MINI-REVIEW

Influence of Isoflavone Intake and Equol-producing Intestinal Flora on Prostate Cancer Risk

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Abstract

Background: The age-adjusted incidence rate of prostate cancer (PCa) has been reported to be lower among Asians than Western populations. A traditional Japanese meal, high in soybean products or isoflavones, may be associated with a decreased risk of PCa. Equol, which is converted from daidzein by human intestinal flora, is biologically more active than any other isoflavone aglycone. Materials and Methods: We reviewed not only recent epidemiological studies on association of isoflavones with PCa risk, but also recent research on human intestinal bacteria responsible for converting daidzein into equol. Studies were systematically searched from the database published within the last 5 years of from 2008-2012. Results: Five out of 6 articles showed significant association of isoflavones with a decreased risk of PCa, and two of them consistently showed that equol-producers carry a significantly reduced risk of PCa. Furthermore, 5 human intestinal bacteria that can convert daidzein into equol were identified in the last 5 years. Conclusions: If equol can reduce risk of PCa, a possible strategy for reducing the risk of PCa may be to increase the proportion of equol-producers by changing the intestinal flora to carrying an equol-producing bacterium with dietary alteration or probiotic technology.

Keywords: Prostate cancer - isoflavones - equol - soybean products - intestinal flora

Asian Pacific J Cancer Prev, **14 (1)**, 1-4

Introduction

The age-adjusted incidence rate of prostate cancer (PCa) has been reported to be lower among Asian populations than among Western populations (Curado et al., 2007). Environmental and/or host factors may contribute to this difference in rates. A considerable number of articles have been published assessing dietary habits in association with PCa risk (World Cancer Research Fund/American Institute for Cancer Research, 2007). We previously reviewed them, focusing on the possibility that the traditional Japanese diet may be associated with a decreased risk of PCa (Mori et al., 2009). A traditional Japanese meal consists of a main dish of rice with additional dishes high in soybean products, fish, and other seafood, as well as low in red meat. Isoflavones, polyunsaturated long chain (n-3) fatty acids, and saturated fatty acids were thought to be micronutrients in biological etiology relevant to soybean products, fish, and red meat, respectively (Mori et al., 2009).

The most abundant isoflavones are daidzein and genistein, which together comprise 0.1-0.3 mg/g soybean (Kim et al., 2008), while glycitein is less abundant. Recent researches show that equol, which is converted from daidzein by human intestinal flora, is biologically more active than any other isoflavone (Yuan et al, 2007; Kim et al., 2008; Setchell and Clerici, 2010). A number of bacteria in intestinal flora have also been reported to be responsible for this conversion (Setchell and Clerici, 2010). If a person could make equol in response to consuming soy isoflavones, they are classified as an equol-producer, and the health benefits of soy-based diets may be greater in equol-producers than in equol-nonproducers. However, equol production by a specific human intestinal bacterium has been yet been epidemiological evaluated with regard to risk of PCa.

Accordingly, we began a case-control study on PCa to elucidate the role of equol and a specific human intestinal bacterium, Slackia sp. NATTS (Tsuji et al., 2010), which converts daidzein into equol, supported by a grant from Japanese Government. We have collected data of serum isoflavones concentration, NATTS identification in faeces, and semi-quantitative frequency of food intake, from both PCa cases and their controls. In this article, we reviewed

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not only recent epidemiological studies on association of isoflavones with PCa risk, but also recent researches on human intestinal bacteria responsible for converting daidzein into equol.

Analytical Epidemiology

Analytical epidemiological studies on the risk of PCa, such as cohort and case-control studies, were identified using the MEDLINE database of the National Library of Medicine, United States. Only studies in English from the database published within the last 5 years of from 2008-2012 were included, because our previous review included publications from 1998-2007 (Mori et al.,2009). The keywords "soybean products," "isoflavones," "equol," and "intestinal flora" were added for the search. The statistical significance was set at a 0.05 level. Odds ratios (ORs) and their 95% confidence intervals (95%CI) were cited if shown in the article. Furthermore, we searched recent researches on human intestinal bacteria responsible for converting daidzein into equol.

Discussion

As shown in Table 1, 6 articles were found by the search. Miyanaga et al. (2012) reported a randomized, double-blind placebo-controlled trial of oral isoflavones (60 mg/day) for 12 months. The isoflavone tablet included daidzein, genistein, and glycitein. Of the 89 patients evaluated by central pathological review, the incidence of high-grade prostatic intraepithelial neoplasia (HG-PIN) showed no difference between the isoflavone and placebo groups. However, for the 53 patients aged 65 years or more, the incidence of HG-PIN in the isoflavone group was higher than in the placebo group (28.0% vs. 57.1%, P=0.031). They concluded that this result may support the role of isoflavones for prostate cancer risk reduction.

Jackson et al. (2010) evaluated the relationship of spot urinary concentrations of isoflavones with PCa in a hospital-based case-control study in Jamaica, collecting urine samples from 175 cases and 194 controls. As a result, compared with the lowest tertile (non producers of equol), men with the highest tertile of equol concentration in urine showed a decreased risk of total PCa (OR, 0.48; 95%CI, 0.26-0.87) and high-grade PCa (OR, 0.29; 95%CI, 0.13-0.60). They concluded that producers of equol may have a reduced risk of PCa.

Park et al. (2009) reported results from a nested case-control study of the Multiethnic Cohort in Hawaii and California in USA, measuring urine samples of 249 incident cases and 404 controls matched for age, race/ethnicity, and so on. Urinary concentration of daidzein was inversely associated with risk of PCa (OR of the highest quintile vs. the lowest, 0.55; 95%CI, 0.31-0.98). They suggested that high intake of isoflavones, as reflected by urinary excretion of daidzein, may be protective against PCa.

Travis et al. (2009) examined plasma concentration of phyto-estrogens in relation to risk of PCa in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition. Concentrations of genistein, daidzein and equol were measured in plasma samples for

Table 1. Summary of Studies Assessing the Influence of Isoflavones on Risk of Prostate Cancer During 2008-2012

First author, reference, and publication year	Country	Study design	Case (or Inte -rvention)	Control (or Cohort)	Duration of follow-up (years)	Items	Contents	Odds ratio	95%CI*	P value
Miyanaga et al., 2012	Japan	Intervention study	42 (Isolavone)	47 (Placebo)	1		High-grade prostatic intraepithelial neoplasia			
							Age≥65years			
						isoflavones	2/17 (11.8%)			
						placebo	0/19 (0.0%)			0.220
							Age≥65years			
						isoflavones	7/25 (28.0%)			
						placebo	16/28 (57.1%)			0.031
Jackson et al., 2010	Jamaica	Case-control study	175	194	Not applicable		Total cancer			
						genistein	highest tertile (vs. lowest)	1.23	0.67-2.56	0.502
						daidzein	highest tertile (vs. lowest)	0.85	0.47-1.54	0.600
						equol	highest tertile (vs. lowest)	0.48	0.26-0.87	0.020
							High-grade cancer			
						genistein	highest tertile (vs. lowest)	1.07	0.53-1.64	0.816
						daidzein	highest tertile (vs. lowest)	0.65	0.36-1.52	0.318
						equol	highest tertile (vs. lowest)	0.29	0.13-0.60	0.001
Park et al., 2009	USA (Hawaii and California)	Nested case-control study	249	404	1.9	genistein	highest quintile (vs. lowest)	0.72	0.40-1.31	0.09
						daidzein	highest quintile (vs. lowest)	0.55	0.31-0.98	0.03
						equol	highest tertile (vs. lowest)	1.32	0.84-2.08	0.08
Travis et al., 2009	Europe	Nested case-control study	950	1,042	15.1	genistein	highest quintile (vs. lowest)	0.71	0.53-0.96	0.032
						daidzein	highest quintile (vs. lowest)	0.82	0.62-1.09	0.368
						equol	highest quintile (vs. lowest)	1.01	0.72-1.41	0.926
Kurahashi et al., 2008	Japan	Nested case-control study	201	402	12.8	genistein	highest tertile (vs. lowest)	0.66	0.40-1.08	0.08
						daidzein	highest tertile (vs. lowest)	0.78	0.49-1.25	0.44
						glycitein	highest tertile (vs. lowest)	0.78	0.48-1.26	0.51
						equol	highest tertile (vs. lowest)	0.60	0.36-0.99	0.04
Park et al., 2008	USA (Hawaii and Los Angels)	Cohort study	4,404	82,483 (Cohort)	8	genistein	highest quintile (vs. lowest)	0.94	0.84-1.04	0.16
						daidzein	highest quintile (vs. lowest)	0.92	0.82-1.02	0.09
						glycitein	highest quintile (vs. lowest)	0.91	0.82-1.01	0.07
						total isoflavone	highest quintile (vs. lowest)	0.93	0.83-1.04	0.17

*95% confidence interval

950 cases and 1,042 matched control participants. As a result, higher concentrations of genistein were associated with lower risk of PCa (OR of the highest quintile vs. the lowest, 0.71; 95%CI, 0.53-0.96). They suggested that higher a intake isoflavones, as reflected by higher concentration of circulating genistein, may reduce the risk of PCa.

Kurahashi et al. (2008) conducted a nested case-control study within the Japan Public Health Center-based Prospective Study. A total of 14,203 men aged 40-69 years who provided blood samples were followed-up from 1990-2005. After a mean of 12.8 years of follow-up, 201 newly diagnosed PCa and two matched controls for each case were analyzed. As a result, the highest tertile of plasma equol was associated with a decreased risk of total PCa (OR, 0.60; 95%CI, 0.36-0.99). They concluded that isoflavones, especially equol, may prevent the development of PCa.

Park et al. (2008) investigated the relationship of isoflavone intake to PCa risk in a nested case-control study within the Multiethnic Cohort Study in Hawaii and Los Angeles. The analyses included 82,483 men who completed a detailed quantitative food frequency questionnaire from 1993-1996. A total of 4,404 PCa cases were recorded during the average follow-up period of 8 years. As a result of this cohort study, the highest quintile of total isoflavone intake, as well as specific isoflavones, were not associated with risk of PCa, although they slightly reduced risk (OR of total isoflavone, 0.93; 95%CI 0.83-1.04). They concluded that isoflavones may not be significant contributors to reduction of PCa risk in this cohort. As they suggested, it might be thought that levels of soybean or isoflavone consumption in their cohort were too low to detect a protective effect against PCa.

In summary, 5 out of 6 cited articles (Kurahashi et al., 2008; Park et al., 2009; Travis et al., 2009; Jackson et al., 2010; Miyanaga et al., 2012) showed significant association of isoflavones with a decreased risk of PCa, and 2 articles (Kurahashi et al., 2008; Jackson et al., 2010) among them consistently showed that equol-producers carry a significantly reduced risk of PCa.

Breinholt and Larsen (1998) found in 1998 for the first time that equol has more potent estrogenic activity than other flavonoids. Moreover, Lund et al. (2004) showed that equol has the ability to specifically bind and sequester 5α-dihydrotestosterone from androgen receptors. They suggested a broad and important usage for equol in the treatment of androgen-mediated pathologies because of this property. In addition, as stated by Setchell et al. (2002), equol is superior to all other isoflavones in its antioxidant activity.

Table 2. List of Identified Intestinal Bacteria Converting Daidzein to Equol During 2008-2012

First author, reference, and publication year	Country	Bacterium strain
Tsuji et al., 2010	Japan	*Slackia* sp. NATTS
Jin et al., 2010	Japan	*Slackia equolifaciens* sp.
Matthies et al., 2009	German	*Slackia* sp. HE8
Maruo et al., 2008	Japan	*Adlercreutzia equolifaciens*
Yokoyama and Suzuki, 2008	Japan	*Eggerthella* sp. YY7918

*Classification, Coriobacteraceae

According to the review published by Setchell and Clerici in 2010 (2010), 13 human intestinal bacteria that can convert daidzein into equol have been described. We picked up some articles from the review (Setchell and Clerici, 2010), and added some others published during the 5 years from 2008-2012. As shown in Table 2, 5 human intestinal bacteria (Maruo et al., 2008; Yokoyama and Suzuki, 2008; Matthies et al., 2009; Jim et al., 2010; Tsuji et al., 2010) have been identified as responsible for converting daidzein into equol so far.

Tsuji et al (2010), one of the co-authors for this review, and his co-workers also isolated an equol-producing bacterium from human faeces, strain NATTS, which was characterized as Gram-positive, non-spore-forming, and rod-shaped. They proposed a novel species of the genus *Slackia*, NATTS. Tsuji et al. (2012) also showed 3 enzymes and their corresponding genes in conversion of daidzein into equol in the bacterium of the *Slackia* sp. NATTS. Jin et al. (2010) isolated an equol-producing bacterium, strain DTE^T, from human faeces, which was characterized as Gram-positive, obligately anaerobic, non-spore-forming, asaccharolytic and rod-shaped. They proposed a novel species of the genus *Slackia, Slackia equolifaciens* sp. nov.

Matthies et al. (2009) isolated an equol-producing bacterium from human faeces, strain HE8, which was characterized as rod-shaped, Gram-positive, and anaerobic. They proposed a novel species of the genus Slackia, Slackia isoflavoniconvertens. Maruo et al. (2008) isolated an equol-producing bacterium from human faeces, which was characterized as asaccharolytic, obligately anaerobic, non-spore-forming, non-motile, and Gram-positive coccobacilli. They proposed a novel species of the genus *Adlercreutzia, Adlercreutzia equolifaciens* sp. nov. Yokoyama et al. (2008) isolated an equol-producing bacterium from human faeces, strain YY7918, which was characterized as non-sporulating, non-motile, and Gram-positive. They proposed a novel species of the genus *Eggerthella, Eggerthella* sp. YY7918.

In summary, 5 human intestinal bacteria that can convert daidzein into equol were identified in the last 5 years. As these intestinal bacteria need to be epidemiologically evaluated with regard to PCa risk, we began a case-control study including NATTS identification in faeces, as previously mentioned.

Concluding Remarks

Although the incidence rate of PCa is low among Japanese men, it has been increasing, probably because of changes in their dietary habits. If isoflavones, especially equol, can reduce risk of PCa, a possible strategy for reducing risk of PCa may be to increase the proportion of equol-producers by changing intestinal flora to carrying an equol-producing bacterium with dietary alteration or probiotic technology.

Acknowledgements

This study was supported by a Grant-in-aid for

Scientific Research (Project Number 221S0001) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

Breinholt V, Larsen JC (1998). Detection weak estrogenic flavonoids using a recombinant yeast strain and modified MCF7 cell proliferation assay. *Chem Res Toxicol*, **11**, 622-9.

Curado MP, Edwards B, Shin HR, et al (2007). Cancer Incidence in Five Continents. Vol. 9. IARC Scientific Publ. No. 160, IARC, Lyon.

Jackson MD, McFarlane-Anderson ND, Simon GA, et al (2010). Urinary phytoestrogens and risk of prostate cancer in Jamaican men. *Cancer Causes Control*, **21**, 2249-57.

Jin JS, Kitahara, M, Sakamoto M, et al (2010). Slackia equolifaciens sp. nov. a human intestinal bacterium capable of producing equol. *Int J System Evol Microbiol*, **60**, 1721-4.

Kim M, Han J, Kim S (2008). Isoflavone daidzein: chemistry and bacterial metabolism. *J Appl Chem*, **51**, 253-61.

Kurahashi N, Iwasaki M, Inoue M, et al (2008). Plasma isoflavones and subsequent risk of prostate cancer in a nested case-control study: the Japan public health center. *J Clin Oncol*, **26**, 5923-9.

Lund TD, Munson DJ, Haldy ME, et al (2004). Equol is a novel anti-androgen that inhibit prostate growth and hormone feedback. *Biol Reprod*, **70**, 1188-95.

Maruo T, Sakamoto M, Ito C, et al (2008). Adlercreutzia equolifaciens gen. nov. sp. nov. an equol-producing bacterium isolated from human faeces and emended description of the genus Eggertbella. *Int J System Evol-Microbiol*, **58**, 1221-7.

Matthies A, Blaut M, Braune A (2009). Isolation of a human intestinal bacterium capable of daidzein and genistein conversion. *Appl Environ Microbiol*, **75**, 1740-4.

Miyanaga N, Akaza H, Hinotsu S, et al (2012). Prostate cancer chemoprevention study: an investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen. *Cancer Sci*, **103**, 125-30.

Mori M, Masumori N, Fukuta F, et al (2009). Traditional Japanese diet and prostate cancer. *Mol Nutr Food Res*, **53**, 191-200.

Park SY, Murphy SP, Wilkens LR, et al (2008). Legume and isoflavone intake and prostate cancer risk: the multiethnic cohort study. *Int J Cancer*, **15**, 927-32.

Park SY, Wilkens LR, Franke AA, et al (2009). Urinary phytoestrogen excretion and prostate cancer risk: a nested case-control study in the multiethnic cohort. *Br J Cancer*, **101**, 185-91.

Setchell KDR, Brown NM, Lydeking-Olesen E (2002). The clinical importance of the metabolite equol-A clue to the effectiveness of soy and its isoflavones. *J Nutr*, **132**, 3577-84.

Setchell KDR, Clerici C (2010). Equol: history, chemistry, and formation. *J Nutr*, **140**, 1355-62.

Travis RC, Spencer EA, Allen NE, et al (2009). Plasma phyto-oestrogens and prostate cancer in the European prospective investigation into cancer and nutrition. *Br J Cancer*, **100**, 1817-23.

Tsuji H, Moriyama K, Nomoto K, et al (2010). Isolation and characterization of the equol-producing bacterium Slackia sp. strain NATTS. *Arch Microbiol*, **192**, 279-87.

Tsuji H, Moriyama K, Nomoto K, et al (2012). Identification of an enzyme system for daidzein-to-equol conversion in Slackia sp. strain NATTS. *Appl Environ Microbiol*, **78**, 1228-36.

World Cancer Research Fund/ American Institute for Cancer Research (2007). Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. American Institute for Cancer Research, Washington DC.

Yokoyama S, Suzuki T (2008). Isolation and characterization of a novel equol-producing bacterium from human feces. Biosci. *Biotechnol Biochem*, **72**, 2660-6.

Yuan JP, Wang JH, Liu X (2007). Metabolism of dietary soy isoflavones to equol by human intestinal microflora-implication for health. *Mol Nutr Food Res*, **51**, 765-81.