

WHEAT LIGNANS: PROMISING CANCER PREVENTIVE AGENTS¹

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16.1 INTRODUCTION

Adequate dietary fiber intakes, particularly fiber from cereal grains, have been associated with a low risk of colorectal cancer (1). Wheat bran appears to protect against tumor development more consistently than other sources of plant fiber in a number of experimental colon cancer studies (2–4). Several hypotheses have been established to explain the link between wheat bran and cancer prevention, including the increase of overall GI transit time, dilution of carcinogenic compounds, release of short-chain fatty acids, and promotion of tumor suppressor signaling, among others (5–8). However, the experimental evidence by using different fiber sources or different doses on colon cancer prevention is controversial (9,10). Especially, a study conducted in the Takemoto laboratory showed that antitumor activities of wheat bran from various wheat cultivars were significantly different even when fiber content was equal (11).

Lignans are a group of the phytochemicals that are composed of phenylpropane dimer linked with a 1,4-diarylbutane structure by β - β bonds. Figure 16.1 shows the chemical structure of a few prominent lignans. Lignans not only present abundantly in flaxseed but also present in various grains such as wheat (12). In wheat, lignans are located in the pericarp and aleurone layers with the highest concentration in wheat bran (13). The main lignan in wheat bran is SDG. Table 16.1 lists the contents of SDG in flaxseed, wheat bran, and some other selected plant foods.

As one group of secondary metabolites, lignans are synthesized via phenylpropanoid pathway. However, the completed biosynthetic pathways to the lignans in wheat are not clear. The Lewis laboratory from the Washington State University

¹This work is supported in part by a USDA Cooperative Project (KS 680-0199184), Agricultural Experiment Station, Kansas State University (contribution No. 07-77-B).

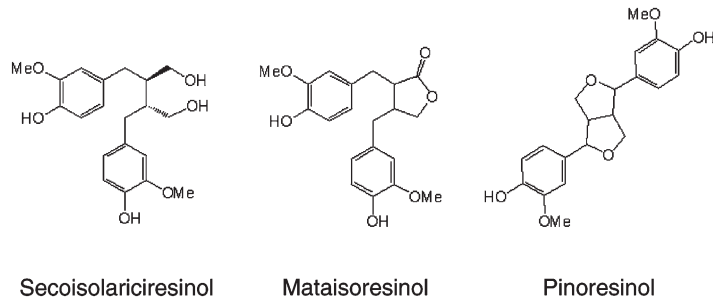


Figure 16.1 Chemical structures of the prominent lignans: secoisolariciresinol, matairesinol, and pinoresinol.

TABLE 16.1 Level of Lignans in Selected Plant Foods

Food	Total lignans, $\mu\text{g/g}$
Whole flaxseed	636–2213 ^a
Flaxseed meal	675 ^b
Flaxseed flour	527 ^b
Wheat bran	0.5–83 ^a
Oat bran	6.5 ^b
Soy bean	8.6 ^b

^aThe contents of lignans in 10 flaxseed samples (unpublished data) and 4 wheat cultivars were measured by HPLC analysis as described in our previous publication (Qu et al. 2005).

^bAdapted from Reference (14).

reported a series of studies of the lignan biosynthesis in *Forsythia intermedia* (15–17). As shown in Fig. 16.2, the biosynthetic pathways to secoisolariciresinol occur via coupling of two coniferyl alcohol molecules to afford pinoresinol. Then pinoresinol undergoes sequential reduction by pinoresinol–lariciresinol reductase (PLR) to generate lariciresinol and secoisolariciresinol (15,16). Our ongoing studies are trying to

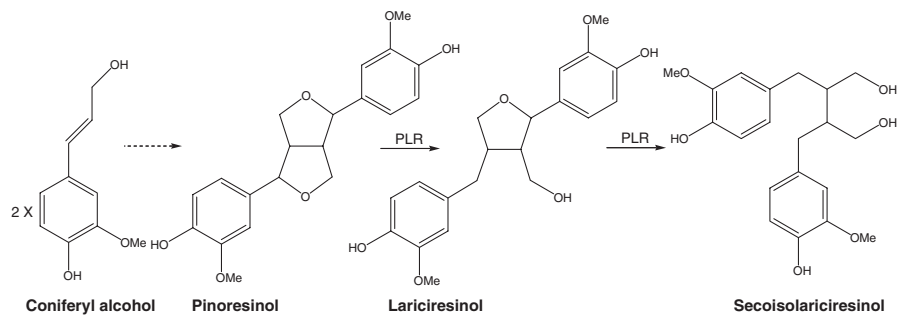


Figure 16.2 Schematic overview of the lignan biosynthesis pathway. Pinoresinol–lariciresinol reductase (PLR) is the last enzyme directly downstream of the biosynthetic pathways from coniferyl alcohol to secoisolariciresinol (modified from Reference 16).

develop genetically engineered wheat lines that have overexpressed PLR and thus may potentially generate a high level of SDG.

16.2 LIGNANS AND CANCER PREVENTION

When SDG is consumed, it is oxidized by intestinal microflora to lignan metabolites including enterodiol and enterolactone (Fig. 16.3). The pharmacokinetics of lignan metabolites after SDG consumption have been reported (18). Therefore, dietary

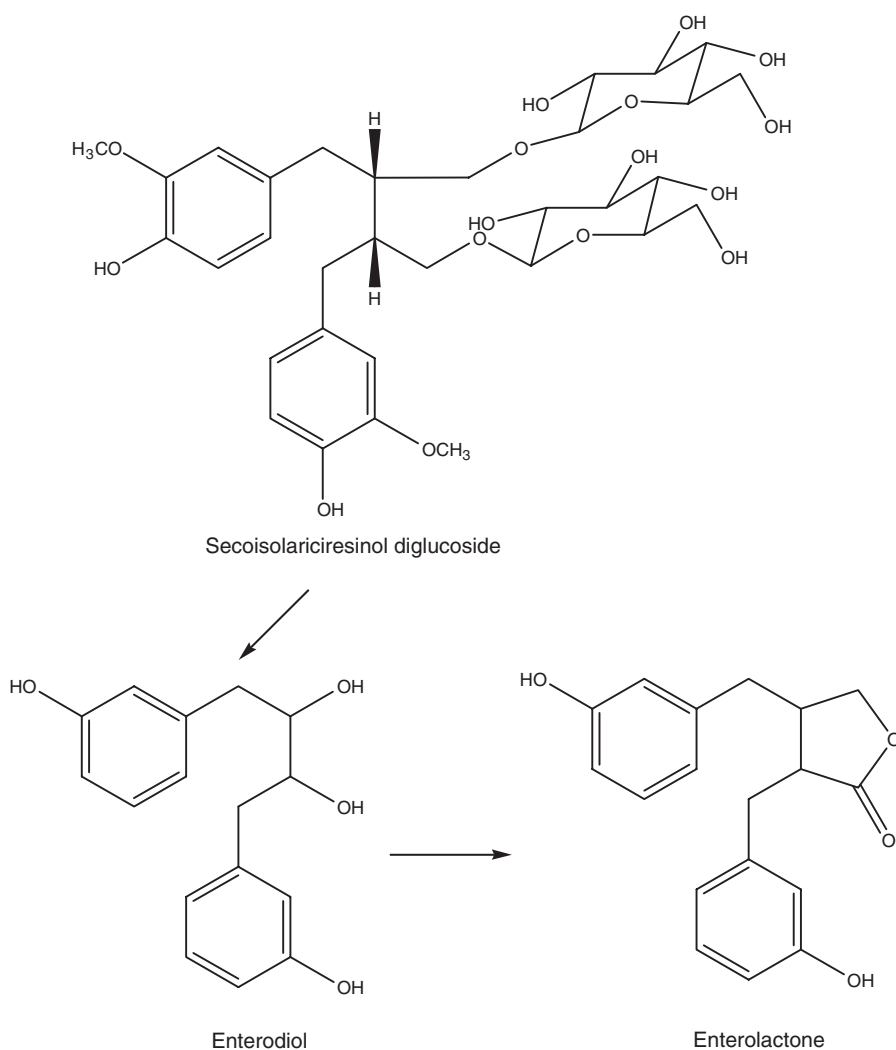


Figure 16.3 Chemical structures of the secoisolariciresinol diglucoside and its mammalian metabolites: enterodiol and enterolactone.

lignans such as SDG may exert their biological effects through their metabolites. Compelling data from epidemiological, clinical, and experimental studies, along with *in vitro* mechanistic studies, have suggested that lignans may be promising for cancer prevention.

16.2.1 Epidemiological and Clinical Studies

Although some controversial data exist, many epidemiological studies have suggested an inverse relationship between lignan consumption and various cancer risks. Adlercreutz and his team in the University of Helsinki have intensively studied the relationship between plasma or urine levels of lignan metabolites and cancer risk since 1981. They found that a lignan-low diet was related to an increased breast cancer risk in case-control and prospective studies (19). A review paper summarized a conclusion that the most support for a cancer preventive role of dietary lignans was observed for premenopausal breast cancer (20). Furthermore, a case-control study conducted in the Netherlands recently demonstrated that a substantial reduction of colorectal adenoma risk was associated with a high plasma level of lignan metabolites (21). Another case-control study from Sweden further supported a reverse correlation of serum levels of lignan metabolites with prostate cancer risk (22). However, the conflicting results have been reported. A case-control study nested within a prospective cohort study by the New York University Women's Health Study did not find a protective role of circulating lignan enterolactone against breast cancer development (23). A prospective Zutphen Elderly cohort study conducted in the Netherlands did not find an association of a total lignan intake with cancer risk (24). Arts and Hollman (25) reviewed three prospective nested case-control studies and three case-control studies and found an inverse association between lignans, and breast cancer, and observed prostate cancer only in case-control studies but not in prospective studies. The conflicting data have been suggested, at least in part, due to inadequate databases used in dietary lignan estimation (26). It is interesting to note that a clinical intervention study by the Thompson laboratory has shown a potential reduction of breast cancer growth in patients supplemented with dietary flaxseed, the richest source of dietary lignans (27).

16.2.2 Experimental Animal Studies

In comparison with epidemiological studies, more consistent results regarding cancer-preventive role of dietary lignans have been demonstrated in animal models. For example, dietary flaxseed at 10% or purified SDG at an equivalent dose during sucking significantly suppressed later 9,10-dimethyl-1,2-benzanthracene-induced mammary tumorigenesis in rats (28). A study conducted in athymic mice carrying LNCaP human prostate cancer xenografts showed that dietary lignan 7-hydroxymatairesinol at 0.3% inhibited the tumor growth significantly (29). A similar model using athymic mice but with MCF-7 human breast cancer xenografts demonstrated dietary flaxseed at 10% attenuated soy protein-stimulated tumor growth (30). Recently, the Thompson laboratory reported that dietary flaxseed at 10% inhibited metastasis but

TABLE 16.2 Inhibition of Azoxymethane (AOM)-induced Formation of Aberrant Crypt Foci (ACF) in F344 Rats by Dietary Secoisolariciresinol Diglucoside (SDG)

Treatment	No. rats with ACF	Total no. ACF/colon
Control diet	6/6	264 ± 43
0.01% SDG	6/6	196 ± 51*

Male F344 rats at 6 weeks old were fed either control or experimental diet containing SDG at 0.01% for 4 weeks. One day after the first dietary treatment, rats were given s.c. injection with 15 mg/kg B.W. of AOM once per week for 2 weeks. At the end of 4-week dietary treatment, rats were terminated under ether euthanasia and the entire colon was resected for 0.2% methylene blue staining. The number of ACF per colon was scored under a light microscope. Values are means ± SD, * $P < 0.05$ versus the controls ($n = 6$).

not the recurrence of estrogen receptor negative human breast cancer cells after excision in nude mice (31).

We also conducted an animal study to assess the effects of dietary lignan SDG on azoxymethane-induced aberrant crypt foci (ACF) formation in rat colons. ACF are morphologically altered crypts, alone or in cluster, first identified by microscopic examination of methylene blue-stained whole-mount preparations of colonic mucosa from azoxymethane-treated rodents (32). Studies demonstrated that ACF in humans are important precursors to human colon cancer (33). As shown in Table 16.2, we found that 0.01% SDG significantly reduced the formation of ACF.

16.3 PLAUSIBLE MECHANISMS OF LIGNANS FOR CANCER PREVENTION

As a group of the phytoestrogens, lignans may act through estrogen receptor-mediated mechanisms. Webb and McCullough (20) have reviewed this potential mechanism in details. They also discussed a potential interaction of the phytoestrogenic activity with other growth hormones as well as antioxidant and antiproliferative activities. In fact, our previous studies and others have shown that enterolactone is a strong antioxidant against human LDL oxidation (34,35). We also found that enterolactone was capable of inhibiting colon cancer cell growth and inducing detoxification enzyme activity (36,37). Furthermore, the chemopreventive mechanisms of lignans and lignan metabolites via antiinflammatory and immunosuppressive activities have been intensively reviewed by Saleem et al. (38).

We assessed the cancer preventive mechanisms of two prominent lignan metabolites on a human colonic cancer cell line SW480. Treatment of SW480 cells with enterolactone and enterodiol, alone or in combination, at 0–40 μM resulted in a dose- and time-dependent decrease in cell numbers (37). While the cytotoxicity as measured by tyran blue staining was not significantly changed, DNA flow cytometric analysis indicated that treatments induced cell cycle arrest at S phase (Fig. 16.4). Furthermore, apoptosis analysis by TUNEL assay showed an increased percentage of apoptotic cells in the treated cells (Fig. 16.5). These results suggest that inhibition of cancer cell growth by lignan metabolites appears to be mediated with cytostatic and apoptotic mechanisms.

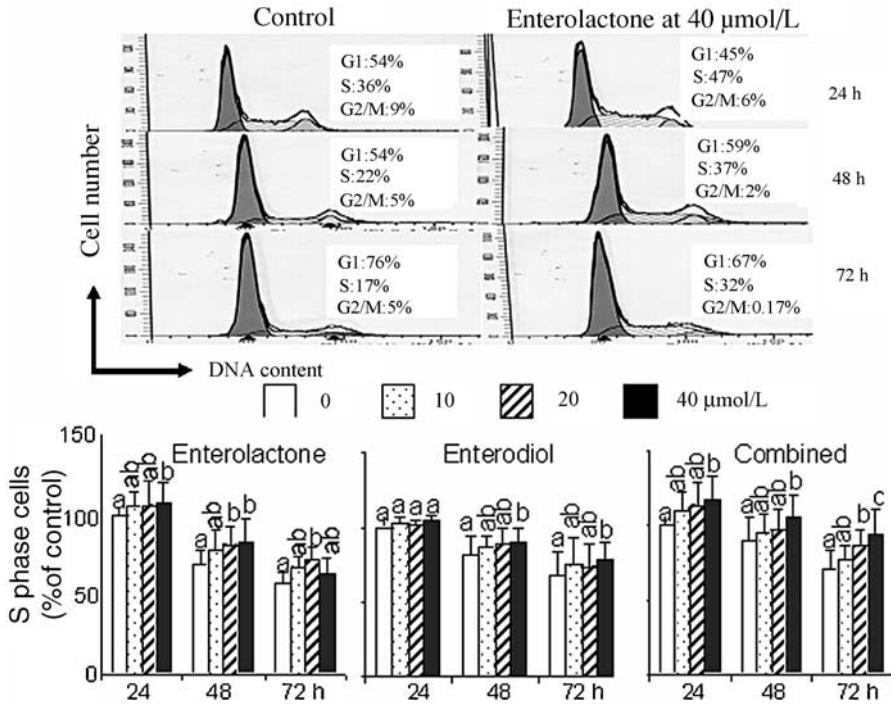


Figure 16.4 Induction of cell cycle arrest at S phase by enterolactone, enterodiol, or their combination. The human colon cancer SW480 cells were cocultured with the indicated lignan metabolites at 0–40 μmol/L for 24–72 h and the cell cycle was measured by DNA flow cytometric analysis. Values are mean ± SD (n = 5–6). Means within a treatment without a common letter differ, P ≤ 0.05. (Adapted from Reference 3.).

Taken together, the observations, direct *in vitro* cell culture studies along with *in vivo* animal experiment, as well as the epidemiological correlation between lignan intake and antitumor activities, suggest that dietary lignans may attribute to the observed cancer prevention. However, most studies so far have been performed by using lignan-rich foods such as flaxseed. We recently conducted a study by measuring lignan contents in four wheat cultivars (e.g., “Madison,” “Ernie,” “Betty” and “Arapahoe”) and tried to link them with antitumorigenesis in APC^{Min} mouse model. APC^{Min} mice carry truncated adenomatous polyposis coli (APC) and thus spontaneously develop multiple intestinal neoplasia (Min). Drankhan et al. (11) applied this model into tumor prevention by feeding wheat bran and found a significant difference in antitumor activity among various wheat cultivars when the fiber contents were equal. As shown in Table 16.3, we compared the SDG contents in four wheat cultivars with antitumor activities in APC^{Min} mice and found a significant correlation between SDG contents and antitumor activities, suggesting lignans may attribute to the observed colon cancer prevention by wheat bran or whole grain products.

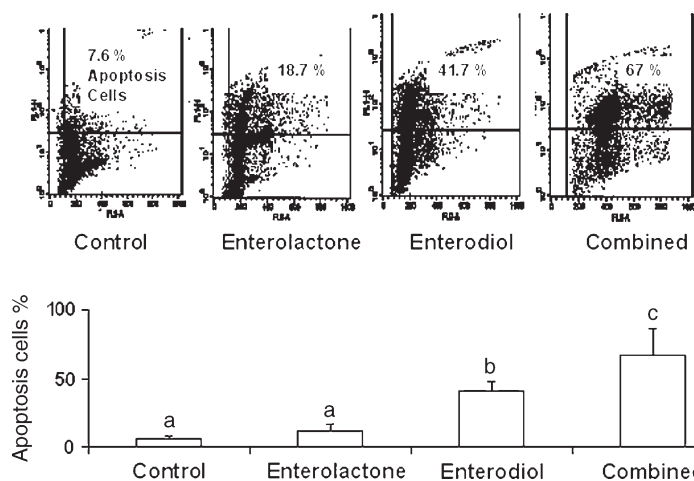


Figure 16.5 Increase of apoptotic cells by enterodiol and the combination of enterodiol and enterolactone. The human colon cancer SW480 cells were cocultured with the indicated lignan metabolites at 40 $\mu\text{mol/L}$ for 72 h and the cellular apoptosis was measured by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay. Values are mean \pm SD ($n = 4$). Means with different alphabetic letters are significantly different, $P \leq 0.05$. (Adapted from Reference 37 with permission.)

TABLE 16.3 Correlation between the contents of secoisolariciresinol diglucoside (SDG) in various wheat cultivars and antitumor activities in APC^{Min} mice

Wheat cultivars	SDG, $\mu\text{g/g}^a$ Means \pm SE ($n = 2-3$)	Antitumor activities ^b , % of inhibition
Madison	82.9 \pm 16.0	58.6 \pm 1.8
Ernie	52.2 \pm 22.4	36.1 \pm 3.5
Betty	42.7 \pm 0.1	23.7 \pm 4.5
Arapahoe	Undetectable	27.2 \pm 2.6
Correlation coefficient	$r = 0.73$ ($P < 0.02$)	

^aThe contents of lignans in four wheat cultivars were measured by HPLC analysis as described in our previous publication (37).

^bThe antitumor activities assessed in APC^{Min} mice were adapted from Drankhan et al. (2003). Briefly, female APC^{Min} mice at 5 weeks old (Jackson Lab, Bar Harbour, ME) were fed wheat bran at 45% in basal diet ($n = 10$ per group). The antitumor activities were calculated based on a formula as follows: (total tumor numbers in mice fed basal diet – total tumor numbers in mice fed wheat bran diet)/total tumor numbers in mice fed basal diet.

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