Original Article

Pycnogenol®, French Maritime Pine Bark Extract, Augments Endothelium-Dependent Vasodilation in Humans

Kenji NISHIOKA[1)], Takayuki HIDAKA[2)], Shuji NAKAMURA[2)], Takashi UMEMURA[1)], Daisuke JITSUIKI[1)], Junko SOGA[2)], Chikara GOTO[1)], Kazuaki CHAYAMA[2)], Masao YOSHIZUMI[1)], and Yukihito HIGASHI[1)]

Pycnogenol®, an extract of bark from the French maritime pine, *Pinus pinaster* Ait., consists of a concentrate of water-soluble polyphenols. Pycnogenol® contains the bioflavonoids catechin and taxifolin as well as phenolcarbonic acids. Antioxidants, such as bioflavonoids, enhance endothelial nitric oxide (NO) synthase expression and subsequent NO release from endothelial cells. The purpose of this study was to determine Pycnogenol®'s effects on endothelium-dependent vasodilation in humans. This was a double-blind, randomized, placebo and active drug study. We evaluated forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and to sodium nitroprusside (SNP), an endothelium-independent vasodilator, in healthy young men before and after 2 weeks of daily oral administration of Pycnogenol® (180 mg/day) ($n=8$) or placebo ($n=8$). FBF was measured by using strain-gauge plethysmography. Neither the placebo nor Pycnogenol® altered forearm or systemic hemodynamics. Pycnogenol®, but not placebo, augmented FBF response to ACh, from 13.1±7.0 to 18.5±4.0 mL/min per 100 mL tissue ($p<0.05$). SNP-stimulated vasodilation was similar before and after 2 weeks of treatment in the control and Pycnogenol® groups. The administration of N^G-monomethyl-L-arginine, an NO synthase inhibitor, completely abolished Pycnogenol®-induced augmentation of the FBF response to ACh. These findings suggest that Pycnogenol® augments endothelium-dependent vasodilation by increasing in NO production. Pycnogenol® would be useful for treating various diseases whose pathogeneses involve endothelial dysfunction. (*Hypertens Res* 2007; 30: 775–780)

***Key Words*: endothelial function, nitric oxide, oxidative stress, Pycnogenol®**

Introduction

Epidemiological studies have shown that the consumption of red wine reduces the morbidity of and mortality from cardiovascular diseases in the general population (*1–3*). This concept is called the French paradox that the French eat a lot of fatty foods but have low rates of cardiovascular disease because of their high consumption of red wine. Red wine's cardioprotective effects are due to the antioxidant properties of polyphenols that are also found, though in much smaller concentrations, in fruits, vegetables, chocolate, coffee and tea.

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis, resulting in cardiovascular and cerebrovascular outcomes (*4*). Indeed, several clinical studies have shown that the degree of endothelial dysfunction is a marker of future cardiovascular events in patients with hypertension, coronary artery disease, or peripheral arterial disease

From the [1)]Department of Cardiovascular Physiology and Medicine and [2)]Department of Medicine and Molecular Science, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan.

Address for Reprints: Yukihito Higashi, M.D., Ph.D., Department of Cardiovascular Physiology and Medicine, Hiroshima University Graduate School of Biomedical Sciences, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan. E-mail: yhigashi@hiroshima-u.ac.jp

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Table 1. Clinical Characteristics of the Control and Pycnogenol® Groups

	Control group		Pycnogenol® group	
	Before	After	Before	After
Body mass index (kg/m^2)	23.2±2.1	23.2±2.1	22.4±2.2	22.4±2.2
Systolic blood pressure (mmHg)	115.6±9.8	113.9±9.6	114.2±10.7	112.8±10.2
Diastolic blood pressure (mmHg)	64.3±6.6	65.1±6.7	62.2±6.1	63.2±6.4
Heart rate (bpm)	67.3±4.8	68.1±5.7	69.1±2.5	68.7±2.6
Total cholesterol (mmol/L)	4.55±0.61	4.71±0.72	4.89±0.65	4.82±0.62
Triglyceride (mmol/L)	1.22±0.63	1.25±0.57	1.14±0.72	1.12±0.62
HDL cholesterol (mmol/L)	1.19±0.17	1.20±0.19	1.27±0.15	1.32±0.10
LDL cholesterol (mmol/L)	2.57±0.51	2.61±0.64	2.94±0.34	2.86±0.24
Glucose (mmol/dL)	4.9±1.0	5.0±1.1	5.1±1.2	5.1±1.0
FBF (mL/min per 100 mL tissue)	5.3±1.2	5.4±1.3	4.4±0.9	4.6±0.8
8-OHdG (ng/mL)	8.8±0.6	8.5±0.7	8.4±0.3	8.6±0.4

HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBF, forearm blood flow; 8-OHdG, 8-hydroxy-2′-deoxyguanosine. All results are presenced as mean±SD.

(*5–7*). Therefore, from a clinical perspective, it is important to select an appropriate intervention that improves endothelial dysfunction in patients with cardiovascular diseases or augments endothelial function even in healthy subjects.

Pycnogenol®, isolated from French maritime pine (*Pinus pinaster* Ait.) bark extract, is a mixture of flavonoids, including the bioflavonoids catechin and taxifolin as well as phenolcarbonic acids (*8–11*). Pycnogenol® is thought to inhibit the degradation of nitric oxide (NO) by inhibiting reactive oxygen species (ROS), leading to the augmentation of endothelial function. However, there is little information about the influence of Pycnogenol® on endothelial function in humans.

To determine Pycnogenol®'s effects on endothelial function, we measured the responses of forearm blood flow (FBF) to the endothelium-dependent vasodilator acetylcholine (ACh) and to the endothelium-independent vasodilator sodium nitroprusside (SNP) before and after Pycnogenol® administration.

Methods

Subjects

We studied 16 healthy Japanese men (mean age, 22.4±2.2 years). They had no history of serious disease, and took no medications for at least 4 weeks before the study. The study protocol was approved by the ethics committee of Hiroshima University Graduate School of Biomedical Sciences. Informed consent for participation was obtained from all subjects.

Study Protocol

This was a double-blind, randomized, placebo and active drug study with parallel group design. Sixteen subjects were randomized for 2 weeks of double-blind treatment with either Pycnogenol® ($n=8$, EBS Co., Tokyo, Japan) at a dose of 180 mg or placebo (control group, $n=8$) once daily in the morning. The vasodilatory responses to ACh (Daiichi Pharmaceutical Co., Tokyo, Japan) and SNP (Maruishi Pharmaceutical Co., Tokyo, Japan) in each group were evaluated at the beginning and end of the 2-week follow-up period. The study began at 8:30 AM. Subjects fasted the previous night for at least 12 h. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature 22°C to 25°C) throughout the study. Thirty minutes after maintaining the supine position, basal FBF was measured. The effects of infusion of ACh and SNP on FBF were then measured. ACh infusion was administered at doses of 3.75, 7.5, and 15 μg/min, and SNP infusion was administered at doses of 0.75, 1.5, and 3.0 μg/min. After administration of ACh and SNP, FBF was measured during the last 2 min of the infusion. These studies were carried out in randomized fashion. Each study proceeded after FBF had returned to baseline. In the preliminary study, after infusion of ACh or SNP, FBF returned to baseline within 30 min. Thus, the end of infusion of ACh or SNP was followed by a 30 min recovery period. Baseline fasting serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatinine, insulin, glucose, and electrolytes were obtained after a 30 min rest period.

To examine Pycnogenol®'s effect on the release of NO, we infused N^G-monomethyl-L-arginine (L-NMMA, Clinalfa Co., Läufelfingen, Switzerland), an NO synthase inhibitor, intra-arterially at a dose of 8 μg/min for 5 min while the baseline FBF and arterial blood pressure (BP) were recorded, and administered ACh (3.75, 7.5, and 15 μg/min). The responses of forearm vasculature to ACh after the infusion of L-NMMA were evaluated at the beginning and end of the 2 weeks of Pycnogenol® administration.

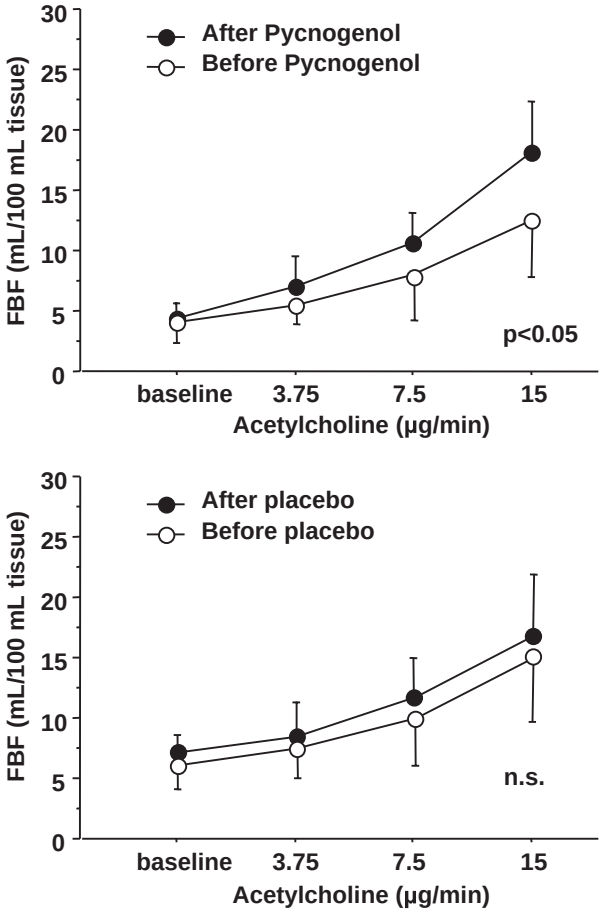

Fig. 1. *The effects of Pycnogenol® or placebo on forearm blood flow (FBF) response to acetylcholine.*

Measurement of FBF

The FBF was measured with a mercury-filled Silastic® strain-gauge plethysmography (EC-5R, D.E. Hokanson, Inc., Issaquah, USA) as previously described (*12*, *13*). Briefly, a strain gauge was attached to the upper left arm, connected to a plethysmograhy, and supported above the right atrium. A wrist cuff was inflated to 50 mmHg above the systolic BP to exclude hand circulation from the measurements taken 1 min before measurement of FBF. To occlude venous outflow from the arm, a rapid cuff inflator (EC-20, D.E. Hokanson, Inc.) was used to inflate the upper-arm–congesting cuff to 40 mmHg for 7 s in each a 15 s cycle. The FBF output signal was transmitted to a recorder (U-228, Advance Co., Nagoya, Japan). FBF was expressed as mL/min per 100 mL of forearm tissue volume. FVR was calculated as the mean BP divided by FBF and was expressed as mmHg/mL/min per 100 mL of forearm tissue volume. Four plethysmographic measurements were averaged to obtain FBF at baseline and after infusion of ACh and SNP. FBF was calculated by two independent observers blinded to the study protocol from the linear portions of plethysmographic recordings. The intraobserver coefficient of variation was 3.0%. We confirmed the reproducibility of FBF responses to ACh and SNP on two separate occasions in 10 healthy males (mean age, 24±4 years). The coefficients of variation were 6.2% and 4.6%, respectively.

Analytical Methods

Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. The EDTA-containing tubes were chilled promptly in an ice bath preceding immediate separation of plasma by centrifugation at 3,100 rpm at 4°C for 10 min. The serum was separated at 1,000 rpm at room temperature for 10 min. The samples were stored at −80°C until assayed. Routine chemical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, triglycerides (TG), creatinine, glucose, and electrolytes. The serum concentration of low-density lipoprotein (LDL) choresterol was determined using Friedewald's method (*14*). The plasma concentration of 8-hydroxy-2′-deoxyguanosin (8-OHdG) was assayed by enzyme-linked immunosorbent assay (ELISA) using 8-OHdG kits (Nihon Yushi Co., Tokyo, Japan).

Statistical Analysis

Results are presented as means±SD. Values of $p<0.05$ were considered significant. The Mann-Whitney *U* test was used to evaluate differences in subjects between before and after treatment. The parameters were compared from before to after treatment with adjusted means by ANCOVA using the baseline date as the covariates. Comparisons of time curves of parameters infusing ACh or SNP were analyzed by two-way ANOVA for repeated measures. The data were processed using the software packages StatView IV (Brainpower, Cary, USA) or Super ANOVA (Abacus Concepts, Berkeley, USA).

Results

Clinical Characteristics

The Table 1 summarizes the baseline clinical characteristics of each group. There were no significant differences in parameters between before and after Pycnogenol® or placebo administration. Neither Pycnogenol® nor placebo altered plasma 8-OHdG levels.

Effect of Pycnogenol® Supplementation on Endothelial Function

The intra-arterial infusion of ACh increased FBF in a dose-dependent manner before and after treatment in the control and Pycnogenol® groups (Fig. 1). Pycnogenol® significantly augmented the FBF response to ACh ($p<0.05$), whereas the placebo did not alter the FBF response to ACh (Fig. 1). The

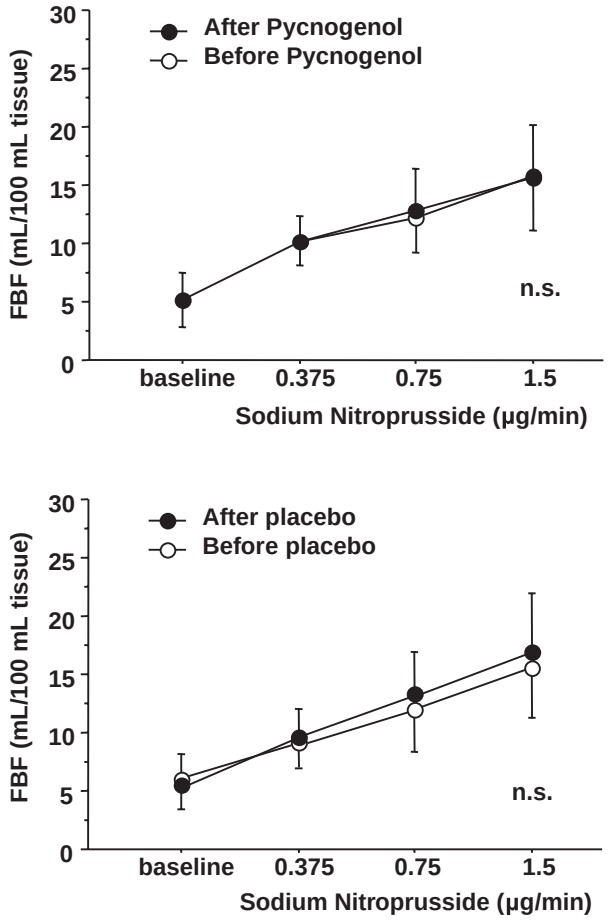

Fig. 2. *The effects of Pycnogenol® or placebo on forearm blood flow (FBF) response to sodium nitroprusside.*

SNP-stimulated vasodilation was similar before and after treatment in each group (Fig. 2). No significant changes were observed in arterial BP or heart rate in any subjects after intra-arterial infusion of either ACh or SNP.

Effect of Pycnogenol® Supplementation on FBF Response to ACh in the Presence of L-NMMA

The intra-arterial infusion of L-NMMA decreased basal FBF significantly, from 6.1±2.3 to 4.3±1.4 mL/min per 100 mL tissue ($p<0.05$) before Pycnogenol® administration and from 5.7±2.0 to 4.2±1.3 mL/min per 100 mL tissue ($p<0.05$). L-NMMA completely abolished the Pycnogenol®-induced augmentation of the FBF response to ACh (Fig. 3). No significant changes in arterial BP or heart rate in any subjects were detected during L-NMMA infusion.

Discussion

In the present study, we demonstrated that 2 weeks of Pycnogenol® administration augmented endothelium-dependent

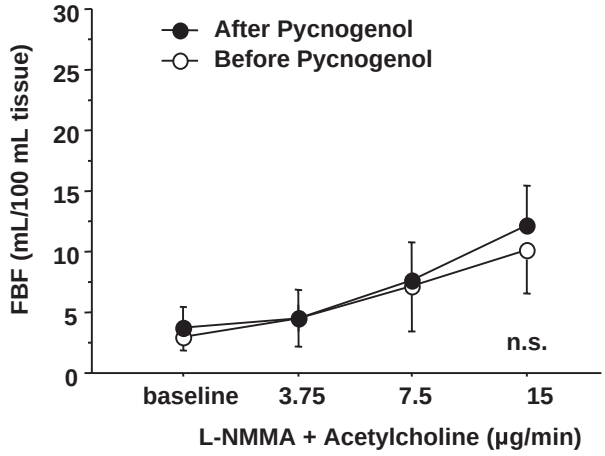

Fig. 3. *The effects of Pycnogenol® on the forearm blood flow (FBF) response to acetylcholine in the presence of N^G-monomethyl-L-arginine (L-NMMA).*

vasodilation but did not alter endothelium-independent vasodilation in healthy young men. Some possible mechanisms by which Pycnogenol® augments endothelial function in humans have been postulated. In the present study, the NO synthase inhibitor L-NMMA substantially inhibited the enhanced response of forearm vasculature to ACh in the Pycnogenol® group, suggesting that the augmentation of NO production is involved in Pycnogenol®-enhanced endothelium-dependent vasorelaxation. Pycnogenol® consists of a concentrate of polyphenols. Antioxidants, such as polyphenols and bioflavonoids, enhance endothelial NO synthase gene expression, resulting in the release of NO from endothelial cells (*15*, *16*). Pycnogenol® may have a direct ability to increase NO production.

A balance between ambient levels of ROS and released NO plays a critical role in the maintenance of endothelial function. In cardiovascular disease, endothelial dysfunction is due, at least in part, to the inactivation of NO by ROS. We recently showed that one mechanism of endothelial dysfunction is an increase in oxidative stress in patients with renovascular hypertension, who are ideal models of excess angiotensin II and the angiotensin II–related increase in oxidative stress (*17*). The levels of endothelium-derived NO production *in vivo* induced by Pycnogenol® should be confirmed. Unfortunately, it is extremely difficult to measure endothelium-derived NO production directly because NO is short-lived *in vivo*. Broere *et al*. (*18*) reported the difficulty of assessing endothelial function by using measurements of NO metabolites, nitrate and nitrite, in healthy young subjects. Recently, Neishi *et al*. (*19*) developed a catheter-type NO sensor to directly measure plasma NO concentration. It has been shown by using an NO sensor that the mechanisms by which angiotensin II impairs endothelial function through increased oxidative stress are both an increase in peroxinitrite production and a decrease in endothelium-derived NO pro-

duction (*20*). The use of a catheter-type NO sensor would enable a more specific conclusion to be drawn about the role of NO bioavailability after Pycnogenol® treatment. Several investigators have shown that Pycnogenol® enhances the antioxidant system and scavenges free radicals (*8–11*, *21–23*). Therefore, we hypothesized that Pycnogenol® augments endothelial function through the scavenging of ROS. However, in the present study, Pycnogenol® did not alter the plasma 8-OHdG level, an index of oxidative stress.

Study Limitations

Previous studies have shown that 8-OHdG is a principal stable marker of hydroxyl radical damage to DNA (*17*, *24*, *25*). The subjects enrolled in those studies included subjects with renal hypertension (*17*, *26*), smokers (*25*), and subjects performing high-intensity exercise (*24*); in all of those studies, the subjects had high levels of oxidative stress. In the present study we measured plasma 8-OHdG levels. It is likely that the measurement of plasma 8-OHdG levels is less sensitive for assessing oxidative stress than is the measurement of urinary excretion of 8-OHdG. The measurement of plasma 8-OHdG levels to assess oxidative stress is not sensitive in young healthy men who have little or no oxidative stress. The measurement of urinary excretion of 8-OHdG may enable us to draw more specific conclusions about the effects of Pycnogenol® on NO inactivation. Further studies are needed to confirm the antioxidant effect of Pycnogenol® on NO bioavailability in patients with high levels of oxidative stress.

Although this was a double-blind, randomized, placebo and active drug study with parallel group design, the number of subjects was small.

In conclusion, these findings suggest that Pycnogenol® augments endothelium-dependent vasodilation through an increase in NO production. It is expected that Pycnogenol® will be useful for the treatment of various diseases in which oxidative stress is involved in the pathogenesis.

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