Biochemical Pharmacology, Vol. 56, pp. 189–195, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00141-5

Induction of NADPH:Quinone Reductase by Dietary Phytoestrogens in Colonic Colo205 Cells

Weiqun Wang,† Lucy Q. Liu,* Carl M. Higuchi‡ and Hongwei Chen**

*Cancer Research Center of Hawaii, and ‡John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96813, U.S.A.

ABSTRACT. Phytoestrogens are a group of naturally occurring diphenolic compounds present in legumes, whole grains, fruits, and vegetables. High consumption of phytoestrogen-rich foods has been linked to a reduced incidence of cancers at many sites. A potential mechanism of dietary anticarcinogenesis involves the induction of detoxifying phase II enzymes such as NADPH:quinone reductase (QR). This study, therefore, examined the ability of six prominent phytoestrogens to affect cellular expression of QR in colonic cells. Colo205 cells were cocultured with various concentrations (0.001 to 10.0 μM) of each phytoestrogen, and then were assessed for cytosolic QR activity, cell growth, and QR mRNA expression. A maximum of 6- to 8-fold induction of QR activity was observed for both enterolactone and genistein, although at high concentrations they showed an adverse effect upon cell growth. The concentrations required to double the specific activity of QR for enterolactone and genistein were about 0.04 and 0.14 μM, respectively. A 2- to 3-fold increase of QR specific activity was found with either biochanin A (1.1 μM) or coumestrol (12.0 μM) treatments. No significant effects were found for daidzein or formononetin treatments. QR induction was further confirmed by using reverse transcription-polymerase chain reaction (RT-PCR) techniques to measure mRNA expression. A significant correlation between the expression of QR mRNA and the corresponding QR activity was observed ($r = 0.76$, $P < 0.001$). The results demonstrated that certain dietary phytoestrogens are capable of QR induction in Colo205 cells by promoting QR mRNA expression, and suggest a novel mechanism by which dietary phytoestrogens may be implicated in colorectal cancer chemoprevention. BIOCHEM PHARMACOL **56**;2:189–195, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. chemoprevention; phytoestrogens; NADPH:quinone reductase; enzyme induction; cell culture; colon

Phytoestrogens are a group of naturally occurring diphenolic compounds that are present in high concentrations in legumes and whole grains as well as in certain fruits and vegetables [1–3]. High consumption of phytoestrogen-rich foods has been linked to a reduced incidence of cancers at many sites including breast, prostate, and colon [4–12]. These epidemiological links seem reasonably established in view of the reported results from animal studies in which specific phytoestrogens have been shown to protect against experimentally induced mammary or other organ cancers [5, 13–17], and because phytoestrogens *in vitro* have been shown to possess many biological properties [18–23] by which they may be involved in the biologic and physiologic process accounting for cancer chemoprevention. However, inconsistent results regarding the proposed anticancer mechanisms of these compounds have been reported [24–28]. Certain phytoestrogens, for example, seem to be more estrogenic than antiestrogenic [25, 26], and the doses required to show the proposed mechanisms far exceed the highest concentrations of these compounds that can be achieved *in vivo* [28]. The question of whether these dietary compounds themselves are protective and/or what is the mechanism(s) by which they are protective still remains.

One proposed mechanism for cellular protection includes induction of phase II detoxification enzymes, which could be a major mechanism for affording cellular protection against the toxic and neoplastic effects of carcinogens [29–31]. Many carcinogens are not in their full carcinogenic forms when first encountered. They are usually metabolized to proximate carcinogens by phase I enzymes, e.g. cytochromes P450 that catalyze oxidative reactions [31, 32]. The oxidized metabolites of potentially carcinogenic xenobiotics are then detoxified by phase II metabolizing enzymes into forms that are relatively inert and even more easily excreted [30]. There is considerable evidence that induction of phase II detoxification enzymes can modulate the threshold for chemical carcinogenesis and then increase cellular resistance to carcinogen exposure [29–32]. QR§ is a main phase II detoxification enzyme (EC 1.6.99.2) that

† Corresponding author (and present address): Dr. Weiqun Wang, Department of Food Science and Human Nutrition, 1127 Nutritional Science Building, Iowa State University, Ames, IA 50011. Tel. (515) 294-0041; FAX (515) 294-6193; E-mail: wwang@iastate.edu.

Received 19 December 1997; accepted 16 March 1998.

§ *Abbreviations:* BHT, 3,5-di-*tert*-butyl-4-hydroxytoluene; CD, concentration required to double the specific activity of QR; QR, NADPH:quinone reductase; and RT-PCR, reverse transcription-polymerase chain reaction.

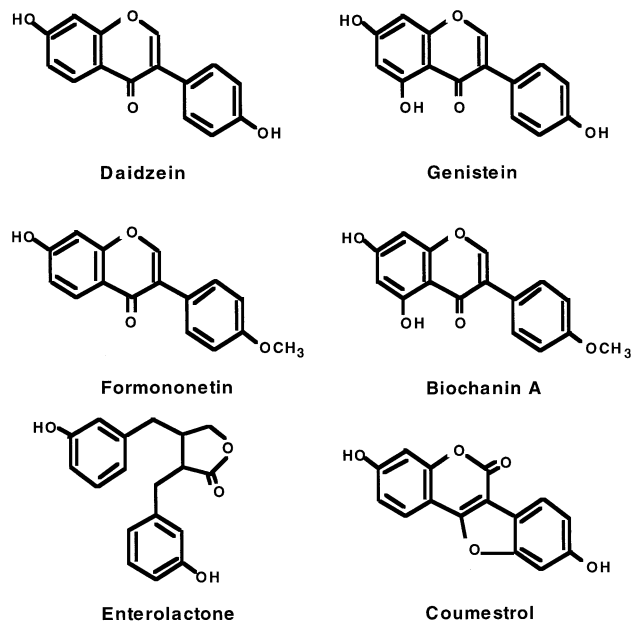

FIG. 1. Chemical structures of six prominent phytoestrogens used in this study.

catalyzes the two-electron reductions of a variety of quinone compounds and, thereby, protects cells against mutagenicity and carcinogenicity resulting from free radicals and toxic oxygen metabolites generated by the one-election reductions promoted by cytochromes P450 and other enzymes [33–35]. As a predominantly cytosolic FAD-containing protein, QR is inducible in the cells of many tissues such as the liver, lung, and colon. Induction of QR by specific dietary components has resulted in protection against carcinogenicity, mutagenicity, and other toxicities caused by carcinogenic xenobiotics [36–41]. Therefore, measurements of the induction of QR activity may provide an efficient approach to understanding the chemopreventive mechanisms of dietary compounds.

To experimentally test the hypothesis that dietary phytoestrogens may confer partial protection against colon cancer by inducing phase II detoxifying enzymes, we investigated the ability of six prominent phytoestrogens (see chemical structures in Fig. 1) including four isoflavonoids (daidzein, genistein, biochanin A, and formononetin), one lignan (enterolactone), and one coumestan (coumestrol) upon QR induction in a human colonic cell line. Additionally, RT-PCR techniques for QR mRNA levels were developed and performed to further confirm the possible regulatory mechanism.

MATERIALS AND METHODS

Chemicals

Biochanin A, daidzein, genistein, and formononetin were purchased from ICN Biomedicals. Enterolactone was provided by Dr. K. Wahala (University of Helsinki). Coumestrol was ordered from Serva Feinbiochemica. All other chemicals and cell culture media, unless indicated otherwise, were purchased from the Sigma Chemical Co.

Cell Culture and Treatments

Colo205, a human colon cancer cell line, was provided by the American Type Culture Collection and was maintained in continuous culture with weekly passage. Experimental cultures were initiated simultaneously in 6-well flat-bottom microculture plates for subsequent QR analysis and in 96-well flat-bottom microculture plates for assessment of proliferation, respectively. In a typical experiment, 100,000 Colo205 cells/mL were introduced into each well initially, grown at 37° in a 5% CO_2 atmosphere for 24 hr, and then induced for another 48 hr by exposure to fresh medium containing serial dilutions of each tested compound. Each test compound at concentrations ranging from 0.001 to 10 μM was supplemented with culture medium (90% RMPI-1640 + 10% fetal bovine serum). All phytoestrogens were solubilized in DMSO, and the final solvent concentration within all compounds containing cultures and the appropriate vehicle control cultures was adjusted to 0.2%.

QR Activity Assay

QR activity was determined as reported in one of our previous publications [41]. In brief, cells were removed from 6-well culture plates after detachment with 0.25% trypsin and 0.03% EDTA solution. One aliquot of cultured cells was suspended in ice-cold (pH 7.4) PBS and homogenized using a Polytron Homogenizer (Brinkmann Instruments, Inc.). After removal of gross debris by centrifugation, the protein content in the supernatants of cell lysates was measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories). The resulting supernatant of cell lysates at 0.4 μg protein was immediately added into the final 200 μL of reaction mixture per well containing 25 mM of Tris-HCl buffer (pH 7.4), 40 μM of 2,6-dichlorophenolindophenol, 0.2 mM of NADPH, 5 μM of FAD, 0.01% Tween-20, and 0.23 μg/μL of bovine serum albumin. Samples that also contained 10 μM of dicumarol served as blanks. Another set of blanks containing all reagents except dicumarol and cell lysates was used to correct for the nonenzymatic reduction of 2,6-dichlorophenolindophenol. The reaction mixture was incubated at 25° for 10 min, and the rate of reduction of 2,6-dichlorophenolindophenol was measured at 590 nm in a Series 750 Microplate Reader (Cambridge Technology, Inc.). Data obtained were calculated against a standard curve of 2,6-dichlorophenolindophenol. Enzyme specific activity was expressed as nanomoles of 2,6-dichlorophenolindophenol reduced per milligram of protein per minute. All samples were assayed in triplicate in three independent experiments.

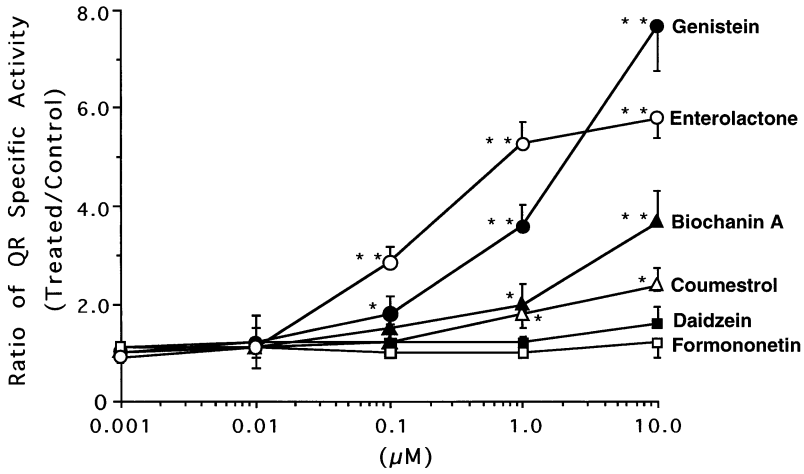

FIG. 2. Potency of induction of QR activity by six phytoestrogens in cultured human colonic cells. Colo205 cells were cocultured with each indicated compound at concentrations between 0.001 and 10 μM for 48 hr. Then the specific activity of QR was measured as described in Materials and Methods. Each value of the ratio of QR specific activity represents the mean ± SD of three independent experiments. *$P < 0.05$ and **$P < 0.01$ vs vehicle control. The mean uninduced activity in the vehicle control cells (0.2% DMSO only) was 67.3 ± 5.1 nmol/mg protein/min.

Proliferative Assay

Cell growth within the 96-well microculture plates was assessed colorimetrically after staining with sulforhodamine B as described in our previous publications [41, 42], and the results are expressed in optical density at 560 nm.

Quantitation of QR mRNA Expression by RT-PCR Techniques

Another aliquot of cells cultured in 6-well culture plates was used for the QR mRNA analysis by RT-PCR techniques. Total RNA was extracted from the cultured cells using a commercial total RNA isolation kit from Ambion, and the following cDNA was reversely transcripted from 2 μg of total RNA by using the first-strand synthesis kit with random decamers from Ambion. One microliter of cDNA products was amplified by a relative quantitative RT-PCR Module using synthesized primers for QR type-1 gene as target gene and 18S primers for 18S rRNA gene as internal standard with 70% 18S primer competimers from Ambion. The primers used for QR type-1 target gene amplification were synthesized by Genosys Biotechnologies, Inc. with sequences reported previously by Traver *et al.* [43] and further confirmed by searching in the GenBank Sequence Database at http://www.ncbi.nlm.nih.gov/BLAST. The sense primer (5′-AGAAGAGCACTGATCGTACTGG 3′) and the antisense primer (5′-CGTAATTGTAAG CAAACTCTCCTATG-3′) amplify a 379-bp region from 60 to 438 bp of QR cDNA. We used a 50-μL PCR reaction with a final concentration of 200 μM of deoxynucleotide triphosphates, 1× RT-PCR buffer (Ambion), a 0.6-μM concentration of each primer, and 1.5 units of *Taq* polymerase. Thermal cycling conditions following an initial denaturation at 94° for 4 min were: 30 sec at 95°, annealing at 65° for 30 sec, and extention at 72° for 30 sec. At the end of 30 cycles, samples were incubated at 72° for 10 min. Twenty microliters of the amplified products was loaded and separated on a 2% Sea Kem ME agarose gel containing 0.5 μg/mL of ethidium bromide. The RT-PCR products were visualized under UV light. For quantitation of QR mRNA expression, densitometric scanning of bands was performed using the Macintosh Image System with NIH Image Software downloaded from http://rsb.info.nih.gov/nih-image/download.html. The relative density of the QR target gene band (379 bp) was normalized to the 18S rRNA band (488 bp), and the mRNA levels were expressed as a ratio between QR target gene and 18S rRNA.

Statistical Methods

The SAS Statistical procedure GLM (SAS Institute Inc.) was applied for statistical analyses. An ANOVA was used to determine differences between the treated and the vehicle control. Covariate-adjusted means were compared by the levels of the independent variable [44]. Linear regression was used to model, the correlation between logarithms of relative QR mRNA levels and QR activities by Pearson's correlation coefficients (r value), using various concentrations at 0.01, 0.1, 1.0, and 10.0 μM for the 6 phytoestrogens ($N = 4 \times 6$). A probability of $P < 0.05$ was considered significant.

RESULTS

To investigate the inducing ability of phytoestrogens upon QR activity, concentration-dependent experiments were performed in the human colonic Colo205 cells. The induction of QR specific activity after a 48-hr exposure to various concentrations (between 0.001 and 10.0 μM) of each phytoestrogen is shown in Fig. 2. The greatest proportionate induction of QR specific activity was observed in the treatments with either enterolactone or genistein. Both of them induced QR activity in a concentration-dependent manner in the concentration range of 0.01 to 10.0 μM with a maximum of 6- to 8-fold induction, although the induction by enterolactone began to plateau at the highest concentration tested. The CDs of enterolactone and genistein were about 0.04 and 0.14 μM, respectively. In the concentration range of 1.0 to 10.0 μM, both biochanin A and coumestrol showed a moderate increase of QR with a

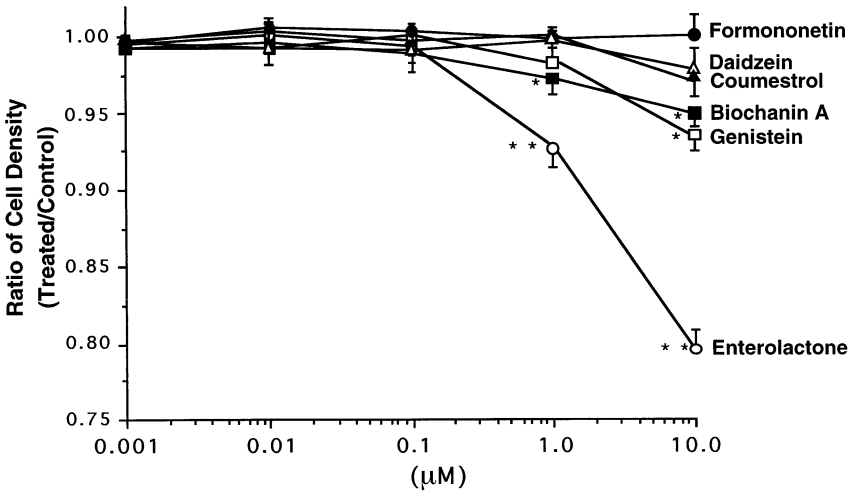

FIG. 3. Toxicity of six phytoestrogens in human colonic cells. Colo205 cells were cocultured with each indicated compound at concentrations between 0.001 and 10 μM for 48 hr. Then the cell density was assessed colorimetrically after staining with sulforhodamine B and expressed as optical density at 560 nm as described in Materials and Methods. Each value of the ratio of cell density represents the mean ± SD of three independent experiments. *$P < 0.05$ and **$P < 0.01$ vs vehicle control.

maximum of 2- to 3-fold stimulation. The CDs for biochanin A and coumestrol were 1.1 and 12.0 μM, respectively. However, no significant effects were found in the treatments with daidzein or formononetin in this *in vitro* model.

The toxic effects upon cell growth, as shown by the sulforhodamine B staining method, are presented in Fig. 3. Enterolactone, genistein, and biochanin A at high concentrations were found to be antiproliferative. The maximum inhibitions were found to be 20% by entarolactone, 7% by genistein, and 4% by biochanin A in comparison with the vehicle control. No significant effects on cell growth were observed in the treatments with daidzein, coumestrol, or formononetin at any of the concentration regimens.

To further investigate the underlying mechanism of induction of QR activity by phytoestrogens, RT-PCR techniques were developed to measure QR mRNA expression in Colo205 cells after treatment with each compound for 48 hr. As illustrated in Fig. 4, QR mRNA was induced significantly by genistein in the concentration range of 0.01 to 10.0 μM. In the same experiment, BHT at 1.0 μM served as a positive control [39]. Similar results were found for the treated cells with enterolactone in the same concentration range of 0.01 to 10.0 μM, but the mRNA levels reached a plateau at the highest concentration tested. Both biochanin A and coumestrol moderately stimulated QR mRNA expression in a concentration range of 1.0 to 10.0 μM, whereas no effect was observed in the treatments with either daidzein or formononetin (data not shown).

To reveal the association of QR mRNA expression with QR activity induction, Pearson's correlation coefficient was determined using the logarithms of relative QR mRNA expression plotted to the corresponding QR specific activity in each of the phytoestrogens tested at the effective concentration range between 0.01 and 10.0 μM. As shown in Fig. 5, a strong linear correlation was observed ($N = 24$, $r = 0.76$, $P < 0.001$).

DISCUSSION

Dietary phytoestrogens are currently receiving increased attention because of their possible role in human cancer prevention. As part of a study focusing on chemopreventive

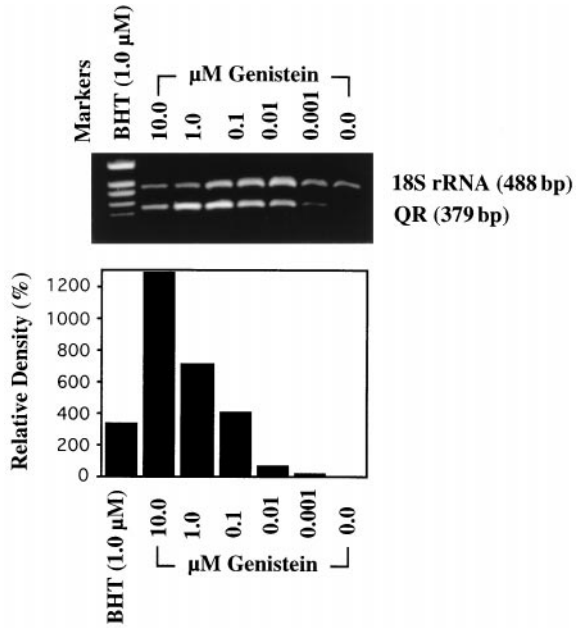

FIG. 4. RT-PCR analysis demonstrating QR mRNA expression in Colo205 cells after a 48-hr treatment by genistein at various concentrations or BHT at 1.0 μM as a positive control. One microliter of cDNA products, which were reversely transcripted from 2 μg of total RNA, was amplified by a relative quantitative RT-PCR Module kit using synthesized primers for QR type-1 gene as target gene and 18S primers for 18S rRNA gene as internal standard, as described in Materials and Methods. Amplified products were separated on agarose gel, and the RT-PCR products were visualized under UV light. For quantitation of QR mRNA expression, densitometric scanning of bands was performed, and the relative density of the QR target gene band (379 bp) normalized to the 18S rRNA band (488 bp) is shown (bottom). The DNA markers of the digested pGEM-3 DNA present bands at 676, 517, 460, 396, and 350 bp, respectively.

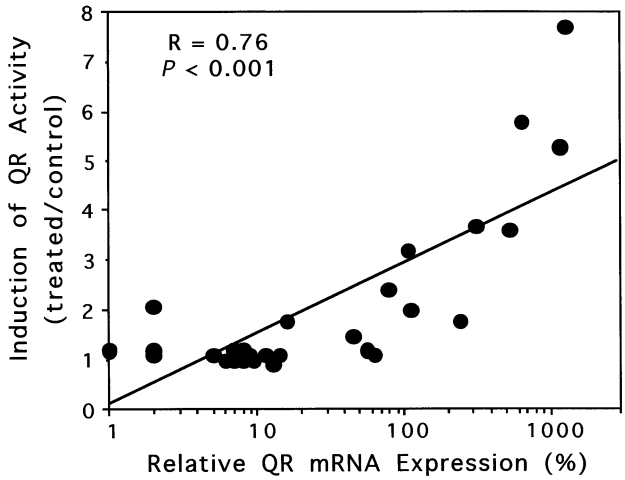

FIG. 5. Correlation between the induction of QR activity and the relative expression of QR mRNA. Colo205 cells were cocultured with various phytoestrogens at concentrations between 0.001 and 10 μM for 48 hr. Then both QR specific activity and mRNA levels were measured simultaneously, as described in Materials and Methods. Pearson's correlation coefficient (*r*) was determined between logarithms of relative QR mRNA expression and QR specific activity in each of the phytoestrogens tested at effective concentrations between 0.01 and 10 μM (N = 24, *r* = 0.76, *P* < 0.001).

mechanisms, we measured the effects of six prominent dietary phytoestrogens upon QR induction in a human colonic Colo205 cell line by using our previously established microtiter-assay method [41]. We found a concentration-dependent 6- to 8-fold induction in enzyme activity with the addition of either enterolactone or genistein at 0.01 to 10.0 μM, and a 2- to 3-fold induction with either biochanin A or coumestrol at 1.0 to 10.0 μM. No significant inducing effect was found for daidzein or formononetin. The CDs of genistein, enterolactone, biochanin A, and coumestrol were found to be 0.04, 0.14, 1.0, and 12.0 μM, respectively. Enterolactone, genistein, and biochanin A at a high concentration, however, showed inhibitory effects upon cell growth. A maximum of 20% inhibition by enterolactone might be the reason to cause a plateau of QR induction at the highest concentration tested. To learn more about the regulatory mechanism, we developed RT-PCR techniques to measure QR mRNA expression in Colo205 cells. Analogous to the induction of enzyme activity was the induction of QR mRNA expression, which again was strongly increased by enterolactone and genistein, moderately stimulated by biochanin A and coumestrol, and little affected by daidzein and formononetin. Significant linear correlation between the QR mRNA expression and the corresponding QR activity was observed. Therefore, the observed induction of QR by phytoestrogens appears to be through the promotion of QR mRNA expression and/or stabilization of mRNA levels.

The different regulatory ability of phytoestrogens tested in this study may be associated with their chemical structures. The 5-hydroxyl group in the isoflavonic phytoestrogens, for example, seems crucial because formononetin and daidzein, which do not possess a 5-hydroxyl group, did not affect QR activity. Genistein strongly induced QR, while daidzein, which lacks only one hydroxyl group at the 5-site, had no effect. That genistein rather than daidzein showed a strong inducing ability is in good agreement with other studies conducted in murine Hepa 1c1c7 cells.* It will be interesting to validate a close structure–function relationship by screening more phytoestrogens or structurally related agents in this *in vitro* model.

To our knowledge, this is the first published study presenting an inducing response of QR to various dietary phytoestrogens in human colonic cells. The significant induction of QR by specific phytoestrogens found in this study appears important, suggesting a novel mechanism through which dietary phytoestrogens may contribute, at least in part, to colon cancer chemoprevention. A point of interest to consider is the concentrations of phytoestrogens used in this exploratory study. The physiologically relevant concentration of active phytoestrogens in human circulating plasma has been estimated to be near 1.0 μM. This makes the results of this study much more interesting, because induction of QR by both genistein and enterolactone was seen at a concentration even lower than 1.0 μM. Although biochanin A and coumestrol required a high concentration of over 1.0 μM to exert inducing ability, it could be reasonably assumed that more phytoestrogen aglycones may be likely to occur in the intestinal tract where phytoestrogen precursors are first hydrolyzed and absorbed. On the other hand, a high concentration of phytoestrogens may be toxic. Of the six phytoestrogens examined, three, especially enterolactone, showed detectable toxicity on cell growth. Cytotoxicity measurements are essential because phase II enzyme inducers may be toxic and/or may be carcinogenic. In view of both enzymatic induction and cytotoxicity, genistein appears especially promising as the best candidate of the six compounds tested for cancer chemoprevention, with stronger QR induction but less cytotoxicity.

In conclusion, a significant induction of QR activity in human colonic Colo205 cells by dietary phytoestrogens has been demonstrated for the first time in this study. The results support the hypothesis that a high dietary consumption of phytoestrogens may confer partial protection against colon cancer by induction of specific detoxifying enzymes such as QR, and suggest a novel mechanism by which dietary phytoestrogens may be implicated in relation to cancer chemoprevention.

We thank Dr. Loic Le Marchand for providing molecular facilities and Dr. Adrian Franke for sponsoring authentic chemicals used in this study. These data have been presented, in part, at the 3rd International Conference on Nutrition and Cancer held in Irvine, California, July

* Lee SK, Song L, Mata-Greenwood E, Kelloff GJ, Steele VE and Pezzuto JM, Modulation of *in vitro* biomarkers of the carcinogenic process by chemopreventive agents. *Proceedings of the Annual Meeting of the American Association for Cancer Research* **38**: 365, 1997.

16–19, 1997. This study was supported, in part, by grants from the National Cancer Institute (R30CA71789) and the Hawaii Community Foundation (955464). Development of QR methodology was supported, in part, by a grant from the National Cancer Institute (K07CA01686).

References

1. Wattenberg LW, Chemoprevention of cancer by naturally occurring and synthetic compounds. In: *Cancer Chemoprevention* (Eds. Wattenberg L, Lipkin M, Boone CW and Kelloff GJ), pp. 19–39. CRC Press, Boca Raton, 1992.
2. Setchell KDR, Naturally occurring non-steroidal estrogens of dietary origin. In: *Estrogens in the Environment. II. Influence on Development* (Ed. McLachlan JA), pp. 619–655. Academic Press, New York, 1979.
3. Price KR and Fenwick GR, Naturally occurring oestrogens in foods—A review. *Food Addit Contam* **2:** 73–106, 1985.
4. Adlercreutz H, Western diet and western disease: Some hormonal and biochemical mechanisms and associations. *Scand J Clin Lab Invest* **50(Suppl):** 3–23, 1990.
5. Messina MJ, Persky V, Setchell KDR and Barnes S, Soy intake and cancer risk: A review of the *in vitro* and *in vivo* data. *Nutr Cancer* **21:** 113–131, 1994.
6. Knight DC and Eden JA, A review of the clinical effects of phytoestrogens. *Obstet Gynecol* **87:** 897–904, 1996.
7. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J and Day NE, Dietary effects on breast-cancer risk in Singapore. *Lancet* **337:** 1197–1200, 1991.
8. Wu AH, Ziegler RG, Horn-Ross PL, Nomura AMY, West DW, Kolonel LN, Rosenthal JF, Hoover RN and Pike MC, Tofu and risk of breast cancer in Asian-Americans. *Cancer Epidemiol Biomarkers Prev* **5:** 901–906, 1996.
9. Mills PK, Beeson WL, Abbey DE, Fraser GE and Phillips RL, Dietary habits and past medical history as related to fatal pancreas cancer risk among Adventists. *Cancer* **61:** 2578–2585, 1988.
10. Severson RK, Nomura AMY, Grove JS and Stemmermann GN, A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Res* **49:** 1857–1860, 1989.
11. Jenab M and Thompson LU, The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. *Carcinogenesis* **17:** 1343–1348, 1996.
12. Steinmetz KA and Potter JD, Food-group consumption and colon cancer in the Adelaide case-control study. I. Vegetables and fruit. *Int J Cancer* **53:** 711–719, 1993.
13. Adlercreutz H, Phytoestrogens: Epidemiology and a possible role in cancer protection. *Environ Health Perspect* **103**(Suppl 7)**:** 103–112, 1995.
14. Giri AK and Lu L-JW, Genetic damage and the inhibition of 7,12-dimethylbenzanthracene-induced genetic damage by the phytoestrogens, genistein and daidzein, in female ICR mice. *Cancer Lett* **95:** 125–133, 1995.
15. Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ and Barnes S, Genistein suppresses mammary cancer in rats. *Carcinogenesis* **16:** 2833–2840, 1995.
16. Murrill WB, Brown NM, Zhang J-X, Manzolillo PA, Barnes S and Lamartiniere CA, Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. *Carcinogenesis* **17:** 1451–1457, 1996.
17. Constantinou AI, Mehta RG and Vaughan A, Inhibition of *N*-methyl-*N*-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. *Anticancer Res* **16:** 3293–3298, 1996.
18. Wei H, Bowen R, Cai Q, Barnes S and Wang Y, Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc Soc Exp Biol Med* **208:** 124–130, 1995.
19. Wang W, Higuchi CM and Zhang R, Individual and combinatory effects of soy isoflavones on the *in vitro* potentiation of lymphocyte activation. *Nutr Cancer* **29:** 29–34, 1997.
20. Peterson G and Barnes S, Genistein inhibition of the growth of human breast cancer cells: Independence from estrogen receptors and the multi-drug resistance gene. *Biochem Biophys Res Commun* **179:** 661–667, 1991.
21. Hirano T, Gotoh M and Oka K, Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. *Life Sci* **55:** 1061–1069, 1994.
22. Constantinou A and Huberman E, Genistein as an inducer of tumor cell differentiation: Possible mechanisms of action. *Proc Soc Exp Biol Med* **208:** 109–115, 1995.
23. Adlercreutz H, Hockerstedt K, Bannwart C, Bloigu S, Hamalainen E, Fotsis T and Ollus A, Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem* **27:** 1135–1144, 1987.
24. Naik HR, Lehr JE and Pienta KJ, An *in vitro* and *in vivo* study of antitumor effects of genistein on hormone refractory prostate cancer. *Anticancer Res* **14:** 2617–2619, 1994.
25. Makela S, Sautti R, Salo L and McLachlan JA, Phytoestrogens are partial estrogen agonists in the adult male mouse. *Environ Health Perspect* **103**(Suppl 7)**:** 123–127, 1995.
26. Whitten PL, Lewis C, Russell E and Naftolin F, Potential advance effects of phytoestrogens. *J Nutr* **125**(Suppl 3)**:** 771S–776S, 1995.
27. Wang W, Tanaka Y, Han Z and Higuchi CM, Proliferative response of mammary glandular tissue to formononetin. *Nutr Cancer* **23:** 131–140, 1995.
28. Zava DT and Duwe G, Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells *in vitro*. *Nutr Cancer* **27:** 31–40, 1997.
29. Wattenberg LW, Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res* **52**(Suppl)**:** 2085S–2091S, 1992.
30. Talalay P, DeLong MJ and Prochaska HJ, Molecular mechanisms in protection against carcinogenesis. In: *Cancer Biology and Therapeutics* (Eds. Cory JG and Szentivanyi A), pp. 197–216. Plenum Press, New York, 1987.
31. Talalay P, Mechanisms of induction of enzymes that protect against chemical carcinogenesis. *Adv Enzyme Regul* **28:** 237–250, 1989.
32. Guengerich FP, Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. *Am J Clin Nutr* **61:** 651S–658S, 1995.
33. Ernster L, DT diaphorase: A historical review. *Chem Scr* **27A:** 1–14, 1987.
34. Lind C, Hochstein P and Ernster L, DT diaphorase properties, reaction mechanism, metabolic function. In: *Symposium on Oxidases and Related Redox Systems* (Eds. King TE, Mason HS and Morrison M), pp. 321–347. Pergamon Press, New York, 1979.
35. Joseph P, Xie T, Xu Y and Jaiswal AK, NAD(P)H:quinone oxidoreductase$_1$ (DT-diaphorase): Expression, regulation, and role in cancer. *Oncol Res* **6:** 525–532, 1994.
36. Benson AM, Hunkeler MJ and Stanley JS, Increase of NAD(P)H:quinone reductase by dietary antioxidants: Possible role in protection against carcinogenesis and toxicity. *Proc Natl Acad Sci USA* **77:** 5216–5220, 1980.
37. Spencer SR, Wilczak CA and Talalay P, Induction of glutathione transferases and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues. *Cancer Res* **50:** 7871–7875, 1990.
38. Helzlsouer KJ and Kensler TW, Cancer chemoprotection by oltipraz: Experimental and clinical considerations. *Prev Med* **22:** 783–795, 1993.
39. De Long MJ, Prochaska HJ and Talalay P, Induction of NAD(P)H:quinone reductase in murine hepatoma cells by

phenolic antioxidants, azo dyes, and other chemoprotectors: A model system for the study of anticarcinogens. *Proc Natl Acad Sci USA* **83:** 787–791, 1986.
40. Prochaska HJ, Santamaria AB and Talalay P, Rapid detection of inducers of enzymes that protect against carcinogens. *Proc Natl Acad Sci USA* **89:** 2394–2398, 1992.
41. Wang W and Higuchi CM, Induction of NAD(P)H:quinone reductase by vitamins A, E and C in Colo205 colon cancer cells. *Cancer Lett* **98:** 63–69, 1995.
42. Higuchi CM and Wang W, Comodulation of cellular polyamines and proliferation: Biomarker application to colorectal mucosa. *J Cell Biochem* **57:** 256–261, 1995.
43. Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA and Rose D, Characterization of a polymorphism in NAD(P)H:quinone oxidoreductase (DT-diaphorase). *Br J Cancer* **75:** 69–75, 1997.
44. Snedecor GW and Cochran WG, *Statistical Methods*, 8th Edn. Iowa State University Press, Ames, IA, 1989.