Vessel Injuries by Sucrose Feed in SHRsp and SHR*

—Natural history and observation by the scanning electron microscope—

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Introduction

Many reports have been published^{1,2)} that vessel injuries or arteriosclerotic changes are developed by the administration of cholesterol or sugar rich feed. And also Yudkin et al.^{3,4)} reported that the patients with ischemic heart diseases had taken much intake of sugar. Although the mechanism of the vessel injuries by sugar administration is not clarified⁵⁾, Kanazawa et al.⁶⁾ described the differences of serum constituents and tissue constituents in lipids, lipoprotein and glycoprotein among standard oriental feed, the feed with sugar and the feed with lard.

To confirm natural history of rats and morphological changes of the vessels before and after sugar administration, the observations by light microscope and scanning electron microscope were carried out for aorta, heart, kidney and brain.

EXPERIMENT I: Natural History in SHRsp and SHR

Subjects and Method

1) 57 SHRsp, 34 SHR and 7 WKY rats fed for 2.5 ± 0.5 months after birth, were used to observe natural history. Systolic blood pressure was 180 mmHg to 200 mmHg and body weight 200 g to 300 g. But blood pressures in WKY rats were at normal levels.

2) The period of observation was one year after administration of arranged diets.

3) All rats which died were autopsied to confirm the cause of death.

4) Cerebral bleeding includes cerebral parenchymal haemorrhage, subarachnoid haemorrhage and other intracranial bleeding which caused death.

5) The subjects were classified into 4 groups by kinds of feed, namely, group 1 (standard feeding): oriental standard feed and water, group 2: oriental standard feed and 10% sucrose solution as drinking water, group 3 (lard feeding): diets which exchanged 40% of the total Calories in oriental standard feed with lard and water, and group 4 (sugar with lard feeding): group 2 + group 3.

Experimental Results

1) Incidence and number of deaths in SHRsp

Incidences (%) of death due to cerebral bleeding are shown in Fig. 1, namely, in standard feeding, 6.7% (male 0/5, female 1/10), in sugar feeding 57.1% (male 4/8, female 4/6), in lard feeding 8.3% (male 1/7, female 0/5) and in sugar feeding with lard feeding 61.5% (male 3/4, female 5/9).

No significant differences were found in the incidence of cerebral bleeding between standard feeding and lard feeding, or sugar feeding and sugar with lard feeding. But the incidence of both feeding groups including sugar in the diet were markedly higher than those of feeding groups without sugar ($p < 0.005$).

2) Incidence and number of deaths in SHR

As shown in Fig. 1, incidence of death in sugar feeding was 57.1% (male 4/7) and in lard feeding 12.5% (male 1/10, female 2/14). The incidence of

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the former was higher than that of the latter ($0.01 < p < 0.02$).

3) Incidence and number of deaths in WKY rats

Number of deaths in 7 WKY rats was 0 for the period.

EXPERIMENT II: Changes of Vessel Wall

Subjects and Method

1) Fifty four of SHRsp and 7 of WKY rats were used for this experiment.

2) The rats were divided into 4 feeding groups. Group 1: WKY rats with standard oriental feed plus 10% sucrose solution as drinking water, group 2: SHRsp with standard oriental feed, group 3: SHRsp with standard oriental feed plus 10% sucrose solution as drinking water and group 4: SHRsp with 40% of total Calorie of standard oriental feed was replaced by lard, plus 10% sugar solution as drinking water.

3) Tissue preparation: aorta, heart, brain, and

kidney of rats fed for 10–12 months were fixed by 10% formalin solution.

These tissues were stained by hematoxylin eosin (HE) for light microscope and furthermore were prepared for the scanning electron microscope. The living rats for 15 months were used for tissue preparation and the rats were killed by bleeding from the carotid artery.

Experimental Results

1) Aorta

i. Light microscopic finding

The elastic lamellae of aorta were compared among the 4 groups in $\times 200$ of light microscope. The elastic lamellae of group 1 were straight and regular, and those of group 2 were slightly tortuous, thick and irregular. Muscle layers of groups 1 and 2 seemed normal. Group 3 showed elastic lamellae with severe tortuosity and irregularity, and some lamellae were much thinner. The widths of muscle layers were not uniform. These in group 4 were thinner in all of them but the layers of lamellae

Fig. 1 Incidence of death by cerebral bleeding in 15 months from birth. Rats after 4 months from birth were fed by 3 kinds of feeds.

Fig. 2 Aortic media ($\times 2,000$) and surface ($\times 4,500$) by scanning electron microscope.

Fig. 3 Heart capillaries ($\times 10,000$) and heart muscle ($\times 10,000$) by scanning electron microscope.

Fig. 4 Brain capillaries and pre-capillaries by scanning electron microscope.

were almost straight, while muscle layers were thick.

ii. Scanning electron microscopic finding by $\times 2,000$ and $\times 4,500$

The scanning electron microscopic findings for elastic lamellae were almost the same as findings in light microscopic observation among the 4 groups. Especially the widths of elastic lamellae of group 3 and group 4 were thinner than the other two groups. Therefore muscle layers in groups 3 and group 4 seemed hypertrophied. And also the aortic surface was observed in the 4 kinds of feeding groups.

The aortic surface in group 1 held regularity of the intima. In group 2, some small craters were found but the intima was regular. In groups 3 and 4, large craters and various irregular intimas were observed (Fig. 2).

2) Coronary artery

Although small capillaries of coronary vessel were observed by scanning electron microscope, no clear differences were found among the four kinds of feeding groups. While in group 1, mitochondria by the myofibrils ranged regularly, in

group 2 the irregularity was recognized and in groups 3 and 4 the changes were more evident (Fig. 3).

3) Cerebral small artery and capillary

i. Small artery (observation by light microscope)

The small artery of groups 1 and 2 did not change so much and in groups 3 and 4 the wall of some meningeal and parenchymal small artery thickened markedly and some of which were homogeneously changed.

ii. Small artery (observation by scanning electron microscope)

The same findings were gotten with the findings of light microscope.

iii. Capillaries (observation by scanning electron microscope)

The capillaries with $3-10\mu$ diameter were observed by scanning electron microscope. As shown in Fig. 4, the walls of capillaries in group 1 are thin and regular. Those in group 2 are slightly thick and in group 3 those are thicker than groups 1 and 2. Those in group 4 showed the thickest wall among the 4 groups (Fig. 4).

Group 1

Group 2

Group 3

Group 4

Fig. 5 Renal glomerulus ($\times 10,000$) by scanning electron microscope.

4) Kidney

i. Light microscopic finding

The sclerotic changes for small artery were slight in groups 1 and 2, but in groups 3 and 4 the changes were most predominant. These vessel walls thickened and the lumens were narrow. Some of the vessel walls were necrosed and some lumens were absent.

ii. Scanning electron microscope

The foot processes of glomerular capillary epithelial cells in the four groups were observed. The foot process of group 1 is regular, long and thin like leaves of fern. The foot process of group 2 is slightly irregular and shorter in length somewhere. And in group 3 the foot process was remarkably irregular and shorter. The length of foot process in group 4 was somewhat shorter and irregular (Fig. 5).

Discussion

In rats of both SHRsp and SHR, the administration of standard oriental feed with 10% sugar solution induced more numbers of cerebral bleeding compared to those of standard oriental and lard feed. But in spite of the administration of sugar feed, the cerebral bleeding did not appear in all WKY rats. All of the SHR and SHRsp had hypertension, whereas cerebral bleeding did not always develop in both diets of standard feed and lard feed as well as sugar feed. These facts suggest that the combinations of sugar feed and high blood pressure play an important role in the development of cerebral bleeding. Yudkin et al.^{3,4)} have shown in epidemiological study that patients with ischemic heart disease had a high intake of sugar. Krichevsky et al.¹⁾ and Murakami et al.⁵⁾ have described vessel injury of the kidney and aorta due to a high sugar intake, also. These reports will support our results.

Murakami et al.⁷⁾ and Palumbo et al.⁸⁾ explained the mechanism of vessel injury with sugar administration from which PAS positive substances and sclerotic changes as well as microaneurysm, intimal thickness and calcification were found in the tissues, and they also reported the development of many kinds of volatile matter in the serum.

By sugar administration, the morphological change of the vessels were described by Murakami⁷⁾ and Yudkin et al.¹⁾ They explained mainly about light microscopic changes such as thickness and edema of epithelial cell of intima. In our experiment, almost the same changes were observed

light microscopically. As shown in Figs. 2 and 3, the elastic lamellae of aorta in SHRsp had marked irregularity by sugar administration, and slight irregularities were found by lard adding, namely, the elastic lamellae became thinner and linear by sugar feeding. The morphological changes of aortic intima were recognized in aged SHRsp with oriental standard feed but the adding of sugar made the changes markedly promoted.

Murakami⁵⁾ reported that much sugar intake induced thickness of intima, necrosis or calcification of media and breakdown of elastic fibre. And also he gave us detailed informations about the changes of various tissues after sugar feeding in rabbits. Especially the changes of coronary artery were marked. But although in our experiment which used SHRsp, the changes of mitochondria were observed in heart muscles, those of coronary capillaries were not so dominant. And the changes of capillaries in kidney and brain were more marked than those in heart. Furthermore, the changes of vessel wall in capillaries with diameter of about 3–5 μ were less than those in precapillaries with diameter of about 10 μ .

Schwartz and Ross⁹⁾ reported that sclerotic changes were observed and occurred easily in the vessels with media. Murakami⁷⁾ described thickness of basement membrane, hypertrophy of mesangium and deposition of fibrinoid substances in the kidney by sugar administration. In our experiment due to observations by scanning electron microscope, marked changes of foot process of glomerulus were found, namely, shortness and fusion of the foot process in SHRsp.

Kanazawa et al.⁶⁾ reported that there were clear differences in serum constituents such as lipids, lipoprotein and binding hexose between sugar feed group and non sugar feed group in SHR. And also Kanazawa et al.¹⁰⁾ announced in natural history of SHR, SHRsp and WKY rats that many intracranial bleedings occurred in groups with sugar feed and hypertension, but bleedings in either only sugar feed or hypertension without sugar feed were not found so much.

Therefore these changes which were explained in our experiment such as in brain, kidney heart and aorta will relate with the high incidence of intracranial bleedings. And it was considered that the changes of serum and tissue constituents which were reported by Kanazawa et al.⁶⁾, will play an important role to such morphological changes

because of close relationships between vessel injury or arteriosclerosis and lipoprotein or binding hexose. Therefore the control of sugar intake and hypertension will prevent the development of vessel injuries.

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Summary

To confirm the relationships between vessel injuries and sugar intake, the following experiments were carried out respectively.

Experiment 1

Natural history of SHRsp and SHR in the administration of various feeds.

Experiment 2

The observations of vessel changes by light microscope and scanning electron microscope in

various feeds.

The incidence of cerebral bleeding was higher in the combination of sugar feed and hypertension than those in other combination. And also the vessel changes of aorta, brain heart and kidney in the same condition were prominent.

Key words: Sugar intake, Vessel injuries, Cerebral bleeding, Hypertension, SHRsp and SHR.