Experimental System for Acute Arterial Fat Deposition

Yamori's Preparation I

Yukio YAMORI,* Ryoichi HORIE,** Hajime HANDA,** and
Masayasu SATO***

Since Yamori showed that some substrains of spontaneously hypertensive rats (SHR) fed on a hypercholesterolemic diet plus 1% salt in drinking water rapidly developed ring-like arterial fat deposition, especially in mesenteric arteries in a week or so (Tr Soc Path Jap 63: 226, 1974), further studies of atherogenesis in various hypertensive rats have been continued in our laboratory.

It was concluded that any hypertensive rats (SHR and experimental-DOCA or renal infarction-hypertensive rats; EHR) when fed on a high-fat-cholesterol (HFC) diet including 20% suet, 5% cholesterol, and 2% of cholic acid, showed rapid ring-like fat depositions which were macroscopically visual in the cerebrobasal arteries by our Barium Contrasted Sudan Staining (BCSS) method (Jap Heart J 16: 332, 1975). These depositions were demonstrated in the other peripheral arteries, such as mesenteric arteries by extirpation of arteries as a whole followed by Sudan staining.

In the present study, fat deposition in the "intracerebral" arteries, morphological findings of chronic fat deposition, and light and electron microscopic findings of the cerebral arteries with fatty degeneration and increased permeability were all studied to show the usefulness of this preparation as a model for atherogenesis.

Material and Method:

(1) Five male stroke-prone SHR (SHRSP, A₁-sb) and 5 male renal infarction hypertensive rats, both at the age of 60 days, with blood pressure over 200 mmHg, were fed on a high-fat-cholesterol diet including 20% suet, 5% cholesterol, and 2% cholic acid for 2 weeks.

They were perfused by 75% barium sulfate with 10% gelatin after physiological saline preperfusion (at 38°C with final systolic pressure of rats through an aortic cannula), followed by Sudan staining of the brain fixed with 10% formalin, i.e., Barium Contrasted Sudan Staining (BCSS) method. Their lateral lenticulostriate arteries were gradually microsurgically extir-

From the Departments of Pathology,* Neurosurgery,** and Psychiatry,*** Faculty of Medicine, Kyoto University, Kyoto.

pated by repeating Sudan staining of the brain for the identification of arterial fat deposits.

(2) Around 50 SHR including A₁-sb, A₁, A₃, B₁, and B₂ were fed on a HFC diet from the age of 60 days until natural death. Their mesenteric arteries and aorta were dissected free from fat, stained by Sudan, and histologically examined.

(3) Five male SHR and renal infarction hypertensive rats at the age of 60 days, with blood pressure over 200 mmHg, were fed on a HFC diet for 2 weeks. Five min, 2 hours, and 20 hours after the injection of 250 mg/Kg horseradish peroxidase (type 2—Sigma), the rats under nembutal anesthesia (30 mg/Kg, i.p.) were fixed by the perfusion through an aortic cannula with an aldehyde fixative, a mixture of paraformaldehyde and glutaraldehyde in cacodylate buffer (pH 7.2). The basilar arteries removed from the brain were incubated at room temperature for 20 min in the diaminobenzidine solution in Tris-Buffer (pH 7.4). Small parts of arteries which colored brown macroscopically were refixed in osmium-tetroxide, dehydrated in graded alcohol and embedded in EPON, and prepared as ultrathin sections for light and electron microscopical observations.

Results:

(1) Three of 5 SHRSP and all of 5 renal infarction hypertensive rats showed ring-like fat deposition even in the lateral lenticulostriate arteries.

(2) All SHR which survived over 80 days on a HFC diet developed aortic fatty streaks; in the all cases with over 1 year survival, atheroma was clearly recognized, while it was observed in 75% of cases with over 250 day survival. Calcifications were detected in many cases with 1 year survival.

(3) Vital microscopic observation: Ring-like depositions of peroxidase reaction products were observed to correspond to the localization of fat deposition, especially to the vasodilated segments of extirpated cerebrobasal arteries.

Light microscopic findings: Two hours after injection: many brown pigments of peroxidase reaction products were noted mainly in the intercellular space of medial smooth muscle cells. Twenty hours after injection: the reaction products were noted diffusely in adventitia.

Electron microscopic findings: Endothelial changes: The most striking change was the appearance of foam cells. Sometimes the vacuoles in foam cells contained homogeneous material or myelin-like structure and sometimes they seemed to be empty. Pinocytotic vesicles were increased in number and often electron dense materials appeared in them 5 min after injection. Changes in the media: Smooth muscle cells which frequently contained various types of fat deposits were observed. Foam cells which contained

numerous large and small vacuoles in the cytoplasm also appeared in the media. Electron dense particles were often seen near the vacuoles 2 hours after injection. In addition to the atrophy of smooth muscle cells, the extracellular space of the media was widened because of an increase in elastin and swollen collagen. Twenty hours after the injection, the electron dense bodies were observed in the cytoplasm of fibrocyte which appeared outside the outer elastic lamella.

Conclusion:

In the present study, (1) intracerebral arteries such as lateral lenticulostriate arteries (which are of the most importance in relation to stroke) as well as cerebrobasal arteries showed ring-like fat deposition which was demonstrated by the BCSS method with microsurgical technique. (2) The final morphological feature in the terminal stage of vascular fat deposition showed severe atheromatous change with calcifications. (3) Some analogousness to human arterial lesions was demonstrated in the light and electron microscopic findings of fatty degeneration even in the cerebral arteries of SHR on a HFC diet. (4) Light and electron microscopic studies using peroxidase clarified that the region of fat deposition in the cerebral arteries showed increased permeability.

Now that these experimental findings have been clarified, this experimental system (Yamori's Preparation I) can be regarded as one of the best experimental systems for the studies on the initiation mechanism in the arterio- or athero-sclerosis of cerebral and other peripheral arteries.

(This study was supported by the Science and Technology Agency of Japanese Government, Ministry of Education, National Institutes of Health, U.S.A. (Grant HL 17754), Japan Society for the Promotion of Science, Japan Heart Foundation, and Japan Tobacco & Salt Public Corporation.)