Jpn J Clin Oncol 2004;34(2)86–89

Comparisons of Percent Equol Producers between Prostate Cancer Patients and Controls: Case-controlled Studies of Isoflavones in Japanese, Korean and American Residents

Hideyuki Akaza[1], Naoto Miyanaga[1], Naomi Takashima[1], Seiji Naito[2], Yoshihiko Hirao[3], Taiji Tsukamoto[4], Tomoaki Fujioka[5], Mitsuru Mori[6], Wun-Jae Kim[7], Jae Mann Song[8] and Allan J. Pantuck[9]

[1]Department of Urology, University of Tsukuba, Tsukuba, Ibaraki, [2]Department of Urology, University of Kyushu, Fukuoka, [3]Department of Urology, Nara Medical School, Kashihara, Nara, [4]Department of Urology, Sapporo Medical University, School of Medicine, Sapporo, [5]Department of Urology, Iwate Medical University, School of Medicine, Morioka, [6]Department of Epidemiology, Sapporo Medical University, Hokkaido, Japan, [7]Department of Urology, Chungbuk National University Hospital, Chungbuk, [8]Department of Urology, Wonju Medical School, Yonsei University, Wonju, Korea and [9]Department of Urology, UCLA School of Medicine, Los Angeles, USA

Received November 5, 2003; accepted December 24, 2003

Background: Our previous case-control study revealed that the Japanese residents in Japan could be divided into those who are able to degrade daidzein, a soybean isoflavone, to equol and those without this ability, and that the incidence of prostate cancer is higher in the latter group.

Methods: We recently conducted a similar case-control study involving not only Japanese residents in Japan but also Korean residents in Korea. The incidence of prostate cancer in Korean residents is known to be close to that of Japanese residents in Japan. On the other hand, American residents in the United States have a markedly higher incidence of prostate cancer as compared to Japanese residents in Japan.

Results: The number of subjects was 295 in Japan (133 patients and 162 controls), 122 in Korea (61 patients and 61 controls) and 45 in the United States (24 patients and 21 controls). The percentage of equol producers among patients and controls was 29% and 46% in Japan ($P = 0.004$) and 30% and 59% in Korea ($P = 0.001$), respectively. The active isoflavone level was markedly lower and the percentage of equol producers was also lower (17% for patients and 14% for controls) for Americans as compared to the Japanese and Koreans.

Conclusions: These results suggest that the ability of producing equol or equol itself is closely related to the lower incidence of prostate cancer. The results also suggest that a diet based on soybean isoflavones will be useful in preventing prostate cancer.

Key words: prostate cancer – isoflavones – equol producer – genistein – daidzein – cancer prevention

INTRODUCTION

A few investigators have reported that soybean isoflavones might play a significant role in the suppression of prostate cancer (1). In a previous study, we compared the serum levels of soybean isoflavones (genistein, daidzein and equol) between Japanese patients with prostate cancer and healthy Japanese volunteers (2). The results suggested that serum genistein and daidzein levels might reflect the dietary habits of individuals to some extent; however, these levels were largely affected by the foods ingested during the latest one-week period and did not specifically reflect the individuals' dietary habits. We also found that some individuals were able to degrade daidzein into equol and others were not. This ability was not related to the blood genistein or daidzein levels. It was also found that the percentage of daidzein metabolizers was significantly lower among patients with prostate cancer.

It is known that the incidence of prostate cancer that can be clinically detected is lower for Japanese, Koreans and other Asians than for Americans and Europeans (3). In the present study, we focused on individuals who were able to degrade daidzein into equol (hereinafter called 'equol producers' rather than 'daidzein metabolizers,' as referred to in our previous report), reviewed data from our previous study in Japan and conducted a case-control study in Korea and the

For reprints and all correspondence: Hideyuki Akaza, Department of Urology, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba-City, Ibaraki 305-8575, Japan. E-mail: akazah@md.tsukuba.ac.jp

Table 1. Subjects and their median age

	Total	Patients	Controls	*P* value
Japan				0.629
No. of subjects	295	133	162	
Median age (range)	68.0 (44–92)	68.0 (52–80)	67.0 (44–92)	
Korea				<0.001**
No. of subjects	122	61	61	
Median age (range)	67.0 (43–89)	70.0 (43–89)	64.0 (54–84)	
USA				0.516
No. of subjects	45	24	21	
Median age (range)	62.0 (43–82)	62.5 (43–79)	60.0 (44–82)	

**Statistically significant ($P < 0.01$) between patients and control.

United States. Furthermore, we analyzed the reproducibility or stability of the ability to degrade daidzein into equol among Japanese subjects for whom the blood levels of soybean isoflavones could be measured twice at a specific interval.

PATIENTS AND METHODS

Informed consent for the study was obtained from all subjects. The subjects were divided into two groups: one group consisted of patients with histologically proven prostate cancer, and the other consisted of age- and geographically-matched cancer-free and urological disease-free male subjects who served as the controls. The prostate cancer patients were required to have been diagnosed for the first time not more than 3 years prior to the study; however, no conditions regarding the details of their therapy or their disease stage were attached. Blood samples were drawn before breakfast and the separated sera were stored at –10°C or lower. These samples were subsequently transported on dry ice to the laboratory of SRL Co. (Tokyo, Japan).

The determination of the concentrations of isoflavones in the serum samples were performed by the method employed in the previous study (2), namely, reversed-phase high performance liquid chromatography-multiple reaction ion monitoring-mass spectrometry (HPLC-MS) method. The assayed isoflavones were genistein, daidzein and equol. Equol producers were defined as having a serum equol concentration above the lower limit of detection of the present assay system, i.e., 0.5 ng/ml. Statistical analyses were performed by Wilcoxon's test (non-parametric), the Kruskal–Wallis test and the chi-square test. A *P* value of <0.05 was defined as representing a statistically significant difference. The experimental subjects in Japan were Japanese. Their serum samples were obtained at the collaborating urology departments. Among the Japanese, in the control group, the number of outpatients was very small as compared to the inpatients. Therefore, in this paper, we made the comparison based on the inpatient.

The stability of daidzein-metabolizing ability was examined in patients (both in- and outpatients) with prostate cancer, being treated at the Tsukuba University Hospital, who had given consent to receive two measurements. Both the patients and the controls of the studies in Korea and the United States were outpatients. All subjects of the Korean study were Koreans residing in Korea. The subjects of the US study were US residents of unconfirmed race living in Los Angeles.

This study was approved by the Institutional Review Board of each institute.

RESULTS

Table 1 summarizes the number of subjects (total population, patients and controls) for each study. The median age of the subjects in the US study was 7–8 years lower than that in the Korean and Japanese studies. The Korean study was initially designed as an age-matched study. However, the median age of the case group had high statistical significance. Table 2 summarizes the median blood levels of genistein and daidzein and the percentage of equol producers in each study. It is noteworthy that the percentage of equol producers was significantly lower for patients with prostate cancer than for the control group in the Japanese and Korean case-control studies. In the US study, extremely low blood levels of soybean isoflavones were noted, as expected. Further, the percentage of equol producers was also markedly low in both the patient group and the control group. Table 3 summarizes the relationship between blood genistein and daidzein levels and the equol-producing ability. In Japan, the genistein level was higher among equol non-producers than among equol producers, and this difference was statistically significant in the control group, which had not been observed in our previous study (2). No other specific relationship was noted between the equol-producing ability and the blood soybean isoflavones levels in the Japanese study. In the Korean study, no specific relationship was noted between the blood soybean isoflavones levels and equol-producing ability. In the US study, blood soybean isoflavones levels were markedly low as shown in Table 2, but genistein and daidzein levels were high among equol producers. The genistein level for both the patient group and the control group and the daidzein level for the total population in the US study significantly differed from those in the other studies. In order to examine the

Table 2. Median blood soybean isoflavones levels and equol producers compared among Japanese, Korean and US subjects

(Patients/Control)	Japan		Korea		USA	
	(*n* = 133/162)	*P* value[a]	(*n* = 61/61)	*P* value[a]	(*n* = 24/21)	*P* value[a]
Daidzein						
Patient	25.6 (1.6–607.1)		29.9 (0.8–371.9)		3.0 (0.5–34.3)	
Control	24.4 (0.7–424.0)	0.273[a]	30.3 (2.3–940.6)	0.518[a]	3.0 (0.6–94.5)	0.592[a]
Genistein						
Patient	84.9 (7.2–1355.8)		67.5 (1.7–973.6)		1.8 (0.6–57.3)	
Control	84.5 (3.4–1056.8)	0.339[a]	64.9 (6.3–448.0)	0.574[a]	2.7 (0.6–140.0)	0.552[a]
Equol producer						
Patient (%)	29		30		17	
Control (%)	46	0.004[b]**	59	0.001[b]**	14	0.826[b]

[a]Wilcoxon's test. [b]Chi-square test. **Statistically significant ($P < 0.01$).

Table 3. Median blood genistein and daidzein levels for equol producers and non-producers

	Japan		Korea		USA	
	Equol +/–	*P* value[a]	Equol +/–	*P* value[a]	Equol +/–	*P* value[a]
Genistein (ng/ml)						
Patients	78.2/106.7	0.747	67.5/69.3	0.623	24.2/1.5	0.012*
Control	80.0/92.7	0.011*	72.9/59.3	0.250	20.4/1.9	0.022*
Total	78.3/103.9	0.033*	69.0/66.0	0.849	20.4/1.6	<0.001*
Daidzein (ng/ml)						
Patients	26.9/32.4	0.639	25.1/30.9	0.724	6.5/1/3	0.089
Control	20.3/27.0	0.567	34.3/21.8	0.244	9.6/2.9	0.242
Total	25.7/30.2	0.379	31.9/29.8	0.713	9.4/2.5	0.044*

[a]Wilcoxon's test. *Statistically significant ($P < 0.05$). Equol +/–, Equol producer/non-producer.

extent to which the equol-producing ability would be stable in individual subjects, we measured the equol level twice at a specific interval. Dual measurements involved 40 patients with prostate cancer. The median interval between two sessions of blood sampling was 569 days. Similar results were observed between the two measurements in 85% of the total number of subjects. The interval of measurement was not significantly different between the subjects showing similar results and those showing discrepancies. The mean genistein and daidzein levels at the first measurement were 149.7 and 70.5 ng/ml, respectively. At the second measurement, they were 270.7 and 109.1 ng/ml, respectively. Thus, both the genistein levels and the daidzein levels were higher at the second measurement than at the first one. This difference appears to be closely associated with the fact that all patients underwent the first measurement during their hospital stay, whereas they received the second measurement at the outpatient clinic. With regard to this, it has been shown that the meals provided for inpatients at the Tsukuba University Hospital contained less soybean isoflavones as compared to those that Japanese usually consume at home.

DISCUSSION

Several questions concerning the metabolism of soybean isoflavones remain unresolved. Equol is known as a metabolite of daidzein; however, the mechanism for its degradation has not yet been clarified. It is thought that equol is primarily degraded at the intestine and that some intestinal bacteria are involved in its biodegradation (4). We previously conducted a case-control study of blood soybean isoflavones levels in Japanese patients with prostatic cancer and cancer-free controls living in Japan (2), and this study yielded a noteworthy finding

Table 4. Dual measurements of equol producing ability in the same subjects

	Median interval (days)	Range (days)	*P* value[a]
Total *n* = 40	569	144–616	
No interval change 34/40 (85%)	574	144–701	0.289
Interval change 6/40 (15%)	477	164–616	

[a]Wilcoxon's test.

that the percentage of equol producers was significantly lower among patients with prostate cancer. Under circumstances in which the intake of soybean isoflavones is likely to be higher in the Japanese than in the Americans and Europeans, the fact that the incidence of prostate cancer is high for the group of individuals who have no equol-producing ability, i.e., the ability to degrade daidzein into equol, may provide an insight to clarify the mechanism for suppression of prostate cancer by soybean isoflavones. In the present study, we attempted a case-control study designed in the same way as the previous Japanese study, involving Koreans residing in Korea, who have similar dietary habits as the Japanese and have low incidence of prostate cancer, and Americans residing in the USA, who have markedly different dietary habits from the Japanese and Koreans and a relatively high incidence of prostate cancer. In the Japanese study, the number of subjects was 295 (133 patients and 162 controls) by adding 126 subjects to the 169 subjects of the previous study (66 patients and 103 controls). In this study, the percentage of equol producers was significantly lower among patients with prostate cancer, which was similar to the previous finding. The Korean study also yielded similar results. In the US study, the results differed markedly from those in the Japanese and Korean studies. In the US study, the serum isoflavones levels were much lower, and the percentage of equol producers was low in both the patient group and the control group. The percentage of equol producers among Japanese and Korean controls was 46% and 59%, respectively, while that among the American controls was only 14%. Since equol is a metabolite of daidzein, we examined the relationship between the blood levels of daidzein and the equol-producing ability in each study. No significant relationship was observed between the two in Japan or Korea; however, the daidzein level of equol producers was significantly high in the total US subjects. In the US study, the blood genistein level was significantly higher (more than 10 times higher) among equol producers than among non-producers in both the patient group and the control group. This relationship was reversed in the Japanese control group. Considering both these studies, these results suggest that acquisition of the equol-producing ability requires ingestion of a certain amount of soybean isoflavones; however, some other factors may determine the equol-producing ability of blood isoflavones levels is above a certain level. This view is endorsed by the results shown in Table 4, i.e., the equol-producing ability remained stable for long time periods, and it was relatively stable even when blood isoflavones levels changed considerably. If conversion of daidzein into equol is regulated by genetic factors, the metabolic ability would remain stable for the life time of an individual. However, currently the metabolism is thought to be regulated by the intestinal microflora. An 85% stability observed in our study is thought to be meaningful, because this data prompts us to study whether it is possible to convert equol non-producers to producers, and whether changing the metabolic activity relates to the carcinogenesis and biology of prostate cancer.

Lampe et al. (7) reported that equol excretors who have positive urine equol had a higher intake ratio of carbohydrates to total energy as well as higher intake of phytoproteins and fiber than those of equol non-excretors in their study on 30 female subjects. In addition, Rowland et al., (8) reported a positive relationship between equol excretors and a high intake ratio of carbohydrates to total energy. However, a report denying such a relation has also appeared (9).

Thus, the reason for a higher ratio of equol producers in Japanese and Koreans should be discussed in relation to not only the amount of intake of isoflavones but also the amount of fiber and ratio of carbohydrates to total energy in the diet.

The results of our study suggest that soybean isoflavones significantly contribute towards preventing the onset of prostate cancer and that a more detailed evaluation of equol producers is needed. It is particularly important to examine whether equol itself is directly involved in the suppression of prostate cancer or the mechanism for degrading daidzein into equol is associated with suppression of prostate cancer. We believe that the results from this study are valuable since they can explain, at least partially the reason for the incidence of prostate cancer in Japan and Korea being markedly different from that in the United States. We suggest that a measure taken to increase equol producers may lead to a decrease in the incidence of clinical prostate cancer.

Acknowledgments

We express our thanks to Prof. Sung Joon Hong, Yonsei University, Seoul, Korea, Sang Eun Lee, Seoul National University, Seoul, Korea, Joung Sik Rim, Wonkwang University, Iksan, Korea and Jin Han Yoon, Dong-A University, Busan, Korea for their help in collection of serum samples from Korea.

This study was supported by a Grant-in-Aid for Scientific Research on Priority Area; Cancer A04, Ministry of Education, Science, Sports and Culture, Japan.

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