23 weeks).

At the 12th week of experiment, eight animals from each group were sacrificed by exsanguination and serum samples were collected. Immediately after exsanguination, aorta, mesenteric arteries and selected organs were perfused from the left ventricle and fixed with 10% neutral buffered formalin [16]. Eight animals from each group at the 4th week and two animals each from groups B and C at the 8th week of experiment were sacrificed by the same methods for monitoring arterial lesions. Paraffin sections were made and stained with hematoxylin and eosin (HE), Gomori's one step trichrome, resorcin fuchsin, periodic acid-Schiff (PAS), alcian blue, phosphotungstic acid hematoxylin (PTAH), and von Kossa. Some frozen sections were stained with Sudan black B and oil red O.

Serum samples were tested for total protein, albumin, Ca, total cholesterol, β -lipoprotein and high density lipoprotein. Total protein was measured by a protein refraction meter (No. 310, Atago Co., Tokyo) and others were analyzed by a spectrophotometer (LKB-7600, AMCO Inc., Tokyo) using commercial kits (Albumin B-test, Wako Pure Chemical Industries, Tokyo; Ca-set, Iatron Laboratories Inc., Tokyo; Determiner-TC "555", Kyowa Medex Co., Tokyo; BL-400 and HDL-555, Eiken Chemical Co., Tokyo).

RESULTS

No animals had any clinical signs throughout the experimental period, showing almost an equal level of final body weight (Fig. 1). There was a gradual decrease in food consumption with a lapse of time, being more

Fig. 1. Body weight. Each point represents a mean with SD range. (n=8)

clear in groups B and C (Fig. 2). The levels of blood pressure were increased in all groups, being highest in the last stage of group C (Fig. 3). The difference, however, was not statistically significant.

As shown in Table 1, total protein and albumin levels of groups B and C were slightly higher than those of group A, and total cholesterol and β -lipoprotein levels of groups B and C were about three times higher than those of group A. On the other hand, no significant difference was seen in Ca or high density lipoprotein level.

The outlines of arterial lesions are shown in Table 2. The changes were essentially the same in groups A and B. In the aorta in both groups A and B, the intima and media were thickened, with rounding of most endothelial cells, underlined by some mononuclear cells. The subendothelium was sometimes expanded with abundant amorphous and slightly eosinophilic material (Fig. 4) as well as some smooth muscle cells (SMCs) with collagen fibers. Fenestration and reduplication of the internal elastic membrane (IEM) were noticed. In the media, there was interstitial edema with accumulation of alcian blue-positive material (Fig. 5) in some cases and collagen fibers were increased in others. Such intimal alterations were always accom-

Fig. 2. Daily food consumption. Each point represents a mean. (n=8)

Fig. 3. Blood pressure. Each point represents a mean with SD range. (n=8)

Table 1. Biochemical values in blood

	Group		
	A	B	C
TP (g/dl)	6.22±0.25	8.15± 0.21	7.87± 0.15
Alb (g/dl)	3.32±0.63	5.07± 1.60	4.27± 0.59
CA (mg/dl)	8.3 ±0.3	8.9 ± 0.7	8.9 ± 0.4
TC (mg/dl)	52.6 ±9.7	173.9 ±29.9	189.3 ±22.9
βLP (mg/dl)	127.6 ±21.1	364.3 ±92.6	400.2 ±42.0
HDL (mg/dl)	17.0 ±1.4	17.2 ± 3.3	14.7 ± 3.7

Table 2. Outline of histopathological findings in arterial system

Group	Aorta				Artery					
	Thoracic		Abdominal		Mesenteric		Coronary		Intrarenal	
	Int.	Med.	Int.	Med.	Int.	Med.	Int.	Med.	Int.	Med.
A	△	△○	△	△●		△		△		△
B	△	△○	△	△●		△		△		△
C	△	▲□●	△□	▲■●	▲□●★	▲●★	△	△	△	△

Int., intima; Med., media; △, thickening; ▲, severe thickening; □, calcification; ■, severe calcification; ○, deposition of mucopolysaccharides; ●, severe deposition of mucopolysaccharides and lipids; ★, inflammatory change.

panied with these prominent medial changes, which were more apparent in the abdominal portion.

In group C, edema as well as fibromuscular plaques were produced in the intima (Fig. 6), and there was deposition of von Kossa-

positive calcium salts at the center of some plaques. Calcified IEM was thickened, disorganized and partially disrupted (Fig. 6). The media were edematous with degenerated elastic membranes, whose changes were similar to those of IEM and with calcified and

Fig. 4. Remarkable expansion of the subendothelium with abundance of slightly eosinophilic material in the thoracic aorta. Group A. HE. $\times 150$.

Fig. 5. Accumulation of alcian-blue positive material in the media of the thoracic aorta. Group A. PAS and alcian blue. $\times 150$.

Fig. 6. Intimal fibromuscular plaque and calcification of medial elastic membranes in the abdominal aorta. Group C. HE. $\times 150$.

Fig. 7. A large calcified mass and cartilaginous metaplasia (arrow head) in the media of the abdominal aorta. Group C. HE. $\times 150$.

necrotic SMCs, being stained positively with either alcian blue or PAS. Deposition of a small amount of lipid was noticed in these areas. Medial calcification sometimes building up a large mass resulted in a protrusion to the endothelium or adventitia, and occasionally cartilaginous metaplasia was found in the vicinity (Fig. 7). Calcification occurred more frequently in the abdominal aorta. No significant changes were seen in the ad-

ventitia.

In groups A and B, changes in large-sized mesenteric arteries were essentially the same as those seen in the aorta. In the media of most medium- and small-sized arteries, slight thickening with proliferation of SMCs was observed. Exceptionally, in one of group A, fibrinoid necrosis with peripheral mononuclear cell infiltration was detected in the media of a small-sized artery (Fig. 8).

Fig. 8. Medial fibrinoid necrosis with mononuclear cell infiltration in the adventitia of a mesenteric arteriole. Group A. HE. $\times 300$.

Fig. 9. Lead pipe-like thickening of mesenteric arteries (arrow heads) in formalin-fixed mesenterium. Group C.

Fig. 10. Calcification of the internal elastic membrane (arrow heads) and slight medial thickening in a small-sized mesenteric artery. Group C. HE. $\times 150$.

Fig. 11. Intimal and medial thickening with proliferation of smooth muscular cells in a mesenteric artery near the pancreas. Group C. HE. $\times 150$.

Macroscopically, in the mesenteric arteries, especially in the pancreaticoduodenal artery, in group C, segmental and lead pipe-like thickening was detected (Fig. 9). Histologically, there were irregular thickening through the whole length of these arteries, with swollen IEM and partial calcification and disruption (Fig. 10). More progressive lesions

were characterized by adventitial proliferation of fibroblasts with collagen fibers (Fig. 11), and marked and irregular thickening of the intima and media with proliferation of SMCs and increased interstitium stained positively with alcian blue, PAS and Sudan black B. SMCs frequently contained lipid droplets (Fig. 12 and 13), but no fibrin was detected.

Fibrin deposition, fibrinoid necrosis (Fig. 14) with muscular hyperplasia and fibrinoid arteritis were frequently observed in some

Fig. 12. Interstitium is positive for PAS and alcian blue in the similar artery as shown in Fig. 11. PAS and alcian blue. $\times 150$.

Fig. 13. Prominent lipid deposition in and around proliferated smooth muscle cells in the deep intima and inner media of a mesenteric artery. Group C. Sudan black B. $\times 150$.

Fig. 14. Accumulation of fibrins in the outer intima and inner media of a mesenteric artery. Group C. PTAH. $\times 300$.

Fig. 15. Typical view of panarteritis. Fibrin exudation and neutrophilic infiltration in the whole of a mesenteric artery. Group C. HE. $\times 150$.

segments of the mesenteric arteries. In severely affected portions, especially in the cranial pancreaticoduodenal artery, the arterial wall totally had leukocytic infiltration (Fig. 15).

In the heart of all groups, focal myocardial necrosis and fibrosis with infiltration of mononuclear cells in the interstitium were observed, and slight proliferation of medial

SMCs was demonstrated in the coronary arteries of the left ventricular wall. In addition, proliferation of SMCs and accumulation of amorphous material were encountered in the intima of coronary arteries of group C.

In the kidney of all cases, glomerulosclerosis and cuboidal metaplasia of Bowman's capsule were occasionally seen. In the intrarenal arteries, medial thickening with muscu-

lar hyperplasia was also observed. In group C, in addition, accumulation of a small amount of amorphous material in the subendothelium was noticed.

No abnormalities were detected in other organs of group A. In all cases of groups B and C, however, venous congestion and hypertrophy of the hypophysis and thyroid glands as well as diffuse fatty change of the liver were observed.

DISCUSSION

As no atheromatous lesions were provoked in any types of arteries in 4 week- or 8 week-treated SHRs, contrary to the experiment reported by Enomoto and Saito [4], cholesterol concentration in the diet was elevated to 3% for the last 4 weeks in our experiment.

No animals showed any clinical signs nor weight loss throughout the experimental period. Systolic blood pressure reached a rather high level in group C with most remarkable arterial lesions. The fact that treatment-related severe arterial changes were observed only in group C despite higher levels of serum total cholesterol and β -lipoprotein in both groups B and C, strongly suggested that the hypercholesterolemia and hyper- β -lipoproteinemia might not be enough to produce apparent arterial lesions in SHRs.

SHRs are known to develop intimal changes in the aorta with advanced age in response to increased endothelial permeability and persistent hypertension [5, 9]. In the present study, changes in the intima of untreated SHRs were almost similar to those already described, except for the presence of medial lesions with an increased amount of interstitial mucopolysaccharide or collagen fibers instead of muscular hypertrophy or hyperplasia described by Limas *et al.* [9]. On the other hand, control SHRs in the present experiment had medial changes in the muscular-type arteries, which were characterized by slight to moderate muscular hyperplasia us-

ually without intimal alterations. Similar findings in SHRs were also described by Hadjiisky and Peyri [5] and Limas *et al.* [9] with emphasis of local factors such as flow dynamics, arterial architecture or tissue vulnerability [13].

In SHRs treated with the high-cholesterol diet and VD, marked lesions were produced in the aorta and mesenteric arteries. The aorta showed intimal fibromuscular plaques and medial sclerosis with calcification and deposition of lipids. In combination with persistent hypertension, large calcified masses were present in the injured media of SHRs without any signs of absorption, indicating an important risk of aneurysm occurring in advanced age. Okawa *et al.* [11] reported that the mesenteric arteries were markedly thickened in the intima and media with proliferation of SMCs in normotensive rats shortly loaded with large doses of cholesterol and VD. In the present study, however, prominent lipid deposition was observed in and around the proliferated SMCs in the deep portion of thickened intima and inner media, and therefore the present mesenteric arterial lesion seems to have possibility to develop into apparent atherosclerosis in future.

These alterations of the aorta and mesenteric arteries appear to be initiated by intimal edema and SMCs necrosis resulting from hypervitaminosis D that caused an enhanced permeability of the endothelium for calcium ion [16] and they also seem to be developed into such subsequent characteristic lesions with emphasis of local factors.

Furthermore, panarteritis or periarteritis nodosa was frequently observed in some segments of the muscular hyperplastic mesenteric arteries treated with high-cholesterol and VD. For the provocation of panarteritis in treated SHRs within 6 months of age, local hypertension and increase in vulnerability due to the treatment seem to be responsible. It is of interest that a high-cholesterol diet and VD might have produced panarteritis in a

younger age than those reported as spontaneous cases in SHR_s [7, 10, 13–14].

In the present study, alterations were never detected in the cerebral arteries [9]. Fatty liver and hypertrophy in the pituitary gland and thyroid glands in the animals treated with or without VD may be closely related to the high concentration of cholesterol in the diet. Bowman's capsule transformation was observed in all animals as already described in SHR_s by Haensly *et al.* [6], and glomerulosclerosis was much less severe in comparison with that in Fawn-hooded rats with congenital hypertension [12].

ACKNOWLEDGEMENTS. The authors are greatly indebted to Professor K. Fujiwara, Department of Veterinary Pathology, the University of Tokyo for his encouragement and support to this work.

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要 約

高血圧発症ラットにおける動脈硬化症: 伊勢川直久・土井邦雄・水谷武夫¹・尾崎 明²⁾・大和田勉²⁾・光岡知足 (東京大学農学部実験動物学教室, 理化学研究所, ¹⁾動物薬理研究室, ²⁾動物試験室) ——12週齢の雄ラット (SHR/NCrj) に12週間高脂肪飼料を与え, 動脈硬化症の発現に対するビタミン D₂ 負荷の影響を臨床および病理学的に観察した。ビタミン D₂ を負荷したラットの血圧は負荷しない例より高値であった。ビタミン D₂ 負荷の有無にかかわらず, 高脂肪飼料給与ラットの血清総コレステロール値およびβ-リポ蛋白値は普通飼料給与対照ラットの約3倍であった。ビタミン D₂ 負荷ラットの大動脈では結合組織と平滑筋の増殖からなる内膜の巣状肥厚, および石灰化を伴った顕著な中膜硬化がみとめられ, 腸間膜動脈では粘液多糖類および脂質の沈着と平滑筋の増殖による内膜・中膜の顕著な不規則肥厚がみとめられ, 一部には結節性汎動脈炎が観察された。