Dietary Effect on Platelet Aggregation in Men with and without a Family History of Essential Hypertension

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SUMMARY Platelet aggregation induced by 5 μM adenosine 5’-diphosphate (ADP) was significantly higher in men with a family history of essential hypertension than in men without such a history when they were fed a low fat-cholesterol diet with low salt. Platelet aggregation activity was remarkably increased in both groups when the diet was changed from low salt into high salt. Platelet aggregation activity was higher in the group with a positive family history of hypertension on the low fat-cholesterol plus high salt diet than in the group without a family history under the same conditions. The activity was slightly increased in both groups when fed a high fat-cholesterol diet with low salt. There was no significant difference in the platelet aggregation between the two groups. The activity was significantly increased in both groups on the high fat-cholesterol diet after the diet was changed from low salt to high salt. Under both the low and high fat-cholesterol diets, the mean blood pressure was significantly elevated in response to excessive salt intake in the group with a family history of essential hypertension, but it was not elevated in the group without such a family history.

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KEY WORDS • salt • genetic disposition • platelet aggregation • essential hypertension • volunteer experiment

PLATELET aggregation is known to be affected by various environmental factors, such as smoking, high fat-cholesterol diet, and fish oil diet. Platelet aggregation is also known to contribute to thrombus formation, especially in diseased arteries, and to be basically involved in major cardiovascular accidents such as cerebral infarction and myocardial infarction. Epidemiological studies of cardiovascular disease have shown that the most common cardiovascular incident in Japan is stroke, while in the United States and European countries it is myocardial infarction. Such a difference stems partly from the marked difference in the life-style of Japanese and Western peoples, particularly in dietary salt intake, which is excessive in the Japanese, and in dietary fat and cholesterol intakes, which are excessive in the West.

In stroke-prone spontaneously hypertensive rats (SHRSP), which develop hypertension in 100% of the population and die mostly (over 95%) of stroke while on a regular diet containing low fat-cholesterol and high carbohydrate, the development of hypertension was accelerated and the stroke incidence markedly increased within a short period of time by excess salt intake. The development of severe hypertension was attenuated, however, when the rats were fed a high fat-cholesterol diet; nevertheless, atherosclerotic fat deposits had formed in the cerebrobasal and mesenteric arteries. These results suggest that the type of hypertensive cardiovascular disease depends on the dietary condition of these genetically hypertensive and stroke-prone animal models.

To clarify the relationship between genetic factors and environmental dietary factors in the pathogenesis of cardiovascular disease, we investigated platelet aggregation activity in men with and without a family history of essential hypertension, under strictly controlled dietary conditions. Preliminary results have been reported in part elsewhere.

Materials and Methods

Healthy male volunteers, aged 20 to 35 years, were divided into two groups: those with a family history of essential hypertension who had at least one parent with...
clinically defined essential hypertension, and those without such a family history (control group).

As shown in Figure 1, two experimental diets were given, which consisted of a low fat-cholesterol diet with a low and then a high salt intake (Experiment 1), and a high fat-cholesterol diet with a low and then high salt intake (Experiment 2).

Experiment 1

Eleven volunteers with and 11 volunteers without a family history of hypertension were placed for 4 weeks on a low fat-cholesterol diet with 6.1 or 25.4 g sodium chloride per day under strictly controlled dietary conditions. Alternating sessions of low salt and high salt intake, each for 1 week, were repeated twice to confirm the reproducibility of the results.

Experiment 2

Seven volunteers with and seven volunteers without a family history of hypertension were placed for 3 weeks on a high fat-cholesterol diet with 6.1 or 26 g sodium chloride per day under strictly controlled dietary conditions. The first 2 weeks were with a low salt intake and the last week was with a high salt intake. Neither smoking nor alcoholic drinking was allowed for 1 week before and during the whole experimental period. In each subject, blood pressure was measured three times at each sitting with a self-recording sphygmomanometer that recorded Korotkoff sounds on thermopaper (Ueda Electronic Company, Ltd., Daito-ku, Tokyo, Japan). After the measurements, the recording papers were checked for Phase I and V sounds independently by two doctors, and the mean of the three readings was determined as the blood pressure value of the day for each individual. The measurement was carried out between 1600 and 1800 hours before supper on Days 3, 5, and 6 of the week, with the subject in the sitting position after at least 20 minutes of rest. Twenty-four-hour urine samples were collected daily to check dietary sodium and potassium intakes, and blood samples were collected every other day to analyze various parameters, such as sodium, potassium, cholesterol, creatinine, triglycerides, urea nitrogen, and hematocrit.

Blood was removed from the brachial vein into a syringe containing 3.8% citrate (1:9, citrate blood) and was centrifuged to obtain platelet-rich plasma (PRP) at 1100 rpm for 10 minutes at room temperature. The pellet was further centrifuged to obtain platelet-poor plasma (PPP) at 3000 rpm for 10 minutes. The platelet number was counted with a Coulter Counter Model ZF (Miami, Florida) and was within the range of $1.5 \times 10^9$ to $6.4 \times 10^9$/mm$^3$. As the platelet aggregation activity was not affected by the number of platelets in this range, we did not adjust the number of platelets strictly at a certain level to measure the activity.

Platelet aggregation in response to various concentration of ADP was studied in PRP (180 µl) with an aggregometer (Niko Bioscience Company, Ltd., Tokyo, Japan) on Days 4 and 8 of the dietary regimen. The determination of platelet aggregation was performed within 3 hours of the blood sampling, and the activity was expressed as the maximal aggregation. Sodium and potassium in the urine and plasma were measured with a flame photometer (Nihon Bunko Flame 30, Jasco Medical Instruments, Inc., Tokyo, Japan). The plasma cholesterol level was measured by an enzymatic method. Blood pressure was expressed as mean arterial pressure. All data were means ± se and statistically analyzed by Student's $t$ test except for specific descriptions.

Results

As shown in Figure 1, the basic diet in Experiment 1 was a low fat-cholesterol diet typical of the Japanese people, while the basic diet of Experiment 2 was a high fat-cholesterol diet typical of Westerners. With the low fat-cholesterol diet in Experiment 1, plasma cholesterol levels were gradually decreased (from 190 ± 6 to 149 ± 6 mg/dl). However, the levels were increased on the high fat-cholesterol diet of Experiment 2 (from 205 ± 6 to 220 ± 8 mg/dl). There was no significant difference in the plasma cholesterol level of the two groups with positive or negative family histories.

Initial mean blood pressure levels were normal in both the positive and control groups: 90 ± 2 and 85 ± 2 mm Hg in Experiment 1, and 86 ± 3 and 79 ± 2 mm Hg in Experiment 2, respectively. Significantly higher blood pressure levels were noted in the positive family
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FIGURE 2. Changes in mean arterial blood pressure (MAP) under low fat-cholesterol (LFC) dietary conditions in men with or without a genetic disposition to essential hypertension (predisposed or non-predisposed group).

FIGURE 3. Changes in mean arterial blood pressure (MAP) under a high fat-cholesterol (HFC) diet in men with or without genetic disposition to essential hypertension (predisposed or non-predisposed group).

history group after the low salt diet in both Experiments 1 and 2 (Figures 2 and 3). Each point in the graphs was the mean blood pressure value for 2 days (the 5th and 6th days) of each week. With both dietary conditions of Experiments 1 and 2, the blood pressure was significantly increased in the positive family history group in response to the high salt intake. However, under high salt intake, there was no blood pressure rise in the group without a family hypertension history.

Sodium excretion in the urine gradually decreased and reached the lowest level on the 3rd day of low salt intake. On the contrary, the excretion was gradually increased and reached a constant amount on the 3rd day of the high salt intake (data not shown). There was no significant difference in sodium excretion between the positive and control groups. Urinary potassium excretion was constant in both groups during low salt and high salt intakes.

Plasma renin activity and aldosterone levels were markedly decreased after a change from low to high salt in both groups. There was no significant difference in plasma renin activity nor in aldosterone level between both groups (data not shown).

Platelet aggregation in Experiment 1 (Figure 4) was dose-dependently increased by various amounts of ADP (1–10 μM). On the last day of the low salt diet, the platelet aggregation was observed to be greater in the group with the positive family history. There was a significant difference in the platelet aggregation induced by 5 μM ADP between the two groups. It was remarkably enhanced in both groups during high salt diet feeding, but was more pronounced in the positive family history group. There was also a significant difference in the platelet aggregation induced by 2 μM ADP between the two groups.

Figure 5 shows platelet aggregation in Experiment 2. As there was no significant difference between the two groups, each point was the mean of all subjects. The value for the low salt diet was the mean of three data points on the 8th, 11th, and 15th days. Platelet aggregation was not decreased in both groups on the high fat-cholesterol diet with low salt; rather, the aggregation induced by 2 μM ADP was increased even under this low salt condition. After a change from low to high salt intake, platelet aggregation was increased transiently; the aggregation induced by 1 or 2 μM ADP was significantly increased on the 4th day of high salt intake, but on the last day it tended to return to the value during the low salt intake.
Discussion

Epidemiological studies have reported that excess sodium intake is a risk factor for hypertension and stroke, and that excess cholesterol intake is a risk factor for hypertension-related cardiovascular disease such as myocardial infarction or stroke. Although accelerated platelet aggregation may be involved in cardiovascular accidents, there is no experimental study about the effect of genetic disposition toward hypertension or of environmental dietary factors on the activity of platelet aggregation in humans.

The activity of platelet aggregation was significantly high in the group with a family history of essential hypertension under the dietary conditions of low fat-cholesterol with low salt. After excess sodium intake, the platelet aggregation activity was remarkably accelerated in both groups, especially in the group with a family history of essential hypertension. The blood pressure was also significantly elevated in the group with the positive family history. These results suggest that platelet aggregation is partly related to genetic disposition toward hypertension and is influenced by excessive sodium intake under low fat-cholesterol dietary conditions.

On the other hand, platelet aggregation activity was not decreased but was rather increased in both groups under the dietary conditions of high fat-cholesterol with low salt for 2 weeks. Excessive sodium intake accelerated the activity of platelet aggregation, although temporarily, even under the high fat-cholesterol dietary regimen. The blood pressure in the positive family history group was significantly higher than in the control group under the high fat-cholesterol plus low salt diet, and it was significantly elevated in the former group in response to excessive salt intake on the high fat-cholesterol diet.

These results indicate that the effect of excessive sodium intake on platelet aggregation is different under low and high fat-cholesterol dietary conditions, and that platelet aggregation is fully activated by a high fat-cholesterol diet even with low salt intake. However, the blood pressure response to excessive salt intake did not differ under the low and high fat-cholesterol dietary conditions. We can conclude that reduction of sodium intake is beneficial in controlling the blood pressure under both low and high fat-cholesterol dietary conditions, but that it is only effective for attenuating platelet aggregation under a low fat-cholesterol diet.

The mechanism that determines the effect of sodium on platelet aggregation and on platelet acceleration in individuals with a predisposition toward hypertension is not yet known. Previously we and other researchers reported that membrane abnormalities related to sodium ion permeability were detected in the erythro-
cytes from genetically hypertensive models (SHR, SHRSP) and essentially hypertensive humans. Such membrane defects may result in intracellular sodium accumulation not only in erythrocytes but also in vascular smooth muscle cells, especially under the condition of excessive sodium intake, through the inhibition of Na⁺,K⁺-ATPase by a possible action of a natriuretic hormone; this then becomes one cause of hypertension. On the other hand, platelet aggregation is also known to be activated by ouabain treatment, which inhibits Na⁺,K⁺-ATPase activity and results in the accumulation of sodium in platelets. Markedly accelerated platelet aggregation by excess salt intake, especially in the positive family history group, might result from the accumulation of sodium in the platelets due to genetic membrane abnormalities related to the disposition to hypertension.

It has been reported that a thromboxane-forming system plays an important role in platelet aggregation. Activation of platelet aggregation under high fat-cholesterol dietary conditions may therefore be due to the augmented formation of thromboxane A₂, which has been observed under such experimental conditions. The results of the present studies led us to conclude that dietary sodium restriction is generally important for preventing hypertension and may also be beneficial for reducing thrombotic cardiovascular disease, especially in populations such as the Japanese whose fat and cholesterol intakes are lower.

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