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REVIEW ARTICLE

Cancer Phytotherapeutics: Role for Flavonoids at the Cellular Level

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Dietary foods and fruits possess an array of flavonoids with unique chemical structure and diverse bioactivities relevant to cancer. Numerous epidemiological studies have validated the inverse relation between the consumption of flavonoids and the risk of cancer. Flavonoids possess cancer blocking and suppressing effects. Flavonoids modulate various CYPs involved in carcinogen activation and scavenging reactive species formed from carcinogens by CYP-mediated reactions. They induce biosynthesis of several CYPs. They are involved in the regulation of enzymes of phase-II responsible for xenobiotic biotransformation and colon microflora. Since cytochromes P450, P-gp and phase-II enzymes are involved in the metabolism of drugs and in the processes of chemical carcinogenesis, interactions of flavonoids with these systems hold great promise for their therapeutic potential. The role of flavonoids also includes the inhibition of activation of pro-carcinogens, inhibition of proliferation of cancer cells, selective death of cancer cells by apoptosis, inhibition of metastasis and angiogenesis, activation of immune response against cancer cells, modulation of the inflammatory cascade and the modulation of drug resistance. This has greatly extended the goal of cancer therapy from eradicating the affected cells to control of the cancer phenotype. Phytotherapy is being used in combination with other therapies as phytonutrients have been shown to work by nutrient synergy.

Keywords: flavonoids; phytotherapy; synergy; CYP; P-gp; drugs.

INTRODUCTION

In the past few years, research in the area of phytotherapy has greatly influenced aspects of nutrition and disease control. The focus and emphasis of phytotherapy is on the phytochemicals in fruits, vegetables and grains apart from herbal medicines. Phytochemicals, also called bioactive compounds, influence physiological or cellular activities resulting in a beneficial health effect. Like nutrients, phytochemicals are not essential for life. They occur in small amounts and have more subtle effect on health (Kris-Etherton *et al.*, 2002). These compounds have great potential to modify the risk of disease. Because of their potential role in the prevention and cure of chronic diseases, there is keen interest in studying the health effects of phytochemicals and unraveling the mechanisms that mediate their effects.

Phytochemicals, being plant derived compounds, are considered pharmacologically safe. More than 5000 phytochemicals have been identified in fruits, vegetables and grains (Liu, 2003). Flavonoids are the most abundant phytochemicals in our diet and provide much of the flavor and color to fruits and vegetables. In the early 1930s, a flavonoid glycoside, rutin, was isolated from oranges and designated as vitamin P. The first observation regarding their biological activities was published in 1936 (Rusznyak and Szent-Gyorgyi, 1936).

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More than 4000 distinct flavonoids have been identified in fruits, vegetables and other plant foods and have been linked to reducing the risk of cancer and other major chronic diseases. Flavonoids and their polymers can alter metabolic processes and have a positive impact on health. Epidemiological studies have shown that frequent consumption of fruits and vegetables is associated with low risks of various cancers (Wattenberg, 1997; Block *et al.*, 1992). Block *et al.* reviewed about 200 epidemiological studies that examined the relationship between intake of fruits and vegetables and the incidence of cancer of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas and ovary. The consumption of fruits and vegetables was found to have a significant protective effect in 128 of 156 dietary studies. The risk of cancer was 2-fold higher in persons with a low intake of fruits and vegetables than in those with a high intake. The protective effect has largely been attributed to flavonoids, which are ubiquitously present in plant-derived foods and are important constituents of the human diet (Knekt *et al.*, 1997; Hertog *et al.*, 1993; Digiovanni, 1990), and an inverse association was shown between flavonoid intake and subsequent lung cancer incidence. In a study on 9959 Finnish men and women aged 15–99 years, consumption of quercetin from onions and apples was found to be inversely associated with the lung cancer risk (Marchand *et al.*, 2000). The onions were effective, particularly against squamous-cell carcinoma. Boyle *et al.* (2000) showed that increased plasma levels of quercetin after a meal of onions was accompanied by increased resistance to strand breakage by lymphocyte DNA and decreased levels of some oxidative metabolites in the urine.

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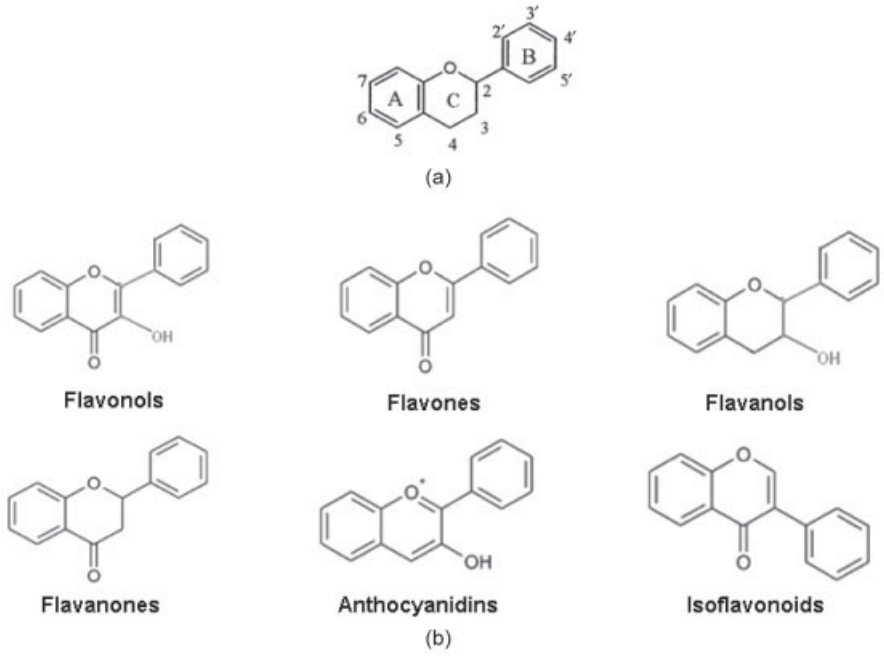

Figure 1. Generic structure of (a) flavonoid and (b) general structure of each class of flavonoid.

CHEMISTRY OF FLAVONOIDS

The generic structure of a flavonoid (Fig. 1a) consists of two aromatic rings (ring A and ring B) linked by three carbons that are usually in an oxygenated heterocyclic ring or C ring (Gary, 2003). Based on differences in the generic structure of the heterocyclic C ring as well as the oxidation state and functional groups of the heterocyclic ring, they are classified as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins and isoflavonoids (Fig. 1b). Within each subclass, individual compounds are characterized by specific hydroxylation and conjugation patterns. Flavonoids are most frequently found in nature as conjugates in glycosylated or esterified forms. There are 80 different sugars identified to bind to flavonoids (Hollman and Arts, 2000). The polyphenolic structure of flavonoids renders them quite sensitive to oxidative enzymes and cooking conditions. Usually, natural flavonoids occur as glycosides (e.g. glucosides, rhamnoglucosides, rutinosides) and their structures can be more complex such as flavonolignans (silybin), catechin esters (epigallocatechin gallate) or prenylated chalcones (xanthohumol). The chemical structure and some activities of several flavonoids are similar to those of naturally occurring estrogens and are frequently assigned as phytoestrogens (Kummer *et al.*, 2001).

The major source of flavonoids includes fruits and fruit products (e.g. citrus fruits, rose hips, apricots, cherries, grapes, black currants, bilberry and apples), vegetables (e.g. onions, green pepper, broccoli, tomatoes, spinach), tea leaves, soybeans and herbs (e.g. *Silybum marianum*, *Alpinia officinarum*, *Hypericum perforatum*) (Barnes *et al.*, 2001). Table 1 gives a list of members of each subclass of flavonoid and their food source.

There are two major sites of flavonoid metabolism in a mammalian body. The first is the colon microflora which (in addition to release of aglycones) degrades flavonoids into phenolic acids and the second is the liver. Whether the whole molecule or the aglycone form of flavonoid is more effective depends on a particular flavonoid and its biological activity (Rice-Evans, 2001).

CELLULAR EVENTS IN CARCINOGENESIS AND CHEMOPREVENTION BY FLAVONOIDS

Chemopreventive substances show their effects by delaying or reversing the process of carcinogenesis at various points. Such mechanisms may be divided as blocking effects and suppressing effects. The earliest stage is the direct inhibition of free radical mediated DNA damage that leads to mutagenesis (Newmark, 1992). The other way is to enhance the ability of target tissues to metabolize mutagens. At the site of first entry, these mutagens are metabolized in phase I reactions by cytochrome P-450 (CYPs) enzymes and are then conjugated (phase II biotransformation) to make them more water soluble and ready for excretion. The phase I and II enzymes occur in the intestinal mucosa as well as the liver. Some of these enzymes are ubiquitously present in other major organs of the body. The flavonoids have been reported to modulate the activities of these enzymes and needs to be explored (Johnson *et al.*, 1994). Another major hurdle in cancer treatment is the drug resistance shown by cancer cells. The drug is transported back by some efflux transporters making the cell resistant. The cells show drug resistance because the efflux pumps maintain a subtoxic concentration of drugs within the cells. P-gp is one of the major players in drug resistance. Flavonoids have been shown to inhibit the P-gp and other efflux transporters.

The stage of tumor progression or promotion, arising after the initiation has taken place is a highly complex process and a least understood one. The

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Table 1. Main groups of flavonoids, the individual compounds, and food sources

Flavonoid sub-class	Compound	Food Source
Flavonols:	Quercetin, Rutin, Quercetrin, Morin, Myricetin, Myricitrin, Galangin, Kaempferol, Kaempferide, Fisetin, Rhamnetin, Isorhamnetin, Spirenoside, Robinin	Lovage leaves, Peppers, Apple skins, Berries, Broccoli, Celery, Fruit peels, Cranberries, Grapes, Lettuce, Olives, Onions, Parsley, Wine, Apricots, Black currant, Capers, Chives, Cocoa, Coriander, Corn poppy, Cress, Dock, leaves, Say thistle, Fennel, leaves, Hart wort, Tarragon, Green tea, Black tea
Flavonones:	Hesperitin, Naringin Naringenin, Eriodictyol Hesperitin, Pinocembrin Likvirtin	Citrus fruit, Citrus peel, Orange, Grapefruit, Lemon, Peppermint, Pummelo, Tangelo, Tangerine, Tangor
Flavones:	Rpoifolin, Apigenin, Tangeretin, Baicalein, Luteolin, Chrysin, Techtochrysin, Diosmetin Diosmin	Parsley spices, Saw thistle, Celeriac, Celeryhearts, Oregano, Parsley, Peppermint, Pepper, Thyme spices, Greek green pie, Queen Anne's Lace
Flavanols:	Catechin, Gallocatechin, Epicatechin, Epigallocatechin, Epicatechin 3-gallate, Epigallocatechin 3-gallate, Theaflavin Theaflavin 3-gallate, Theaflavin 3,3' digallate, Thearubigins	Green tea, Black tea, Oolong tea, Red wine, Barley, Berries, Broad beans Buck wheat, Grapes, Rhubarb stalks
Anthocyanins	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin,	Berries, Cherries, Elderberries, Onions, Beats, Grapes, Raspberries Red/Black grapes, Red wine, Strawberries, Tea, Fruit peels with dark pigments
Isoflavones:	Genistein, Daidzin, Glycitein, Formononetin	Soy, Soy flour, Soybeans Miso, Tofu, Tempeh, Soy milk

intervention at this point requires a multi-targeted therapy that promotes antiproliferation and pro-apoptotic changes in cancer cells and should act as antimetastatic and antiangiogenic to suppress the spread of cancer. There are substantial evidences from various *in vitro* studies to show that flavonoid combinations are effective at all the stages of cancer listed above. Figure 2 summarizes the effects of flavonoids as blocking and suppressing effects on multistage carcinogenesis.

Flavonoids are highly specific in their action on some key regulatory enzymes and receptors in our body. Flavonoids have also been reported to modulate P-glycoprotein (Pgp), the MDR (multidrug-resistance) protein. In addition to CYPs, flavonoids are also involved in the regulation of enzymes of phase II responsible for the xenobiotic biotransformation [e.g. glutathione *S*-transferase (GST), UDP-glucuronyl transferase (UGT), *N*-acetyltransferase] and colon microflora (Walle *et al.*, 2001). Since cytochromes P450, P-gp and phase II enzymes are involved in the metabolism of drugs and in the processes of chemical carcinogenesis, interactions of flavonoids with these systems hold great promise of their therapeutic potential. These activities may be beneficial in detoxification, in chemoprevention or in drug resistance suppression. They also inhibit many enzymes that are the targets in anticancer treatment, e.g. eukaryotic DNA topoisomerase I, Cox I and II and estrogen 2- and 4-hydroxylases.

Flavonoids as modulators of cytochrome P450 and phase-II enzymes

Among the proteins that interact with flavonoids, cytochromes P450 (CYPs), monooxygenases metabolizing xenobiotics (e.g. drugs, carcinogens) and endogenous substrates (e.g. steroids), play a prominent role (Hodek *et al.*, 2002). Flavonoids by interacting with P450 enzymes reduce the activation of procarcinogen substrates to carcinogens which makes them putative anticancer substances (Mukhtar *et al.*, 1988; Tsyrlov *et al.*, 1994; Guengerich, 1988). An inhibitory capacity of flavonoids with respect to CYP activities has been extensively studied because of their potential use as agents blocking the initiation stage of carcinogenesis (Doostdar *et al.*, 2000; Henderson *et al.*, 2000; Chan *et al.*, 1998; Zhai *et al.*, 1998). *In vivo* and *in vitro* studies have shown that flavonoids can enhance or inhibit the activities of certain P450 isozymes (Obermeier *et al.*, 1995; Trela and Carlson, 1987; Friedman *et al.*, 1985; Lasker *et al.*, 1984; Havsteen, 1983).

CYPs interact with flavonoids in at least three different ways (Hodek *et al.*, 2002): (i) flavonoids induce biosynthesis of several CYPs; (ii) they modulate (stimulate or inhibit) enzymatic activities of CYPs; and (iii) flavonoids are metabolized by several CYPs. Synthetic and naturally occurring flavonoids are effective inhibitors of four CYPs involved in the metabolism of

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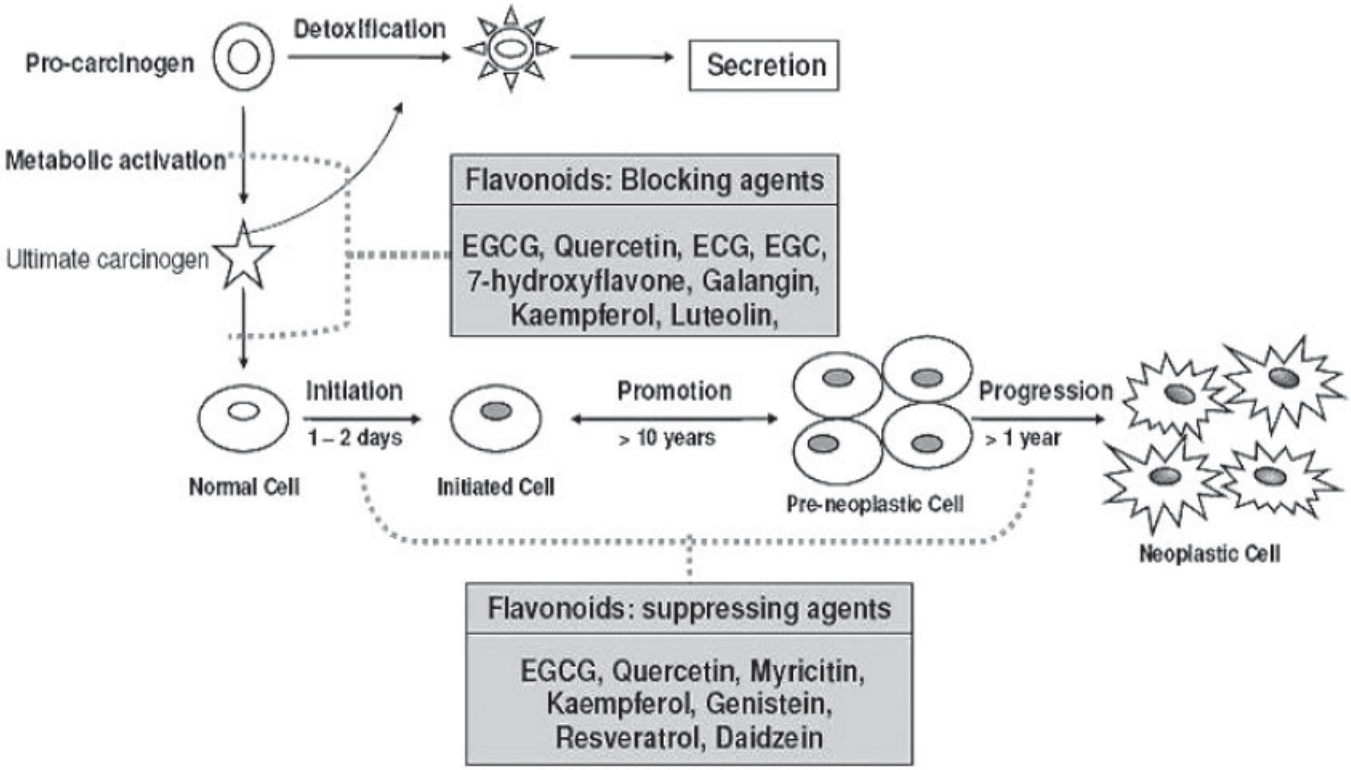

Figure 2. Flavonoids as blocking and suppressing agents of multistage carcinogenesis. Figure adapted from Surth (1999).

xenobiotics: CYP1A1, 1A2, 1B1 and 3A4, and one steroidogenic cytochrome P450 (CYP19). The activation of aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor is associated with the elevation of activities of CYP1 family enzymes (CYP1A1, 1A2 and 1B1) that are responsible for activation of carcinogens such as benzo[a]pyrene (B[a]P), 7,12-dimethylbenz[a]anthracene (DMBA), aflatoxin B1 and meat derived heterocyclic aromatic amines (Omiecinski *et al.*, 1999). Increased expression of CYP1A1 in the lungs increases the risk of lung cancer (McLemore *et al.*, 1990; Guengerich, 1988) as well as colorectal cancer (Sivaraman *et al.*, 1994). CYP1A2 has role in tobacco-related cancers (Smith *et al.*, 1996). 7-Hydroxyflavone and galangin are the potent inhibitors of CYP1A1 and CYP1A2 (Zhai *et al.*, 1998), respectively, thereby block the process of carcinogenesis.

Many flavonoids act as blocking agents of AhR. The inhibition of gene expression of CYP1 family enzymes through blocking AhR plays an important role in the cancer chemopreventive properties of flavonoids. Quercetin, one of the most abundant naturally occurring flavonoids, binds as an antagonist to AhR and consequently inhibits benzo[a]pyrene, DMBA and aflatoxin B1 by altering the expression of CYP1A1, 1A2 and 1B1. This inhibition results in reduced B[a] P-DNA adduct formation (Kang *et al.*, 1999). Kaempferol also prevents CYP1A1 gene transcription induced by the prototypical AhR ligand, TCDD (Ciolino *et al.*, 1999). However, certain flavonoids (diosmin, diosmetin, galangin) are AhR agonists, increasing CYP1 expression and consequently carcinogen activation capacity. But these compounds strongly inhibit activities of the expressed enzymes. For instance, treatment of cells with diosmetin caused a dose-dependent expression of CYP1A1 mRNA, however, an extensive decrease in the formation of CYP1A1-mediated DNA adducts from DMBA was observed (Ciolino *et al.*, 1998).

On the basis of available data on flavonoid-CYP interactions, it can be deduced that flavonoids possessing hydroxyl groups inhibit CYP-dependent monooxygenase activity, whereas those lacking hydroxyl groups can stimulate the enzyme activity. In the study by Tsyrlov *et al.* (1994), quercetin inhibited the metabolism of aryl hydrocarbons but stimulated the activity of cDNA expressed human CYP 1A2. In another study, 7,8-benzoflavone has been reported as a stimulator of CYP3A4 activity (Bo *et al.*, 2001; Ueng *et al.*, 1997; Guengerich *et al.*, 1994) and an inhibitor of human CYP1A1, 1A2 (Tassaneeyakul *et al.*, 1993) and activation of CYP3A4. This shows that a flavonoid can have different effects on different CYP activities. Thus, flavonoids can either inhibit or activate human cytochromes P450 depending upon their structures, concentrations and experimental conditions.

Beneficial properties of various flavonoids include inhibition of CYPs involved in carcinogen activation and scavenging reactive species formed from carcinogens by CYP-mediated reactions. Induction of CYP activity by flavonoids proceeds via various mechanisms, including direct stimulation of gene expression through a specific receptor and/or CYP protein, or mRNA stabilization (Shih *et al.*, 2000, Lin and Liu, 1998).

UGT and GST are two major phase-II detoxifying enzymes, which protect cells against both endogenous and exogenous carcinogens by glucuronidation and nucleophilic addition of glutathione to a variety of different substrates, respectively (Fisher *et al.*, 2001; Talalay and Fahey, 2001; Tukey and Strassburg, 2000; Mannervik and Danielson, 1998). Flavanones and flavones increase the activities of GST and UGT (Canivenc-Lavier *et al.*, 1996). Green tea catechins have

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been shown to activate mitogen-activated protein kinases. This activation results in the stimulation of transcription of phase II detoxifying enzymes through the antioxidant responsive element (Yu *et al.*, 1997). In addition, flavonoids, being structurally similar to estrogen, show an estrogenic or antiestrogenic activity. Like natural estrogens, they can bind to the estrogen receptor and modulate its activity. They also block CYP19, a crucial enzyme involved in estrogen biosynthesis. Soy isoflavones have been studied extensively for estrogenic and antiestrogenic properties. Other flavonoids have been much less tested for steroid hormone activity. Luteolin and naringenin display the strongest estrogenicity, while apigenin shows a relatively strong progestational activity (Zand *et al.*, 2000). Flavonoids in the human diet may reduce the risk of various cancers, especially hormone-dependent breast and prostate cancers, as well as preventing menopausal symptoms.

Flavonoids as modulators of P-glycoprotein and MRP

Resistance of cancer cells to chemotherapy is a major obstacle to the success of cancer chemotherapy and has been closely associated with treatment failure. P-glycoprotein (Pgp), a plasma membrane ABC (ATP-binding cassette) transporter, interferes with drug bioavailability and disposition, including absorption, distribution, metabolism and excretion, affecting the pharmacokinetics and pharmacodynamics of many herbal and synthetic drugs (Sun *et al.*, 2004; Cummins *et al.*, 2003; Mizuno *et al.*, 2003). Increased or over expression of P-gp is often involved in cancer cell resistance to chemotherapy. Some isoflavones and flavones have been found to be active against P-gp (Ferte *et al.*, 1999).

Quercetin has been known to inhibit ATP-dependent drug efflux (Shapiro and Ling, 1997; Scambia *et al.*, 1994). The flavone, luteolin and its 7-O-b-D-glycopyranoside have shown to inhibit multi-drug-resistance (mdr) transporter by interacting with their ATP binding domains (Nissler *et al.*, 2004). The prenylated flavonoids strongly inhibit drug interactions and nucleotide hydrolysis and may serve as potential modulators of multidrug resistance (Di Pietro *et al.*, 2002).The *in vitro* everted gut studies conducted by Chen *et al.* (2002) indicated that phellamurin, a prenylated flavonoid glycoside, significantly inhibited the function of intestinal Pgp. Many isoflavone and flavone compounds have been found to be active against P-gp (Ferte *et al.*, 1999). On the other hand, Pgp in normal tissues may serve as a cellular defense mechanism against naturally occurring xenobiotics. Due to the potential importance of Pgp in cellular defense against environmental carcinogens (Phang *et al.*, 1993; Yeh *et al.*, 1992), cancer chemopreventive properties of flavonoids by modulating the P-gp activity resulted in flurry of research in the area. Flavonoids may up-regulate the activity of Pgp. Several commonly occurring flavonoids, especially, quercetin, kaempferol and galangin at micromolar concentrations stimulated the efflux of adriamycin in Pgp-expressing HCT-15 colon cells (Tsyrlov *et al.*, 1994). Moreover, quercetin and genistein potentiate the effects of adriamycin and aunorubicin, respectively, in a multidrug-resistant MCF-7 human breast cancer cell line (Chieli *et al.*, 1995; Scambia *et al.*, 1994). Among the various cell targets of genistein, ABC transporters were also identified. Genistein was found to be a modulator of non-P-gp mediated multidrug resistance, not affecting Pgp MDR cells (Versantvoort *et al.*, 1994). In a study, quercetin, at low concentrations, stimulated the activity of Pgp, whereas at high concentrations it inhibited Pgp (Mitsunaga *et al.*, 2000). Critchfield *et al.* (1994) conducted a study to evaluate the effects of flavonols on Pgp activity in rat hepatocytes by assessing the transmembrane transport of Pgp substrates such as rhodamine-123 and doxorubicin. The results indicated that flavonols strongly up-regulate the activity of Pgp in cancer cell lines. At the same time, they may modulate differently the transport of putative Pgp substrates in normal rat hepatocytes. This differential nature of flavonoids to up-regulate P-gp activity in normal cells and at the same time down-regulate it in cancerous cells can be of high significance in cancer therapy.

The membrane protein mediating the ATP-dependent transport of lipophilic substances conjugated to glutathione, glucuronate or sulfate, have been identified as members of the multidrug resistance protein (MRP) family. A soybean isoflavone genistein was found to be an inhibitor on the basis of its effect on drug accumulation in MRP1-overexpressing cells.

Flavonoids as tumor suppressing agents

Certain members of the flavone, flavonol, flavanone and isoflavone classes possess antiproliferative effects in different cancer cell lines (Kuntz *et al.*, 1999). The antitumor activity of several flavonoids (pinostrobin, quercetin, myricetin, morin) is attributed to their efficiencies to inhibit topoisomerase I and II (Sukardiman *et al.*, 2000; Constantinou *et al.*, 1995). Flavonoids might slow down cell proliferation as a consequence of their binding to the estrogen receptor (Primiano *et al.*, 2001). Complete growth retardation of androgen-independent human prostatic tumor cells was observed when they were treated with kaempferol (Knowles *et al.*, 2000).

Flavonoids possess protein kinase inhibitory activities (Gamet-Payrastre *et al.*, 1999; Cushman *et al.*, 1991; Ferriola *et al.*, 1989; Geahlen *et al.*, 1989). Alternatively, flavonoids can affect cancer cells by triggering the process of apoptosis (Galati *et al.*, 2000. Flavonoids are also potent inhibitors of mitogen signaling processes by affecting various kinase activities (Reiners *et al.*, 1998). Genistein and daidzein are the two major soy isoflavones. Genistein, like other isoflavones, displays a remarkable estrogenic activity. Genistein is also a specific and potent inhibitor of tyrosine kinases and it interferes with many biochemical pathways (Polkowski and Mazurek, 2000).

Ahmad *et al.* reported that EGCG induced apoptosis and cell cycle arrest in human epidermoid carcinoma cells A431 (Ahmad *et al.*, 1997). They also found that the apoptotic response of EGCG was specific to cancer cells only. The study conducted by Liang *et al.* (1997) showed that EGCG suppresses extracellular signals and cell proliferation by binding to epidermal growth factor receptor. The findings by Liang *et al.* (1997) suggest that EGCG blocks the cellular signal transduction pathways and might result in inhibition of tumor formation.

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EGCG has been reported to block the induction of nitric oxide synthase by inhibiting the process of binding of NF-κB to inducible nitric oxide synthase (iNOS) (Lin *et al.*, 1997).

The certain members of flavone, flavonol, flavanone and isoflavone classes possess antiproliferative effects in different cancer cell lines (Kuntz *et al.*, 1999). The polyphenols from tea, particularly green tea, have strong antiproliferative capacity. Valcic *et al.* (1996) studied the antiproliferative effects of six green tea catechins ((+)-gallocatechin, (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin gallate, and (–)-epigallocatechin gallate) on four different human cancer cell lines. In their study, all catechins strongly inhibited proliferation. EGCG was found to be most effective inhibitor in MCF-7 breast cancer cells, HT-29 colon cancer cells and UACC-375 melanoma cells.

Quercetin causes cell cycle arrest in the G_0/G_1 phase in human leukemic T-cells and gastric cancer cells (Yoshida *et al.*, 1990, 1992). In human leukemic T-cells quercetin reversibly blocked the cell cycle at a point 3–6 h before the start of DNA synthesis and it suppressed DNA synthesis to 14% in gastric cancer cells. In gastric cancer cells it specifically induced the G1 phase arrest. It also arrests the cell cycle in the G2/M phase (Choi *et al.*, 2001; Koide *et al.*, 1997). Richter *et al.* (1999) in their study on colorectal cancer cells reported that quercetin induced growth inhibition and cell loss, and cells were preferentially retained in the S phase. It was more effective than apigenin, fisetin, robinetin and kaempferol. This effect was attributed to the inhibition of EGF receptor kinase by quercetin.

FLAVONOIDS AS ANTIOXIDANTS

The antioxidant capacity of a compound is based on its structural features, such as the number and position of double bonds, hydroxyl-groups and modification like linkage to sugar-moieties (Kozikowski *et al.*, 2003). Flavonoids are potent antioxidants and thereby inhibit cell growth. The inhibition of cell growth depends on the capacity of the compound to scavenge free radicals. Flavonoids act as antioxidants by chelating redox-active metals and by scavenging free radicals. It has been reported that flavonoids with 4–6 OH groups act as strong antioxidants in an aqueous milieu, whereas those with more or fewer OH groups show low or no antioxidant activities (Rice-Evans *et al.*, 1995; Rice-Evans and Miller, 1996). Moreover, it was found that OH groups in the ortho-position at ring B as well as the double bond between C2 and C3, together with the carbonyl function in ring C are important structural determinants for the antioxidant effects of flavonols (Bors *et al.*, 1990). Flavones, isoflavones and flavanones act as antioxidants against peroxyl and hydroxyl radicals and in the presence of Cu^{2+}, serve as prooxidants (Cao *et al.*, 1997). Tea polyphenols have shown strong antioxidant activity, both *in vitro* and *in vivo*. Rietveld and Wiseman (2003) reported that the majority of the human intervention studies in which the biological antioxidant properties of tea polyphenols have been studied demonstrate an increase in plasma antioxidant potential after the consumption of green tea as well as black tea. An increase in the blood antioxidant potential leads to reduced oxidative damage to macromolecules such as DNA and lipids.

The carbonyl and hydroxyl groups of flavonoids can chelate bivalent metals (Fe, Cu) which make them unavailable for redox cycling reactions (Bravo, 1998), and inhibit lipid peroxidation (Afanas'ev *et al.*, 1989). Flavonoids also function as terminators of free radicals by donation of electrons to form stable products. Flavonoids are very effective scavengers of hydroxyl and peroxyl radicals (Bravo, 1998) as well as quenching superoxide radicals and singlet oxygen (Jovanovic and Simic, 2000, 1990).

An important property of flavonoids is that they do not affect β-carotene, vitamin C and E, which are attributed for the endogenous antioxidant protection system of the body (Pietta and Simonetti, 1998).

Flavonoid glycoside molecules such as rutin, and the glycoside of quercetin, are less potent as antioxidants (Vinson *et al.*, 1995). The intracellular antioxidant efficacy of flavonoid glycosides depends on their capacity to react against ROS. The recycling potential of the cell also plays an important role in the antioxidant status of the flavonoid glycosides (Kagan and Tyurina, 1998).

STRUCTURE–FUNCTION RELATIONSHIP OF FLAVONOIDS

The structure–function relationship of flavonoids has been studied extensively to provide an inspiration for the design of a rational drug and/or chemopreventive agent for future pharmaceuticals.

The number and position of hydroxyl groups are determining factors for isoflavone and flavone activity. The influence of OH substitution on MDR modulating activity appeared to be highly dependent on the position of the substitution (Ferte *et al.*, 1999). For example, the presence of an unsubstituted OH group at position 5 appears to be preferable in the case of resistance mediated by P-gp. This behaviour could be related to the presence of an intramolecular hydrogen bond between this phenol and the adjacent ketone group. This creates a structure which brings an additional lipophilic contribution to the flavonoid nucleus. Lipophilicity by itself does not determine MDR modulating activity, but may be regarded as a favorable parameter within a homologous series.

Based on CYP1A1 and 1A2 inhibitory studies, the structure–function relationship of flavonoids can be explored. The CYP1A1 active site has a preference for binding of 7-hydroxyl-substituted flavones (Zhai *et al.*, 1998). A prerequisite for binding to CYP1A2 is the presence of multiple hydroxyl groups (preferably two in positions 5 and 7) on the flavone skeleton and an additional hydroxyl-substitution of C2 in the case of flavonols (e.g. morin). Planar molecules with a small volume/surface ratio turn out to possess high inhibitory activity of CYP1A2. That is why flavanones and flavanes (missing the C2, C3 double bond), having a phenyl group (B ring) nearly perpendicular to the rest of the molecule, showed a decreased inhibitory efficiency. Glycosylation, as well as the presence of several hydroxyl groups and/or addition of methoxy-groups, results in a drastic decrease in their inhibitory activities. Based on the observation that catechins had no effect on

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the CYP enzyme activity, the oxo-group (position C4) in the C ring is also an essential factor for enzyme inhibition (Moon *et al.*, 1998). The most potent CYP1A2 inhibitor is chrysin (5,7-dihydroxyflavone) followed by apigenin (5,7,4_-trihydroxyflavone) and morin (3,5,7,2_, 4_-pentahydroxyflavone) (Lee *et al.*, 1998). For CYP1B1, acacetin (3,5,7,2,4-pentahydroxyflavene) seems to be the most potent and selective inhibitor with an IC_{50} which is more than 10 times lower than that of CYP1A1 and 1A2 (Doostdar *et al.*, 2000). Similarly, hesperetin (5,7,3_-trihydroxy-4_-methoxyflavone) is a selective inhibitor of expressed CYP1B1 in lymphoblastoid microsomes. Prenylated flavonoids from hops are highly effective inhibitors of CYP1 family enzymes. At 0.01 mM concentration, prenylated chalcone, xanthohumol, almost completely inhibited CYP1A1 and totally eliminated CYP1B1 activity (Henderson *et al.*, 2000). The most effective inhibitors of CYP1A2 were 8-prenylnaringenin and isoxanthohumol. These findings are in agreement with the suggested similarities of the binding sites of CYP1A1 and 1B1 when compared with that of CYP1A2.

It has been stated that the large hydrophobic substituent at position 7 elicits a higher affinity for CYP1A2 than does the hydrophilic hydroxyl substituent, whereas the hydroxyl substitutions at positions 3 and 5 increase the binding affinity. A molecular model of human CYP1A2 supports this interpretation (Dai *et al.*, 1998).

FLAVONOID–DRUG INTERACTIONS

After the first report of grapefruit juice–drug interaction was published in 1989, flavonoid–drug interactions have received increasing attention. CYP3A4 is a predominant cytochrome P450 enzyme present in hepatic and intestinal cells of humans. It is responsible for the metabolism of about 50% of therapeutic agents, as well as the activation of some carcinogens. Flavonoids act as effector molecules of CYP3A4, resulting in either activation or inhibition of the substrate modulated by it. Due to this, interaction of flavonoids with CYP3A4 becomes important. Depending on the structure of flavonoid, simultaneous administration of flavonoids and clinically used drugs may result in altered pharmacokinetics of the drug increasing their toxicity or in the decline of their therapeutic effect (Tang and Stearns, 2001). Besides the well known synthetic 7,8-benzoflavone, several other flavonoids, e.g. flavone, tangeretin, were described as enzyme stimulators (Backman *et al.*, 2000; Maenpaa *et al.*, 1998; Ueng *et al.*, 1997). In contrast, there is a group of CYP3A4 inhibitors represented by flavonolignan, silymarin, a component of milk thistle extracts, and I3, II8-biapigenin and hyperforin of St John's wort extracts (Obach, 2000; Venkataramanan *et al.*, 2000). Likewise, naringenin (5,7,4′-trihydroxyflavanone), a flavonoid present in grapefruit juice, exerts also an inhibitory effect on CYP3A4. Naringenin and bergamottin (furocoumarin) are considered to be involved in impaired hepatic metabolism of certain drugs coadministered with a high uptake of grapefruit juice (Bailey *et al.*, 2000; He *et al.*, 1998). However, very little data are available on *in vivo* CYP cooperativity compared with *in vitro* experiments with enzymes or cell cultures.

SYNERGESTIC ACTION OF FLAVONOIDS

Information regarding the possible synergistic or antagonistic biochemical interactions among flavonoids, other polyphenols and nutrients contained in fruits and vegetables is scarce. Knowledge on the potential interactions among these compounds may help to define the efficiency of polyphenol-containing foods in cancer prevention as related to the structure–function activity of the compounds. The combination of apigenin with sulforaphane resulted in a synergistic induction of UGT1A1 mRNA up to 12-fold and also GSTA1 in CaCo-2 cells (Svehlikova *et al.*, 2004). Mertens-Talcott *et al.* studied the synergistic effect of Quercetin and ellagic acid on cell cycle arrest and apoptosis in MOLT-4 human leukemia cells (Mertens-Talcott *et al.*, 2003). They found that the two compounds together had a greater effect on the cells than individual ones.

Genistein and diadzein together showed better antitransformation activity in neoplastic cells in the study conducted by Franke *et al.* (1998). However, individually they had a lower effect. Quercetin and resveratrol at a micromolar range along with ethanol synergistically suppressed iNOS gene expression and nitric oxide production in RAW264.6 cells (Chan *et al.*, 2000). Elattar *et al.* reported that combining 50 μM of resveratrol with 10, 25 and 50 μM of quercetin resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis in oral squamous carcinoma cells (SCC-25) (Elattar and Virji, 1999). From the study they concluded that resveratrol or a combination of resveratrol and quercetin, in concentrations equivalent to that present in red wines, are effective inhibitors of oral squamous carcinoma cell (SCC-25) growth and proliferation.

Studies conducted by Scambia *et al.* indicate that quercetin may increase the effectiveness of some chemotherapeutic agents such as doxorubicin, adriamycin, diamminedichloroplatinum (II) and cisplastin (Scambia *et al.*, 1990, 1992, 1994). Quercetin and genistein both increased the concentration of daunorubicin in some multidrug-resistant cell lines (Versantvoort *et al.*, 1993). Genistein *in vitro* increased the concentration of cisplatin in resistant cell lines (Marverti and Andrews, 1996). Flavonoids are also reported to have a protective effect on ascorbic acid (Harper *et al.*, 1969) and thereby enhance the antioxidant activity of ascorbic acid. Clemetson and Anderson studied the correlation between the antioxidant property of ascorbic acid and flavonoids (Clemetson and Anderson, 1966; Clemetson, 1989). They examined the effect of 34 different flavonoids on the oxidation of ascorbic acid at physiological pH. They found that compounds possessing 3′,4′-OH groups of the B ring and the 3-hydroxy-4-carbonyl grouping of the g-pyrone ring exhibited significant antioxidant activity on ascorbic acid. Accordingly, quercetin and rutin were found to have a greater ascorbic acid-protective capacity than the other flavonoids in the study (Hughes and Wilson, 1977). In this way, flavonoids can enhance the effects of ascorbic acid. Tangeretin, found in citrus fruits, completely blocked the inhibitory effect of tamoxifen on mammary cancer in mice (Bracke *et al.*, 1999). An *in vitro* study showed tamoxifen and genistein synergistically inhibit the growth of estrogen

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receptor negative breast cancer cells (Shen *et al.*, 1999). The synergistic effects of flavonoids with other flavonoids and chemotherapeutic agents are *in vitro* reports. To determine whether flavonoids can exhibit the same effects *in vivo* needs to be investigated. It is now widely believed that the actions of dietary supplements alone do not explain the observed health benefits of diets rich in fruits, vegetables and whole grains, because, taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects (Yusuf *et al.*, 2000; Ommen *et al.*, 1996; Stephens *et al.*, 1996). The benefits of eating fruits and vegetables may be much greater than are the effects of any of the individual antioxidants, as various vitamins, minerals, and phytochemicals in these whole foods may act synergistically (Brown *et al.*, 2001).

DISCUSSION

The biological activities of flavonoids and the synergistic action shown by them with other phytonutrients or drugs make them ideal candidates in alternative cancer therapies. Flavonoids can block the initiation or reverse the promotion stage of multistep carcinogenesis. They can also halt or retard the progression of precancerous cells into malignant ones. The chemopreventive effects that most flavonoids exert are likely to be the sum of their effect on several distinct mechanisms working inside the cell, e.g. quercetin and EGCG show a wide array of mechanisms, from antioxidant property to inhibition of cell signals. Though the flavonoids have been the focus of research since the 1930s, many of them have been used in traditional medicine for thousands of years in Eastern countries. Various epidemiological studies substantiate their role as anticancer agents. However, very little information is available on the efficacy, safety and the possible risks of flavonoids in the human body or their adverse reaction with drugs.

Efforts are required to quantitate the amounts of different flavonoids in an assortment of foods and medicinal plants. The effective physiological levels of flavonoids individually and in combination are to be assessed. Their pharmacokinetic properties and bioavailability studies are to be extensively studied. This type of data should bring out the potential of phytotherapy in combating this dreadful disease.

Modulation of CYPs, Pgp and MRP by flavonoids may be used beneficially in detoxication, chemoprevention or suppression of drug resistance. With careful monitoring, it should be possible to regulate the systemic availability of some anticancer agents provided the drug–drug interactions are properly documented.

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