Abstract

Flavones and isoflavones may play a prominent role in cancer prevention since these compounds are found in numerous plants that are associated with reduced cancer rates. This article reviews recent epidemiological and animal data on isoflavones and flavones and their role in cancer prevention. It covers aspects of the bioavailability of these dietary constituents and explores their mechanism of action. Human epidemiology data comes primarily from studies in which foods rich in isoflavones or flavones are associated with cancer rates. This approach has been particularly useful with isoflavones because of their abundance in specific foods, including soy foods. The bioavailability of flavones and isoflavones has been shown to be influenced by their chemical form in foods (generally glycoside conjugates), their hydrophobicity, susceptibility to degradation, the microbial flora of the consumer, and the food matrix. Some information is available on how these factors influence isoflavone bioavailability, but the information on flavones is more limited. Many mechanisms of action have been identified for isoflavone/flavone prevention of cancer, including estrogenic/antiestrogenic activity, antiproliferation, induction of cell-cycle arrest and apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, and changes in cellular signaling. It is expected that some combination of these mechanisms will be found to be responsible for cancer prevention by these compounds. Compelling data suggest that flavones and isoflavones contribute to cancer prevention; however, further investigations will be required to clarify the nature of the impact and interactions between these bioactive constituents and other dietary components. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Flavones; Isoflavones; Cancer; Epidemiology; Bioavailability; Mechanisms

Abbreviations: ACF, aberrant crypt foci; Apc, adenomatous polyposis coli; AOM, azoxymethane; DMBA, dimethylbenz(a)anthracene; GI, gastrointestinal; GST, glutathione-S-transferase; LDL, low-density lipoprotein; MNU, methylnitrosourea; ODMA, O-desmethylangolensin; QR, NAD(P)H:quinone reductase (EC 1.6.99.2); TPA, 12-0-tetradecanoyl-phorbol-13-acetate.

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1. Introduction

Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of human cancers at many sites (Block et al., 1992; Messina et al., 1998; Steinmetz & Potter, 1991a). There are many plausible mechanisms by which intake of vegetables and fruits may prevent carcinogenesis. Plant foods contain a wide variety of anticancer phytochemicals with many potential bioactivities that may reduce cancer susceptibility (Waladkhani & Clemens, 1998; Wattenberg, 1992a, 1992b; Steinmetz & Potter, 1991b; Adlercreutz, 1990). Flavonoids and isoflavonoids are especially promising candidates for cancer prevention (Bravo, 1998; Kuo, 1997; Potter & Steinmetz, 1996; Knight & Eden, 1996; Hollman et al., 1997; Knekt et al., 1997; Adlercreutz, 1995).

Flavonoids are plant secondary metabolites, present in all terrestrial vascular plants. Flavonoids are defined chemically as substances composed of a common phenylchromanone structure (C6–C3–C6), with one or more hydroxyl substituents, including derivatives (Table 1). In marked contrast to flavonoids, isoflavonoids possess a 3-phenylchroman skeleton that is biogenetically derived from the 2-phenylchroman skeleton of the flavonoids. Isoflavonoids are found in plants of the subfamily *Papilionoideae* of the *Leguminosae*, which includes soybeans (Harborne, 1989). Flavonoids and isofla-

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Flavonoids occur commonly as ester, ether, or glycoside derivatives or mixtures thereof, and embrace over 4000 compounds (Harborne, 1989). In mammals, flavonoids and isoflavonoids occur only through dietary intake. The average daily human intake of flavonoids in the United Kingdom and the United States has been estimated to be 20 mg to 1 g. These compounds are present in fruits, vegetables, grains, nuts, tea, and wine (Pierpoint, 1986).

Flavonoids and isoflavonoids have shown many biological properties that may account for cancer chemoprevention (Bravo, 1998; Kuo, 1996; Birt et al., 1999; Messina et al., 1998; Adlercreutz, 1995). In recent years, considerable attention has been paid to their abilities to inhibit the cell cycle, cell proliferation, and oxidative stress, and to induce detoxification enzymes, apoptosis, and the immune system. In view of heightened interest in the biological effects of flavonoids and isoflavonoids, the time was appropriate to review the current knowledge of the epidemiology, anticarcinogenic activity, bioavailability, and potential mechanisms of action of flavonoids and isoflavonoids. Building upon this foundation will facilitate development of new strategies and approaches for cancer control.

2. Epidemiology

Studies relating flavonoid and isoflavonoid intake to cancer rates have assessed the relationship of food groups rich in these compounds to cancer risk. Currently, no intervention trials have been conducted. A large body of data has demonstrated the importance of plant intake in reducing cancer risk (Steinmetz et al., 1994). Because flavonoids and isoflavonoids are found in particular foods, studies relating specific foods to cancer have been used to develop hypotheses on the importance of flavonoids and isoflavonoids in cancer prevention.

Flavonoids are widely distributed in plants, but little quantitative information is available on their presence in foods, and, thus, only a few studies have attempted to assess the relationship between consumption of foods rich in flavonoids and the prevention of cancer. The expanding data on the impact of flavonoids in the prevention of animal carcinogenesis and the impact of flavonoids on a plethora of endpoints that have been associated with cancer prevention have led investigators to attempt to associate the intake of these compounds with human disease patterns. A study of this type is being conducted in Zutphen, The Netherlands. In this study, a cohort of 878 men were followed beginning in 1960 for 25 years, and a report was published by Hertog et al. in 1994. This study associated the intakes of 5 flavonoids — quercetin, kampherol, myricetin, apigenin, and luteolin — with the incidence or mortality from all causes of cancer or with the mortality from alimentary or respiratory tract cancers. In this study, flavonoids were found to be primarily from tea, apples, and onions (Hertog et al., 1992, 1993). Flavonoid intake was not associated with all site cancer outcomes or with the rates of cancer in the alimentary or respiratory tract (Hertog et al., 1994). However, the intake of flavonoids from vegetables and fruits was only inversely associated with the risk of alimentary and respiratory cancers, suggesting that constituents of fruits and vegetables other than flavonoids were probably important in the lower rates of cancer associated with these foods. In the 7 countries that were included in the Zutphen study, a 25% decrease in coronary heart disease mortality could be explained by flavonoid intake, but cancer risk was not associated with flavonoid intake (Hertog et al., 1995).

A much larger and more recent study from Finland that followed a cohort from 1967–1991 further investigated the association between flavonoid intake and human cancer (Knekt et al., 1997). This study provides stronger evidence for a protective role of flavonoids against cancer. The incidence of cancer at all sites was inversely associated with flavonoid intake, and this association was primarily due to the lower rates of lung cancer in the groups with the highest flavonoid intake (relative risk of 0.54 with a 95% confidence interval of 0.34–0.87, in comparison with the lowest quartile). Fig. 1 shows the relative risk of cancer of the lung, stomach, and colorectum in the three panels by flavonoid quartile. The protection was greatest in individuals who were under 50 years of age (relative risk of 0.33 with a 95% confidence interval of 0.15–0.77) and in non-smokers (relative risk of 0.13 with a 95% confidence interval of 0.03–0.58) (Knekt et al., 1997). Furthermore, this association was found not to be due to other nutrients that have been studied for their ability to prevent cancer, such as vitamin E, vitamin C, or β-carotene.

Another recent study used the same database on the flavonoid composition of foods (Hertog et al., 1992, 1993) to assess dietary intakes of 4 flavonoids (quercetin, kampherol, myricetin, and luteolin), and specific carotenoids (α-carotene, β-carotene, lutein, and lycopene) in relation to lung cancer in Spanish women (García-Closas et al., 1998). They observed weak associations between the greatest lycopene or kampherol intakes and the least lung cancer in women, but the $P$ values did not reach statistical significance (0.15 and 0.10, respectively).

This line of research can be criticized for the limited number of flavonoids assessed in the fruits and vegetables, inaccuracies in the analytical methods employed, or the small size of the investigations (generally a few hundred cancer cases). However, these early investigations suggest the need for additional associative human studies. We need to know more about the constituents of fruits and vegetables, the variability in these constituents, and the factors that influence the constituents in order to improve interpretation of studies of this type. The reported relationship between flavonoid intake and cancer may be caused by some other constituent in fruits and vegetables. We must remember the lesson learned when, based solely on associative evidence, it was assumed that the protective constituent in fruits and vegetables was β-carotene. Intervention trials have demon-
Isoflavonoids are much more narrowly distributed in foods, with soybeans being the primary human food that is rich in these compounds. Because of this, the data associating soybean consumption with reduced cancer rates has been used as evidence for a role of isoflavones in cancer prevention. However, it is critical to keep in mind that soybeans are also a rich source of trypsin inhibitor (Kennedy, 1995), other proteins with health benefits, phosphatidyl inositol, saponins, and sphingolipids, all of which have potential health benefits. All of these soybean constituents demonstrate tumor preventative properties in animal models (Fournier et al., 1998; Birt, 2001). We recently demonstrated that 20% by weight of dietary soy protein significantly reduced rat intestinal mucosa levels of polyamine, a biomarker of cellular proliferation for colorectal cancer risk. However, 0.1% of soy isoflavones (genistein and daidzein at a 1:1 mixture) did not affect polyamine levels (Wang & Higuchi, 2000). Recent reviews have emphasized the complexity of soy foods and the difficulty in assuming that associations that suggest protective properties of soy foods are due to single constituents (Fournier et al., 1998; Birt, 2001).

Because of the association between diets in Japan and China and lower rates of cancers, such as those of the breast, prostate, and colon, than in Europe and the United States, many investigators have assumed that this is due to soy food consumption in Japan and China. Other factors in the Asian diet may be responsible. For example, a case control study of gastric cancer in China (Hu et al., 1988) found that Chinese cabbage seemed to play an important role in protecting against this common cancer. Consumption of fermented and salted soy paste was positively associated with gastric cancer (Hu et al., 1988). Further, a case control study of lung cancer in Chinese women in Hong Kong indicated that fresh fruit and fish consumption was associated with lower cancer rates (Koo, 1988). When considering adenocarcinoma or large cell lung tumors, they observed lesser rates of disease in people with greater consumption of leafy green vegetables, carrots, tofu, fresh fruit, and fresh fish. Finally, Severson et al. (1989) prospectively studied prostate cancer in Hawaiian men of Japanese descent. Prostate cancer rates were least among men who consumed diets abundant in rice ($P = 0.017$) and tofu (modestly protective, $P = 0.054$) and low in seaweed ($P = 0.017$). These studies in Asian populations suggest that soy foods, the predominant source of isoflavones, are often associated with reductions in cancer rate, but they do not consistently appear to be the primary protective component of the Asian diet.

In reviewing the evidence for dietary soybeans as a protective factor against breast cancer, Wu et al. (1998) noted the difficulties in assessing the relationship between the level of intake and protection. Trying to combine studies in Asia with studies from the West is particularly difficult, since soy food consumption in Asia is much greater than in the West. Furthermore, case control and prospective epidemiological investigations that have provided a suggestion of protection against cancer by soy foods have not provided adequate information on the bioactive constituents in the soy foods, the portion size, or the other components that may be protective in the diets of people who eat soy foods. These investigators summarize the data on soy intake and breast cancer as suggesting, but not consistently, that soy foods provide some protection against breast cancer.

Because of these difficulties in relating reported intake of soy-based foods with cancer rates, many investigations have attempted to use biomarkers of soybean intake as surrogate markers of soy food intake. Considering that the purpose of this review is to consider the role of isoflavones in cancer prevention, it is indeed fortuitous that the biomarkers used are usually isoflavones. Unfortunately, the use of isoflavones as a marker for soy food intake leads...
many casual readers to assume that isoflavones are the sole active constituents in soy foods. Isoflavones may be markers for another constituent or for a combination of constituents that are the agents responsible for the cancer prevention. Keeping this in mind, it is intriguing that some recent investigations provide support for isoflavones as protective against cancer. For example, Zheng et al. (1999) assayed urinary isoflavonoids in a case control study of breast cancer in Shanghai. Interestingly, urinary excretion of total phenols and all individual isoflavonoids, particularly glycetin, was lower in breast cancer patients than in controls. The adjusted odds ratio for breast cancer was 0.14 (relative risk of 0.02–0.88 with a 95% confidence interval) for women with the highest urinary excretion of phenol and total isoflavonoids in comparison with women in the lowest tertile.

3. Anticarcinogenesis

Studies of cancer prevention in experimental animals have assessed the impact of a wide variety of flavonoids and a select few isoflavones for their efficacy in inhibiting cancer in a number of animal models.

As in other studies of dietary prevention of cancer, models of breast and colon cancers have been prominent in assessing cancer prevention by flavonoids and isoflavonoids. Citrus flavonoids were the focus of studies by So et al. (1996). They determined the impact of hesperetin and naringenin from oranges and grapefruit, respectively, and baicalein, galangin, genistein, and quercetin from non-citrus sources on MDA-MB-435, a human breast carcinoma cell line. They assessed the inhibition of cell proliferation in culture, and all of the flavonoids showed low cytotoxicity (>500 μg/mL for 50% cell death). The IC_{50} (concentration that inhibited cell proliferation by 50%) was lowest for baicalein (5.9 μg/mL), intermediate for quercetin, hesperetin, naringenin, and galangin (10.4–56.1 μg/mL), and highest for genistein (140 μg/mL). Combinations of flavonoids (1:1) generally were effective in inhibiting cell proliferation at lower doses of total flavonoid (IC_{50}, 4.7–9.2 μg/mL). Studies with orange juice, grapefruit juice, naringenin, and naringin, the glycosylated form of naringenin, assessed the inhibition of mammary tumor development in 7,12-dimethylbenz(a)anthracene (DMBA)-treated female Sprague–Dawley rats. In two experiments, the greatest inhibition of cancer generally was observed with orange juice (double-strength reconstitution in place of drinking water) or naringin (0.24- to 100-g diet)-supplemented rats, but there was considerable variability between experiments (So et al., 1996). Further studies by this laboratory demonstrated that these compounds in media were effective against both estrogen receptor-positive and estrogen receptor-negative breast cancer cells (IC_{50}, 1–18 μg/mL) and that double-strength orange juice was more effective than double-strength grapefruit juice in preventing the development of DMBA-induced mammary cancers in rats (Guthrie & Carroll, 1998).

The soybean isoflavones genistein and daidzein have been studied extensively for anti-breast cancer activity because of their estrogen receptor antagonist and agonist activities. Studies by Constantinou et al. (1996) assessed the ability of genistein and daidzein injections (0.8 mg daily for 180 days) against N-methyl-N-nitrosourea-induced mammary tumors in Sprague–Dawley rats. Genistein and daidzein moderately reduced the number of tumors, but only marginally reduced the tumor incidence. They assessed topoisomerase activity and protein tyrosine kinase activity in normal and tumorous glands, and found that while these activities were elevated in tumors, isoflavonoid treatment did not alter this elevation. Thus, the inhibition of cancer by isoflavones was independent of influences on topoisomerase or protein tyrosine kinase (Constantinou et al., 1996). Further research by this group with cultured human breast cancer cells demonstrated the inhibition of growth of estrogen receptor positive (MCF-7) or estrogen-receptor negative (MDA-MB-468) cells by 30–150 μmol/L genistein and that this inhibition was paralleled by increased expression of maturation markers (Constantinou et al., 1998). In addition, treatment of these cells with genistein for 6 days with 30 μmol/L before implantation into nude mice decreased the growth of these cells in the animal. These investigators suggested that the inhibition of human cancer cell growth by genistein was unrelated to the estrogenic activity of this compound.

Another approach to assessing genistein in the prevention of breast cancer was used by Lamartiniere et al. (1995). Considering that neonatal estrogen was known to inhibit both spontaneous and chemically induced breast cancer, and that diet early in life has been suspected of playing a role in human breast cancer, these investigators treated neonatal rats with 5-mg genistein on days 2, 4, and 6 postpartum. They then induced mammary tumors with DMBA on day 50, and observed a delay in the development of tumors and a reduction in the number of tumors in the rats that were pretreated with genistein (Lamartiniere et al., 1995). Parallel studies determined that pre-pubertal administration of 500 μg/g body weight genistein with the DMBA protocol similarly inhibited breast cancer development. Finally, recent studies demonstrated that exposure of rats to genistein (0, 25, and 250 mg/kg diet) from conception to 21 days postpartum prior to treatment with DMBA at 50 days postpartum resulted in a dose response inhibition of mammary tumors. These investigators further reported that mammary glands at 21 and 50 days of age had fewer terminal end buds and fewer undifferentiated terminal ductal structures (Fritz et al., 1998). Further research demonstrated that neonatal administration of 5 mg/pup genistein was adverse to normal ovarian follicular development, but that pre-pubertal genistein (500 μg/g body weight) did not appear to be toxic (Lamartiniere et al., 1998a, 1998b).
In concert with parallel estrogen or genistein in early life protecting against breast cancer, further studies determined that genistein (750 ppm in the diet), like estrogen, when administered during tumor development, enhanced tumor growth of estrogen-responsive tumors (Hsieh et al., 1998). Genistein (10 nM to 10 μM) was found to enhance the proliferation of MCF-7 human breast cancer cells, both in vitro and in ovariectomized athymic mice. Genistein (1 μM) acted as an estrogen agonist in that it induced pS2, estrogen-responsive gene expression. These results suggest that caution should be used in considering cancer prevention by soybean isoflavones in humans. This is a particular concern because high-potency isoflavone preparations are now available to consumers as dietary supplements.

Studies with the drug flavone acetic acid (125–500 mg/kg) demonstrated that compounds in the flavonoid class could kill transplantable rodent colon tumors (Kal et al., 1992). Parallel studies were conducted with rats with transplantable radiation-induced lung tumors, rats with rhadomyosarcoma BA, and mice with colon 38 tumor. The rat lung and rhadomyosarcoma BA tumors did not experience a delay in growth, but the mouse colon tumor was inhibited by intraperitoneal injections of flavone acetic acid (125–150 mg/kg).

Several investigations have assessed the ability of flavonoids to inhibit azoxymethane (AOM)-induced mouse or colon tumors. Dietary quercetin (0.5–5%) and rutin (4%) were found to inhibit hyperproliferation and dysplasia and to reduce colon tumor incidence in AOM-treated mice (Deschner et al., 1991, 1993). Interestingly, the glucoside form, rutin, was generally less effective than quercetin in colon cancer prevention (Deschner et al., 1991). Kawamori et al. (1995) assessed the flavonoid liquiritin in comparison with a number of other natural phenolic compounds, and determined that while liquiritin treatment (0.02% in the diet) reduced some indices of proliferation, it increased others, and it did not inhibit aberrant crypt foci (ACF), an early, preneoplastic lesion. Tanaka et al. (1997) determined that diosmin and hesperidin, both alone (1000 ppm) and in combination (900:100 ppm, respectively), inhibited indices of cell proliferation, ACF, and colon cancer. The combination of these agents did not increase their efficacy.

Recent research in our laboratory assessed the impact of the ubiquitous flavonoid apigenin on cell proliferation and cell cycle in human colon cancer cell lines (Wang et al., 2000). SW480 [mutant ras and p53 and truncated adenomatous polyposis coli (Apc) genes], HT-29 (mutant p53 and truncated Apc genes), and Caco-2 (mutant p53 and truncated Apc genes) were cultured with apigenin (0–80 μM), and cell number and cell cycle were assessed in parallel studies. These studies suggested that the SW480 cells, with all of the noted genes mutated, were the most sensitive to apigenin-induced cell cycle arrest in G2/M and to the inhibition of cell proliferation. Further studies determined that apigenin (0.1%) in the diet of CF-1 mice that were pretreated with AOM inhibited ACF. However, thus far, we have no evidence for the inhibition of colon carcinogenesis, although we have conducted three sequential carcinogenesis experiments (Au et al., unpublished).

Genistein, the most extensively studied soybean isoflavone, was found to inhibit AOM-induced colonic ACF at doses of 75 and 150 mg/kg (Pereira et al., 1994). Recent studies with soy flakes, soy flour, genistein, and Ca^{2+} demonstrated that soy flakes, soy flour, and genistein reduced ACF and that genistein (0.015%) caused the greatest reduction (Thiagarajan et al., 1998). Finally, using the min mouse model that carries a mutant Apc gene, Sorensen et al. (1998) demonstrated that while the nonsteroidal anti-inflammatory drug Sulindac inhibited intestinal tumors, mice given 2 different soy isolates, either isoflavone (475 mg/kg diet) or soy protein, did not experience a reduction in intestinal tumors.

The complexity of the relationship between topical flavone and 7,8-benzo[ghi]flavone on polycyclic hydrocarbon skin carcinogenesis was revealed in studies reported by Alworth and Slaga (1985). The purpose of this research, at least in part, was to attempt to identify enzyme induction/inhibition profiles that would be characteristic of inhibitors of 12-0-tetradecanoyl-phorbol-13-acetate (TPA)-promoted polycyclic hydrocarbon carcinogenesis. The results demonstrated that 7,8-benzo[ghi]flavone inhibited or enhanced tumor development, depending on the polycyclic hydrocarbon initiator, and that flavone applications were generally inhibitory at higher (4500 nmol) topical doses, but enhancing at low doses (450 nmol). The flavonoids robinetin, quercetin, and myricetin were assessed in a parallel report for their impact on polycyclic hydrocarbon initiation (Chang et al., 1985). These flavonoids were found not to be tumor promoters when given alone. Quercetin and robinetin (250 nmol) had little effect on tumor initiation by benzo[a]pyrene, but they weakened inhibition initiated by a diol epoxide of benzo[a]pyrene (Chang et al., 1985). A recent report noted that munetone, an isoflavonoid from a tropical shrub, inhibited ornithine decarboxylase activity and thus, would be expected to inhibit carcinogenesis in the skin of mice (Lee et al., 1999).

A series of 14 flavonoids were examined for their ability to inhibit the Epstein–Barr virus early antigen activation by TPA (Konoshima et al., 1992), and 5,7,2′-tri hydroxy flavone and 5,7,2′,3′-tetrahydroxy flavone (85 nmol) were found to be particularly active and also to inhibit tumor promotion in the DMBA-initiated, TPA-promoted model of skin carcinogenesis. Recent investigations have focused on the inhibition of skin carcinogenesis in the DMBA/TPA model by the flavonoid silymarin (Lahiri-Chatterjee et al., 1999). Exceptional inhibition of skin tumor promotion was observed when silymarin (3.6 and 12 mg) was applied prior to each treatment with the tumor promoter TPA (75% reduction in incidence and 97% reduction in tumor multiplicity with 12-mg applications). Further, studies by this group have demonstrated the parallel inhibition of numerous markers of cellular proliferation by silymarin.
The Birt laboratory (Birt et al., 1986, 1997; Wei et al., 1990) conducted a series of investigations into the inhibition of chemically induced (DMBA/TPA-treated) and UV light-induced skin carcinogenesis by apigenin in mice (Fig. 2 shows the UV-light study). Parallel studies in the Pelling laboratory with cultured epidermal cells (Lepley et al., 1996) suggested that the inhibition of skin carcinogenesis might be due to the induction of cell cycle arrest by apigenin, as is thoroughly discussed in Section 5.3. Interestingly, the cellular concentrations of apigenin that are achieved in mouse skin following topical treatment with doses of apigenin (5–10 μmol) that inhibit carcinogenesis are similar to cellular concentrations that are achieved in cultured cells that exhibit cell cycle arrest (20–30 μM) (Lepley et al., 1996).

Studies by Wei et al. (1998) focused on the ability of the isoflavone genistein to inhibit skin tumorigenesis. In an anti-initiation protocol, genistein (10 μM) was applied prior to DMBA, and both tumor incidence and multiplicity were reduced. This was associated with blockage of DMBA-induced bulky DNA-adduct formation (Wei et al., 1998). In two anti-promotion protocols, genistein was applied prior to TPA application, and papilloma multiplicity was reduced, but incidence was not altered. Genistein did not modify the proliferation marker ornithine decarboxylase, but it did inhibit oxidative and inflammatory events (Wei et al., 1998). Thus, it appears that while most evidence points to flavonoids inhibiting skin tumor promotion through the inhibition of cellular proliferation, the isoflavone genistein may act by an alternative mechanism.

The inhibition of chemically induced oral cancer by flavonoids was assessed in the rat 4-nitroquinoline 1-oxide-induced model (Makita et al., 1996; Tanaka et al., 1997b). Dietary chalcone, 2-hydroxychalcone, and quercetin were administered in the diet (500 mg/kg), either during or following an 8-week treatment protocol with the carcinogen, and tumor rates were reduced in all flavonoid groups (Makita et al., 1996). In the second experiment, diosmin and hesperidin were fed alone (1000 mg/kg each) or in combination (900 and 100 mg/kg, respectively) using the above protocols, and all groups experienced a reduction of oral cancers (Tanaka et al., 1997b). In both of these studies, the inhibition of oral carcinogenesis was associated with an inhibition of cell proliferation markers (Makita et al., 1996; Tanaka et al., 1997b).

Flavonoid inhibition of hepatocarcinogenesis by aflatoxin B1 (Nixon et al., 1984), and phenobarbital treatment following diethylnitrosamine initiation (Lee, K. W. et al., 1995) was assessed. Aflatoxin carcinogenesis in trout was inhibited by 50 and 500 ppm β-naphthoflavone, but this inhibition was not dependent upon the induction of the mixed-function-oxidase system (Nixon et al., 1984). Soybean isoflavone extract [containing 920 or 1840 μmol (240 or 480 mg) total isoflavones/kg diet, with approximately equal proportions of genistein and daidzein] was fed during phenobarbital treatment following initiation, and the devel-

![Fig. 2. Inhibition by topical apigenin of squamous cell carcinoma induced by UV light in SK-1 mouse skin. UV light was administered for 11 weeks, with a cumulative dose of 40 J/cm². Apigenin treatment at 5 μmol (P < 0.1) or at 10 μmol (P < 0.01) before UV light treatment increased tumor free survival. Modified from Birt et al. (1997).](image-url)
opment of γ-glutamyltransferase and placental glutathione-S-transferase (GST) positive foci was inhibited by both doses of isoflavone extract fed for 3 months. However, feeding isoflavone extract for 11 months showed that while the isoflavones inhibited altered hepatic foci in the presence of phenobarbital, the high dose increased the development of preneoplastic foci promoted by isoflavones alone. This study demonstrated the importance of dose and time of exposure in the prevention of carcinogenesis by isoflavones, and suggested that a narrow margin of safety may exist for isoflavones in cancer prevention.

The inhibition of prostate cancer growth and development has been the focus of several investigations. Prostate cancer, like breast cancer, is observed less frequently in Asia than in Western cultures, and because of this, there has been some interest in the ability of soybeans and genistein to prevent the development of this disease. Unfortunately, the absence of representative models for prostate carcinogenesis has impeded research in this area. Studies conducted on the ability of diets containing soy flour to inhibit tumor growth were reported by Zhang, J.-X. et al. (1997). Feeding 33% by weight of the diet as soy flour resulted in a 30–40% reduction in the growth of transplanted Dunning R3327 prostatic adenocarcinoma in rats. Furthermore, studies with cultured prostate cancer cell lines suggested that genistein (1 μg/mL and 100 ng/mL, respectively) was cytotoxic to the rat prostate cell line MAT-lylu and the human prostate cancer cell line PC-3. However, genistein added to the drinking water (0.07–0.285 mg/kg/day) failed to inhibit the growth of MAT-lylu cells implanted into rats (Naik et al., 1994). In studies where prostate lesions were induced by diethylstilbestrol treatment of male rats for 3 days following birth, 7% soybean feeding reduced the development of severe dysplasia at 9 months, but soybean feeding did not significantly reduce prostatic dysplasia at 12 months (Makela et al., 1995). Furthermore, the ability of soybean isoflavones to inhibit methylnitrosourea (MNU)-induced prostate seminal vesicle adenocarcinomas in Lobund–Wistar rats was inhibited by feeding high isoflavone (1.69 mg/g)-supplemented soy diet before initiation by MNU compared with the same diet low in isoflavones (Pollard & Luckert, 1997). Studies using the 3,2′-dimethyl-4-aminobiphenyl and testosterone propionate model in rats indicated that feeding a soybean isoflavone mixture containing 74% genistein and 21% daidzein, at total doses of 100 and 400 ppm, reduced the incidence of adenocarcinoma in the prostate and seminal vesicles by 50% compared with rats fed control diet (Onozawa et al., 1999). It is noteworthy that the relevance of the diethylstilbestrol, MNU, and 3,2′-dimethyl-4-aminobiphenyl/testosterone propionate rodent models to human prostate cancer is not clear.

Another model of carcinogenesis that was studied with flavonoids for potential prevention was 20-methylcholanthrene-induced mouse sarcomas (Elangovan et al., 1994). Fibrosarcoma induction in mice was inhibited by dietary quercetin and luteolin (1% in the diet), administered either during initiation or promotion (Naik et al., 1994).

The anti-metastatic potential of genistein and daidzein was assessed using the pulmonary metastasis model of B16 BL6 murine melanoma cells injected into C57BL/6 mice (Li et al., 1999). Mice were pre-fed diets containing isoflavones (113,225,450 or 900 μmol/kg) for 2 years before and after intravenous injection of the melanoma cells, and the isoflavone-containing diets inhibited the number of lung metastases in a dose-dependent manner (Li et al., 1999). These intriguing data need to be followed up with different model systems and methods of administering the flavonoids.

4. Bioavailability and metabolism of dietary flavonoids and isoflavonoids

4.1. Application of general principles of bioavailability to flavonoids and isoflavonoids

Bioavailability is defined operationally and pharmacologically as the proportion of the compound administered intravenously that appears in plasma over time (measured as area under the curve) when the compound is administered orally. This represents the proportion of the compound that is absorbed from the gastrointestinal (GI) tract. Bioavailability from a nutritionist’s viewpoint is often expressed as the proportion of an ingested dose that is excreted in urine compared with the proportion excreted in feces over time. However, lipid-soluble compounds would not be directly excreted in urine, but they would appear as their more water-soluble metabolites. Notably, for lipid-soluble compounds, some fraction would enter fat stores, so ingestion versus excretion will not tell the full story of the biological fate of those compounds. Either plasma or urinary contents of a compound may reflect its absorption from the GI tract. Many compounds are extensively metabolized, and their total bioavailability is reflected in the amounts of the parent compound plus all bioactive metabolites. Compounds may undergo extensive modification within the GI tract before initial absorption or after biliary excretion before reabsorption, further metabolism, possible degradation, and ultimate excretion. All compounds will be excreted from the body over time, but this time course is considerably longer for highly lipophilic compounds that are not metabolized than for hydrophilic compounds. The time course of excretion depends on the compound’s metabolism to more water-soluble conjugates, which are excreted through urine or bile. Flavonoids and isoflavonoids are not very soluble in either water or organic solvents. Even in their best solvents (ethanol, methanol, acetone, trile), they are only moderately soluble. Flavonoids and isoflavonoids are present in foods mostly as glycosidic conjugates, which are more water soluble than the parent aglycones, and may require enzymatic cleavage of the sugar moiety by mammalian or microbial glucosidases before absorption. The phenolic
moieties of these compounds may be conjugated by UDP-glucuronosyltransferases to form glucuronides or by sulfotransferases to form sulfates. The glucuronide and sulfate conjugates are more readily transported in the blood and excreted in bile or urine than are the parent aglycones. The spectrum of conjugation products may be species- and gender-dependent. These metabolites are not necessarily biologically inert. Much work remains in order to determine the metabolic fates and bioavailabilities of specific flavonoids and isoflavonoids. The solubility, metabolic fate of compounds due to endogenous and exogenous biotransformation, and interaction of the compounds with other dietary components all determine isoflavonoid and flavonoid bioavailability (Hendrich et al., 1998a, 1998b).

4.2. Apparent absorption and disposition kinetics of flavonoids and isoflavonoids

The absorption of isoflavonoids by the GI mucosa seems to partly depend on the relative hydrophobicity/hydrophilicity of these compounds. There is no evidence for facilitated or active transport of isoflavonoids. Isoflavone aglycones are of appropriate molecular weight (250 g/mol) to permit their diffusion. Isoflavone glycosides predominate in foods, but isoflavone glycosides have not been detected in human blood plasma or urine (Xu et al., 1994, 1995; King & Bursill, 1998). Human intestinal or gut microfloral glycosidases seemingly cleave these moieties before the isoflavonoids can be absorbed. Isoflavone aglycones elute in the following order under the conditions we commonly use for reverse-phase HPLC of these compounds: daidzein, then slightly later glycitein, and much later genistein (Wang & Murphy, 1994; Zhang et al., 1999b; Xu et al., 1994). Although genistein has three hydroxyl groups and daidzein only two, a hydrogen bond is created in genistein’s structure, increasing its hydrophobicity compared with daidzein. Consistent with its greater hydrophobicity, genistein seems to be retained in the human body for longer times than is daidzein. Humans fed soymilk containing 13% more genistein than daidzein (predominantly as their glycosides) had apparent peak plasma genistein concentrations of 50% greater than peak daidzein concentrations (at 6 hr after dose). Genistein plasma concentrations were 2-fold greater than daidzein, even at 24 hr after dosing (Zhang et al., 1999b). However, genistein seems to be less well-absorbed overall than is daidzein because the relative proportion of ingested daidzein excreted in urine is 2- to 3-fold greater than is the proportion of ingested genistein excreted in urine in numerous studies of isoflavone disposition after soy food feeding (Xu et al., 1994, 1995, 2000; Tew et al., 1996; Zhang et al., 1999b). This difference in urinary disposition may be due to greater gut microbial degradation of genistein than of daidzein (Xu et al., 1995) after the initial biliary excretion of the compounds (Sfakianos et al., 1997), rather than to the compounds’ solubility differences. Daidzein and genistein are of similar apparent absorbability, as reflected in percentage of ingested dose that is excreted in urine [48% vs. 47% of ingested dose of daidzein or genistein, respectively, after either soymilk or soy germ (glycitein-rich) was fed to human subjects] (Zhang et al., 1999b, erratum published in 2001). This was expected from their relatively similar retention times during HPLC. For unknown reasons, the relative retention of genistein in blood plasma is similar to that of daidzein in men and significantly less than daidzein in women (Zhang et al., 1999b). Genistein degradation by gut microorganisms has not been studied. Relative absorption and disposition of isoflavones seems to be determined by relative solubility and susceptibility to degradation by gut microorganisms.

Flavonoids are seemingly more widely dispersed in the food supply and are of more varied composition than are isoflavones. For these reasons, their absorption and metabolism have been less completely characterized. Recent reviews have covered flavonoid bioavailability (Wiseman, 1999; Hollman & Katan, 1997, 1998). Studies of quercetin disposition in ileostomy patients showed the apparent absorption of quercetin to be 3-fold greater (50% of ingested dose) after ingestion of quercetin predominantly in glycosidic form from onions than that of a similar dose of quercetin aglycone or of quercetin rutinoside (17 and 24% of ingested doses, respectively) (Hollman et al., 1995). While the urinary excretion of quercetin differed among treatments to the same extent, the total quercetin excreted was < 0.3% of the ingested dose. Thus, nearly all of the absorbed quercetin must have remained in the body over the 13 hr of measurement, suggesting that quercetin has a long elimination half-life. However, a randomized crossover design study showing greater human plasma concentrations of quercetin glucoside than of quercetin rutinoside described plasma elimination half-lives of 22 and 28 hr for these compounds, respectively (Hollman et al., 1999). To obtain a complete picture of quercetin bioavailability, urinary and fecal (or ileostomy) excretion and plasma concentrations over time need to be compared within the same study. Aziz et al. (2000) studied human plasma and urinary contents of the flavonols quercetin and isorhamnetin after ingestion of onions by five subjects. Peak plasma concentrations for quercetin and quercetin and isorhamnetin glucosides occurred at 1.5–2 hr after ingestion. Over 24 hr, urinary excretion of isorhamnetin glucoside (a minor onion flavonol) was 17%, but the urinary excretion of quercetin and quercetin glucoside, the major onion flavonols, was < 1% of ingested dose. Thus, for these flavonols, the glucoside form seems to be absorbed intact, but quercetin is absorbed to a very limited extent. Thus, the general, although limited, data at this time suggests that flavonoids may be far less bioavailable to humans than are isoflavonoids. Ingestion of flavonoids and isoflavonoids results in micromolar plasma concentrations of these compounds. The biological effects of these compounds should be studied within this concentration range for nutritional relevance. The effects of fla-
vonoid glucosides, but probably only isoflavone aglycones, should be investigated.

These compounds may also be investigated as potential pharmaceuticals. A Phase 1 clinical and pharmacokinetic trial was conducted with the synthetic flavonoid flavone acetic acid (Havlin et al., 1991). This agent has been extensively studied for its therapeutic potential against solid tumors. Although the mechanism of action of flavone acetic acid is unknown, it has been shown to induce DNA damage in tumors, augment natural killer (NK) immunity, and act as an anti-angiogenesis factor (Zwi et al., 1989; Havlin et al., 1991). Thirty-eight patients were treated weekly for 4 weeks with doses from 0.33 to 12.5 g/m². Hypertension was identified as a dose-limiting effect with treatment at doses of 10 g/m² and above. Linear pharmacokinetic parameters were observed, and a dose of 8 g/m² was recommended for Phase 2 trials (Havlin et al., 1991).

4.3. Mammalian biotransformation of isoflavones and flavonoids

Isoflavones and flavonoids may be rapidly and predominately glucuronidated in the GI mucosa, if genistein can be considered to be a model for all of these phenolic compounds (Zhang et al., 1999a). Further, glucuronidation occurs in the liver. Genistein undergoes biliary excretion, with more than 70% of a dose recovered in bile within 4 hr after dosing in rats. Although genistein may be well-absorbed initially, a maximum of 25% of an oral genistein dose would be eliminated in rat urine. About 20–25% of an oral dose of genistein (predominantly as its glucoside from soy foods) is recovered in human urine (Watanabe et al., 1998; Zhang et al., 1999b). Genistein may be excreted to a greater extent in bile than are daidzein or glycitein, based on urinary recovery data in rats (Sfakianos et al., 1997) and humans (Xu et al., 1994, 1995; Zhang et al., 1999b). The 7-0-glucuronides of daidzein and genistein follow the retention of the parent isoflavones (genistein glucuronide being the more hydrophobic of the two) (Zhang et al., 1999a). Greater biliary excretion of genistein than daidzein (mostly as conjugates) would be expected because the biliary route would be somewhat more hydrophobic than the urinary route of excretion.

The presence of hydroxylated and methylated genistein metabolites correlated positively with inhibition of cancer cell proliferation, but genistein sulfates were not associated with antiproliferative effects of genistein, suggesting that some types of metabolism of the isoflavones may be crucial for their action (Peterson et al., 1998). Genistein and daidzein glucuronides were an order of magnitude less estrogenic than their respective isoflavone aglycones, as seen in their binding to mouse uterine cytosolic estrogen receptor (Zhang et al., 1999a). The isoflavone glucuronides also modestly enhanced human NK cell activity in vitro, exerting effects similar to genistein aglycone. The glucuronides were effective and nontoxic over a wider range of concentration than was genistein. As little as 0.1 to 0.5-μM isoflavone or isoflavone glucuronide enhanced NK cell activity, levels that are achievable in plasma after consumption of soy foods (Xu et al., 1994, 1995; Zhang et al., 1999a).

Phenolics are probably rapidly conjugated by UDP-glucuronosyltransferases and/or sulfotransferases. Such conjugates seem not to be biologically inert, but may be less toxic than the aglycone forms (Zhang et al., 1999a). It may prove important to know the proportion of the total flavonoid or isoflavone absorbed that is converted into specific conjugated forms. Aziz et al. (2000) did not specifically measure isorhamnetin or quercetin conjugates. It is possible, for example, that isorhamnetin conjugates co-chromatograph with the isorhamnetin glucoside, which might significantly change the picture of the biologically active forms of these compounds. In vitro studies of flavonoid and isoflavone effects might be misleading, unless the metabolism of the parent compound and the effects of the metabolites are considered. Characterization of physiologically relevant metabolite levels and patterns of metabolism is likely to be necessary to determine the mechanisms of action of flavonoids and isoflavones.

4.4. Gut microbial biotransformation of isoflavones and flavonoids

Isoflavone and flavonoid activity may also be altered by microbial biotransformation. Equol, an estrogenic isoflavone, is produced from daidzein in some individuals after a few days of repeated exposure to soy foods (Setchell et al., 1984). One of 6 men fed soy for several weeks was an equol producer (Lu et al., 1995), but 4 of 6 women fed soy for several weeks were equol producers (Lu et al., 1997). Thirty-five percent of the men and women (n=30 per gender) excreted equol after 3 days of soy feeding, with no significant gender difference (Lampe et al., 1998). After feeding soy flour to 12 subjects for 3 days, equol production was inversely related to the production of O-desmethyldiogenolsenin (ODMA), another daidzein metabolite produced during gut fermentation (Kelly et al., 1993). Several other urinary isoflavone metabolites were detected, and they were likely to have been derived from gut microbial fermentation, including 6-hydroxy-ODMA, dehydro-ODMA, dihydrogenistein, two isomers of tetrahydrodaidzein, and dihydrodaidzein. The health significance of these metabolites is unclear. However, in a breast cancer case-control study in Shanghai, breast cancer patients had lower urinary levels of the two major isoflavone metabolites, ODMA and equol, as well as of the three major soy aglycones, in comparison with matched controls (Zheng et al., 1999). Any isoflavone metabolite present in measurable amounts might be at least a qualitative biomarker of soy intake. Estrogenic isoflavone metabolites, e.g., equol, could be useful in clarifying
the mechanisms of soy’s action, i.e., whether estrogenicity was especially important or not. Quantifying soy intake and health effects by examining the isoflavones and their metabolites is complicated by the gut microbial diversity that seems to be at least partly responsible for major differences in isoflavone metabolism among individuals. Likewise, flavonoids are known to be metabolized by gut microflora (Griffiths & Smith, 1972). Metabolism by gut microflora is likely to affect the bioavailability and effects of phenolics in general.

Interindividual variation in isoflavone metabolism and degradation by gut microorganisms seems to follow certain patterns. When 7 women were fed 3 soymilk meals over 1 day, 2 women consistently showed 10- to 20-fold greater fecal excretion of isoflavones in feces compared with the other 5 subjects. This paralleled 2- to 3-fold greater urinary and plasma levels of isoflavones in the “high excretors” compared with the other subjects (Xu et al., 1995). In vitro isoflavone disappearance by a human fecal sample was shown to occur rapidly for both genistin and daidzein (degradation half-lives of 3.3 and 7.5 hr, respectively). The more rapid degradation of genistin may account for the seemingly lesser absorption of genistin than of daidzein (Xu et al., 1994, 1995; Zhang et al., 1999a, 1999b). Rat intestinal bacteria degrade flavonoids and isoflavonoids with a 5-OH group, such as genistein, to a much greater extent than structures lacking the 5-OH group, such as daidzein (Griffiths & Smith, 1972). This might partly account for the reduced bioavailability of some flavonoids, as well as genistein, in comparison with daidzein. Human gut microorganisms seem to possess similar ability, based on relative apparent absorption of the compounds and their relative fecal degradation (Xu et al., 1995).

Twenty men and women were sorted into three distinct isoflavone disappearance phenotypes when examined using in vitro assays with fresh fecal samples. For example, disappearance rate constants for genistin were 0.023 hr⁻¹ (high degrader, n = 5), 0.163 hr⁻¹ (moderate degrader, n = 10), and 0.299 hr⁻¹ (low degrader, n = 5) (Hendrich et al., 1998a, 1998b). These phenotypes were relatively constant when reexamined 10 months later. Disappearance rate constants for genistin were 0.049 hr⁻¹ (high degrader, n = 5), 0.233 hr⁻¹ (moderate, n = 4), and 0.400 hr⁻¹ (low, n = 5). Twelve of 14 subjects who remained in the study after 10 months maintained their initial isoflavone disappearance phenotype. Eight men of varying degradation phenotypes who were fed soymilk showed plasma isoflavone contents that correlated negatively and significantly (P < 0.05) with the disappearance rate constants, r = −0.88 for daidzein and r = −0.74 for daidzein (Wang, 1997). Part of the interindividual variability in isoflavone disposition might be accounted for by variation in gut microbial isoflavone disappearance rate. When subjects of moderate disappearance phenotypes were chosen for an isoflavone bioavailability study, the variation between subjects in urinary excretion of isoflavones was about 7-fold (Zhang et al., 1999b). In 14 randomly selected subjects fed different doses of soy protein for a 9-day interval, within treatment variation between subjects in isoflavone excretion was about 13-fold (Karr et al., 1997). Selection for isoflavone disappearance phenotype may lessen inter-subject variation.

Thirty-five Caucasian and 35 Asian women were studied to characterize factors influencing isoflavone disappearance phenotype (Zheng, 2000). The Asian subjects (mostly recent immigrant Chinese) were more likely to express high rates of isoflavone disappearance. Although Asian and Caucasian subjects differed significantly in body size (Caucasian > Asian), physical activity (Caucasian > Asian), red meat intake (Asian 3-fold greater than Caucasian), dairy intake (Caucasian > Asian), and insoluble dietary fiber intake (Caucasian, 3 g per day, vs. Asian, 1 g per day), none of these differences affected isoflavone disappearance phenotype. Asians of the low-rate isoflavone disappearance phenotype showed 3-fold greater urinary excretion (apparent absorption) of genistein after soymilk feeding than did Asians of the high isoflavone disappearance rate phenotype and all Caucasians. Asians having low rates of isoflavone disappearance from in vitro incubations of fecal samples had significantly more rapid gut transit time (40 hr) than did Asians of the high-rate isoflavone disappearance phenotype (60 hr) or Caucasians (both phenotypes > 80 hr). This suggests that gut transit time might significantly affect isoflavone and flavonoid bioavailability. Perhaps certain microorganisms normally present in some human guts affect gut transit time, just as some pathogenic organisms can, but to a lesser extent and with benign or even beneficial results. More rapid transit time might prevent flavonoid-degrading organisms from growing and/or exerting their effects to a great extent.

Isoflavone and flavonoid-degrading microorganisms in vivo in humans remain to be identified. Some Clostridia strains cleave the C-ring of flavonoids and isoflavonoids (Winter et al., 1989). Clostridia are absent from the GI tracts of some individuals, and may be introduced during meat consumption (Mitsuoka, 1982). Isoflavones can be broken down into other compounds such as monophenolics. For example, p-ethylphenol is a nonestrogenic metabolite of genistein that is identified in ruminants (Verdeal & Ryan, 1979). Methyl p-hydroxyphenylactate is a flavonoid metabolite that blocks nuclear estrogen receptor binding, inhibiting the growth of MCF-7 breast cancer cells in vitro (Markaverich et al., 1988). Further support for involvement of intestinal microorganisms in flavonoid metabolism comes from a study of 11 humans fed a standardized horsetail (Equisetum arvense) extract containing quercetin and caffeic acid derivatives (Graefe & Veit, 2000). Hippuric acid (benzoate conjugated with glycine) and dihydroferulic acid were the main urinary metabolites collected during 8 days of extract dosing. This suggests significant bacterial metabolism of the extract components and the need to characterize biological effects of various bacterial metabolites of flavonoids and isoflavones.
Characterization of their metabolism by gut microorganisms may provide insights into the mechanisms of action of isoflavones and flavonoids. Gut microorganisms might be key determinants of the bioavailability and efficacy of these compounds.

4.5. Influence of other dietary components on isoflavone bioavailability

Bioavailability of dietary constituents to some extent depends upon interactions with other dietary components. For example, isoflavones are associated with soy protein. The food matrix for isoflavones does not seem to affect isoflavone bioavailability, although more study is warranted. Isoflavone disposition from several soy foods was compared when single meals of tofu, tempeh, cooked soybeans, or texturized vegetable protein were fed to women in a randomized crossover design (Xu et al., 2000). Urinary recoveries of isoflavones over 24 hr did not differ with soy food form (total recovery averaged 46% of ingested daidzein and 15% of ingested genistein among all soy foods), nor did plasma isoflavones. Women also showed similar urinary isoflavone disposition whether they were fed tofu (high in malonyl glycosides) or texturized vegetable protein (high in acetyl glycosides) (Tew et al., 1996). These studies suggest that isoflavone aglycones, glycosides, and glycosides with different side chains are similar in bioavailability. However, when men were fed soybean pieces or tempeh for 9 days, tempeh isoflavones (mostly aglycones) seemed to be absorbed twice as well as were isoflavones from soybean pieces, as reflected in urinary excretion, although the overall isoflavone bioavailability (as urinary excretion, 5–10%) was lower in this study than in several other studies (urinary total isoflavone excretion of 25–50%) (Hutchins et al., 1995). In rats fed equimolar doses of genistein or genistin, the plasma content at 10–50 hr after dosing and total urinary isoflavone excretion were similar, although genistein was more rapidly absorbed than was genistin (King et al., 1996). It is possible that isoflavones from fermented soy foods, such as tempeh, natto, or miso, would be somewhat more rapidly absorbed because the isoflavone aglycone content would be relatively increased in comparison with unfermented soy foods. Based on limited evidence, isoflavone glycosides and aglycones may be similar in overall bioavailability.

Other dietary factors seem to have little influence on isoflavone bioavailability. Choice of background diet did not affect isoflavone bioavailability when soymilk was fed at three specific times on a single day to women given three different background diets (diets provided totally by the investigators versus self-selected diets eaten at the specific times versus ad libitum diets) in a randomized crossover design. No significant differences were noted among treatments with respect to plasma or urinary isoflavone content (Xu et al., 2000). In 30 men and 30 women who were fed soy protein powder for 4 days, greater dietary fiber and total carbohydrate intake was associated with equol production in women, but not in men who consumed greater total dietary fiber than did women (Lampe et al., 1998). In women, 40 g of dietary wheat fiber consumed during a single day significantly suppressed urinary genistein excretion by 20% after a soy milk meal compared with 15 g dietary fiber (Tew et al., 1996). Thus, wheat fiber has only a modest effect on isoflavone bioavailability. The effects of other dietary factors on isoflavone bioavailability remain to be studied. Given the structural similarities between flavonoids and isoflavones, dietary ingredient interactions might be expected to have little influence on flavonoid bioavailability, as shown for isoflavones. However, this remains to be seen.

5. Potential mechanisms for flavonoid and isoflavonoid inhibition of cancer

5.1. Estrogenic and antiestrogenic activity

The estrogenic activity of isoflavonoids was first noted in the 1940s when clover pastures rich in isoflavones were proposed to be responsible for infertility of sheep in Western Australia (Bennets et al., 1946). Since then, a list of isoflavonoids, including genistein, daidzein, and formononetin, have been shown to be estrogen agonists in various animal models (Moule et al., 1963; Farnsworth et al., 1975). By using an estrogen receptor-dependent transcriptional response assay, Miksicek (1993) reported that commonly occurring flavonoids also had estrogenic activity. Of the flavonoids and isoflavonoids tested in this sensitive assay, the order of estrogenicity was genistein > kampherol > naringenin > apigenin > daidzein > biochanin A > formononetin > luteolin > fisetin > catechin/taxifolin > hesperetin (Miksicek, 1995). Some properties of soy isoflavones, such as preventing osteoporosis (Anderson, 1999), improving menopausal symptoms (Brezinski et al., 1997), and lowering cholesterol levels (Lichtenstein, 1998), may suggest an estrogen-related mechanism.

The estrogenic potency of all the reported isoflavonoids and/or flavonoids is extremely weak, 10^3- to 10^5-fold less than 17-ß-estradiol (Davis et al., 1998). Since the circulating concentrations of isoflavone aglycones may be 100-fold greater than estradiol, isoflavones and flavonoids may be antiestrogenic, depending on the level of natural estrogens. Isoflavonoids and/or flavonoids might counteract endogenous estrogens through competitive binding to estrogen receptors. The relative binding affinity of these compounds for the estrogen receptor (as their aglycone forms) is 0.05–1% of the binding affinity of 17-ß-estradiol (Shutt & Cox, 1972). We examined the effect of formononetin on mammary glandular tissue and found that the estrogenic potency of formononetin appears extremely weak and that it is proportional to its estrogen receptor-binding capacity (Wang et al., 1995). On the basis of both weak estrogenic activity and low estrogen receptor-binding capacity, isoflavonoids
and/or flavonoids might be unlikely to exert significant cancer prevention via antiestrogenic mechanism(s). However, a recent study of isoflavone estrogenicity in vivo (mouse uterine growth assay) and in vitro (mouse uterine cytosolic estrogen receptor binding) showed that glycitein was about 3-fold more potent than genistein in vivo, but about 20-fold less potent than genistein in vitro (unpublished). This suggests that bioavailability in vivo and in vitro may differ significantly. The efficacy of isoflavones and flavonoids as estrogen agonists or antagonists in vivo requires much more study.

5.2. Antiproliferation

Disregulated proliferation appears to be a hallmark of increased susceptibility to neoplasia. Cancer prevention is generally associated with inhibition, reversion, or retardation of cellular hyperproliferation. It is well known that dietary flavonoids and isoflavonoids behave as general cell growth inhibitors. One of their biological properties in plants, in fact, is providing resistance to fungal or bacterial growth as phytoalexins (Dewick, 1988; Bohm, 1980). Although most flavonoids and isoflavonoids appear nontoxic to humans and animals, they have been demonstrated to inhibit proliferation in many kinds of cultured human cancer cell lines. Kandaswami et al. (1991), for example, reported antiproliferative effects of four citrus flavonoids (quercetin, taxifolin, nobiletin, and tangeretin, at 2–8 μM) on squamous cell carcinoma HTB43. Piantelli et al. (1993) demonstrated antiproliferative activity of 10 μM quercetin on meningioma cells. Kuo (1996) showed antiproliferative potency of 5 flavonoids and 2 isoflavonoids (0–100 μM) on colon carcinoma HT29 and Caco-2 cell lines with apoptosis induction mechanisms. Hirano et al. (1994) found inhibitory effects of 8 of the 28 tested flavonoids on leukemia HL-60 cells (IC₅₀ < 100 ng/mL) via a nontoxic mechanism. Le Bail et al. (1998) suggested antiproliferative activity of certain flavonoids and genistein at high concentrations (50 μM) on breast cancer MCF-7 through estrogen receptor-independent mechanisms. Kawai et al. (1999) investigated antiproliferative activities of 27 citrus flavonoids, at 40 μM, on several tumor cell lines, including lung carcinoma A549 and gastric TGCBC11TKB cancer cells, and found that they inhibited proliferation of cancer cell lines, but that they did not significantly affect proliferation of human normal cell lines. Fotisis et al. (1997) studied the inhibition of cell proliferation and angiogenesis by flavonoids in 6 different cancer cell lines, and noted that the IC₅₀ of active flavonoids was in the low micromolar range, physiologically available concentrations. The influence of flavonoids on lymphocyte proliferation was studied by Lee, S. J. et al. (1995). They observed a nonreversible inhibition of concanavalin A-induced lymphocyte proliferation by quercetin and apigenin, with IC₅₀ of 10 μM and 3 μM, respectively. Booth et al. (1999) reported that genistein and synthetic isoflavone analogues, at 0.1–25 μg/mL, inhibited intestinal epithelial cell proliferation and induced apoptosis in vitro. An inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids was reported by So et al. (1996).

Although the antiproliferative effects of flavonoids and isoflavonoids in cultured cells appear well established, relatively little data have been published regarding the antiproliferative activity in vivo, and virtually nothing is known regarding the clinical relevance of this bioactivity.

5.3. Cell cycle arrest and apoptosis

The observed antiproliferative properties of flavonoids and isoflavonoids suggests that these compounds may inhibit the cell cycle or induce apoptosis. Indeed, checkpoints at both G1/S and G2/M of the cell cycle in cultured cancer cell lines have been found to be perturbed by flavonoids and isoflavonoids. Traganos and co-workers (1992) demonstrated that genistein, at 5–20 μg/mL, produced cell cycle arrest at both the G1/S and G2/M phases in the human myelogenous leukemia HL-60 line and the lymphocytic leukemia MOLT-4 cell line. A later study by Matsukawa et al. (1993) reported that genistein, up to 60 μM, arrested human gastric cancer cells at G2/M. Studies conducted in a non-small-cell lung cancer cell line demonstrated that genistein, at 30 μM, induced G2/M arrest through p21 up-regulation and apoptosis induction (Lian et al., 1998). Zhou et al. (1998) demonstrated that isoflavones (genistein, genistin, daidzein, and biochanin A, at 0–50 μM) inhibited growth of murine and human bladder cancer cell lines by inducing cell cycle arrest, apoptosis, and angiogenesis (Zhou et al., 1998). Other studies regarding the induction of apoptosis by genistein at similar concentrations were reported in human prostate cancer cell lines (Kyle et al., 1997) and in Jurkat T-leukemia cell line (Spinozzi et al., 1994).

Quercetin (30–100 μM), a widely distributed flavonoid, was reported to block the cell cycle at G1/S in human colonic COLO320 DM cells (Hosokawa et al., 1990) and leukemic T-cells (Yoshida et al., 1992), and to trigger apoptosis (Wei et al., 1994b). Wei and co-workers (1994a) reported the induction of apoptosis by quercetin, at 50 μM or higher, in several tumor cell lines, resulting in nuclear fragmentation, condensation of nuclear chromatin, and a subdiploid peak by DNA flow cytometry. Previous studies conducted in our laboratory demonstrated that apigenin (0–80 μM), another widely distributed flavonoid, significantly induced a reversible G2/M arrest in keratinocytes (Lepley et al., 1996), fibroblasts (Lepley et al., 1996; Lepley & Pelling, 1997), and colonic carcinoma cell lines (Wang et al., 2000). Studies on silymarin (0–75 μM) found that perturbations in cell cycle progression may account for the anticarcinogenic effects on human prostate carcinoma DU145 cells (Zi et al., 1998) and epidermoid carcinoma A431 cells (Giles & Wei, 1997). Cell cycle arrest and
induction of apoptosis could be functionally related to activation of p53 (Plaumann et al., 1996) and/or inhibition of cell cycle kinase activity (End et al., 1987; De Azevedo et al., 1996; Kyle et al., 1997). Recently, we compared the effects of apigenin (0–80 μM) on cell growth and cell cycle arrest in three human colonic cancer cell lines, SW480, Caco-2, and HT-29 (Wang et al., 2000). The effects on both cell cycle arrest and cell growth inhibition were strongest in SW480 cells, moderate in HT-29 cells, and weakest in Caco-2 cells (Fig. 3). The differential effectiveness of cell growth inhibition and cell cycle arrest in response to apigenin in the three cell lines tested might be related to their status of functional p53 and/or ras genes. The results suggest that cells with mutations in genes critical to colon cancer development may be more sensitive to apigenin. This implies that flavonoids may be more effective in controlling growth of tumors with certain mutational spectra.

5.4. Antioxidation

Flavonoids and isoflavonoids might protect against cancer and/or heart disease through inhibition of oxidative damage (Omenn, 1995). Oxidation of DNA is likely to be an important cause of mutations that potentially can be reduced by dietary antioxidants. Chemically, flavonoids and isoflavonoids are one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in vitro cell culture or cell free systems by scavenging superoxide anion (Robak & Gryglewski, 1988), singlet oxygen (Husain et al., 1987), lipid peroxyl radicals (Torel et al., 1986), and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (Lewis, 1993; Shahidi et al., 1992). Flavonoids themselves become free radicals in the process, but their conjugated structure allows the remaining orbital electron to be relatively inactive (Hudson & Lewis, 1983). The antioxidant activity and the structure–activity relationships of flavonoids have been investigated intensively in many in vitro systems (Lien et al., 1999; Yamanaka et al., 1997; Foti et al., 1996; Ratty & Das, 1988). Antioxidant activities of isoflavonoids are also reported (Arora et al., 1998; Mitchell et al., 1998). Quercetin, luteolin, and genistein have been shown to inhibit oxidative DNA damage induced by UV light irradiation in HL-60 cells with IC$_{50}$ < 1 μM (Giles & Wei, 1997; Cai et al., 1997). The antioxidant activity of flavonoids was suggested to be related to the number and position of hydroxyl groups (Noroozi
et al., 1998). However, relatively few data have been published regarding their in vivo roles.

Theoretical underpinnings for the efficacy of flavonoids as antioxidants in vivo come from the inhibition of low-density lipoprotein (LDL) oxidation. Elevated LDL oxidation has been associated with coronary artery disease. An inverse association of the dietary intake of phenolic flavonoids from red wine, with the risk of developing cardiovascular diseases, has been observed in French populations (Frankel et al., 1993; Renaud & De Lorgeril, 1992). We recently examined the antioxidant efficiency of 26 common dietary flavonoids, isoflavonoids, and phenolic acids (20–200 μM) in a human ex vivo LDL-oxidation model (Wang & Goodman, 1999). The antioxidative efficiency of dietary flavonoids and isoflavonoids appears to be associated not only with their reductive capacity, but also with their protein-binding properties. It is suggested that the availability of flavonoids at the oxidative site on LDL may block oxidative attack and prevent LDL oxidation in vivo. Potential protein binding and interference with oxidative processes by flavonoids and isoflavonoids may explain their ultimate antioxidant roles in vivo (Wang & Goodman, 1999).

5.5. Induction of detoxification enzymes

Another proposed mechanism for protection against cancer by dietary flavonoids and isoflavonoids may include induction of Phase II detoxification enzymes in cells. Modification of cellular detoxification enzymes could be a major mechanism for protecting against the toxic and neoplastic effects of carcinogens (Talalay, 1989; Wattenberg, 1992b; Talalay et al., 1995). Many environmental carcinogens require metabolism to their fully carcinogenic forms. They are often metabolized to proximate carcinogens by Phase I enzymes, e.g., cytochromes P450, which catalyze oxidative reactions (Talalay, 1989). The oxidized metabolites of potentially carcinogenic xenobiotes are then detoxified by Phase II metabolizing enzymes into the forms that are relatively inert and more easily excreted (Talalay et al., 1995). 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 (Talalay, 1989; Wattenberg, 1992b; Talalay et al., 1995). Both NAD(P)H:quinone reductase (EC 1.6.99.2) (QR) and GST (EC 2.5.1.18), for example, are Phase II detoxifying enzymes. They are widely induced in many cells coordinately with xenobiotic exposures. QR is a major enzyme of xenobiotic metabolism that carries out obligatory two-electron reductions and thereby protects cells against mutagenicity and carcinogenicity resulting from free radicals and toxic oxygen metabolites generated by the one-electron reductions catalyzed by cytochromes P450 and other enzymes (Ernster, 1967). GST detoxifies a number of carcinogenic electrophiles by catalysis of the conjugation with reduced glutathione (Chas-seaud, 1979). While some flavonoids, such as chrysin, apigenin, luteolin, kaempferol, quercetin, myricetin, and naringenin, have high potencies and selectivities for inhibition of CYP1A isoforms (IC50 <4 μM) (Kanazawa et al., 1998), induction of either QR or GST activity has been noted for certain dietary antioxidant flavonoids, such as quercetin (50–100 μM in cultured cells or 1% in murine diet) and epicatechin (25–100 μM) (Wang & Higuchi, 1995; Gordon et al., 1991; Benson et al., 1980; Rodgers & Grant, 1998; Nijhoff et al., 1993).

We measured the effects of 4 prominent isoflavones upon QR induction in a human colonic Colo205 cell line, and found a dose-dependent induction in enzyme activity up to 6- to 8-fold after addition of genistein, at 0.01–10.0 μM, and up to 2- to 3-fold induction with biochanin A, at 1.0–10.0 μM. The observed induction of QR by isoflavones appears through the promotion of QR mRNA expression and stabilization of mRNA levels (Wang et al., 1998). Current scientific opinion suggests that the optimal conditions for preventing carcinogen activation would couple inhibition of Phase I metabolism with activation of Phase II metabolism, and we are presently not aware of flavonoids or isoflavones that have been shown to have this favorable induction/inhibition profile.

5.6. Regulation of host immune function

The role of the host immune function has become increasingly important in our understanding of the mechanisms that are involved in cancer prevention. The enhancement of host immune function by dietary antioxidants may be beneficial to cancer prevention (Hughes, 1999; Appelbaum, 1992; Lowell et al., 1990; Watson, 1986). Since phagocytic cells produce reactive oxygen species as part of the body’s defense against infection, adequate intake of antioxidants is required to prevent damage by oxidants to the immune cells themselves. Middleton and Kandaswami have demonstrated that a number of immune cell systems do not appear to be affected significantly by flavonoids, notably quercetin, while they are resting (Middleton, 1998). However, once a cell becomes activated by a physiological stimulus, a flavonoid-sensitive substance is generated and interaction of flavonoids with that substance dramatically alters the outcome of the activation process (Middleton, 1998; Middleton & Kandaswami, 1992). By using a murine model, we demonstrated that the isoflavone daidzein administrated orally at doses of 20–40 mg/kg body weight stimulated murine nonspecific immunity, activated humoral immunity, and enhanced cell-mediated immunity (Zhang, R. et al., 1997). In addition, we examined the influence of daidzein, genistein, and combinations of these isoflavones on the proliferation of murine splenocytes activated with mitogens and on the secretion of IL-2 and IL-3. We found that daidzein at physiologically relevant concentrations (0.01–10 μM) potentiates lymphocyte activation, suggesting that
the immunostimulatory effects of daidzein may be involved in cancer chemoprevention (Wang et al., 1997). Although the clinical relevance of this finding is not clear, we also observed that daidzein and genistein glucuronides and genistein (in nutritionally relevant concentrations, 0.1–10 μM) enhanced activation of NK cells in vitro (Zhang et al., 1999a). The glucuronides were less toxic to NK cells than was genistein, which inhibited NK activity at 50 μM.

5.7. Other mechanisms

Numerous additional mechanisms have been suggested for flavonoid and/or isoflavonoid inhibition of carcinogenesis. Certain studies have associated changes in protein phosphorylation of cancer cell lines with growth inhibition by flavonoids and isoflavonoids. For example, apigenin, kampherol, and genistein, at 25 μM, reversed the transformed phenotype of v-H-ras-transformed NIH 3T3 cells that was associated with a reduction in phosphotyrosine content in the cells (Kuo et al., 1994). Further work from the same group suggested that apigenin, at 12.5 μM, inhibits mitogen-activated protein kinase (Kuo & Yang, 1995). Gamet-Payrastre et al. (1999) recently reported that some flavonoids such as quercetin blocked particular isoforms of phosphoinositide 3-kinase or protein kinase C and their downstream-dependent cellular responses (Gamet-Payrastre et al., 1999). Furthermore, a number of flavonoids were shown to be topoisomerase antagonists, of which myricetin, soflavone, inhibited either Topo I or Topo II (Constantinou et al., 1997). The inhibition by genistein (20–100 μM) on c-myc oncogene expression in colon cancer cell lines was suggested to be related to the inhibition of tyrosine kinase activity rather than to the inhibition of topoisomerase II (Heruth et al., 1995). Genistein (30–200 μg/mL) was also reported to induce differentiation (Constantinou et al., 1990) and inhibit angiogenesis (IC50 = 150 μM) (Fotsis et al., 1993). Although dietary flavonoids and isoflavonoids are usually nontoxic, some flavonoids such as quercetin were reported to be potent mutagens (Friedman & Smith, 1984; Czeczot et al., 1990), and some flavonoids and isoflavonoids, such as kampherol and genistein, at 1 μM, were identified as estrogenic agonists (Miksicke, 1993, 1995). The evidence for potentially harmful effects of flavonoids and/or isoflavonoids is limited and has been generally discussed in Section 3.

Considering the great variety of dietary flavonoids, it appears extremely unlikely that any one substance is responsible for all of the associations seen between plant foods and cancer prevention. The specific mechanisms of action of most flavonoids and isoflavonoids, with respect to cancer prevention, are not clear yet, but appear to be varied, complementary, and/or overlapping. Although many individual flavonoids have been shown to inhibit cancer cell growth, the ability of flavonoid or isoflavonoid combinations to prevent cancer progression has not been adequately studied. Clinical studies using these dietary agents are scarce. Further investigations into the potential role of flavonoids and isoflavonoids in cancer prevention and/or therapy are warranted.

References


Middleton, Jr., & J. B. Harborne (Eds.), *Plant Flavonoids: Advances in Research and Application* (pp. 139–157). Amsterdam: Elsevier Science Publishers B.V.


So, F. V., Guthrie, N., Chambers, A. F., Moussa, M., & Carroll, K. J.


