Pathogenesis of Acute Arterial Fat Deposition in Spontaneously Hypertensive Rats

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The selectively-bred substrains of spontaneously hypertensive rats with a greater vulnerability to vascular lesions rapidly developed arterial fat deposition within 1 or 2 weeks as well as a greater hypercholesterolemic response when fed on high fat cholesterol diet including 20% of suet, 5% of cholesterol and 2% of cholic acid. The ring-like arterial fat deposition at the branches of superior mesenteric arteries and cerebrobasal arteries, which was found to be good indices for the deposition of intrarenal or coronary arteries, was not observed in normotensive rats fed on high fat cholesterol diet for 3 months, greatly delayed in SHR under antihypertensive treatment and accelerated by 1% salt loading in drinking water. The horseradish peroxidase infused intravenously 1 to 4 hours before sacrifice leaked in ring-like forms which corresponded to the fat deposit in mesenteric arteries. The incorporation of 3H-proline infused 4 hours before sacrifice was enhanced in the mesenteric arteries with the fat deposition. These results clearly indicated that hypertension was a great contributory factor to rapid arterial fat deposition, which was caused by an increased vascular permeability and enhanced the arterial collagen formation, the initiation process of arterio- or atherosclerosis.

ARTERIAL fat deposition, which might be the initiation process of atherogenesis, the basic vascular lesions for myocardial infarction or cerebral softening in man, is rarely observed in normotensive rats fed on high fat cholesterol diet even for 3 months or so. However, the substrains of spontaneously hypertensive rats (SHR)1,2 rapidly developed hypercholesterolemia as well as ring-like fat deposits in mesenteric arteries after 1 or 2 weeks of high fat cholesterol diet feeding3,4 and these findings substantiated the clear substrain difference in cholesterolemic responses2,5 and vascular vulnerability6,7 to hypercholesterolemic diets reported in SHR.

In the present studies we analyzed various influential factors on arterial fat deposition in SHR, and investigated its mechanism in relation to vascular permeability as well as its sequelae in arterial collagen synthesis.

MATERIALS AND METHODS

More than 200 rats of the various substrains (A1-sb, A3, B1, C) of SHR and Wistar-Kyoto rats (WK) at the age of 60 days were fed on high fat cholesterol diet (20% of suet, 5% of cholesterol and 2% of cholic acid) with or without 1% salt in drinking water for 7–10, 14–20, 50–70 days and over 150 days. A group of SHR was treated

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Key Words:
Spontaneously hypertensive rats
High fat cholesterol diet
Horseradish peroxidase
3H-proline incorporation into arterial collagenous protein
Atherosclerosis
Vasoconstriction and vasodilatation

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This study was supported by Science and Technology Agency of Japanese Government, the Ministry of Education, Japan Society for Promotion of Science and Japan Monopoly Cooperation.

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Fig. 1. Arterial fat deposition in SHR with and without hypertension.

Left: Mesenteric arteries with multiple sudanophilic rings in a 4-month-old SHR fed on hypercholesterolemic diet for 52 days. (Final tail blood pressure; 205mmHg. Serum cholesterol level when sacrificed; 303mg/dl).

Right: Mesenteric arteries with only a few sudanophilic rings in a 4-month-old SHR fed on hypercholesterolemic diet plus drinking water containing apresoline (80mg/L) for 52 days. (Tail blood pressure, kept under 150 mmHg during cholesterol feeding. Serum cholesterol level when sacrificed; 338mg/dl).
Fig. 2. Effect of hypercholesterolemic diet on arterial fat deposition.

- F3 obtained from cross breeding between A1 and A3
- Wistar-Kyoto

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with apresoline (80 mg/L) in drinking water during hypercholesterolemic diet feeding.

Blood samples were collected in hematocrit capillary tubes by the partial incision of the tail 1 and 2 weeks after the hypercholesterolemic diet feeding or by decapitation at the end of experiments. Serum cholesterol level was measured by Zurkowski's method. Whole branches of mesenteric arteries dissected free from fat in physiological saline and 20–30 μ thick sections of the kidney and the heart embedded in gelatin, prepared by a thermo-electro-freezing microtome (Komatsu Electron Inc.), were stained with Sudan III.

For the detection of increased vascular permeability, some rats under Nembutal anesthesia (40 mg/kg, i.p.) were perfused through an aortic canule with 200 ml of heparinized physiological saline with the perfusion pressure equal to the final systolic pressure of the rat, 2 to 4 hours after the intravenous infusion of 40 mg/kg of horseradish peroxidase (type II) in physiological saline, and then perfused with 5% glutaraldehyde in 1/15 M phosphate buffer (pH 7.4) for 20 min. Mesenteric arteries were extirpated and stained by diaminobenzidine reaction for the demonstration of the localization of peroxidase activity.

Collagenous and noncollagenous proteins of mesenteric arteries and aorta from the SHR fed on hypercholesterolemic diet for 4 weeks and the control SHR, both sacrificed by decapitation 4 hours after the injection (0.7 μCi/g, i.v.) of 3H-proline (L-proline-T(G), 63 Ci/m mole, Daiichi Pure Chemical Co., Ltd.), were extracted after Skosey et al. to determine the 3H-proline incorporation into these proteins as specific radioactivities of hydroxyproline or protein, respectively.

**RESULTS**

(1) Intensified hypercholesterolemic responses in SHR-substrains.

Serum cholesterol levels (M ± SE) at the age of 60 days before hypercholesterolemic diet feeding were significantly lower in male SHR (A1-sb: 91 ± 4 mg/dl [32], B1: 110 ± 26 [7]) compared with age-matched male normotensive WK rats (148 ± 3 [13]). The numbers of cases examined were indicated in the parentheses. This difference was also noted between female SHR (A1-sb: 98 ± 8 [41], B1: 118 ± 6 [12]) and WK (162 ± 7 [5]). However, hypercholesterolemic diet plus 1% salt in drinking water for 1 week greatly increased serum cholesterol levels in male SHR (A1-sb: 507 ± 29 [33], B1: 385 ± 44 [8]) but not so much in male WK (235 ± 9 [7]). These hypercholesterolemic responses were augmented in female SHR (A1-sb: 781 ± 30 [37], B1: 582 ± 36 [12]) and WK (491 ± 34 [5]), respectively. Such a strain difference in hypercholesterolemic response was somewhat less but still obvious when serum cholesterol levels were checked at the 2nd week after salt in drinking water was deprived at the end of the 1st week of hypercholesterolemic diet feeding; males: A1-sb: 479 ± 42 [20], B1: 451 ± 49 [8], WK: 278 ± 28 [6], females: A1-sb: 808 ± 32 [19], B1: 708 ± 33 [11], WK: 525 ± 167 [5].

(2) Acute arterial fat deposition in SHR with or without hypertension (Fig. 1, 2).

In SHR, especially, A1-sb substrain, arterial fat deposition in mesenteric arteries was noted even 1 week after hypercholesterolemic diet feeding with salt in drinking water. Ring-like sudan positive fat deposits were microscopically noted in the intima and media of mesenteric arteries and the number of the deposits increased so long as SHR were continuously fed on the hypercholesterolemic diet (Fig. 1). Such fat deposits in mesenteric arteries were never noted in normotensive WK fed on hypercholesterolemic diet even for 3 months.

Similar ring-like fat deposition was observed in main extracerebral arteries such as basilar or medial cerebral arteries, although the number of rings was less than those in mesenteric arteries. Small branches from the aorta such as spermatic or intercostal arteries also showed ring-like sudanophilia. However, aorta was free from fat deposition except at the intima near the base of aortic valves. Small sized intrarenal arteries (interlobular arteries) and arterioles (vas afference) as well as coronal arteries from the orifice to small branches showed fat deposits mainly at the intima and media.

Ring-like fat deposition was never observed in the vein, although whole the branches of portal veins were dissected and stained for sudanophilia together with mesenteric arteries.

(3) Relationship between vascular permeability and arterial fat deposition (Fig. 3).

In SHR on hypercholesterolemic diet, which was perfused and sacrificed 4 hours after the intravenous infusion of peroxidase, mesenteric arteries showed ring-like peroxidase positive bands, which corresponded well to the fat deposits revealed by the Sudan III staining following the peroxidase reaction. Peroxidase positive

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bands were noted also ring-like, although less clear in SHR without hypercholesterolemic diet feeding.

(4) Effect of hypercholesterolemic diet on $^3$H-proline incorporation into the collagenous protein of vasculatures in SHR (Fig. 4).

$^3$H-proline incorporation into the collagenous protein of mesenteric arteries with fat deposits
in SHR fed on hypercholesterolemic diet was increased compared with fat free mesenteric arteries in control SHR. However, such a significant increase in $^3$H-proline incorporation was not noted in the aorta free from fat deposits in SHR fed on hypercholesterolemic diet.

**Discussion**

Rats are commonly thought to be not suitable animals for experimental atherogenesis because their arteries, especially aorta, are resistant to fat deposition even under hypercholesterolemic diet feeding. It takes usually over 6 months of hypercholesterolemic diet feeding before arterial fat depositions develop into detectable sizes at arteries. However, SHR fed on hypercholesterolemic diet developed severe arterial fatty lesions within 6 months. It was further confirmed that some substrains of SHR developed a greater hypercholesterolemic response as well as severe arterial fat deposition within 2 months. As previously reported in abstracts, the present study confirmed that such substrains of SHR as A1s showed a significantly greater hypercholesterolemic response when fed on hypercholesterolemic diet plus 1% salt in drinking water even for 1 week than normotensive rats of Wistar-Kyoto and that they concomitantly developed obvious ring-like fatty deposits at the branches of mesenteric arteries within a week, an extremely short period compared to hitherto reported experiments. Moreover, the macroscopic observation of the sudanophilic rings of mesenteric arteries was found to be a simple method for estimating arterial fat deposits generally, because amount of fatty deposits in mesenteric arteries seemed to be correlated to the grade of the fat deposition in renal or coronary arteries. Consequently, we used the rapid fat deposition in mesenteric arteries as the indices of arterial fat deposition in the further experiments.

Our analyses on the influences on such a rapid arterial fat deposition clearly showed that hypertension was a great contributory factor to arterial fat deposition which was accelerated by an increased salt intake. As arterial fat deposits were never noted in SHR fed on ordinary stock diet, *systemic factors* for acute arterial fat deposition are obviously hypertension and hypercholesterolemia. However, the substrain differences in vascular vulnerability to hypercholesterolemic diet in SHR suggested that not only differences in the level of hypercholesterolemia or hypertension but also some other differences in the characteristics of vascular wall such as an increased vascular permeability or an alteration of physical characteristics might be involved in the rapid arterial fat deposition in SHR.

As for the local factors for acute arterial fat deposition...
deposition, the present study indicated that the
sudanophilic rings corresponded to the perox-
idase positive bands showing increased vascular
permeability. Such a ring-like peroxidase positive
reaction of vascular wall was noted in mesenteric
arteries free from fat deposits in SHR and seemed
to be intensified at the fatty lesions in SHR fed
on hypercholesterolemic diet.17 Therefore, the
localized increased in vascular permeability was
likely the cause as well as the sequela of ring-like
fat deposits. Such a localized increase in vascular
permeability was already observed to occur in
relation to vasospasm especially in vasodilated
segments of mesenteric arteries in the acute ex-
periment of Giese.18 Moreover, Ichijima observed
rather stable vasoconstriction in the mesenteric
arteries of SHR after the development of hyper-
tension under vital microscope and confirmed it
by a freeze-substitution method.19 Some addi-
tional findings of our recent observation by similar
technique also indicated that the segmental in-
crease of vascular permeability was related to
vasoconstriction and vasodilatation.17 There-
fore, in summary of these observations, ring-like
arterial fat deposition develops at the sites of
segmental increase of vascular permeability fol-
lowing such a functional and reversible alteration
of arterial wall as vasoconstriction and dilatation.
Once the smooth muscles of media are degener-
ated by fat deposition, the wall with degenerated
media is dilated by the high blood pressure, and
vascular permeability increases further at the sites
of vasodilatation, where an increased fat insula-
tion from plasma accelerates the fat deposition.
Such a kind of ‘vicious circle’ is supposed to be
the base of extremely rapid arterial fat deposition
in hypertensive rats. Although acute arterial fat
deposition itself in rats is never the synonym of
atherosclerosis and morphologically different
from fully developed atheromatous lesions, it is
supposed to be related to the initiation process of
sclerotic alteration of arteries, because the present
study proved the increased collagenous protein
synthesis in mesenteric arteries with fat deposits.
Through the degeneration or necrosis and reactive
fibrosis of arterial walls, the initially reversible
alteration of vascular wall with fat deposits
following functional vasoconstriction and dilata-
tion becomes irreversible and is supposed to
develop into irreversible organic alteration of vas-
cular wall, that is, arterio- or atherosclerosis. This
study raised the importance of functional vascular
change as the initiation mechanism for the chronic sclerotic alteration of vascular wall.

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**Discussion:**

*Chairman, Dr. OGINO (Kyoto Univ.): Now open for discussion*

Dr. NISHIMORI (Nagasak Univ.): My previous observations in the arterial system of the rat in vivo are consistent with your opinion that segmental vasoconstriction and vasodilatation are involved in the development of vascular lesions.

According to your report, fat deposition was facilitated at the sites of vascular lesion. Wouldn’t the vascular lesion itself participate in vascular permeability?

Fat readily deposits to the rabbit aorta, while it rarely does to the rat aorta as was seen in your study. Are there any differences between the rabbit and the rat in tissue structure or metabolism of the artery?

Dr. YAMORI (Kyoto Univ.): Fat deposition is related to vasoconstriction and vasodilatation. Acute deposition particularly develops at the sites of vasodilatation. It should be noted that functional changes in the vessel could trigger an irreversible change, i.e. atherosclerosis.

Cholesterol is vasculotoxic in such vessels that fat readily deposits in their media. In the vessels in whose media fat does not deposit, it is possible that collagen synthesis may be an appropriate stimulus to protect the vessel wall.

Dr. IKEDA (Tokyo Univ.): The lesions shown in large arteries outside the brain and in the cerebral infarction are considerably different from those which constitute human cerebral infarction.

In which size of arteries did you find fibrinoid necrosis?

Did you observe, in the size of vessels, the segmental constriction and dilatation which were seen in the mesenteric artery?

Permeation of Indian ink, when injected in an early stage, into the mesenteric arteries has been recognized in the rabbits with Goldblatt hypertension. This process requires an increase in the permeability of vessel wall besides “push-in” factor due to the blood pressure.

Dr. YAMORI: We have studied the permeability using Indian ink (Perikan). Peroxidase seemed to be more convenient, since it is more permeable due to the particle size and produces manifest reaction.

Dr. KOKUBU (Ehime Univ.): You have a hypercholesterolemia-prone strain of SHR. It is possible that SHR may differ from the normal control in cholesterol metabolism (hepato-intestinal cycle). General cholesterol metabolism should also be taken into account.

Dr. YAMORI: Concerning the cause of the intensified reactive hypercholesterolemia in the strain of SHR, cholesterol metabolism has not been fully studied.

Dr. IGARASHI (St. Luke Hosp.): Female SHR is more prone than the male to hypercholesterolemia when fed with hypercholesterolemic diet. How do you consider about that?

Did you see this kind of intersex difference in the fat deposition to the vessels?

Did you find any aging effect on these pathological processes?

Dr. YAMORI: An intersex difference was observed in the vascular fat deposition. The degree of the fat deposition, however, would not necessarily reflect the higher cholesterol level in the female, because the female has lower blood pressure than the male. The most evident fat deposition in the vessels was seen in pregnant rats, indicating a definite hormonal effect. We are going to analyze it.

Aging effects were also observed considerably. We have an evidence of an increased vascular permeability in an early stage of hypertension in SHR. Provided the vascular permeability is higher in immature vessels, we should consider maturation of the vessel wall in terms of a longer period.

Dr. OGINO: Orifices of the arterial branches
are also well known as predilection sites of atherosclerosis. Microtrauma or metabolic abnormalities in the arterial wall, induced by mechanical stress of turbulence of blood stream may predispose to increased infiltration or deposition of serum lipid in your study.

How do you think about these possibilities in your experiment on SHR?

Dr. YAMORI: The fat deposition in the artery of SHR in this study may be induced by more other factors than turbulence of blood stream at the orifice of arterial branch. Including the mechanism of the constant localization of constriction or dilatation of the vessel, we are studying the differences in composition of the media, innervation or receptors in the arterial wall.

Dr. OGINO: Rat is well known as an animal unsusceptible to atherosclerosis. Dr. YAMORI succeeded in experimental production of fat deposition in the small arteries of SHR. I hope his study is advanced to make the succesful production of similar atherosclerosis in the aorta or coronary artery trunk as clinically observed in the patient with myocardial infarction or ischemic heart diseases.