The Effect of Vitamin E on Platelet Kinetics of Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

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Summary The platelet survival time was shortened in stroke-prone spontaneously hypertensive rats (SHRSP) at 10 weeks of age on feeding the regular diet but it was normalized on administering the vitamin E-supplemented diet. Platelet survival time was normal in stroke-resistant spontaneously hypertensive rats (SHRSR) at 10 weeks of age on feeding the regular diet but it was shortened when supplying the vitamin E-free diet. The maximal uptake of ⁷⁵Se-selenomethionine by platelets was increased in SHRSP at 10 weeks of age on feeding the regular diet. It was further increased in SHRSP on feeding the vitamin E-free diet. On the other hand, the increased maximal uptake of ⁷⁵Se-selenomethionine by platelets was normalized in SHRSP on feeding both the vitamin E-supplemented diet and the vitamin E-supplemented diet after administering the vitamin E-free diet. Therefore, we concluded that the deficiency of vitamin E brought about the shortening of platelet survival time and enhanced platelet production.

Key Words vitamin E, SHRSP, SHRSR, ⁷⁵Se-selenomethionine, platelet kinetics

SHRSP is the substrain of SHR obtained by Okamoto *et al.* in 1974 and is widely used as the best animal model for the human stroke (1). It has been reported that the serum lipid peroxide is increased with aging in SHRSP (2). Serum lipid peroxide which is induced by vitamin E deficiency impairs vascular endothelial cells and participates in increasing platelet aggregation, atherosclerosis and apoplexy. Hence, it is suspected that platelet consumption is increased at the damaged endothelial cells by adherence and aggregation of platelets in vitamin E deficiency. However, there are few reports extant on the effect of vitamin E on platelet economy related to interaction between platelet function and vessel wall alteration *in vivo*. Platelet kinetic study is introduced primarily to detect the early phase of

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microangiospasm and thrombus formation caused by aggregation of platelets and to predict the occurrence of thrombotic cerebral hemorrhage in stroke-prone animals (3). The present study was carried out to investigate the effect of vitamin E on the platelet kinetics of SHRSP using ^{75}Se -selenomethionine (^{75}SeM)-labeled platelets (4).

MATERIALS AND METHODS

Sex-matched SHRSP ($A_{1\text{-sb}}$ substrain) and SHRSR (B_1 substrain) were used. The mean body weight was 130 g in SHRSP and 143 g in SHRSR at 10 weeks of age, and 300 g in SHRSP at 9–12 months of age. The mean systolic blood pressure was 163 mmHg at 10 weeks of age and 206 mmHg at 9–12 months of age in SHRSP.

1. *The effect of vitamin E on platelet kinetics.* SHRSP and SHRSR were divided into 4 groups. The rats of the first group were fed on the regular diet as controls. The rats of the second group were fed on the vitamin E-free diet (Oriental Yeasts Co.: 38% cornstarch, 25% of vitamin E-free casein, 10% of α -starch, 8% of powdered filter-paper, 6% of lard, 6% of inorganic salts, 5% of granular sugar, 2% of mixed vitamins without vitamin E; the vitamin E content of the diet was less than 0.002 mg per g) mixed with 5 percent fish oil as polyunsaturated fatty acids from 6 to 10 weeks of age. The rats of the third group were fed on the vitamin E-supplemented diet (*dl*- α -tocopherol nicotinate was added at the rate of 234 mg per kg to vitamin E-free diet) from 7 to 10 weeks of age and for 4 weeks at 9–12 months of age. The rats of the last group were fed on the vitamin E-free diet from 6 to 10 weeks of age and the vitamin E-supplemented diet from 11 to 14 weeks of age. Adhesion of platelets to glass beads was measured by modifying the method of Salzman (5). Briefly, one-half ml of blood was drawn from the abdominal aorta through a glass bead column in exactly 10 sec. The adhesion ratio of platelets was calculated from that of platelet counts before and after passage through the glass bead column. The platelet kinetics were measured using ^{75}SeM -labeled platelets. ^{75}SeM , specific activity about 54 mCi/mg, was obtained from the CEA-IRE-SOLIN. It was injected through the tail vein in a dose of 2 μCi per 100 g. Rats were killed and samples were obtained daily after infusion up to the 6th day. Four-ml blood samples were obtained from the abdominal aorta with 1.5% EDTA. The platelets were separated at 4°C by centrifugation at 1,000 rpm for 20 min followed by recentrifugation of their platelet-rich plasma at 2,400 rpm for 20 min. After these platelets had been washed once with saline, the radioactivity of the gamma emission from samples was counted by an Automatic Gamma Scintillation System Model 4224 (Nuclear Chicago). The percent incorporation of ^{75}SeM into total platelets was calculated from the circulating total blood volume (6). The platelet survival time was calculated in days between the interval from the 50% point of the ascending, to the 50% point of the descending slope of the ^{75}Se -labeled platelet reappearance curve.

2. *The effect of erythrocyte transfusion on platelet production.* SHRSP at 10 weeks of age fed on a vitamin E-free diet from 6 weeks of age were used. These rats

were divided into two groups. One was the donor group and the other the recipient group. Two-ml packed erythrocytes obtained from the donor group rats were transfused through the femoral vein into the recipient group rats and on the next day $2 \mu\text{Ci}$ of ^{75}SeM per 100 g body weight was injected through the tail vein. After 3 days the percent incorporation of ^{75}SeM into total platelets and total erythrocytes was calculated.

The value of serum vitamin E was measured by the fluorometric method (7).

RESULTS

Physical and hematological changes

No significant difference was seen in body weight in these groups of rats. Among the hematological effects of vitamin E deficiency observed in SHR, mild anemia developed in the vitamin E-free diet group (Ht 37–45%, mean 40.6%), which was eliminated on subsequent feeding of the vitamin E-supplemented diet (Ht 40–50%, mean 44.1%). The platelet counts did not change among these three groups (Fig. 1).

Platelet adhesion

A decreasing tendency of platelet adhesion was observed in the vitamin E-

Fig. 1. The effect of vitamin E on the value of hematocrit and platelet counts. Mild anemia is seen in rats of the group fed on the vitamin E-free diet from 6 to 10 weeks of age, but it is normal in rats of the group fed on the vitamin E-free diet, and subsequently, the vitamin E-supplemented diet. The platelet counts did not vary among these three groups. The bars show the mean values.

Fig. 2. The effect of vitamin E on platelet adhesion. The adhesion ratio of platelets is calculated from that of platelet counts before (open circles) and after (closed circles) glass bead column passage as shown on the left side of this figure. The mean value of this ratio is 30% in the rats of the group fed on the regular diet, 38% in the rats of the group fed on the vitamin E-free diet and 17% in the rats of the group fed on the vitamin E-free diet, and subsequently, the vitamin E-supplemented diet, as shown on the right side of this figure.

supplemented diet group after feeding the vitamin E-free diet (Fig. 2).

The vitamin E value in serum

The value of serum vitamin E was markedly decreased in the vitamin E-free diet group (mean 0.14 mg/100 ml), but it was subsequently normalized by feeding the vitamin E-supplemented diet (0.58 mg/100 ml). On the other hand, there was no significant difference between SHRSP and SHRSR in regular diet groups (0.55 mg/100 ml, 0.59 mg/100 ml, respectively) (Fig. 3).

Effect of vitamin E on platelet survival time

Platelet survival time of SHRSP was 3.7 days and that of SHRSR 4.8 days at 9–10 weeks of age in both groups fed on the regular diet (Fig. 4). The value was 3.0 days in the vitamin E-free diet group of SHRSR (Fig. 5) and 4.7 days in the vitamin

Fig. 3. The value of serum vitamin E in SHRSP and SHRSR fed on the vitamin E-free diet and the vitamin E-free diet, and subsequently, the vitamin E-supplemented diet. The value of serum vitamin E is markedly decreased in SHRSR fed on the vitamin E-free diet. There is no significant difference between SHRSP (open circles) and SHRSR (closed circles) fed on the regular diet.

Fig. 4. Kinetic study on ^{75}Se -labeled platelets in SHRSP and SHRSR fed on the regular diet. The platelet survival time of SHRSP ($A_{1-sb}F_{38}$, female) at 9–10 weeks of age fed on the regular diet is 3.7 days (open circles). The platelet survival time of SHRSR (B_1F_{37} , female) at 9–10 weeks of age fed on the regular diet is 4.8 days (closed circles). The curves show the reappearance of the ^{75}Se -labeled platelets in peripheral blood. Each circle represents the radioactivity (cpm) per 10^9 platelets. The value of platelet survival time is calculated from the ^{75}Se -labeled platelets reappearance curve as described in "MATERIALS AND METHODS."

Fig. 5. Kinetic study of ^{75}Se -labeled platelets in SHRSR fed on the vitamin E-free diet. The platelet survival time of SHRSR at 9–10 weeks of age fed on the vitamin E-free diet is 3.0 days (closed circles). The platelet survival time of SHRSR fed on the regular diet as control is 4.8 days (open circles).

Fig. 6. Kinetic study of ^{75}Se -labeled platelets in SHRSP fed on the vitamin E-supplemented diet. The platelet survival time of SHRSP at 9–10 weeks of age fed on the vitamin E-supplemented diet is 4.7 days (closed circles). The platelet survival time of SHRSP fed on the regular diet as control is 3.7 days (open circles).

E-supplemented diet group of SHRSP (Fig. 6) at 9–10 weeks of age. The maximal uptake of ^{75}SeM by total platelets was observed on the 3rd day in every group.

Effect of vitamin E on platelet production

The maximal uptake of ^{75}SeM by total platelets was increased compared with

Table 1. The comparison of maximal uptake of ^{75}Se -selenomethionine by platelets.
10 W SHRSP A_{1-sb}F₃₉ Female

	Percent incorporation into total platelets (mean $\pm$ SD)	Statistical significance
Regular diet	0.119 $\pm$ 0.010 ($n=8$)	
Vit. E-free diet	0.192 $\pm$ 0.030 ($n=8$)	$p < 0.01^*$
Vit. E-free subsequently Vit. E-supplemented diet	0.063 $\pm$ 0.015 ($n=8$)	$p < 0.01^*$
Vit. E-supplemented diet	0.074 $\pm$ 0.013 ($n=8$)	$p < 0.01^*$
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	10 W SHRSR	B ₁ F ₃₈ Female
	Percent incorporation into total platelets (mean $\pm$ SD)	Statistical significance
Regular diet	0.071 $\pm$ 0.016 ($n=10$)	
Vit. E-free diet	0.119 $\pm$ 0.018 ($n=8$)	$p < 0.01^*$
Vit. E-supplemented diet	0.072 $\pm$ 0.023 ($n=8$)	NS
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	9-12 M SHRSP	A _{1-sb} F ₃₇ Male
	Percent incorporation into total platelets (mean $\pm$ SD)	Statistical significance
Regular diet	0.174 $\pm$ 0.023 ($n=6$)	
Vit. E-supplemented diet	0.135 $\pm$ 0.027 ($n=6$)	$p < 0.05^*$

* Significantly different from the value of regular diet (t -test).

that of the regular diet groups in both vitamin E-free diet groups of SHRSP (0.192 $\pm$ 0.030 vs. 0.119 $\pm$ 0.010, $p < 0.01$) and SHRSR (0.119 $\pm$ 0.018 vs. 0.071 $\pm$ 0.016, $p < 0.01$). It was decreased compared with that of the regular diet group in both the vitamin E-supplemented diet group (0.074 $\pm$ 0.013 vs. 0.119 $\pm$ 0.010, $p < 0.01$) and the vitamin E-free diet group subsequently fed on the vitamin E-supplemented diet (0.063 $\pm$ 0.015 vs. 0.119 $\pm$ 0.010, $p < 0.01$) in SHRSP. This decrease of maximal uptake of ^{75}SeM by total platelets compared with that of the regular diet group was also seen in the vitamin E-supplemented diet group of SHRSP at 9-12 months of age (0.135 $\pm$ 0.027 vs. 0.174 $\pm$ 0.023, $p < 0.05$). On the other hand, there was no significant difference between the vitamin E-supplemented diet group and the regular diet group in SHRSR (0.072 $\pm$ 0.023 vs. 0.071 $\pm$ 0.016, NS) (Table 1).

Effect of erythrocyte transfusion on platelet production

The uptake of ^{75}SeM by total erythrocytes on the 3rd day was decreased in the

Table 2. The comparison of uptake of ^{75}Se -selenomethionine by erythrocytes and platelets.

	10 W SHRSP	$A_{1-sb}F_{39,44}$ Female
	Percent incorporation into total erythrocytes (mean $\pm$ SD)	Statistical significance
Vit. E-free diet	1.063 ± 0.334 ($n=9$)	
Vit. E-free diet and erythrocyte transfusion	0.775 ± 0.103 ($n=9$)	$p < 0.05^*$
	10 W SHRSP	$A_{1-sb}F_{39,44}$ Female
	Percent incorporation into total platelets (mean $\pm$ SD)	Statistical significance
Vit. E-free diet	0.192 ± 0.030 ($n=8$)	
Vit. E-free diet and erythrocyte transfusion	0.199 ± 0.035 ($n=9$)	NS

* Significantly different from the value of Vit. E-free diet (t -test).

vitamin E-free diet group of rats which were transfused with packed erythrocytes compared with the control vitamin E-free diet group of rats which were not transfused (0.775 ± 0.130 vs. 1.063 ± 0.334 , $p < 0.05$). However, the maximal uptake of ^{75}SeM by total platelets showed no significant difference between these two groups of rats (0.199 ± 0.035 vs. 0.192 ± 0.030 , NS) (Table 2).

DISCUSSION

Many studies have been made on the effect of vitamin E on platelet function *in vitro*. Vitamin E is said to be an effective inhibitor of platelet aggregation and release reaction (8–9).

Platelet aggregation is increased in vitamin E-deficient rats (10). Clinically, it is also seen in vitamin E-deficient infants (11–14). In vitamin E deficiency, the occurrence of increased platelet aggregation *in vivo* is suspected by some researchers.

Nafstad reported that endothelial damage and thrombosis, and myocardial infarction were seen in vitamin E-deficient pigs (15).

Lake *et al.* reported that NEM-induced lipid peroxide was increased in platelets of vitamin E-deficient infants (16).

Prostacyclin, which inhibits platelet adhesion and aggregation, is decreased in the vascular endothelial cells of vitamin E-deficient rats (17).

In this experiment the platelet survival time was shortened in the vitamin E-free diet group of SHRSR whose platelet survival time was normal when fed on the

regular diet at 9–10 weeks of age. The platelets of vitamin E-deficient rats seemed excessively fragile from the analysis of the concave curve in the disappearance phase of ^{75}SeM -labeled platelets (Fig. 5). The platelet production shown as the maximal uptake of ^{75}SeM by total platelets was increased in SHRSP and SHRSR in the vitamin E-free diet groups. It was thought that the platelet production increase reflected an overall increase in bone marrow activity associated with anemia in vitamin E deficiency. The production of erythroid series in the bone marrow was suppressed by transfusing packed erythrocytes, but increased production of platelets in vitamin E-deficient rats was not. From these results, it was proved that the anemia in vitamin E-deficient rats that appeared by 10 weeks of age did not cause this increase of platelet production.

The effect of vitamin E deficiency on platelet kinetics was the shortening of platelet survival time and the increase of platelet production in SHRSP and SHRSR. The shortening of platelet survival time was the result of increasing lipid peroxide in the platelets and decreasing prostacyclin in the vascular endothelial cells of vitamin E-deficient rats.

The increase of platelet production was the result of compensating the increased platelet consumption at the damaged endothelium with enhanced platelet aggregation.

On the other hand, the effect of vitamin E-supplementation on platelet kinetics was the correction of the shortened platelet survival time and suppression of the increased platelet production in SHRSP. The platelet survival time was normalized in the vitamin E-supplemented diet group of SHRSP whose platelet survival time was shortened as the result of vascular alteration on feeding the regular diet at 9–10 weeks of age. Normalizing the shortened platelet survival time of SHRSP was the result of inhibiting platelet aggregation *in vivo*. Platelet production was suppressed to normal because of decreasing platelet consumption at the damaged endothelium in the vitamin E-supplemented diet group of SHRSP whose platelet production was increased by feeding the regular diet at 9–10 weeks of age. Furthermore, the further increased platelet production in the vitamin E-free diet group of SHRSP was subsequently corrected to normal by feeding the vitamin E-supplemented diet. Therefore, it is effective *in vivo* to use vitamin E as an antiplatelet drug when the platelet survival time is shortened because of vascular lesion.

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