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ANIMAL AND HUMAN STUDIES ON THE ROLE OF SOYBEAN, RICE AND NUTS CONSUMPTION IN HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS

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Abstract

Soybean, rice and nuts are the staple foods in South East Asia. In order to clarify the function of these foods in relation to coronary heart diseases, we have done a series of experiments using animals and humans. Soybean protein preparations (SPI) in comparison with an animal protein, casein (CAS), resulted in reducing atherosclerotic lesion development in apolipoprotein (apo) E deficient mice that develop an advanced lesion similar to that in humans. This action was attributed to the protein, since the ethanol-extracted SPI (EE-SPI), from which isoflavones and saponins removed, lowered the lesion development in apo E-deficient mice. However, The EE-SPI, compared to the SPI, resulted in a decrease of mRNA for key proteins of cholesterol metabolism (low density lipoprotein receptor and cholesterol 7α-hydroxylase) in hypercholesterolemic rats, followed by an elevation of the serum cholesterol level, indicating a contribution of isoflavones to the serum cholesterol level. Rice protein (RPI) was also effective to lower the lesion development in apo E deficient mice. Both the SPI and RPI led to an increased level of the serum NO_2/NO_3, metabolites of NO. This effect appeared to be attributed to their high content of arginine. Besides, C57BL/6J mice fed a diet containing whole grain rice had an elevation of the serum high-density lipoprotein cholesterol. Furthermore, rice bran oil and the unsaponifiable fraction that is rich in plant sterols were effective to lower serum cholesterol levels in hypercholeserolemic rats. Finally, we tested if walnuts consumption would be hypocholesterolemic for Japanese. A diet containing walnuts effectively lowered the serum total and low density lipoprotein cholesterol in Japanese men and women. These animal and human experiments would be relevant to advocate publics to keep consuming diets including soybean, rice and nuts for their healthful life.

Key words: soy protein, rice protein, walnuts, hypercholesterolemia, atherosclerosis

INTRODUCTION

Soybean, rice and nuts are staple foods in South East Asia. Comparative studies between Japanese and Japanese-Americans indicate that food habits strongly influence the prevalence of coronary heart diseases, indicating that environmental factors, together with a genetic background, are intimately involved in the onset of these diseases (1). Nevertheless, food habits in Japan and South East Asia are getting modernized. In particular, rice consumption in Japan was getting decreased during the last 50 years (2). In this context, it is important to demonstrate a role of these traditional foods in risk reduction for coronary heart diseases, thereby advocating healthful diets to publics. Alternatively, compared with soybean and rice, consumption of nuts in Japan has not been so popular, but the amounts imported from overseas are getting increased. Therefore, it appears to be worthwhile to pursue their role in healthful diet. In order to clarify the physiological function of these foods, we have done a series of experiments in animals and humans.

MATERIALS AND METHODS

Animal studies

Experimental animals: The following animals – apolipoprotein (apo E)-deficient mice, C57BL/6J mice, exogenously hypercholesterolemic (ExHC) rats and SD rats – were used for this study. These experiments were carried out under the guidelines for Animal Experiment in the Faculty of Agriculture and the Graduate Course, Kyushu University, Fukuoka, Japan and the Law (No. 105) and Notification (No. 6) of the Government of Japan.

Diets: The animals were fed the AIN-93G based-purified diet (3), which was supplemented the following ingredients – soy protein isolate (SPI, Fuji Oil Co., Osaka, Japan), ethanol-extracted SPI (EE-SPI), rice protein isolate (RPI) prepared by the method of Morita & Kiriyama (4), arginine, rice grain powder, rice bran oil and sterols-rich fraction prepared from rice bran oil. More detailed information is described in the Result and Discussion section.

Analyses: Serum and liver lipids, aortic lesions and mRNAs for low density lipoprotein (LDL) receptor, cholesterol 7 -hydroxylase, and apo A-IV, B and E were determined as described (5-7). Serum nitric oxide (NO) metabolites and urinary isoprostanes were determined by commercially available kits. Serum autoantibody titers against oxidized LDL or aldehyde-modified albumin were determined by the method developed by us (Iwamoto et al, Biosci. Biochem. Biotechnol., in press).

Human studies

Subjects and experimental design: Twenty Japanese women and 20 men participated and completed the experiments. A controlled, single-blind, crossover design was used. All subjects consumed the average Japanese diet (reference diet) during a 5-day run-in period, according to the dietary allowance for 20 to 39 years olds based on the fifth edition of the Recommended Dietary Allowance in Japan (8). This phase was followed by an 8-week experimental period in which they followed each of two consecutive diets for 4 week. One group consumed the walnut diet during the first period and reference diet during second period; the other group followed the diets in reverse order. The study protocol was approved by the University Hospital, Faculty of Medicine, Kyushu University, in accordance with the Helsinki Declaration of 1975 as revised in 1983 and 2000, and all subjects gave informed consent.

Diets: During the 61 day of the study, they received all of their meals at the nutrition-research kitchen of the university. Daily menus were formulated so that there were two levels of energy intake, ranging from 10.0 to 11.1 MJ per day for men, and from 8.37 to 9.20 MJ per day for women. The experimental diets were identical except that the walnut diet substituted two servings of walnuts per day (25 or 27 g per serving, or 52 g of walnuts per 10.0 MJ) for portions of some foods in the reference diet. The walnuts were provided by the California Walnut Commission (Sacrament, CA, USA). Walnuts were served in several ways; mixed in salads or cooked in dinner entrees. Walnuts contributed 50, 8.5 and 12.6 % of the total fat, protein and fiber, respectively, of the walnut diet.

Measurements: Blood lipids were measured by the commercially available kit. Serum apo A-I and B were determined by turbidimetric assay. Blood pressure was measured with a random-zero sphygmomanometer.

Statistical analyses: Statistical analyses included two-tailed *t* test, ANOVA or Duncan's new multiple range test.

RESULTS AND DISCUSSION

Antiatherogenic effect of dietary SPI and RPI in apo E-deficient mice

Apo E-deficient mice were fed the AIN-93G diet containing 20% CAS, SPI and RPI for 9 week. The CAS-containing diet resulted in an increased atherosclerotic lesion area in the abdominal aorta than did the SPI- or RPI-containing diet. The major component of SPI is protein, but it also includes other components such as lipids, saponins and isoflavones. In the subsequent study, we fed the mice

on the diet containing EE-SPI that was treated with ethanol to remove isoflavones and saponins from SPI. The mice fed the EE-SPI lowered the lesion volume of the aortic valve to the extent similar to those fed the intact SPI. These results indicate an active role of the protein in preventing the lesion development. However, it remains a possibility that isoflavones might alleviate the lesion development in ovariectomized animals or postmenopausal women.

Active component of SPI and RPI that lowers the lesion development

Both SPI and RPI include higher proportion of arginine and lower proportion of methionine than did CAS (Arginine: 7.4, 8.1 and 3.5% for SPI, RPI and CAS. Methionine: 1.4, 2.2 and 2.8% for SPI, RPI and CAS). Based on the content of arginine or methionine, we prepared CAS-based diet that was supplemented arginine to the amount equivalent to that in SPI, or SPI-based diet that supplemented methionine to the amount equivalent to that in CAS. The arginine-supplemented group, in comparison with the CAS group, lowered the lesion volume, but stile had greater lesion volume than did the SPI group. The methionine supplementation did not result in significant effect on the lesion development.

Mechanism of antiatherogenic effect of dietary SPI and RPI

Cholesterol: In accordance with our previous study (5), dietary SPI or RPI had no significant effect on the level of the serum total and high density lipoprotein (HDL) cholesterol in apo E-deficient mice.

Nitric oxide: Arginine is the precursor of NO that may exert beneficial effect on preventing the initiation and development of atherosclerosis (9). We first confirmed in the following experiment that adequate NO synthesis is prerequisite for preventing apo E-deficient mice from developing the lesion development. The mice administered N^{w}-nitro-L-argininemethylurea (L-NAME) that is the inhibitor for NO synthesis had a decreased level of serum NO_2/NO_3 (metabolites of NO), accompanied with an increased lesion volume of the aortic valve. Subsequently, we measured the serum NO metabolites in apo E-deficient mice fed the diet containing CAS, SPI, RPI and amino acids (arginine, methionine). Excess consumption of methionine has been reported to result in more homocystine, which would lead to the development of atherosclerotic lesion (10). Both the SPI and RPI groups had an elevation of the serum NO_2/NO_3 than did the CAS group. The mice fed the arginine-supplemented CAS diet, in comparison with the CAS group, increased the metabolites, but the level was lower than that in the SPI or RPI group. The methionine supplementation had no effect on the NO_2/NO_3 level. The mice fed the arginine-supplemented diet had as much serum free

arginine level as the SPI or RPI group. These results indicate that dietary arginine in a form of SPI is superior to the free form for NO production.

Oxidative stress: Apo E-deficient mice have been reported to contain a measurable level of autoantibodies against oxidized LDL or aldehyde modified albumin (11) and isoprostanes in urines (12): that are considered to be potential markers of oxidant stress in atherosclerotic diseases. The mice fed the arginine-supplemented CAS diet, in comparison with the CAS group, had a comparable level of the serum autoantibody titers against oxidized LDL and urinary isoprostanes. However, the arginine-supplemented group had a lower level of autoantibody titer against aldehyde-modified albumin, indicating that arginine derived from free form or SPI can alleviate oxidative stress through NO-dependent and/or NO-independent action (9).

Role of soybean and rice in serum cholesterol level

Since serum cholesterol (LDL and HDL) level is one of the most reliable biomarkers for coronary heart diseases, presenting publics information on food components that affect the level in an appropriate animal model stile has a considerable value.

Soybean isoflavones: Previous studies including us repeatedly showed hypocholesterolemic action of SPI in animals (13) and humans (14). Since commercially available SPI preparations include isoflavones, a group of phytoestrogens (5), we fed ExHC rats, which were isolated from the SD strain and sensitive to dietary cholesterol (15), on the diet containing SPI or EE-SPI for 2 week. The EE-SPI group had higher level of the serum cholesterol than did the SPI group. The former group lowered the hepatic abundance of mRNA for LDL receptor and cholesterol 7 -hydroxylase. Furthermore, the rats fed the diet containing both CAS and isoflavone-rich fraction prepared from soybean germ had a lower level of the serum cholesterol than did the CAS group, but the level was stile higher than the SPI group. These results indicate that hypocholesterolemic action of SPI was attributed to the protein and, to some extent, the other components of SPI.

Whole rice and rice bran oil (RBI): It is not known if the whole rice consumption influences the level of serum cholesterol in animals and humans. We prepared a AIN-93G-based diet containing powdered rice grain at the level of 0, 20 and 40%, and fed C57BL/6J mice on the diet for 8 week. The rice supplementation resulted in an increased body weight. Alternatively, the rice-fed mice had an increased level of the serum HDL cholesterol, accompanied without alteration of the LDL and very low density lipoprotein cholesterol level. Interestingly, the serum glucose level was lowered when rich supplemented at 20% level. Subsequently, we evaluated again the effect of rice bran oil on the

serum cholesterol concentration in ExHC rats (7). We confirmed the hypocholesterolemic action of rice bran oil and the active principle was the plant sterols.

Walnuts: Research reported from Western society suggests that consumption of nuts – almonds, brazilnuts, cashews, hazelnuts, macadamia, peanuts, pecans, pistachios, and walnuts – is negatively correlated to the incidence of coronary heart diseases (16). This beneficial effect is in part attributed to their cholesterol lowering action. Accordingly, we tested if it would be true for Japanese. We chose walnuts since the consumption is gradually increasing in Japan. A diet containing walnuts effectively lowered serum total (3.8 and 4.9% for men and women, respectively) and LDL (8.9 and 10.6% for men and women, respectively) cholesterol in Japanese. This hypocholesterolemic effect appeared to be attributed to the relatively high content of α-linolenic acid in the oil fraction. However, female apo E-deficient mice fed a diet containing walnut oil rather had an increased lesion volume in the aortic valve than those fed a diet containing linoleic acid-rich safflower oil, suggesting that the beneficial effect of walnuts appears not to be limited to the content of α-linolenic acid. Accordingly, the value of walnuts in healthful diet seems to reside in when they are consumed as a whole.

Possible biomarkers for coronary aterosclerosis

According to the definition by FDA (1999; http://www.cfsan.fda.gov/~dms/guidance.html#lab.), a biomarker is a measurement of a variable related to a disease that may serve as an indicator or predictor of that disease. This is the case for serum cholesterol in that high levels are generally accepted as a predictor of risk for coronary heart disease. In the present study, the experiments with using apo E-deficient mice allowed us to raise biomarkers other than cholesterol for coronary heart disease, because there was a positive or negative correlation between the severity of arterial lesion and HDL-cholesterol ($r = -0.5$), urinary isoprostanes ($r = 0.33$), serum NO metabolites ($r = -0.48$) or autoantibody titer against aldehyde-modified albumin ($r = 0.38$). Therefore, further studies are required for establishing these variables as a biomarker for life style-related disease, in particular coronary heart disease.

In conclusion, these animal and human experiments confirm that diets including soybean, rice and nuts (walnuts) provide healthful diet to publics.

References

1. Marmot, M.G., Syme, S.I., Kagan A., Kato, H., Cohen, J.B., and Belsky, J. : Epidemiological

studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Prevalence of coronary and hypertensive heart disease and associated risk factors, Am. J. Epidemiol. 102, 514-525 (1975)

2. National Nutrition Survey of Japan : Annual Report: Health Service Bureau. Ministry of Health and Welfare, Daiichi Shuppan, Tokyo (2000)
3. Reeves, P.G., Nielsen, F.H., and Fahey, G.C.Jr. : AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, J. Nutr. 123, 1939-1951 (1993)
4. Morita, T., and Kiriyama, S. : Mass production method for rice protein isolate and nutritional evaluation, J Food Sci. 58: 1393-1396 (1993)
5. Ni, W., Tsuda, Y., Sakono, M., and Imaizumi, K. : Dietary soy protein isolate, compared with casein, reduces atherosclerotic lesion area in apolipoprotein E-deficient mice, J. Nutr. 128: 1884-1889 (1998)
6. Ni, W., Yoshida, S., Tsuda, Y., Nagao, K., Sato, M., and Imaizumi, K. : Ethanol extracted soy protein isolate results in elevation of serum cholesterol in exogenously hypercholesterolemic rats, Lipids 34: 713-716 (1999)
7. Nagao, K., Sato, M., Takenaka, M., Ando, M., Iwamoto, M., and Imaizumi, K. : Feeding unsaponifiable compounds from rice bran oil does not alter hepatic mRNA abundance for cholesterol metabolism-related proteins in hypercholesterolemic rats, Biosci. Biotechnol. Biochem. 65: 371-377 (2001)
8. Recommended Dietary Allowance for the Japanese : 5th Revision, Health Service Bureau, Ministry of Health and Welfare, Japan, Daiich Shuppan, Tokyo (1995)
9. Wu, G., and Meininger, C.J. : Arginine nutrition and cardiovascular function, J. Nutr. 130: 2626-2629 (2000)
10. Duell, P.B., and Malinow, M.R. : Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. Curr. Opin. Lipidol. 8: 28-34 (1997)
11. Palinski, W., Ord, V.A., Plump, A.S., Breslow, J.L., Steinberg, D., and Witztum, J.L. : Apo E-deficient mice are a model of lipoprotein oxidation in atherogenesis: demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum, Arterioscler. thromb. 14: 605-616 (1994)
12. Praticò, D., Tangirala, R.T., Rader, D.J., Rokach, J., and FitzGerald, G.A. : Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in apo E-deficient mice, Nature Med.

4: 1189-1192 (1998)

13. Sakono, M., Fukuyama, T., Ni, W., Nagao, K., Ju, H.R.., Sato, M., Sakata, N., Iwamoto, H., and Imaizumi, K. Comparison between dietary soybean protein and casein of the inhibiting effect on atherogenesis in the thoracic aorta of hypercholesterolemic (ExHC) rats treated with experimental hypervitamin D. Biosci. Biotechnol. Biochem. 61: 514-519 (1997)
14. Anderson, J.W., Johnstone, B.M., and Cook-Newwell, M.E. : Meta-analysis of the effects of soy protein intake on serum lipids, N. Engl. J. Med. 333: 276-282 (1995)
15. Imaizumi, K., Nagatomi, A., Sato, M., Tominaga, A., and Sugano, M. : Cholesterol metabolism in ExHC (exogenously hypercholesterolemic) rats, Biochim. Biophys. Acta 1123: 101-109 (1992).
16. Dreher, M.L., and Maher, C.V. : The traditional and emerging role of nuts in healthful diets, Nutr. Rev. 54: 241-240 (1996)