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Emerging Research on Equol and Cancer[1–3]

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Abstract

Mechanisms of action of equol described using in vitro studies suggest possible effects of this compound in relation to cancer risk. However, experimental data are lacking with regard to the effects of S-(-)-equol (a gut bacterial product of daidzein), racemic equol, or even daidzein on tumorigenesis in vivo. Rodent studies, using racemic equol or daidzein in equol-producing animals, suggest that equol exposure does not stimulate mammary tumor growth, but there is little evidence that it is protective either. Racemic equol has been shown to inhibit skin carcinogenesis in hairless mice. Epidemiologic studies of associations between urinary or plasma isoflavone concentrations and breast cancer risk in women have reported no association nor increased risk associated with higher equol measures in low-soy–consuming populations but have reported a trend toward decreased cancer risk with increased equol in Asian populations. These population-based differences have been reported for prostate cancer too. Several studies in Asian men report lower equol concentrations or a lower prevalence of equol-producers among men with prostate cancer compared with controls, whereas studies in European populations report no association. Studies using intermediate biomarkers of cancer risk and susceptibility in humans also have examined the effects the equol-producer phenotype in relation to soy intake with varying results. Overall, the role of equol in relation to cancer remains unclear. With the availability of R- and S-equol, animal studies of carcinogenesis and human intervention studies addressing effects of the equol enantiomers on intermediate biomarkers may help to ascertain the role of equol in cancer risk. J. Nutr. 140: 1369S–1372S, 2010.

Introduction

Equol is a chiral molecule that can exist as 2 distinct optically active isomers, R- and S-equol. The enantiomer S-(-)-equol, is the product of gut bacterial metabolism of the soy isoflavone daidzein. Several actions of equol, including its estrogenic and antioxidant properties and its proliferative and antiproliferative effects, suggest that exposure to the compound may have implications for cancer risk [reviewed in (1,2)]. However, results of in vitro studies can be influenced by whether R- or S-equol or the racemic mixture are used. For example, in binding assays, S-equol had a high and preferential binding affinity for estrogen receptor (ER)[4] β, whereas R-equol bound more weakly with a preference for ERα (3). Further, compared with the racemic mixture, S-equol had no antigenotoxic or antioxidant effects in breast cancer cell lines (2). The objective here was to summarize the available animal studies of equol and tumorigenesis, to update our 2005 review of the epidemiologic literature of equol exposure and cancer risk (1), and to discuss the complexities of conducting research in this area.

Animal studies

Experimental data are lacking with regard to effects of S-(-)-equol, racemic equol, or even daidzein on tumorigenesis. Rodent studies, using racemic equol or daidzein in equol-producing animals, suggest that R- and S-equol combined do not stimulate mammary tumor growth, but there is little evidence that these compounds provide a protective effect either. Lamartiniere et al. (4) reported that in rats, dimethylbenz(a) anthracene-induced mammary tumorigenesis was not affected by feeding daidzein-containing diets. Further, Ju et al. (5) showed that dietary racemic equol administered at 3 doses [250, 500, and 1000 ppm (1.03, 2.06, and 4.12 mmol/kg diet, respectively)] to ovariectomized athymic mice did not stimulate growth of implanted estrogen-dependent human breast tumor

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[4] Abbreviations used: EPIC, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; OR, odds ratio.

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(MCF-7) cells, increase tumor cell proliferation, or induce estrogen-responsive pS2 expression, despite stimulating growth of MCF-7 cells in vitro. Findings such as these point to the challenges of translating in vitro results to effects in vivo and speak to the need for more in vivo studies that allow for the integration of pharmacokinetic and other factors that may affect the biologic response.

Topical application of racemic equol has been shown to reduce the proportion of tumors progressing from benign papillomas to malignant squamous cell carcinoma and reduce the average diameter of lesions in hairless mice treated with solar-simulated UV radiation and/or dimethylbenz(a)anthracene (6). Further, in mice treated with solar-simulated UV radiation, racemic equol topically applied prior to UV treatment reduced DNA damage as measured by cyclobutane pyrimidine dimers, whereas equol applied after UV treatment did not increase the rate of dimer removal (7). Whether there are differences in effects of the specific equol enantiomers on tumorigenesis remains to be established.

Epidemiologic studies of equol and cancer

The association between equol production and cancer risk in humans has not been extensively characterized. Because of the lack of commercially available dietary equol supplements, human exposure to equol historically has been exposure to S-(-)-equol as a result of gut bacterial conversion of daidzein to equol. In 2005 in a review of the literature, Atkinson et al. (1) identified 8 studies of equol and cancer. The studies in Asian men tended to report lower equol concentrations or a lower prevalence of equol producers among men with prostate cancer compared with controls (8–10). The studies of breast cancer yielded inconsistent results, with reports of nonsignificant lower equol excretion in breast cancer cases than controls in Asian and Asian-American populations (11,12), a significant trend toward lower risk of breast cancer across increasing quartiles of equol excretion in an Australian study (13), and higher urine and serum equol associated with breast cancer in the UK (14). In a case-control study of women with histologically confirmed cervical squamous intraepithelial lesions (cases) and normal cytology (controls), plasma equol concentrations were positively associated with cervical squamous intraepithelial lesions risk for the highest relative to the lowest quartile level (15). Some of the limitations of these studies included small sample sizes, insufficient statistical power, and lack of controlled evaluation of equol-producer status.

Since 2005, additional, larger studies have examined the relationship between equol measures and risk of prostate, colon, and breast cancer (**Table 1**). They continue to report mixed results. A recent study in Japanese men reported reduced risk of prostate cancer across tertiles of plasma equol and genistein (16). This association was limited to men with localized disease. In a study of the Multiethnic Cohort, a cohort including men in 5 ethnic/multi-racial groups (i.e., African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites), Park et al. (17) reported a nonsignificant association between prostate cancer risk and tertiles of urinary equol. Odds ratios (OR) (95% CI) for the second and 3rd tertiles compared with the lowest tertile were 0.89 (0.58–1.37) and 1.32 (0.84–2.08), respectively (P-trend = 0.08). There was no significant interaction of urinary equol by race/ethnicity or any difference by tumor characteristics.

In European populations, 2 large studies conducted in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts reported no association between equol and risk of prostate cancer and colon cancer (18,19). Two studies of breast cancer, also in EPIC cohorts, similarly reported no association between equol measures and overall breast cancer risk (20,21); however, among ER-positive cases in the Norfolk cohort, urinary equol was associated with a slightly higher risk [OR (95% CI) = 1.07 (1.01–1.112); P = 0.013] in the 95 cases compared with the 329 controls.

Observational studies of equol and disease outcomes, such as the ones described above, present particular challenges. They require sufficient habitual exposure to daidzein to allow for bacterial production of equol. In Asian cohorts, the primary source of daidzein in observational studies is soyfoods and, among individuals excreting measurable amounts of equol, equol excretion is soy protein dose-dependent (22). Therefore, in a population with a range of soy intakes, it is often difficult to tease out whether equol itself is associated with disease risk, whether equol is serving as an additional measure of daidzein or genistein exposure or of soy food intake in general, or whether

TABLE 1 Summary of studies since 2005 evaluating associations between urinary or circulating equol measures and cancer risk[1]

Study population	Cases	Controls	Findings	Reference
Japanese	Prostate: 201	402	Highest tertile for plasma equol associated with decreased risk of total cancer [OR (95% CI) = 0.60 (0.36–0.99); P-trend = 0.04]; association stronger when confined to cases with localized disease [OR (95% CI) = 0.43 (0.22–0.82); P-trend = 0.02].	(16)
Multiethnic cohort, US	Prostate: 249	404	NS trend for higher prostate cancer risk with higher urinary equol (nmol mg^{-1} creatinine; (P-trend = 0.08).	(17)
EPIC cohort, Europe	Prostate: 950	1042	No association between plasma equol and prostate cancer risk.	(18)
EPIC cohort, Norfolk, UK	Prostate: 191 cases (serum); 152 (urine)	815 (serum); 665 (urine)	No association between serum or urine equol and prostate cancer risk.	(19)
EPIC cohort, Norfolk, UK	Colon and rectum: 214 (serum); 146 (urine)	877 (serum), 686 (urine)	No association between serum or urine equol and colorectal cancer risk.	(19)
EPIC cohort, The Netherlands	Breast: 383 (296 postmenopausal)	383	No association between plasma equol and breast cancer risk.	(20)
EPIC cohort, Norfolk, UK	Breast: 219 (serum); 198 (urine)	891 (serum), 7971 (urine)	No association between serum or urine equol and breast cancer risk overall; however, among ER+ cases, urinary equol (μg $mmol^{-1}$ creatinine) associated with higher risk [OR (95% CI) = 1.07 (1.01–1.112); P = 0.013].	(21)

[1] EP, Equol producer; NS, nonsignificant.

equol is a marker of harboring a particular gut bacterial community (1). To address some of these issues, statistical approaches are needed that adjust for overall soy or isoflavone exposure before testing for equol effects. Studies of equol exposure are more problematic in Western Europe and the US where soy intake is very low. Even among individuals in the highest quantiles of exposure in these populations, equol in blood and urine is low and likely to be below clinically relevant levels (23). Equol exposure in some Western populations also may be due to dietary intake of equol from animal and dairy sources, rather than from daidzein from soy foods (24).

Equol phenotype and intermediate biomarkers in human studies

Studies using intermediate biomarkers of cancer risk and cancer susceptibility in humans have also examined the effect of equol-producer phenotype. Similar to the studies of cancer outcomes in humans, these studies also reflect exposure to S-(-)-equol. In an observational study of postmenopausal women phenotyped for equol production, Fuhrman et al. (25) reported a significant interaction between equol-producer phenotype and soy intake in association with mammographic density (a biomarker of breast cancer risk) despite no independent associations of phenotype or soy intake individually. In contrast, an intervention study testing the effects of soy protein on mammographic density showed no effect of equol-producer phenotype (26). An isoflavone supplement intervention in men with a personal or family history of colorectal adenoma showed that circulating insulin-like growth factor-I decreased in equol producers but not nonproducers (27). Further, the serum insulin-like growth factor-I change was inversely associated with serum equol concentration. In another study of postmenopausal women, a stronger effect of isoflavone supplementation (900 mg/d for 84 d) on estrogen-responsive genes in peripheral lymphocytes was observed among equol producers compared with nonproducers (28). These studies suggest that there may be a differential response to isoflavones dependent on equol-producer phenotype; however, results are not consistent across studies.

Summary

The role of equol in relation to cancer remains unclear. To date, animal studies using either daidzein or racemic equol are few and there are no studies of S-(-)-equol specifically. The number of epidemiologic studies of equol exposure and cancer risk in humans is also limited and the studies are difficult to interpret. These studies have had to rely on measurement of circulating or urinary equol concentrations in populations routinely consuming soy. The ideal test would be a randomized, placebo-controlled intervention trial of supplemental equol with cancer endpoints. However, given the lack of preclinical data and the lack of consistent effects of equol-producer phenotype in soy-isoflavone intervention studies, such an undertaking is unwarranted. With the recent availability of sufficient amounts of R- and S-equol, animal studies of carcinogenesis and human intervention studies that address directly the effects of the equol enantiomers on intermediate biomarkers of cancer risk may help to further ascertain the effects of the equol-producer phenotype and equol itself.

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