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The antithrombotic activity of mini-type tomatoes is dependent on the particular variety and the stage of harvest. Lycopene content does not contribute to antithrombotic activity

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ABSTRACT

The prevention of arterial thrombotic disease has a high priority in developed countries. We have focused our studies on the antithrombotic activity of those fruits and vegetables with the potential to prevent the disease, and the present study was undertaken as part of a series of investigations to examine beneficial fruits and vegetables. For this purpose, suitable laboratory tests as well as diets have been devised. In the current investigation, we have classified various tomato varieties with antithrombotic properties, and we now have extended our overall data to include more than ten antithrombotic varieties of fruits and vegetables. A method designed to measure shear-induced platelet activity (the Global Thrombosis Test, GTT) was used to assess haemostasis *in vitro* and a He-Ne laser-induced thrombosis technique was utilized to examine arterial thrombogenesis *in vivo*. Concentrations of the antioxidant, lycopene, were also measured. Three mini-type tomato varieties, coded "Cin", "Pik" and "Caec", and one medium-type variety, coded "K", were harvested at different stages of maturity. All mini-type varieties demonstrated antithrombotic activity at an early (green) stage. The antithrombotic activity decreased with the maturation of "Cin" and "Caec" but remained constant at all stages of maturity with "Pik". The medium variety, "K", did not possess antithrombotic activity. Lycopene was not detected at any stage in any of the tomato varieties, suggesting that this antioxidant did not contribute to antithrombotic activity. The present results indicated that the antithrombotic activity of tomatoes is dependent on the particular variety and stage of maturity, and that this activity is not due to lycopene.

Keywords: Tomato; Lycopene; Global Thrombosis Test (GTT); Shear-Induced Platelet Aggregation

1. INTRODUCTION

"Lifestyle-related atherothrombotic diseases" represent the major cause of death in many developed countries and the prevention of cardiovascular diseases of this nature is widely regarded as an important and urgent social task. We have focused on studies designed to identify fruits and vegetables with antithrombotic activity with the potential to prevent disorders of this nature. We have devised laboratory tests to classify a number of antithrombotic fruit and vegetable varieties [1-8], and the present study extends this data to include different varieties of tomato.

Platelets play a pivotal role in arterial thrombotic diseases including myocardial infarction and stroke. Platelet-function *in vitro* is commonly assessed using platelet aggregometry, which measures platelet aggregation induced by various chemical agonists *in vitro*. In clinical practice, however, tests using native, non-anticoagulated blood in the presence of physiological shear force are likely to be much more relevant to the *in vivo* environment than those using anticoagulated blood and chemical platelet agonists. The present study was undertaken,

therefore, as part of a series of investigations to examine antithrombotic fruits and vegetables with a shear-induced platelet function *in vitro* test using non-anticoagulated (native) blood together with a helium-neon laser induced *in vivo* thrombosis method [9-14]. Concentrations of the antioxidant, lycopene, were also examined.

We have demonstrated the potential of mini-type varieties of tomato, and have shown that antithrombotic properties may be dependent on the particular variety and stage of maturity. Our results indicated that these antithrombotic properties were not related to the concentration of the antioxidant, lycopene [15].

2. MATERIALS AND METHODS

2.1. Animals

Male Wistar 13-week-old ST rats (Japan SLC Co. Ltd., Hamamatsu, Japan) and 10-week-old male C57BL/6 mice (Japan SLC Co. Ltd., Hamamatsu, Japan) were purchased one week before the scheduled experiments. Animals were maintained in compliance with the "*Guiding Principles for the Care and Use of Animals in the field of Physiological Sciences*", published by Physiological Society of Japan. The protocol was approved by the Animal Experiment Committee of Kobe Gakuin University.

2.2. Tomatoes

Three kinds of mini-type tomato varieties coded as "Cin", "Pik" and "Caec" and medium-type variety, coded "K", were harvested at different stages of maturity (green, pink and mature) from the same field in Kobe City, Japan.

2.3. Preparation of Tomato Filtrate

Several washed tomatoes from each variety were crushed, with skin, blended and the juice obtained by centrifugation at 3000 rpm for 15 min at 4˚C. The supernatant was filtered (pore size 0.5 μm; Schleicher & Schuell GmbH., Dassel, Germany) and the clear filtrates were stored at –80˚C until use.

2.4. *In Vitro* Assessment of Platelet Activity and Endogenous Thrombolytic Activity by the Global Thrombosis Test (GTT)

The GTT test (Montrose Diagnostics Ltd., UK) has been described in detail earlier [10-13,16,17]. In brief, native blood is passed through four narrow channels where platelets are activated by the high shear stress (145 dyne/cm^2). Down-stream from this point, the flow is slow and turbulent, and platelets aggregate and form thrombin. Consequently, fibrin-stabilized platelet aggregates (thrombi) cause occlusion and stop blood flow. The instrument detects the time interval (d, sec) between consecutive blood drops. At the start, blood flow is rapid and hence (d) is small. Subsequently, the flow rate decreases and hence (d) increases. When the actual (d) exceeds 15 seconds (before the flow is completely arrested), the instrument displays "Occlusion Time (OT, sec)". Subsequently, flow is restored due to thrombolysis, and is indicated by the detection of the first blood drop (Lysis Time (LT), sec).

In the present investigations, blood was withdrawn from the abdominal aorta of rats 30 minutes after Nembutal anaesthesia (60 mg/kg, im) and diluted with saline in 1:1 ratio. 0.4 ml tomato filtrate or 0.4 ml saline (control) were added to 3.6 ml of the diluted blood, mixed and transferred to the GTT test tube within 15 sec after withdrawal of blood. Four blood containing various concentrations of tomato filtrates and control were assessed in four channels simultaneously and the tests were repeated six times (n = 6) for each sample.

2.5. Helium-Neon (He-Ne) Laser-Induced Thrombosis *in Vivo* Model in Mice

The Helium-Neon laser-induced thrombosis method has been previously described in detail [14]. In brief, the left femoral artery of an anaesthetized mouse was exposed and Evans blue dye was injected intravenously. The center of the carotid artery was irradiated with the laser beam, and the formation of thrombi at the site of irradiation was monitored and recorded on videotape. Calculation of thrombus size has been described earlier [14]. In outline, images of thrombus formation were computer-analysed at intervals of ten seconds. The area of thrombus was delineated and the mass of thrombus calculated by multiplying gray scale and area using Image J software (Image Processing and Analysis Java version 1.30, National Institutes of Health, Maryland, USA). Thrombotic status was expressed as the total sum of thrombus mass after the first 10 minutes of irradiation.

2.6. Antithrombotic Effect of Tomato Filtrates after Oral Administration

Filtrates and controls (distilled water) (7.7 ml/kg × 2) were orally administered to the animals through a gastric tube. A second dose using the same volume of filtrate or water was given 30 min later. Mice were anaesthetized and the laser-induced thrombosis experiments commenced 90 min after the second oral treatment. The antithrombotic or prothrombotic effects were assessed by measuring the total thrombus size. Antithrombotic activity was related to smaller thrombus formation.

2.7. Lycopene Content

Lycopene content of tomato filtrates was commer-

cially measured by HPLC (Japan Food Research Laboratories, Tokyo, Japan).

2.8. Statistical Analysis

OT and LT were analyzed by repeated ANOVA, followed by post hoc (Dunnett). Thrombus size in the laser-induced experiments was analysed by ANOVA and followed by post hoc (Dunnett). Values were expressed as means ± SEM. $P < 0.05$ was considered as statistically significant. Analysis was performed by statistical package software (Unistat Light 5.6, Unistat Ltd., London, UK).

3. RESULTS

3.1. Effect of Tomato Variety Filtrates and Harvest Phase on Platelet Activity and Endogenous Thrombolytic Activity *in Vitro*

The results are shown in **Table 1**. At the green stage, all varieties except "K" significantly inhibited platelet activity (OT). At the pink stage, "Pik" and "Caec" inhibited OT but "Cin" and "K" did not. At the mature stage, only "Pik" inhibited this measurement. These results demonstrated that inhibition of OT was dependent on the particular variety and stage of maturity of the different tomatoes.

The effects on thrombolysis were difficult to determine because both inhibition of OT and enhanced thrombolysis caused less haemostatic plug mass formation.

3.2. Antithrombotic Effects of Tomatoes at the Mature Stage Assessed by the He-Ne Laser-Induced Thrombosis Test

The antithrombotic activity of the different tomato varieties was assessed by laser-induced thrombosis *in vivo* (**Figure 1**). The variety "Pik" demonstrated antithrombotic activity. In contrast, the variety "K" showed no antithrombotic activity. The results indicated that platelet reactivity *in vivo*, assessed by the He-Ne laser-induced platelet-rich thrombosis test, was consistent with that observed by the shear-induced platelet test *in vitro*.

3.3. Lycopene Concentration in Various Tomato Varieties

Lycopene, which appears to be the most readily available carotenoid in tomatoes, was measured in the various varieties at the early and full mature stages. The results are shown in **Table 2**. Little lycopene was detected in any of the mini and medium-type varieties, although full-size market tomatoes had significant amounts. The results indicate that antithrombotic activity was not related to lycopene in the mini-type tomatoes used in the present experiments.

3.4. Maturation Phase and Antiplatelet Thrombotic Activity Assesses by Global Thrombosis Test (GTT)

Results are shown in **Figure 2**. Antiplatelet activity was maximum at the early green phase and decreased with maturation. With the "Pik" variety, antiplatelet activity remained consistent from the green stage to the mature stage. In contrast, antiplatelet activity decreased with maturation in "Cin" and "Caec" varieties. Antiplatelet activity was not observed at any stage with the "K" variety.

4. DISCUSSION

In our on-going series of investigations on the role of nutrition in the pathogenesis of arterial thrombotic diseases, we have targeted especially dietary fruits and vegetables with antithrombotic activity. In addition, we have utilized novel, shear-induced platelet-rich thrombosis tests using non-anticoagulated blood rather than conventional tests dependent on agonist-induced platelet aggregation and thrombotic markers using anticoagulated blood. The innovative shear-induced platelet reactivity test Global Thrombosis Test (GTT) also enabled the concurrent assessment of endogenous thrombolytic activity. Tests of this nature have facilitated the classification of different varieties of mulberry, carrot, potato and onion according to antithrombotic activity [4-6,8]. Furthermore, we have confirmed that systemic antithrombotic activity depends on a balance between platelet reactivity and thrombolytic activity [4-6,8]. Our findings offer the challenging possibility that regular daily diets containing particular antithrombotic fruits and vegetables could help to prevent arterial thrombotic diseases.

In our thrombosis/thrombolysis animal model, the GTT is designed to measure shear-induced platelet reactivity *in vitro*, and the He-Ne laser-induced thrombosis technique is used to assess hemostasis *in vivo*. In the present study, the concentration of the antioxidant lycopene was also measured in three mini-type tomato varieties, coded "Cin", "Pik" and "Caec", and one medium-type variety, coded "K". In our previous studies, we tested antithrombotic activity using Haemostatometry, an early test of shear-induced platelet function. We demonstrated that tomato varieties could be classified into subgroups according to antiplatelet activity and that antiplatelet activity decreased with maturity or colour change from green to red [1]. Since that time, techniques to assess platelet reactivity have been considerably improved, and the GTT in particular provides valuable quantitative data in this respect. In our current studies, the GTT identified

Table 1. Effects of raw filtrates from three mini-type and one medium-type tomato varieties and three harvest stages on shear-induced platelet thrombosis (OT) and endogenous thrombolysis (LT).

(a)

Variety	Dilution	Occlusion time (OT)	Lysis time (LT)
Cin (green)	control	342.4 ± 25.8	1120.3 ± 84.2
	×1	605.0 ± 70.8*	ND
	×3	100.1 ± 11.1*	ND
	×10	181.1 ± 54.1	1228.8 ± 351.0
Pik (green)	control	340.8 ± 11.5	1060.5 ± 46.6
	×1	668.4 ± 121.2*	ND
	×3	ND*	ND
	×10	333.4 ± 15.1	1570.0 ± 121.7**
Caec	control	356.6 ± 14.6	1232.5 ± 73.9
(green)	×1	811.3 ± 41.4**	ND
	×3	177.7 ± 23.6**	ND
	×10	272.5 ± 31.4	1335.2 ± 408.9
K	control	325.5 ± 17.5	1353.0 ± 128.5
(green)	×1	264.6 ± 55.6	ND
	×3	324.8 ± 14.1	1419.0 ± 423.1
	×10	336.6 ± 15.7	1319.0 ± 90.9
Cin	control	372.6 ± 21.7	1218.2 ± 222.7
(pink)	×1	303.3 ± 39.8	ND
	×3	80.7 ± 4.7**	1118.7 ± 559.4
	×10	235.5 ± 57.2	1319.4 ± 326.0
Pik	control	334.1 ± 28.4	974.0 ± 75.2
(pink)	×1	895.4 ± 56.6**	ND
	×3	371.0 ± 79.3	ND
	×10	ND	ND
Caec	control	304.2 ± 11.5	1188.8 ± 93.7
(pink)	×1	739.6 ± 173.3*	ND
	×3	200.4 ± 26.4	ND
	×10	322.9 ± 207.6	1653.0 ± 130.4
K	control	342.1 ± 9.4	1090.8 ± 69.1
(pink)	×1	346.5 ± 33.2	ND
	×3	ND	ND
	×10	238.0 ± 21.4*	993.4 ± 136.4

(b)

Cin	control	348.5 ± 15.6	1396.0 ± 135.0
(mature)	×1	301.3 ± 44.1	ND
	×3	245.1 ± 135.8	2834.5 ± 271.5*
	×10	593.1 ± 173.5	822.8 ± 229.9
Pik	control	351.8 ± 11.8	1139.8 ± 106.3
(mature)	×1	683.1 ± 139.8*	ND
	×3	147.7 ± 26.8	886.0 ± 560.7
	×10	ND	ND
Caec	control	335.3 ± 21.9	1085.5 ± 81.9
(mature)	×2	159.2 ± 38.7	421.2 ± 342.2
	×4	387.2 ± 156.9	734.5 ± 327.8
	×10	453.0 ± 102.9	1219.3 ± 147.9
K	control	307.3 ± 13.4	1003.8 ± 83.9
(mature)	×1	387.1 ± 40.0	ND
	×3	180.7 ± 99.9	1441.5 ± 50.5
	×10	271.2 ± 41.2	788.6 ± 138.1

*$P < 0.05$, **$P < 0.01$ versus control (saline), ND: not determined.

Table 2. Lycopene concentration in the tomato varieties at different stages of maturity (micrograms/g wet weight of tomato fruit).

	Cin	Pik	Caec	K	Full-size tomato
Premature	<0.2	<0.2	<0.2	<0.2	
Mature	<0.2	<0.2	<0.2	<0.2	8.8 - 42

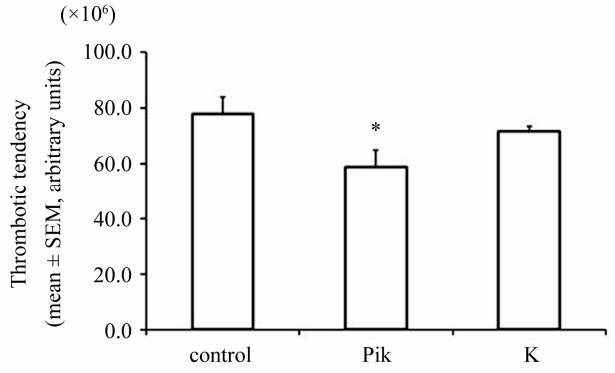

Figure 1. Antithrombotic activity of two varieties at the mature stage was demonstrated by He-Ne laser-induced thrombosis *in vivo*. $^{*}P < 0.05$ versus control (water).

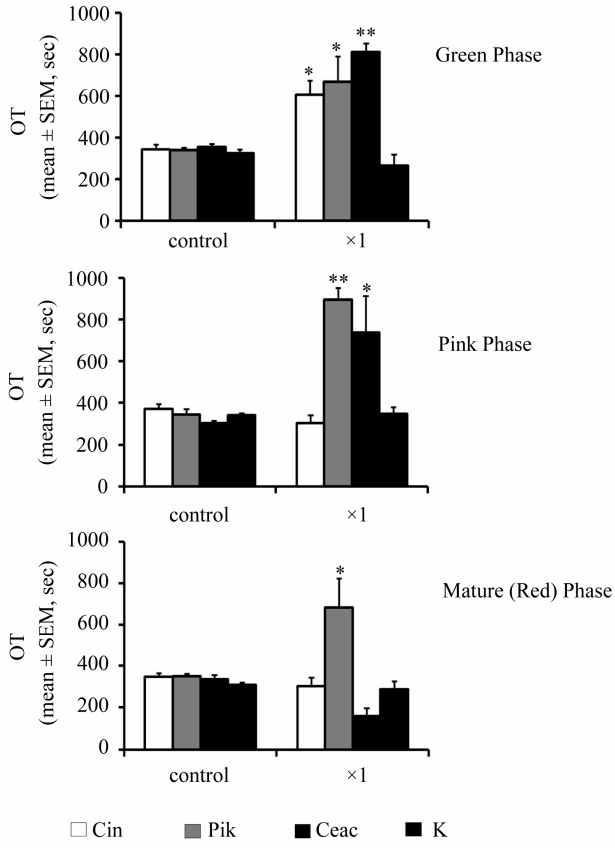

Figure 2. Anti-platelet activity of tomato varieties at different stages of maturity was demonstrated by the Global Thrombosis Test. $^{*}P < 0.05$ versus control (water). Mini-type: Cin, Pik, Caec; medium-type: K, $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (saline), ND: not determined.

some mini-type tomato varieties that had significant antiplatelet activity. We demonstrated that these anti-platelet effects were retained in one variety but were not evident in other varieties at the mature stage.

Lycopene is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It appears to be one of the most potent antioxidants among dietary carotenoids. Previous reports have suggested that dietary intake of tomatoes and tomato products containing lycopene may be associated with a decreased risk of chronic diseases, such as cancer and cardiovascular disease. Our current results indicate, however, that lycopene at least at the concentrations found in our tomato extracts, did not contribute to the antithrombotic characteristics of the natural mini-tomato products. Further studies are required to examine the potentially beneficial properties of purified lycopene.

Thrombotic diseases are amongst the leading causes of death in developed countries. The identification of antithrombotic food products could enhance the promotion of healthy diets and contribute to a reduction in the incidence of life-related diseases. The reproducible and sensitive point-of-care test, the GTT, might provide a valuable means for assessing the specific benefits of a healthy life-style.

5. CONCLUSION

The present study shows that the antithrombotic activity of different tomatoes is dependent on the particular variety and stage of maturity.

REFERENCES

[1] Yamamoto, J., Taka, T., Yamada, K., Ijiri, Y., Murakami, M., Hirata, Y., Naemura, A., Hashimoto, M., Yamashita, T., Oiwa, K., Seki, J., Suganuma, H., Inakuma, T. and Yoshida, T. (2003) Tomatoes have natural antithrombotic effects. *British Journal of Nutrition*, **90**, 1031-1038. doi:10.1079/BJN2003994

[2] Yamada, K., Naemura, A., Sawashita, N., Noguchi, Y. and Yamamoto, J. (2004) An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis. *Thrombosis Research*, **114**, 213-220. doi:10.1016/j.thromres.2004.06.007

[3] Naemura, A., Mitani, T., Ijiri, Y., Tamura, Y., Yamashita, T., Okimura, M. and Yamamoto, J. (2005) Anti-thrombotic effect of strawberries. *Blood Coagulation & Fibrinolysis*, **16**, 501-509.

doi:10.1097/01.mbc.0000184737.50594.a8

[4] Yamamoto, J., Naemura, A., Ura, M., Ijiri, Y., Yamashita, T., Kurioka, A. and Koyama, A. (2006) Testing various fruits for anti-thrombotic effect: I. Mulberries. *Platelets*, **17**, 555-564. doi:10.1080/09537100600759295

[5] Yamamoto, J., Naemura, A., Ijiri, Y., Ogawa, K., Suzuki, T., Shimada, Y. and Giddings, J.C. (2008) The antithrombotic effects of carrot filtrates in rats and mice. *Blood Coagulation & Fibrinolysis*, **19**, 785-792. doi:10.1097/MBC.0b013e3283177b19

[6] Ichiura, D., Naemura, A., Ura, M., Mori, M. and Yamamoto, J. (2009) Antithrombotic effect of potato in animal experiments. *Food*, **3**, 8-12.

[7] Kinugasa, C., Naemura, A., Hyodo, K., Nakai, Y., Katsuta, M. and Yamamoto, J. (2011) Experimental antithrombotic effects of sesame seed whole grains and extracts. *Blood Coagulation & Fibrinolysis*, **22**, 526-531. doi:10.1097/MBC.0b013e328347b085

[8] Hyodo, K., Horii, I., Nishino, M., Giddings, J.C. and Yamamoto, J. (2011) The antithrombotic effects of onion filtrates in rats and mice. *Health*, **3**, 319-325. doi:10.4236/health.2011.36055

[9] Ratnatunga, C.P., Edmondson, S.F., Rees, G.M. and Kovacs, I.B. (1982) High-dose aspirin inhibits shear-induced platelet reaction involving thrombin generation. *Circulation*, **85**, 1077-1082. doi:10.1161/01.CIR.85.3.1077

[10] Yamamoto, J., Yamashita, T., Ikarugi, H., Taka, T., Hashimoto, M., Ishii, H., Watanabe, S. and Kovacs, I.B. (2003) Gorog Thrombosis Test: A global *in-vitro* test of platelet function and thrombolysis. *Blood Coagulation & Fibrinolysis*, **14**, 31-39. doi:10.1097/00001721-200301000-00007

[11] Yamamoto, J. and Kovacs I.B. (2003) Shear-induced *in-vitro* haemostasis/thrombosis tests: The benefit of using native blood. *Blood Coagulation & Fibrinolysis*, **14**, 697-702. doi:10.1097/00001721-200312000-00001

[12] Kovacs, I.B., Gorog, D. and Yamamoto, J. (2006) Stimulated spontaneous thrombolysis: A new therapeutic challenge. *Journal of Thrombosis and Thrombolysis*, **21**, 221-227. doi:10.1007/s11239-006-6579-0

[13] Kovacs, I.B. and Yamamoto, J. (2006) Review: Spontaneous thrombolysis. A forgotten determinant of life or death. *Clinical and Applied Thrombosis and Hemostasis*, **12**, 358-363. doi:10.1177/1076029606291410

[14] Ijiri, Y., Miura, M., Hashimoto, M., Fukunaga, C., Watanabe, S., Kubota, A., Oiwa, K., Okuda, T. and Yamamoto, J. (2002) A new model to evaluate the diet-induced prothrombotic status, using He-Ne laser-induced thrombogenesis in the carotid artery of apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. *Blood Coagulation & Fibrinolysis*, **13**, 497-504. doi:10.1097/00001721-200209000-00004

[15] Rao, A.V. and Agarwal, S. (2000) Role of antioxidant lycopene in cancer and heart disease. *Journal of American College of Nutrition*, **19**, 563-569.

[16] Saraf, S., Christopoulos, C., Salha, I.B., Stott, D.J. and Gorog, D.A. (2010) Impaired endogenous thrombolysis in acute coronary syndrome patients predicts cardiovascular death and nonfatal myocardial infarction. *Journal of American College of Cardiology*, **55**, 2107-2115. doi:10.1016/j.jacc.2010.01.033

[17] Gorog, D.A., Yamamoto, J., Saraf, S., Ishii, H., Ijiri, Y., Ikarugi, H., Wellsted, D.M., Mori, M. and Yamori, Y. (2011) First direct comparison of platelet reactivity and thrombolytic status between Japanese and Western volunteers: Possible relationship to the "Japanese paradox". *International Journal of Cardiology*, **152**, 43-48. doi:10.1016/j.ijcard.2010.07.002

