A Rice Variety (HYJA-Ri4) Enhances Arterial Thrombus Formation in an Animal Model of Thrombosis

Aki Naemura^a Yoshinobu Ijiri^b Mitsuhiro Kodama^c Yoshinori Honda^c
Junichiro Yamamoto^a

^aLaboratory of Physiology, Faculty of Nutrition and Cooperative Research Center of Life Sciences, Kobe Gakuin University, Kobe, ^bDepartment of Food Nutritional Science, Faculty of Liberal Arts, Osaka Shoin Women's University, Higashiosaka, and ^cHyogo Rokko Agricultural Cooperatives, Hyogo, Japan

Key Words

Atherosclerosis • Cardiovascular disease • Diet • Rice • Stroke • Whole grain

Abstract

Epidemiological evidence exists for a causative role of inappropriate diets in the development and clinical outcome of thrombotic diseases. Our previous studies have shown that some fruits and vegetables have antithrombotic activity. As rice is a principal ingredient of everyday food in Japan, the aim of the present study was to determine rice varieties with antithrombotic effect. Western-style high-fat diet containing flour prepared from whole-grain rice of different varieties was fed to atherosclerosis-prone (apolipoprotein E- and low density lipoprotein receptor-deficient) mice for 3 months. A laser-induced carotid-artery thrombosis model was used. None of the five tested rice varieties showed an antithrombotic effect. The effect of HYJA-Ri1, HYJA-Ri2, HYJA-Ri3 and HYJA-Ri5 rice on experimental thrombus formation did not differ significantly from the controls. However, HYJA-Ri4 rice significantly enhanced the thrombotic reaction ($p = 0.0078$), thus having a prothrombotic effect. Our findings suggest that HYJA-Ri4 rice should not be included in an antithrombotic diet.

Copyright © 2009 S. Karger AG, Basel

Introduction

Prevention of lifestyle-related atherothrombotic diseases such as myocardial infarction and stroke is an important and urgent social task in many countries. Epidemiological studies have provided evidence for the causative role of inappropriate diets in the development and clinical outcome of thrombotic diseases [1–3].

Mortality from cardiovascular diseases has been found to be significantly lower in the French population, who otherwise consume a high-fat diet, than in other countries where a similarly high-fat diet is consumed [4]. It was suggested that the French's habitual and large amount of red wine consumption was responsible for the lower cardiovascular mortality [5]. The so-called 'French paradox' stimulated experimental studies for finding antithrombotic fruits, vegetables, cereals and other foods.

A specific variety of grapes and grape juice (Welch's natural purple grape juice) showed particularly significant antithrombotic effect [6]. Juice made from a specified raw onion also showed antithrombotic activity [7]. In contrast to Welch's grape juice, orange juice purchased from the market and grapefruit juice prepared without special attention to the variety had no significant anti-

thrombotic effect [8]. We have screened vegetables and fruits for antithrombotic activity by paying special attention to their varieties and demonstrated that antithrombotic activity differs from variety to variety in tomato, onion, strawberry, mulberry, carrot and potato [9–15].

The aim of the present study was to screen rice varieties for antithrombotic activity after long-term intake of flour prepared from the tested rice varieties.

Materials and Methods

Rice

Non-glutinous rice varieties (HYJA-Ri1, HYJA-Ri2, HYJA-Ri3, HYJA-Ri4 and HYJA-Ri5) were planted in May or June and harvested in the paddy in the Kande-cho area, Kobe, Japan, in October 2004. Whole-grain white rice was stored at 4°C until use and ground just before the experiments.

Animals

Double-homozygous apolipoprotein E- and low-density lipoprotein receptor-deficient mice (DK mice, 129 × C57BL/6J background) were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA) and bred in the Animal Units of the Kobe Gakuin University. Mice were kept in air-conditioned rooms (22.5 ± 0.5°C and 50 ± 5% humidity) with 12-hour light-dark cycles. Animals had free access to diet and were fasted overnight before the test. Drinking water was available ad libitum. Animals were kept in compliance with previously established guidelines published by the Physiological Society of Japan [16] and the experiments were approved by the Animal Experiment Committee of the Kobe Gakuin University.

Diets

Diets were prepared from the different varieties of whole-grain rice flour and purified materials on the basis of the Western-style high-fat diet model as described previously [17]. Whole-grain flour was used as a carbohydrate source and the contents of protein, oil and dietary fiber in the diets were adjusted using the Standard Tables of Food Composition in Japan [18]. Diets were stored in a freezer at –30°C until use. The DK mice were conditioned with a commercial solid diet (CE-2; Clea Japan, Tokyo, Japan) and kept on the test diets (table 1) from the age of 6 weeks for 3 months.

Laser-Induced Mouse Carotid-Artery Thrombosis Model

The He-Ne laser-induced thrombosis method has already been described in detail [17, 19]. Briefly, a polyethylene tube was inserted into the left femoral artery of Nembutal-anesthetized mice, and the carotid artery on one side was exposed. The mouse was placed on a special microscope stage. Evans blue dye (30 mg/kg) was injected intra-arterially, and the center of the exposed carotid artery was irradiated with a laser beam. Thrombus formation at the site of irradiation was monitored under epi-illumination and simultaneously recorded on videotape using a CCD camera. The coefficient of variation of this test was 17–28% [17].

Table 1. Ingredients of rice and control diets

Ingredients	Control diet g	Rice diet g
Rice powder	0.0	39.7
Casein	23.2	20.3
Cystine	0.3	0.3
Corn starch	36.2	0.0
Sucrose	9.95	9.95
Soy oil	3.0	2.6
Butter	7.5	7.5
Beef tallow	10.0	10.0
Cellulose	5.0	4.8
Mineral mix	3.5	3.5
Vitamin mix	1.0	1.0
Choline bitartrate	0.25	0.25
Tert-butylhydroquinone	0.0041	0.0041
Cholesterol	0.05	0.05
Total	100.0	100.0

The calories of control and rice diet were 1,837 and 1,820 kJ/100 g diet, respectively.

Calculation of Thrombus Size

A computer image of the forming thrombus was taken every 10 s. The thrombus mass was delineated on the screen and the size was determined. Image analysis was performed using Image J software (Image Processing and Analysis Java version 1.30; National Institutes of Health, Bethesda, Md., USA). As an occlusive thrombus cannot form in a big artery with high flow, the size of the thrombus increased and decreased periodically reflecting embolization. The thrombotic status was defined by the total sum of thrombus sizes obtained every 10 s for 10 min from the start of irradiation. An increase or decrease in the total sum indicated increased or decreased thrombotic status, respectively.

Statistical Analysis

Results were analyzed by ANOVA, followed by Dunnett's post hoc test using the commercially available Unistat Statistical Package (version 5.5.09 Light; Unistat, London, UK). Values were expressed as means ± SEM. $p < 0.05$ was considered the limit of significance.

Results

Body Weight and Dietary Intake

Changes in body weight over the period of feeding are shown in figure 1. Dietary intake was similar in all groups. Body weights in the rice diet groups were slightly higher than those of the controls, but the difference was only significant in mice on the HYJA-Ri3 diet.

Fig. 1. Change in body weight in mice fed different rice variety diets. Means $\pm$ SEM. * $p < 0.05$; $n = 9$ – 10 /group.

Fig. 2. Effect of rice varieties on thrombogenicity measured by a He-Ne laser-induced mouse carotid artery thrombosis test after intake of Western-style diet containing the flours from different rice varieties for 3 months. $n = 8$ – 10 /group. Means $\pm$ SEM.

Effects of Rice Diets on Thrombotic Risk

Results are shown in figure 2. Compared to the control diet, feeding mice with HYJA-Ri1, HYJA-Ri2 and HYJA-Ri5 rice did not significantly change the thrombotic response to laser irradiation. Diet containing HYJA-Ri3 slightly but not significantly enhanced the thrombotic reaction while in mice on HYJA-Ri4 the thrombotic reaction was significantly ($p = 0.0078$) enhanced.

Discussion

In this study, we employed the He-Ne laser-induced thrombosis animal model, which is a close approximation of the pathological process of arterial thrombosis in humans. This test is based on the principle that irradiation of an artery with laser selectively damages the endothelium and results in the formation of platelet-rich thrombi [17, 19].

Rodents are known to be resistant to experimental atherothrombosis. C57BL/6J mice are comparatively sensitive to diet, and a high-fat diet can induce fatty streak lesions but not atherothrombosis [20]. The pathogenesis of atherosclerotic lesions in the arteries of apolipoprotein E-deficient mice resembles that of humans, and especially homozygous animals are more sensitive to diets than the heterozygous or wild-type C57BL/6 mice [21]. Earlier, we found that apolipoprotein E and low-density

lipoprotein receptor double-deficient mice are sensitive to diets and therefore a suitable model for assessing the thrombotic risk. Laser irradiation of the artery of such animals resulted in an intensive, measurable thrombotic reaction [17, 22].

The current experiments using diet-sensitive mice showed that the thrombotic reaction in mice fed with HYJA-Ri1; HYJA-Ri2 and HYJA-Ri5 rice did not differ significantly from that observed in mice on the control diet. In animals kept on a diet containing HYJA-Ri3, the thrombotic reaction was slightly, but not significantly intensified. In mice kept on a diet containing HYJA-Ri4, the laser-induced thrombotic reaction was significantly enhanced. Accordingly, this rice variety was considered prothrombotic.

There are epidemiological findings that whole-grain intake is associated with a reduced risk of atherothrombotic disease [23–25]. Whole-grain intake is also recommended for the prevention of metabolic syndromes [26]. Animal experiments have suggested that the anti-atherogenic effect may be due to antioxidants in the outer layer of colored rice [27, 28]. Rice proteins rich in arginine and poor in methionine might also be involved in the anti-atherogenic effect [29].

The current control high-fat diet contains nutritionally minimal requirements. The nature of chemical(s) responsible for the prothrombotic properties of the RYJA-Ri4 variety is unclear and requires further investigation.

Rice diet enhanced growth compared with the controls. This tendency to increased growth (statistically not significant) went hand in hand with the enhanced thrombogenicity. Fast growth or fast accumulation of fat might contribute to the prothrombotic property.

In conclusion, out of the five tested rice varieties, none showed an antithrombotic effect. The effect of four varieties on the thrombotic reaction did not differ significantly from the controls while one variety (HYJA-Ri4) significantly intensified the thrombotic reaction and was considered having a prothrombotic effect.

References

- 1 Vinereanu D: Risk factors for atherosclerotic disease: present and future. *Herz* 2006;31 (suppl 3):5–24.
- 2 Gállego J, Martínez Vila E, Muñoz R: Patients at high risk for ischemic stroke: identification and actions. *Cerebrovasc Dis* 2007; 24(suppl 1):49–63.
- 3 Lutsey PL, Steffen LM, Stevens J: Dietary intake and the development of the metabolic syndrome: the Atherosclerosis Risk in Communities study. *Circulation* 2008;117:754–761.
- 4 Ulbricht TL, Southgate DA: Coronary heart disease: seven dietary factors. *Lancet* 1991; 338:985–992.
- 5 Renaud S, De Lorgeril M: Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 1992;339:1523–1526.
- 6 Demrow HS, Slane PR, Folts JD: Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation* 1995;91:1182–1188.
- 7 Briggs WH, Folts JD, Osman HE, Goldman IL: Administration of raw onion inhibits platelet-mediated thrombosis in dogs. *J Nutr* 2001;131:2619–2622.
- 8 Keevil JG, Osman HE, Reed JD, Folts JD: Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J Nutr* 2000;130:53–56.
- 9 Yamamoto J, Taka T, Yamada K, Ijiri Y, Murakami M, Hirata Y, et al: Tomatoes have natural antithrombotic effects. *Br J Nutr* 2003; 90:1031–1038.
- 10 Yamada K, Naemura A, Sawashita N, Noguchi Y, Yamamoto J: An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis. *Thromb Res* 2004;114:213–220.
- 11 Naemura A, Mitani T, Ijiri Y, Tamura Y, Yamashita T, Yamamoto J: Antithrombotic effect of strawberries. *Blood Coagul Fibrinolysis* 2005;16:501–509.
- 12 Naemura A, Ohira H, Ikeda M, Koshikawa K, Ishii H, Yamamoto J: An experimentally antithrombotic strawberry variety is also effective in humans. *Pathophysiol Haemost Thromb* 2006;35:398–404.
- 13 Yamamoto J, Naemura A, Ura M, Ijiri Y, Yamashita T, Kurioka A, Koyama A: Testing various fruits for antithrombotic effect: I. Mulberries. *Platelets* 2006;17:555–564.
- 14 Yamamoto J, Naemura A, Ijiri Y, Ogawa K, Suzuki T, Shimada Y, Giddings JC: The antithrombotic effects of carrot filtrates in rats and mice. *Blood Coagul Fibrinolysis* 2008; 19:785–792.
- 15 Ichiura D, Naemura A, Ura M, Mori M, Yamamoto J: Antithrombotic effect of potato in animal experiments. *Food* 2009;3:8–12.
- 16 Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences. *J Physiol Soc Jpn* 2004;66:x-xiii.
- 17 Ijiri Y, Miura M, Hashimoto M, Fukunaga C, Watanabe S, Kubota A, et al: A new model to evaluate the diet-induced prothrombotic state, using He-Ne laser-induced thrombogenesis in the carotid artery of apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. *Blood Coagul Fibrinolysis* 2002;13:497–504.
- 18 Resources Council, Science and Technology Agency: Report of the Resources Survey Committee, Science and Technology Agency, Tokyo, Japan. *Standard Tables of Food Composition in Japan (in Japanese)*, ed 4. Tokyo, Printing Bureau, Ministry of Finance, 1982, vol 87, pp 38–291.
- 19 Kovacs IB, Tigyi-Sebes A, Trombitas K, Gorog P: Evans blue: an ideal energy-absorbing material to produce intravascular microinjury by He-Ne gas laser. *Microvasc Res* 1975;10:107–124.
- 20 Nishina PM, Lowe S, Verstuyf J, Naggert JK, Kuypers FA, Paigen B: Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J Lipid Res* 1993;34:1413–1422.
- 21 Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R: ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb* 1994;14:133–140.
- 22 Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest* 1993;92:883–893.
- 23 Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, et al: Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* 1999;70:412–419.
- 24 Steffen LM, Jacobs DR Jr, Stevens J, Shahar E, Carithers T, Folsom AR: Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* 2003;78:383–390.
- 25 Sahyoun NR, Jacques PF, Zhang XL, Juan W, McKeown NM: Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. *Am J Clin Nutr* 2006;83:124–131.
- 26 Newby PK, Maras J, Bakun P, Muller D, Ferrucci L, Tucker KL: Intake of whole grains, refined grains, and cereal fiber measured with 7-d diet records and associations with risk factors for chronic disease. *Am J Clin Nutr* 2007;86:1745–1753.
- 27 Ling WH, Cheng QX, Ma J, Wang T: Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *J Nutr* 2001;131:1421–1426.
- 28 Ling WH, Wang LL, Ma J: Supplementation of the black rice outer layer fraction to rabbits decreases atherosclerotic plaque formation and increases antioxidant status. *J Nutr* 2002;132:20–26.
- 29 Ni W, Tsuda Y, Takashima S, Sato H, Sato M, Imaizumi K: Anti-atherogenic effect of soya and rice-protein isolate, compared with casein, in apolipoprotein E-deficient mice. *Br J Nutr* 2003;90:13–20.