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Effects of α-Tocopherol and Tocotrienols on Blood Pressure and Linoleic Acid Metabolism in the Spontaneously Hypertensive Rat (SHR)

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Both α-tocopherol and a 1:1.7 mixture of α-tocopherol and tocotrienols at a 0.2% dietary level significantly depressed the age-related increase in the systolic blood pressure of spontaneously hypertensive rats (SHRs) after 3 weeks of feeding. The aortic production of prostacyclin was increased 1.5 times both by α-tocopherol and a tocotrienol mixture, suggesting a possible relevance to their hypotensive effect. These vitamins did not influence the Δ6- and Δ5-desaturase activities of liver microsomes, but fatty acid profiles of the liver phospholipids predicted a reduction of linoleic acid desaturation. These effects were in general more clear with tocotrienols than with α-tocopherol. Platelet aggregation by 5 μM ADP remained uninfluenced. Thus, tocotrienols may have effects on various lipid parameters somewhat different from those of α-tocopherol.

It has been reported that dietary palm oil and palm olein, both of which are low in linoleic acid and high in palmitic acid, did not increase an arterial thrombotic tendency and they rather tended to decrease platelet aggregation even compared with sunflower oil containing a large proportion of linoleic acid.[1] Since palm oil contains approximately 50% saturated fatty acids, this observation may be an exception to the general concept that saturated fat is thrombotic. In this context, tocotrienols, which are exclusively rich in palm oil, have been considered as one of the effective components.[2]

In vitamin E-deficient rats, a decrease in the proportion of linoleic acid and an increase in arachidonic acid in tissue phospholipids have been reported.[3] Also, α-tocopherol suppressed thrombosis by balancing prostacyclin (PGI_2) and thromboxane A_2 (TXA_2).[4] The evidence thus indicates that α-tocopherol may have some effect on linoleic acid desaturation and, hence, on eicosanoid production. In addition, the antihypertensive action of α-tocopheryl nicotinate has been reported in the spontaneously hypertensive rat (SHR).[5] On the other hand, it has been found in the feeding experiment using chickens that tocotrienol inhibited cholesterol synthesis and reduced serum cholesterol level,[6] suggesting that tocotrienol has an effect different from tocopherol. Only limited information is, however, available regarding the effect of tocotrienols on other parameters. The present study, therefore, was undertaken to compare the effect of α-tocopherol and tocotrienols on the blood pressure and linoleic acid metabolism of SHRs.

Materials and Methods

Animals and diets. Male SHRs (SHR/NCrj, 8 weeks old) were purchased from Japan Charles River Co., Kanagawa, and acclimatized for 7 days in a room maintained at 20—23°C with a 12-hr light-dark cycle (lights on 08:00—20:00). The rats were divided into 3 groups of 6 each according to the different diets. The control diet was prepared according to the formula recommended by American Institute of Nutrition[7] and contained (in percentage by weight) 20% casein (Wako Pure Chemical Industries Ltd., Osaka), 5% corn oil (Ajinomoto Co., Tokyo), 1% vitamin mixture (AIN^{TM76}), 3.5% mineral mixture (AIN^{TM76}), 0.2% choline bitartrate, 0.3% DL-methionine, 5% cellulose, 15% corn starch, and sucrose to 100%. DL-α-Tocopherol (Toc; Nacalai Tesque, Kyoto) or an α-tocopherol-tocotrienol mixture (Toc3; 36% α-tocopherol, 24% α-tocotrienol, 27% γ-tocotrienol, and 11% δ-tocotrienol, supplied by the Palm Oil Research Institute of Malaysia, Kuala Lumpur) were added to the control diet at the 0.2% level, respectively. The rats were given these diets *ad libitum* for 6 weeks. At the end of the feeding period, the rats were anesthetized with diethyl ether, and 9 ml of blood was collected from the abdominal aorta in a syringe containing 1 ml of 3.8% trisodium citrate.

Measurement of systolic blood pressure. The systolic blood pressure in the rat tail was measured every week for 5 weeks with a sphygnomanometer (MK-100, Muromachi Kikai Co., Tokyo) in a chamber maintained at 33°C.

Measurement of the Δ6- and Δ5-desaturase activity of liver microsomes. The Δ6- and Δ5-desaturase activity of liver microsomes was measured by the method of Svensson.[8] The enzymatic reactions were measured with 100 nmol of the substrates and approximately 1 mg of microsomal protein with incubation at 37°C for 20 min. In this condition, the enzymes were saturated with the substrate, and the reactions were essentially linear with a microsomal protein concentration and with incubation time.[8] Microsomal protein was measured by the method of Lowry *et al.*[9]

Fatty acid composition of the issue lipids. Plasma and liver lipids were extracted by the method of Folch *et al.*,[10] and their lipid fractions were separated by thin-layer chromatography.[11,12] The fatty acid composition of each lipid fraction was analyzed by gas-liquid chromatography on a SILAR 10C column.[13]

Aortic production of prostacyclin (PGI_2) and platelet aggregation. To measure the aortic production of PGI_2, the thoracic aorta (approximately 25 mg) was incubated in Krebs-Henseleit bicarbonate buffer (pH 7.4) at 25°C for 30 min, and the concentration of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in the medium was measured by a radioimmunoassay, using a commercial kit (NEK-008, New England Nuclear, Boston, MA).[14] The concentration of 6-keto-$PGF_{1\alpha}$ was determined in a range of around 50% of the normalized percentage bound (% B/B_0) for the standard according to the instruction manual provided by the supplier. ADP (5 μM)-induced platelet aggregation (% of maximum aggregation) was measured with an automated platelet aggregation

Abbreviations: PGI_2, prostacyclin; TXA_2, thromboxane A_2; SHR, spontaneously hypertensive rat; Toc, DL-α-tocopherol; Toc3, α-tocopherol–tocotrienol mixture; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PC, phosphatidylcholine; PI, phosphatidylinositol; CE, cholesterol ester.

analyzer (AGGRECORDER II, Kyoto Daiichi Kagaku Co., Kyoto).

Statistical analyses. Data were analyzed by Duncan's new multiple-range test to determine the exact nature of the differences among groups.[15]

Results

Growth and liver weight

No statistically significant differences were found in the body weight gain and food intake among the groups (Table I). Compared with the control group, the relative liver weight of the Toc group was heavier, whereas it was comparable in the Toc3 group. During the 3rd to 4th weeks, two rats in the Toc group died for unknown reasons.

Systolic blood pressure

As shown in Fig. 1, the systolic blood pressure measured in the tail increased gradually with feeding period in all groups of rats, but the degree of increase was significantly lower in the Toc and Toc3 groups than in the control group after 2 to 3 weeks.

Δ6- and Δ5-Desaturase activity of the liver microsomes

The Δ6- and Δ5-desaturase activity of the liver microsomes is shown in Table II. No statistically significant effect of Toc and Toc3 was apparent with the Δ6- and Δ5-desaturase activity.

Fatty acid composition of the tissue lipids

In the liver phosphatidylcholine (PC), the proportion of linoleic acid was significantly higher, whereas that of arachidonic acid was lower in the rats fed with the Toc or Toc3 diets than in those fed with the control diet (Table III). Therefore, the desaturation index expressed as the ratio of $(20:3n-6+20:4n-6)/18:2n-6$ tended to be lower in the former two groups than in the latter, and the difference between the Toc3 and control groups was statistically significant. The proportion of docosahexaenoic acid $(22:6n-3)$ decreased significantly in the rats with the Toc diet, but not in the rats fed with the Toc3 diet. In the liver phosphatidylinositol (PI), no significant change was apparent in the fatty acid composition after feeding either the Toc or Toc3 diets (data not shown). The polyunsaturated fatty acid composition of plasma PC was also comparable among the groups (data not shown). The proportion of linoleic acid in the plasma cholesterol ester

Table I. Effects of α-Tocopherol and Tocotrienols on the Growth Parameters of SHR

Group	Body weight (g)		Food intake (g/day)	Relative liver weight (g/100 g body wt.)
	Initial	Gain		
Control	236±4	92±4	17.8±0.3	3.84±0.06[a]
Toc	237±4	90±1	17.8±0.4	4.15±0.10[b]
Toc3	237±3	95±4	18.0±0.3	3.99±0.08[ab]

Mean±SE of 4 to 6 rats. Toc, α-tocopherol; Toc3, α-tocopherol and tocotrienol mixture.

[ab] Values without a common superscript letter are significantly different at $p<0.05$.

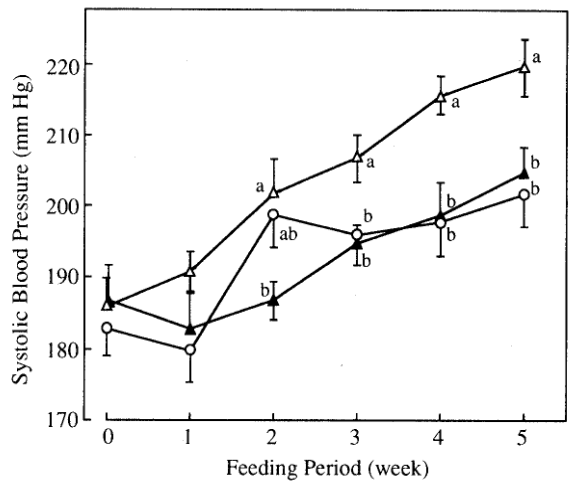

Fig. 1. Effects of α-Tocopherol and Tocotrienols on the Systolic Blood Pressure of SHR.

The systolic blood pressure was measured with a sphygnomanometer in a chamber maintained at 33°C. △ control, ○ α-tocopherol, ▲ α-tocopherol–tocotrienol mixture. Each value represents the mean±SE of 4 to 6 rats.

[ab] Values without a common superscript letter are significantly different at $p<0.05$.

Table II. Effects of α-Tocopherol and Tocotrienols on the Δ6- and Δ5-Desaturase Activity of Liver Microsomes

Group	Activity (pmol/min·mg protein)	
	Δ6-Desaturase	Δ5-Desaturase
Control	164±21	709±145
Toc	156±54	585±237
Toc3	133±22	635±113

Mean±SE of 4 to 6 rats. Toc, α-tocopherol; Toc3, α-tocopherol and tocotrienol mixture.

Table III. Effects of α-Tocopherol and Tocotrienols on the Polyunsaturated Fatty Acid Composition of Liver Phosphatidylcholine

Group	Fatty acids (weight %)					Desaturation Index*
	18:2n-6	20:3n-6	20:4n-6	22:5n-6	22:6n-3	
Control	9.1±0.7[a]	0.4±0.0	35.2±1.1[a]	1.0±0.1	3.3±0.2[a]	4.1±0.5[a]
Toc	11.5±1.0[b]	0.5±0.0	32.3±0.6[b]	0.9±0.2	2.3±0.2[b]	2.9±0.3[ab]
Toc3	11.2±0.2[b]	1.0±0.4	33.2±0.4[ab]	0.8±0.1	3.0±0.1[a]	3.1±0.1[b]

Mean±SE of 4 to 6 rats. Toc, α-tocopherol; Toc3, α-tocopherol and tocotrienol mixture. *$(20:3n-6+20:4n-6)/18:2n-6$.

[ab] Values without a common superscript letter are significantly different at $p<0.05$.

Table IV. Effects of α-Tocopherol and Tocotrienols on the Polyunsaturated Fatty Acid Composition of Plasma Cholesterol Ester

Group	Fatty acids (weight %)				20:4/18:2
	18:2n-6	20:4n-6	22:5n-3	22:6n-3	
Control	13.6±0.3[a]	67.1±1.2	0.8±0.1	0.2±0.1[a]	5.0±0.2[a]
Toc	14.1±0.4[a]	66.4±1.4	0.9±0.3	0.5±0.1[b]	4.7±0.2[ab]
Toc 3	15.5±0.2[b]	65.2±0.6	0.6±0.2	0.3±0.1[ab]	4.2±0.1[b]

Mean±SE of 4 to 6 rats. Toc, α-tocopherol; Toc3, α-tocopherol and tocotrienol mixture.

[ab] Values without a common superscript letter are significantly different at $p<0.05$.

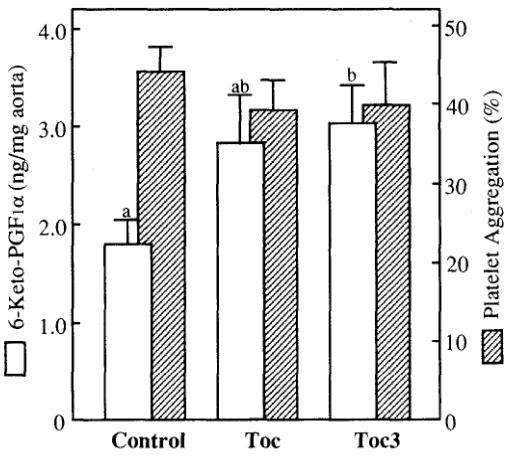

Fig. 2. Effects of α-tocopherol and Tocotrienols on the Aortic Production of Prostacyclin and Platelet Aggregation in SHR.

Toc, α-tocopherol; Toc3, α-tocopherol–tocotrienol mixture. Each value represents the mean ± SE of 4 to 6 rats. Prostacyclin was measured as 6-keto prostaglandin $F_{1\alpha}$. Platelet aggregation was measured after induction by 5 μM ADP and is expressed as % of maximum aggregation.
[ab] Values without a common superscript letter are significantly different at $p<0.05$.

(CE) was significantly higher in the Toc3 group than in the control and Toc groups, whereas that of arachidonic acid tended to be slightly lower in the former than in the latter two groups (Table IV). The desaturation index for linoleic acid was therefore significantly lower in the Toc3 but not Toc group than in the control group.

Aortic production of PGI_2 and platelet aggregation

The aortic production of PGI_2, measured as 6-keto-$PGF_{1\alpha}$, was about 1.5 times higher in the Toc and Toc3 groups than in the control group, and the difference between the Toc3 and control groups was statistically significant (Fig. 2). The extent of platelet aggregation by 5 μM ADP was comparable among the groups (Fig. 2).

Discussion

An antihypertensive effect of α-tocopherol and tocotrienols was apparent in the present study. There is evidence that vitamin E lowers blood pressure, although not always consistently.[5,16,17] This effect of vitamin E on blood pressure may at least in part be related to a change in the production of vasodilative PGI_2, since the production was approximately 1.5 times higher in rats fed with Toc or Toc3 than in the control rats. However, α-tocopherol and tocotrienols had no effect on the PGI_2 production when conventional male Sprague-Dawley rats were fed with a diet containing 0.5% cholesterol and 0.2% sodium cholate,[18] probably due to the suppressive effect of dietary cholesterol.[19] The action of Toc and Toc3 seems to be attributable to their antioxidative effect, as hydroperoxy fatty acids inhibit PGI_2 synthesis.[20] Although the aortic production of PGI_2 was increased, no effect on the platelet aggregation by ADP was seen, in contrast to the previous observation with human platelets.[21] Since the platelet production of TXA_2 induced by collagen but not by ADP was increased significantly in vitamin E-deficient rats,[22] a study on the effect of collagen on platelet aggregation would provide further information.

In the present study, the effect of Toc and Toc3 on the metabolism of linoleic acid to arachidonic acid was apparent according to the fatty acid pattern of liver PC. When considering the abundance of PC in rat liver phospholipids, it seems likely that PC preferentially provides arachidonic acid for the production of two series of prostaglandins. In liver PC, the desaturation index, expressed as the ratio of the metabolites to the parent molecule, $(20:3n-6+20:4n-6)/18:2n-6$, tended to be lower in the Toc and Toc3 groups than in the control group, and the difference between the Toc3 and control groups was statisticaly significant. Although it is not necessarily appropriate for a direct comparison, the present result is inconsistent with the previous observation in Sprague-Dawley rats fed with cholesterol-enriched diets; the supplementation of the tocotrienol mixture to palm oil did not influence the fatty acid profile of liver PC.[18] This may again be attributable to the effect of dietary cholesterol.[23] However, the Δ6- and Δ5-desaturase activity of the liver microsomes were comparable among the groups in the present study. Since the fatty acid profile of aortic phospholipids in general resembles that of the plasma and liver phospholipids,[24] it is probable that Toc and Toc3 influenced the fatty acid profile of aortic phospholipids in a similar way to the case of serum and liver phospholipids. Therefore, Toc and Toc3 appear to be effective for regulating the production of prostaglandins rather than the metabolism of linoleic acid to arachidonic acid.

In conclusion, both α-tocopherol and tocotrienols suppressed an age-dependent rise in the systolic blood pressure. The aortic production of PGI_2 was increased by α-tocopherol and tocotrienols, suggesting their possible relevance to an antihypertensive effect. However, platelet aggregation by ADP was not influenced. It is difficult to distinguish the effect of tocotrienols from that of α-tocopherol with the present experimental protocol, but the former seems to have been more effective than α-tocopherol at least in increasing the prostaglandin production and altering the fatty acid profile of the tissue phospholipids.

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