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Research Article

Association between Isoflavones and Colorectal Cancer Risk: A Case-Control Study in Japanese

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Abstract

Background: Isoflavones, a component of soy-foods, may play a role in the inhibition of human colon cancer cell growth and the induction of apoptosis. However, the relationship between isoflavones and colorectal cancer risk remains unknown in epidemiological studies. Equol, an isoflavone metabolite produced by colonic bacteria has been shown to have stronger antioxidant effects than its precursor daidzein.

Objectives: This case-control study examined whether plasma concentration, isoflavones intake, and presence of *Slackia* sp. strain NATTS, an equol-producing bacteria, may influence colorectal cancer risk.

Methods: We retrospectively identified 74 cases of newly diagnosed colorectal cancer and 71 hospital controls enrolled, from 2016 to 2018. Isoflavones were assessed by plasma concentrations and administration of a food frequency questionnaire. *Slackia* sp. strain NATTS in feces was measured. An unconditional regression model was used to estimate odds ratios (OR_s) and 95% confidence intervals (CI_s) for colorectal cancer in relation to plasma isoflavones concentrations.

Results: After adjustment for confounders, plasma equol showed no association with colorectal cancer risk: however, plasma genistein, daidzein, and glycitein risk of colorectal cancer (highest tertile vs. lowest: for genistein, OR_s = 0.29, 95%CI_s: 0.12-0.70, *p*-trend = 0.007; daidzein, OR_s = 0.31, 95%CI_s: 0.13-0.75, *p*-trend = 0.009; glycitein, OR_s = 0.25, 95%CI_s: 0.10-0.63, *p*-trend = 0.004). No statistically significant associations were observed between dietary genistein and daidzein, and *Slackia* sp. strain NATTS and colorectal cancer risk.

Conclusion: Plasma concentrations of genistein, daidzein, and glycitein may contribute to the risk of colorectal cancer.

Keywords: Case-control study; colorectal cancer; isoflavones; Japanese

Introduction

In Japan in 2018, colorectal cancer was the most prevalent type of cancer in Japan and the second leading cause of diagnosed cancer death in both women and men [1]. Westernization of dietary habits is considered a key contributing factor in the increased of colorectal cancer and includes increased consumption of red and processed meat, an established risk for colorectal cancer [2].

Soybean, a food traditionally consumed in Japan, is low in fat and rich in isoflavones [3]. Glycoside forms of isoflavones including genistin, daidzin, and glycitin, are hydrolyzed by intestinal microbiota to form genistin, daidzin, and glycitin activated metabolic products known as aglycones [4]. Isoflavone aglycones are thought to exert protective effects against the development of colorectal cancer. In a previous study, genistein was shown to promote the inhibition of human colon cancer cells growth [5,6] and has also been reported to induce apoptosis in LoVo and HT-29 human colon cancer cells via inhibition of the NF-κB pathway [7]. Multiple epidemiological studies have reported that the consumption of dietary isoflavones is associated with a reduced risk of developing colorectal cancer [8-10]. Furthermore, plasma isoflavones concentration has been shown to be associated with a decreased risk of colorectal cancer in nested case-control studies [11]. Conversely, other studies found no associations between isoflavone intake and colorectal cancer risk [12,13].

Among the aglycones, daidzein is most likely to be metabolized to equol through the enzymatic activity of specific intestinal colonic bacteria [14-16]. Although equol has been reported to show greater antioxidant activity than daidzein [4], to the best of our knowledge, only one epidemiological study to date has assessed the association between equol and colorectal cancer risk [12]. Plasma isoflavone concentrations reflect isoflavone consumption and subsequent metabolism by colonic bacteria [17]. It remains possible that isoflavone intake and plasma concentration may have differential effects with respect to colorectal cancer risk. It is therefore important to examine isoflavone intake in parallel with plasma concentration, and levels of equol-producing bacteria in the intestine

Hence, the aim of the present study was to examine associations among plasma isoflavone concentration; isoflavone intake; *Slackia* sp. strain NATTS, an equol-producing bacteria in the intestinal microbiota; and colorectal cancer risk.

Materials and Methods

Study participants

Individuals newly diagnosed with colorectal cancer between 2016 and 2018 following colonoscopy at the department of surgery, surgical oncology, and science at Sapporo Medical University Hospital were eligible for participation in this study. At the time of inclusion, no participants had undergone chemotherapy or radiation therapy as treatment for colorectal cancer. Control participants were selected in the same period as outpatients from the department of cardiovascular, renal, and metabolic medicine, and had diagnoses of hypertension, cardiovascular disease, arrhythmia, hyperlipidemia, Graves' disease, or diabetes. Exclusion criteria were clinical history of large intestine disease, cancer undergoing treatment, and planned colonoscopy or fecal occult blood test.

No participants were on dietary restrictions or had received warfarin potassium or antibiotic treatment within 1 month prior to fecal sample collection. Informed consent was obtained from cases and controls. This study was approved by the Sapporo Medical University Ethics Committee (reference no. 312-149).

Measurements of plasma isoflavones

Fasting blood samples (8 mL) were obtained from all participants. In patients with colorectal cancer, blood samples were collected immediately following diagnosis. For control patients, samples were obtained at the next outpatient visit. Plasma levels of genistein, daidzein, glycitein, and equol were measured using triple quadrupole tandem liquid chromatography mass spectrometry with <0.5 ng/mL limit of detection. All measurements were performed at Sumika Chemical Analysis Service (Osaka, Japan).

Measurements of *Slackia* sp. strain NATTS

Fecal samples (1 g) were collected from all participants at home. Colorectal cancer patients provided fecal samples prior to surgery, while control participants brought the fecal sample to the next outpatient visit.

Fecal samples were frozen at −20 ˚C immediately after collection. Using the reverse transcription quantitative polymerase chain reaction method, the 16S rRNA gene fragments of *Slackia* sp. strain NATTS and related strains were amplified from the total RNA extracted from feces so as to determine the number of species present. Analysis of fecal samples was performed at the Yakult Central Institute (Tokyo, Japan).

Questionnaire assessment

All patients answered a validated [18,19] self-administered semi-quantitative food frequency questionnaire (FFQ) at home, as described previously [20]. Briefly, daily intake frequency and portion size were assessed for the 1-year period prior to diagnosis for patients with colorectal cancer and during the 1-year period before study inclusion for control participants.

A lifestyle questionnaire was also administered and included

age, height, weight, and smoking status. Body Mass Index (BMI) was calculated as weight divided by height. The following clinical data were also obtained from medical records: histological type, cancer site, tumor marker levels (serum carcinoembryonic antigen (CEA) and cancer antigen (CA19-9)), and clinical stage of colorectal cancer at diagnosis.

Statistical analysis

Student's t-test was used to compare the average (standard deviation) of continuous variables, the Chi-square test was used to compare proportions of categorical variables between the groups, and the Mann-Whitney U-test was used to compare medians (interquartile ranges) of continuous variables (alcohol consumption, plasma concentrations of isoflavones, energy intake, and dietary isoflavones) for case and control patients.

Dietary consumption of genistein and daidzein was adjusted by total energy employing a residual method [21]. At first, we categorized the plasma concentrations and dietary consumption of isoflavones into tertiles based on the control patients, and calculated the odds ratios (OR_s) and 95% confidence intervals (CI_s) of the risk of colorectal cancer. Linear trends were estimated using the median values for each tertile. To assess the association between plasma concentrations of isoflavones, dietary consumption of isoflavones, and colorectal cancer, we used unconditional logistic regression models where the lowest category was the reference category. In multivariable analyses, we first performed adjustment for the first model (age and sex) and thereafter we adjusted for the second model (age, sex, BMI, smoking status, alcohol consumption; or for age, sex, BMI, smoking status, alcohol consumption, and energy intake).

P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

Results

Seventy-four patients with colorectal cancer (mean age 64 years, range 27-88 years) and 71 control patients (mean age 65 years, range 32-87 years) were included in this study. The distribution of characteristics between the case and control participants is shown in Table 1. Significantly higher plasma concentrations of genistein, daidzein, and glycitein, but not of equol were found in control participants compared with case participants, in addition to a significantly higher dietary consumption of genistein and daidzein. Energy intake was significantly greater in the case participants compared with the controls. No other significant differences were observed between the two groups.

Variable	Cases (n = 74)	Controls (n = 71)	p value[a]
Age (years, mean± SD)	63.7 ± 12.4	64.5 ± 11.8	0.694
Sex [n (%)]			
Female	36 (48.6%)	34 (47.9%)	0.927
Male	38 (51.4%)	37 (52.1%)	
Body mass index (kg/m^2, mean ± SD)	23.6 ± 3.6	24.2 ± 3.2	0.466
Smoking status [n (%)]			
Never smoker	27 (36.5%)	34 (47.9%)	0.156
Former smoker	32 (43.2%)	30 (42.3%)	
Current smoker	15 (20.3%)	7 (9.8%)	
Alcohol consumption[b,e] (g/day)	73.54 [18.04–552.94]	56.40 [9.72–280.53]	0.101
Plasma concentrations of isoflavones[c] (ng/ml)			
Genistein[b]	37.15 [6.61–85.13]	64.30 [26.60–146.00]	0.001
Daidzein[b]	2.86 [0.55–10.75]	10.90 [2.91–21.50]	<0.001
Glycitein[b]	0.28 [0–0.93]	0.89 [0.35–2.15]	<0.001
Equol[b]	0 [0–0.12]	0 [0–0.85]	0.500
Equol [n(%)]			
Non-producer[d]	56 (75.7%)	50 (70.4%)	0.476
Producer	18 (24.3%)	21 (29.6%)	
Slackia sp. strain NATTS [n (%)]			
Not detected[e]	62 (83.8%)	60 (84.5%)	0.905
Detected	12 (16.2%)	11 (15.5%)	
Energy intake[b] (kcal/day)	1824.74 [1463.23–2233.92]	1602.14 [1278.27–1957.01]	0.038

Dietary genistein[b,f] (mg/day)	22.51 [11.74–37.95]	27.11 [20.47–45.14]	0.024
Dietary daidzein[b,f] (mg/day)	13.60 [7.71–22.62]	16.35 [12.54–26.28]	0.021

[a]p value for Student's t-test, Chi-square test, or Mann–Whitney U test
[b]Value are medians [25th percentile–75th pecentile]
[c]All values below the detection limit <0.10 ng/mL were regarded as zero
[d]Values below the detection limit <0.10 ng/mL were regarded as non-producer
[e]Values below the detection limit <3.00 log10 cells/g feces were regarded as not-detected
[f]Alcohol consumption, dietary genistein, and daidzein were energy adjusted.
SD, standard deviation

Table 1: Characteristics of patients (cases) with colorectal cancer and control participants.

The diagnosis findings for the 74 case patients are presented in Table 2. Pathological classification (Union for International Cancer Control, 7th edition) was as follows: 6 patients with stage 0, 38 patients with stage Ⅰ or ⅡA, 4 patients with stage ⅡB or ⅢA, 18 patients with stage ⅢB or ⅢC, 8 patients with stage ⅣA or ⅣB.

Variables	Cases (n = 74)
Cancer site [n (%)]	
Colon cancer	31 (42.7%)
Rectal cancer	43 (57.3%)
Tumor marker at the time of diagnosis[a]	
CEA ng/ml	3.05 [1.70–6.83]
CA19-9 U/ml	9.10 [5.70–13.83]
Pathological classification (UICC 7th edition) [n (%)]	
0	6 (8.1%)
I	24 (32.4%)
IIA	14 (18.9%)
IIB	1 (1.4 %)
IIIA	3 (4.1 %)
IIIB	16 (21.6%)
IIIC	2 (2.7%)
IVA	6 (8.1%)
IVB	2 (2.7%)

aValues are medians [25th percentile-75th pecentile]

CEA, carcinoembryonic antigen; CA19-9, cancer antigen 19-9; UICC, Union for International Cancer Control

Table 2: Characteristics of diagnosed colorectal cancers.

The association between plasma concentration, dietary consumption of isoflavones and risk of colorectal cancer is shown in Table 3. Plasma concentrations of genistein, daidzein, and glycitein were significantly associated with reduced colorectal cancer risk. Adjustment for the potential confounders, age and sex (first model), resulted in OR_s and 95% CI_S for the high versus low tertiles of genistein, daidzein, and glycitein of 0.31 (95% CI_s, 0.13 −0.71), 0.29 (95% CI_s, 0.12 −0.67), and 0.23 (95% CI_s, 0.10 −0.55), respectively. Furthermore, multivariable adjusted OR_s and 95% CI_s in the second model showed a similar trend with adjusted age and sex for

genistein 0.29 (95% $CI_{s,}$ 0.12 −0.70), daidzein 0.31 (95% $CI_{s,}$ 0.13 −0.75), and glycitein 0.25 (95% $CI_{s,}$ 0.10 −0.63).The tertile categories of plasma concentrations of genistein, daidzein, and glycitein had a significant dose-dependent association with colorectal cancer risk after adjustment for the first model (*p* for trend = 0.008, 0.004, and 0.001, respectively) and the second model (*p* for trend = 0.007, 0.009, and 0.004, respectively). However, no association was observed between risk of colorectal cancer and plasma equol concentrations or dietary isoflavones.

Variable[a]	Cases (n = 74)	Controls (n = 71)	OR (95%CI)[b]	OR (95% CI)c
Plasma concentrations of isoflavones[d]				
Genistein (ng/mL)				
<38.50	41	23	1.00	1.00
38.50–116.99	20	24	0.47 (0.21–1.02)	0.51 (0.23–1.13)
≥117.00	13	24	0.31 (0.13–0.71)	0.29 (0.12–0.70)
p for trend			0.008	0.007
Daidzein (ng/mL)				
<4.66	44	23	1.00	1.00
4.66–15.69	17	24	0.37 (0.17–0.82)	0.41 (0.18–0.92)
≥15.70	13	24	0.29 (0.12–0.67)	0.31 (0.13–0.75)
p for trend			0.004	0.009
Glycitein (ng/mL)				
<0.45	46	23	1.00	1.00
0.45–1.45	17	24	0.35 (0.16–0.79)	0.39 (0.17–0.88)
≥1.46	11	24	0.23 (0.10–0.55)	0.25 (0.10–0.63)
p for trend			0.001	0.004
Equol (ng/mL)				
<0.10	56	50	1.00	1.00
0.10–0.84	9	10	0.81 (0.31–2.17)	0.64 (0.22–1.82)
≥0.85	9	11	0.74 (0.28–1.96)	0.86 (0.32–2.34)
p for trend			0.561	0.803
Dietary isoflavones[e]				
Genistein (mg/day)				
<22.54	37	23	1.00	1.00
22.54–37.27	18	24	0.45 (0.20–1.03)	0.62 (0.26–1.51)
≥37.28	19	24	0.47 (0.21–1.06)	0.60 (0.25–1.42)
p for trend			0.081	0.270
Daidzein (mg/day)				
<13.66	37	23	1.00	1.00
13.66–22.35	18	24	0.46 (0.20–1.05)	0.70 (0.28–1.74)
≥22.36	19	24	0.48 (0.21–1.07)	0.64 (0.27–1.51)
p for trend			0.075	0.321

[a]Tertile distribution based on controls
[b]Adjusted for age and sex
[c]Adjusted for age, sex, body mass index, smoking status, and alcohol consumption; or for age, sex, body mass index, smoking status, alcohol consumption, and energy intake
[d]Detection limit is <0.10 ng/mL
[e]Dietary isoflavones were energy adjusted.

Table 3: Odds ratios (ORs) and 95% confidence intervals (CIs) for associations with plasma concentrations and dietary of isoflavones.

Discussion

In the present study, the association of both plasma concentration and intake of isoflavones with colorectal cancer risk was evaluated. In this study, high plasma concentrations of genistein, daidzein, and glycitein were inversely associated with risk of colorectal cancer. However, consumption of genistein and daidzein, plasma equol concentration, and *Slackia* sp. strain NATTS were not found to be associated with colorectal cancer risk.

A previous European prospective cohort study reported that plasma isoflavone concentration was not associated with lower colorectal cancer risk [12]. Although inverse associations with isoflavone intakes and colorectal cancer have been reported in a case-control study [11,22], no such association was observed in our present case-control study or in two previous cohort studies [13,23,24]. These studies present the relationship between either plasma concentration or isoflavone intake and risk of colorectal cancer. Taken together, findings from epidemiologic studies to date have been inconsistent.

In our study, it is unclear why plasma isoflavone concentration but not isoflavone intake was found to be associated with colorectal cancer risk, although several explanations are possible. First, a previous study investigated the association between the FFQ used in our study and serum levels of genistein and daidzein, and showed correlation coefficients of 0.33 and 0.31, respectively [18]. In the present study, correlation coefficients were also low: 0.18 for daidzein and 0.20 for genistein (data not shown). Second, the consumption of genistein and daidzein reflect dietary intake over 1 year, a long-term measure in contrast with levels measured in the blood. Indirect dietary survey methods such as the FFQ used in the present study may underestimate the intake of some foods or nutrients [25,26]. Consequently, we speculate that any association between isoflavone intake and colorectal cancer risk may not be statistically significant. Third, the metabolism of ingested isoflavone is associated with bacteria in the small and large intestine [27]. Host colonic bacteria affect metabolism after the consumption of isoflavones [28]. Soybean foods contain glycosides, daidzin, genistin and glycitin, which are not easily absorbed because of their large molecular size [29]. As primary metabolism, glycoside isoflavones are hydrolyzed to aglycones by beta-glucosidases, enzymes produced by bacteria in the small intestine. However, it is assumed that a portion of glycosides is converted into aglycones by bacteria in the colon [30]. Isoflavones, are subsequently absorbed, and transported to the liver where they are conjugated by liver enzymes. Although the conjugated isoflavones are excreted into the intestine via the bile, they are deconjugated by the colonic microbiota and reabsorbed through the enterohepatic circulation [28].

Plasma isoflavone concentration after soybean consumption might be related to the activity of host intestinal microbiota. Hence, it is possible that isoflavone intake dose not correlate with plasma isoflavone concentration. Future studies are necessary to investigate the relationship between intestinal microbiota and colorectal cancer risk.

Among the isoflavones, plasma equol concentration showed no preventive effect on colorectal cancer. Equol is produced from daidzein in 50% -60% of Asian populations and in 20%−30% of Western populations [31]. *Slackia* sp. strain NATTS and related strains are major equol-producing bacterial species that are detected in 40% of healthy Japanese individuals [14]. As shown in Table 1, our study showed that 16.2% of colorectal cancer patients and 15.5% of control participants had *Slackia* sp. strain NATTS and related strains in their fecal samples, indicating that both cases and controls in this study had a lower detection rate of equol-producing bacteria compared with healthy individuals. It has been reported that equol-producers have a favorable cardiovascular risk profile and lower burden of aortic atherosclerosis [32,33]. The control patients in this study included patients with cardiovascular disease. In these contexts, the proportion of equol-producers in both cases and controls in the present study was lower than that of healthy subjects, and no difference in proportion was observed between the two study groups.

Several limitations of this study should be acknowledged. First, this case-control study involved a small sample size. Second, plasma isoflavone concentrations were measured only once after the diagnosis of colorectal cancer in case patients, although no case patient had previously received treatment for colorectal cancer. Third, selection bias existed because case patients and hospital controls were recruited from outpatients, meaning that dietary habits of case patients may have been influenced by the diagnosis of colorectal cancer; moreover, controls did not represent healthy participants. Finally, recall bias regarding dietary intake may have influenced the data collected in the FFQ.

Conclusion

These results are suggestive of a possible relationship between high plasma levels of isoflavones and reduced colorectal cancer risk in Japanese patients. However, further studies on intestinal microbiota isoflavones and colorectal cancer risk are needed to confirm these findings.

Conflict of interest: The authors declare that there are no conflicts of interest associated with this study.

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