Chapter 23
Flavonoids in Cancer Prevention and Therapy: Chemistry, Pharmacology, Mechanisms of Action, and Perspectives for Cancer Drug Discovery

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23.1 Introduction

Among the numerous products available from plants, the flavonoid superfamily plays a central role by its large number of molecules (over 6000) and also by the role these products occupy in the normal physiology of plants. Flavonoids are secondary plant metabolites involved in several biological processes (e.g., germination, UV protection, insecticides) and are also involved in the attraction of pollinating agents via the vivid colors of the anthocyanin pigments found in flowers (e.g., blue, purple, yellow, orange, and red) [1–3]. Flavonoids are found in the normal human diet composed of green vegetables, onions, fruits (apples, grapes, strawberries, etc.), beverages (coffee, tea, beer, red wine) [4, 5], and isoflavonoids are mainly found in soya bean-derived products [6].

Flavonoids are being studied intensely partly because of a renewed interest in medicinal plants used in folk medicine [7], and also because of the so-called "*French paradox*," which is generally

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M. Alaoui-Jamali (ed.), *Alternative and Complementary Therapies for Cancer*, DOI 10.1007/978-1-4419-0020-3_23,

thought to be linked to the Mediterranean diet (rich in fruits, vegetables, and red wine) which appears to protect against cardiovascular diseases in spite of its relatively high content in saturated fat [8]. In addition, several epidemiological studies have shown that diets rich in fruits and vegetables are generally associated with a lower cancer incidence [9–11].

The daily consumption of flavonoids is highly variable among different countries. Inasmuch as most of the human intake of flavonoids is based only on the consumption of a few flavonoids, the actual daily intake of flavonoids is probably superior to the reported estimates in the range of 3–68 mg/day, with a median value of 23 mg/day [12]. Other authors estimate the daily consumption of flavonoids (e.g., polyphenols) to be about 150–1000 mg/day [13]. A recent study in France has shown that fruits (mainly apples and strawberries) and vegetables (e.g., potatoes, lettuce, onions) account for about 28% of the daily intake in polyphenols, and that the total consumption would be over 300 mg/day [14]. Because fruits, vegetables, tea, coffee, and red wine are all rich in flavonoids, the focus of several research teams is now to identify which flavonoid is responsible for a given pharmacological effect and to better understand its molecular mechanism of action.

In this review, after a reminder of the flavonoid chemical structures, we briefly mention the main pharmacological activities of this class of compounds, and focus thereafter on the flavonoids of interest in the prevention and therapy of cancer.

23.2 Chemical Structures of Flavonoids

Flavonoids are composed of a 15 carbon atoms comprising 2 cycles of 6 carbon atoms linked by a 3 carbon chain (rings A and B, Fig. 23.1). Except for the chalcones and aurones, the 3 carbon bridge usually forms a benzo-γ-pyrone ring (ring C). All flavonoids are classified according to the substituents encountered on the different cycles and the saturation degree of the C ring. Three classes of flavonoids can be distinguished: the flavonoids (or 2-phenylbenzopyranes), the isoflavonoids (or 3-phenylbenzopyranes), and the

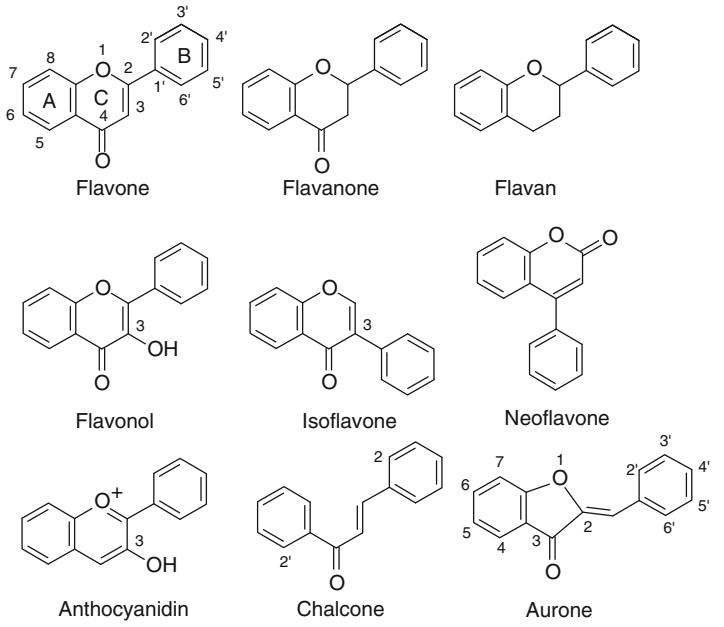

Fig. 23.1 Main flavonoid classes

neoflavonoids (or the 4-phenylbenzopyranes) [15]. The flavonoids are further classified according to the structure of the C heterocycle (if present), in the following groups: flavones, flavanones, flavans, flavonols, chalcones, and anthocyanidins (Fig. 23.1).

In fruits and vegetables, flavonoids can be found as the free aglycones or more frequently linked with a sugar. The flavones and the flavonols (3-hydroxyflavones) are the most frequently found flavonoids (e.g., quercetin, kaempferol, myricetin, apigenin) (Fig. 23.2). Flavonones (e.g., naringenin), flavanols (e.g., catechin, dihydroflavonols, dihydrokaempferol, dihydroquercetin), and the dihydroflavan-3,4-diols (leucopelargonidol, leucocyanidol) have a natural distribution less important than flavones and flavonols. In nature, the flavonoids can also be found as biflavonoids which are O- or C-dimers of flavones, flavonols, flavanones, dihydroflavonols, and sometimes of isoflavones [16, 17].

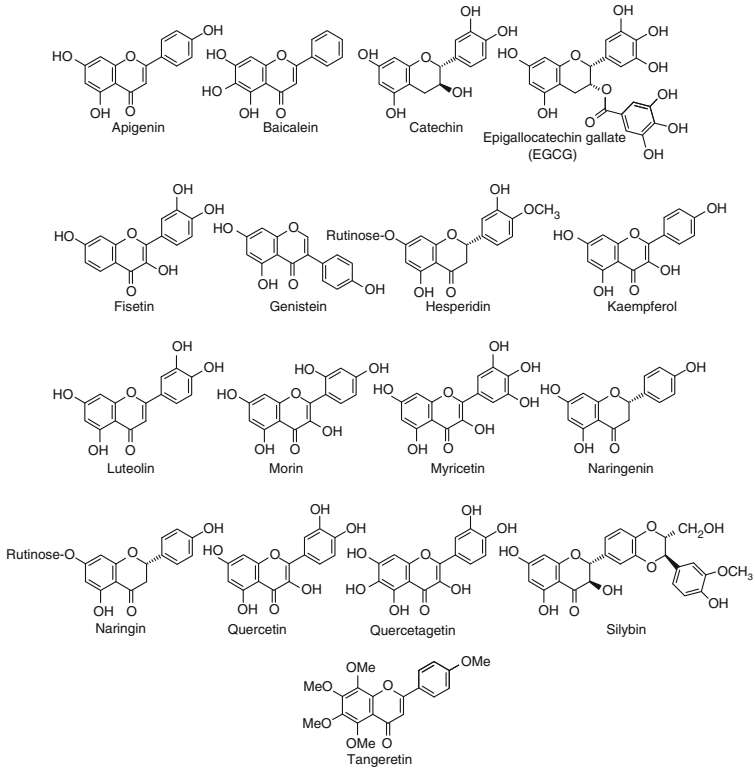

Fig. 23.2 Stuctures of some flavonoids mentioned in this review

23.3 Flavonoid Metabolism

Natural flavonoids in their glycosidic forms are absorbed in the intestines. The glycosidic portion plays an important role in absorption, as was shown with quercetin glycosides which are better absorbed (52%) compared to the quercetin aglycone (24%) [18].

Flavonoid metabolism is considered to play an important role for the expression of its several biological activities [19]. The hepatic cytochrome P450s (CYP) can hydroxylate flavonoids often at the C5 and C6 positions on ring A, on C3 of ring C, and

also on C3' and C4' of ring B. In humans, CYP1A2, CYP3A4, and CYP2C9 are mostly involved in flavonoid hydroxylation [20]. Phase II metabolism (conjugation) involves glucuronidation, sulfatation, and methylation. The metabolism by the intestinal flora is also important and can lead to demethylation and ring fission [21]. Major differences in metabolism may exist between laboratory animals and humans, as was recently shown with the synthetic flavone-8-acetic acid which is thought to be activated in vivo in mice to anticancer active metabolites [22]. It was also recently shown that aminoflavone was activated to antiproliferative active metabolites through sulfatation [23].

23.4 Flavonoid Pharmacological Activities

Several mammalian enzymatic systems have been reported to be inhibited by flavonoids, for example, kinases, topoisomerase I, glutathione S-transferase, cytochrome P450s, aromatase, and so on. [24]. This large number of enzymatic systems affected by flavonoids is probably responsible for the rather large pharmacological activities reported for this class of agents that we briefly mention below before focusing on flavonoids as chemopreventive and chemotherapeutic anticancer agents.

Cardiovascular diseases: A recent study has shown that chronic administration of polyphenols from red wine in rats can prevent hypertension and vascular dysfunction [25]. In humans, a flavonoid-rich diet (e.g., tea, onion, apple, etc.) has been linked to a significant reduction in cardiovascular morbidity and mortality in several studies [26, 27]. Flavonoid and isoflavone intake were found to be the main phytochemicals contributing to the low incidence of coronary heart disease in Japanese women [28].

Antioxidant: Among the numerous pharmacological properties of flavonoids, their antioxidant action is probably the most studied. Free radicals such as the hydroxyl ($OH^{\bullet}$), the superoxyde anion ($O_2^{\bullet -}$), and the peroxylipidic radicals may be scavenged by flavonoids, mainly by the flavonoids bearing a C3 hydroxyl group (flavonols). Flavonoids can also chelate

metal ions. The antioxidant hypothesis is, however, being challenged because compounds with similar antioxidant properties may present different biological effects [29]. It has also been reported that flavonoids can also scavenge the NO radical [30]. This radical is formed by several cell types (e.g., endothelial cells and macrophages) and its release is due to the NO synthase activity which is important in vascular tone regulation. Because some flavonoids can inhibit cyclooxygenase, this could explain the quercetin effect in counteracting the vasodilatation of NO on the vascular endothelium [31, 32]. In this context, it is worth pointing out that some synthetic flavones have been reported to downregulate both iNOS expression and NO expression in leukemia cells [33].

Vascular protection: Polyphenols have been shown to increase the formation of NO by endothelial NO synthase. Flavonoids have also been shown to contribute to the normalization of the vascular permeability [34, 35].

Hepatoprotection: Flavonoid extracts from *Silybum marianum* have been used in folk medicine against liver diseases in the form of a complex mixture comprising silybin which would act on the hepatocyte membrane to prevent the uptake of toxic compounds and would stimulate hepatocyte regeneration [36]. The hepatoprotective effects of silybin and quercetin have been shown in the rat model administered a toxic dose of paracetamol (acetaminophen) [37].

Antiallergic: Certain flavonoids, for example, quercetin, can be antiallergenic by inhibiting enzymes involved in the histamine release from mastocytes and basophils (cAMP phosphodiesterase and Ca^{++} ATPase) [38, 39].

Anti-inflammatory: The immune modulation of flavonoids appears to rely on their inhibition of eicosanoids and histamine formation and on their inhibition of free radical scavenging effects [40]. Several flavonoids can modify the metabolism of platelet arachidonic acid in vitro. Myricetin and quercetin can block cyclo-oxygenases and lipoxygenases action. Hesperidin is anti-inflammatory in a rat model of inflammation induced by carragenin or dextran. The interest in flavonoids as anti-inflammatory compounds is also

underlined by their lack of gastric toxicity frequently encountered with other anti-inflammatory drugs [24, 41].

Antiulcer: Some flavonoids can protect the gastric mucosa against ulcer-causing compounds. For example, hypolaetin-8-glucose, a flavonoid found in *Sideritis* species is considered as an active antiulcer compound. Naringin and quercetin are also antiulcerogen in the gastric ulceration induced by ethanol in the rat. The antiulcer properties of quercetin have been attributed to its mucus production activity [42]. In addition, quercetin can inhibit the growth of the ulcer-forming bacteria *Helicobacter pylori* and can decrease the production of chlorhydric acid by the gastric parietal cells [43].

Antibacterial activity: Several flavonoids have been shown to possess antibacterial activity, for example, apigenin, galangin, chrysin, naringin, epigallocatechin gallate, luteolin, quercetin, and kaempferol [44]. The activity of apigenin and galangin against both sensitive and antibiotic-resistant strains of *Staphylococcus aureus, Enterococcus faecium*, *Escherichia coli*, and *Pseudomonas aeruginosa*, is particularly noteworthy [45].

Antiviral activity: An important activity of some flavonoids is the inhibition of the human immunodeficiency virus (HIV). For example, acacetin, apigenin, baicalein, chrysin, hinokiflavone, myricetin, quercetagetin, robinetin, robustaflavone, and quercetin, were reported to be involved in HIV entry, infection, transcription, or replication in mammalian cells [46–50].

23.5 Flavonoids in Cancer Prevention

Numerous review articles have already been published concerning flavonoid involvement in the prevention of carcinogenesis and treatment of cancer [51–53, 24]. In the following paragraphs, we mainly focus on the mechanisms of action of flavonoids involved in cancer prevention and treatment. Figure 23.3 presents the main flavonoid actions potentially involved in the prevention and therapy of cancer.

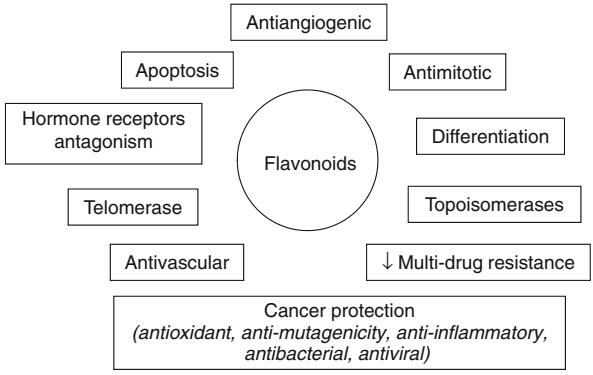

Fig. 23.3 Some mechanisms of action of flavonoids involved in the prevention and therapy of cancer

23.5.1 In Vitro Antimutagenicity

Several flavonoids have been reported to be antimutagenic. Quercetin can inhibit the mutagenic effect of benzopyrene, a powerful carcinogen of the polycyclic aromatic hydrocarbon family, in bacterial systems of mutagenesis [54], and can also prevent the nuclear damages in mouse colon epithelial cells [55]. Galangin (3,5,7-trihydroxyflavone) and other flavonoids have shown anticlastogenic effects in vitro and in vivo in bleomycin or benzopyrene models [56]. Mutagenesis induced by diol-epoxide of benzopyrene (*bay region*) can also be inhibited by hydroxylated flavones [57]. Several synthetic flavones have also shown antimutagenic activity in the Ames test [58].

The prevention of carcinogenesis by flavonoids is thought to be due to the inhibition of a covalent bond between a reactive metabolite and DNA. It has been shown that polyphenols could prevent the covalent link between DNA and carcinogens (e.g., polycyclic aromatic hydrocarbons) by inhibiting enzymes involved in their activation, such as cytochrome P450s 1A1 and 1B1 [59–61]. In addition, the cytochromes' P450s protein expression can be blocked by flavonoids thus preventing the formation of DNA reactive mutagens [62–64]. Flavonoids were also shown to induce phase 2 drug-metabolizing enzymes involved in carcinogens' detoxification mechanisms, such as

UDP-glucuronosyltransferase (UGT), NAD (P) H-quinone oxydoreductase, and glutathione S-transferase [65–67].

23.5.2 Cancer Prevention in Animal Models

Several flavonoids have been shown to prevent cancer in animal models [68]. Methoxylated flavones, for example, the 5,7-dimethoxyflavone and 3',4'-dimethoxyflavone can prevent the formation of colon cancer at the initiation stage [61], and some synthetic 3-nitroflavones were also shown to prevent the formation of colon aberrant crypt foci in the rat model [69]. At the promotion stage, the 5,7-dimethoxyflavone and the 5,7,4'-trimethoxyflavone were found more active compared to their unmethylated counterpart, that is, chrysin and apigenin, respectively [70]. Anthocyanins can prevent colon cancer induced by 1,2-dimethylhydrazine [71].

Orally administered quercetin was shown to inhibit the DMBA-induced carcinogenesis in hamsters and rats [72, 73]. Likewise, orally administered flavone, flavanone, tangeretin and quercetin can inhibit the initiation and promotion of aflatoxin B1-induced hepatocarcinogenesis [74]. It has also been recently reported that orally administered fisetin, 2,2'-dihydroxychalcone or apigenin can significantly inhibit prostate cancer progression in TRAMP mice and prolong survival [75, 76]. Silybin is also active in the prevention and treatment of prostate cancer in animals and clinical studies are currently ongoing [77]. Silybin has been shown to prevent skin cancer in animal models, and its use in humans has been suggested because of its low toxicity [78]. Structure-activity relationships have shown that the ortho-dihydroxyphenyl on ring B appears essential for activity in the prevention of skin cancer [79]. Several green tea extracts have demonstrated the inhibition of skin carcinogenesis by oral ingestion or topical application [80, 81]. Compounds extracted from green tea such as epigallocatechin gallate (EGCG) and theaflavins can inhibit tumorigenesis induced by cisplatin and NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) [82, 83].

The mechanism of action of flavonoids with regard to their preventive effects of chemically induced cancers are generally attributed to their modulation of phase I and phase II metabolic enzymes,

thus preventing the formation of DNA-reactive species by cytochromes P450 and favoring their elimination by phase II enzymes (reviewed in [84]).

It is noteworthy that certain flavonoids and isoflavonoids can also exhibit activity in hormone-dependent mammary and prostate cancers. Aromatase, an enzyme involved in these cancers can be inhibited by flavonoids such as 7-methoxyflavone and 7,4'-dimethoxyflavone, which are potent inhibitors of this enzyme [85]. In addition, the inhibition of aromatase activity could result in a decrease of estrogenic levels in women and could be involved in the prevention of breast cancer [86]. Genistein and daidzein (isoflavones found in soya beans) can also inhibit hormone-dependent or independent mammary and prostate cancers in mice [87, 88]. Based on preclinical studies, Cross et al. have recently suggested the clinical use of phytoestrogens (e.g., genistein) in the prevention and therapy of colorectal, breast, and prostate cancers [89].

23.5.3 Cancer Prevention in Humans

Although the evidence supporting cancer prevention is still controversial in humans, probably because of the inherent difficulties to conduct this type of epidemiological studies, several reports have nonetheless shown beneficial effects of a polyphenol-rich diet in preventing certain types of cancer and to considerably lower the risk of dying from this disease [90].

In humans, important geographical differences in the incidence of prostate cancer appear to indicate that environmental factors are involved. Among these factors, a diet rich in polyphenols appears linked to a lower incidence of prostate cancer [91]. Results from several clinical studies indicate that soybean isoflavones administration appears to favorably affect prostate-specific antigen levels, and these observations should be an impetus for further clinical trials [92]. In addition, a recent large prospective study in European men found that higher concentrations of circulating genistein are indeed associated with a lower risk for prostate cancer [93].

Lung cancer incidence has also been reported to be lower in persons with high intake of flavonoids [94, 95]. A recent review of

epidemiological evidence has shown a small beneficial association between a lower incidence of lung cancer with tea and flavonoids consumption ([9], and references therein).

Epidemiological studies have long identified that breast cancer incidence is lowest in most Asian countries compared to Western countries, and that women of Asian origin eating a Western diet have the same breast cancer incidence as Western women. In breast cancer, it was also observed that the intake of flavones appears to protect against mammary tumors [11]. A recent study has also found that a dietary pattern characterized by frequent consumption of vegetables, fruits, fish, soybean curd, and low fat intake is associated with a reduced risk of breast cancer in Japanese women [96].

Ovarian cancer was also recently reported to be prevented by the consumption of tea and broccoli (containing kaempferol). If additional prospective studies confirm these results, this could lead to an important advance for ovarian cancer prevention [10].

Concerning the anthocyanins, these compounds were clearly shown to be cancer-protective in animal models, but human epidemiological studies have thus far not revealed a protective role of these molecules [97].

As stated above for the animal studies, the cancer-preventive effects of flavonoids in humans is also probably due to the modulation of phase I and phase II metabolic enzymes (reviewed in [84]). Also, the flavonoids could prevent cancer via their anti-inflammatory properties, because there is a growing consensus that inflammation probably plays a major role in cancer initiation [98].

23.6 Flavonoids in Cancer Therapy

23.6.1 Antimitotic Effects

Several authors have reported that certain flavonoids can interfere with tubulin polymerization in vitro and cause a cell arrest in mitosis [99–104]. The study of 79 flavonoid analogues of centaureidin (3,6,4'-trimethoxy-5,7,3'-trihydroxyflavone) has disclosed the structure-activity relationships (SAR) with regard to

cytotoxicity and interaction with tubulin: the most active compounds were the ones with hydroxyl groups at C3' and C5, and also with methoxylated groups at C3 and C4' [99].

Chalcones can also possess antimitotic activity, as demonstrated with the (E)-1-(2,5-dimethoxyphenyl)-3-[4-(dimethylamino) phenyl]-2-methyl-2-propene-1-one. This latter compound is active at only 4 nM in vitro in the HL60 human leukemia, and also in vivo in the B16 melanoma and L1210 leukemia models [105].

23.6.2 Apoptotic Effects

Catechins from green tea can induce prostate cancer cells apoptosis with the following order of potency: ECG (epicatechin-3-gallate) > EGCG (epigalocatechin-3-gallate) > EGC (epigallocatechin) > EC (epicatechin) [106]. Colon cancer cells can also enter apoptosis by exposure to baicalein, myricetin, genistein, and bavachanin [107]. B16 melanoma cells are mostly sensitive to chalcones: isoliquiritigenin > butein = phloretin [108]. Human HL60 leukemia cells are sensitive to apoptosis induced by apigenin, quercetin, myricetin, and kaempferol. EGCG has also been shown to cause cell death via a mechanism involving the inhibition of telomerase [109].

Quercetin-induced apoptosis appears to be due to a cell cycle arrest in S phase and to the inhibition of thymidilate synthase [110]. Another action of flavonoids on cancer cells is their effect on the thioredoxin system which exerts an antioxidant action and acts on cell proliferation and viability. This system is overexpressed in tumors, and it has recently been shown that myricetin and quercetin can inhibit this system with IC_{50} in the nanomolar range [111].

No clear-cut SARs are apparent for flavonoid induction of apoptosis, because the experimental data mentioned above seem to be highly dependent on the cancer cell line considered [53].

23.6.3 Differentiation

Kawaii et al. have shown that HL60 human leukemia cells can undergo differentiation upon flavonoid exposure [112]. The

glycosides are less active than their corresponding aglycones, and the presence of the C2–C3 double bond is needed for activity, as well as a methoxy group in C3 and a catechol group on phenyl B. The most active flavonoids were the ones bearing the following substituents: 3-OH; 5,6,7,8,3′,4′-OMe > 5,7,3′,4′-OH > 5,6,7,8,4′-OMe [112]. Genistein, apigenin, luteolin, and quercetin were also found to induce HL60 cells differentiation [113]. Because the isoflavone genistein and its corresponding flavonoid apigenin are equipotent for the differentiation effect, the phenyl position in C2 or C3 position does not appear to be important for this activity. The double bond at the C2–C3 is needed for differentiation, as well as an unopened C ring because chalcones are inactive [113].

23.6.4 Topoisomerase Inhibition

Topoisomerases I and II are ubiquitous essential enzymes involved in DNA topology and are overexpressed in several tumors [114, 115]. Some flavonoids were reported to inhibit topoisomerase I, for example, EGCG, quercetin, fisetin, kaempferol, apigenin, and acacetin. SAR necessary for this anti-topoisomerase I activity is the absence of a sugar, a C2–C3 double bond, an oxo at C4, an hydroxyl at C3, C7 and C4', and 2 hydroxyl on phenyl B [116, 117, 114, 118–120].

Topoisomerase II can be inhibited by quercetin, quercetagetin, myricetin, baicalein, kaempferol, luteolin, fisetin, genistein, catechin, and EGCG [120, 121]. In addition to the double bond at C2–C3 and the C4 oxo, the presence of an hydroxyl group at the 5, 7, 3′ and/or 4′ positions is needed for topoisomerase II activity.

23.6.5 Multidrug Resistance

Multidrug resistance is a major problem encountered in cancer chemotherapy due to the overexpression of a membrane transport system of the ABC (P-glycoprotein, Pgp) type that can pump the anticancer agent out of the cell using ATP as energy source [122]. Because this resistance type concerns several classes of anticancer

drugs (e.g., anthracyclines, vinca alkaloids, taxanes, epipodophyllotoxins), the development of compounds that can inhibit this Pgp is important. Some flavonoids have been shown to modulate the Pgp, for example, quercetin, kaempferol, apigenin, myricetin, kaempferide, and naringenin [123, 124]. For this activity, a C5 hydroxy, a C4 oxo, and methoxy groups have been shown to be prerequisites.

A molecular mechanism of action for the reversal of multidrug resistance has been recently put forward involving the direct interaction of the flavonoid (EGCG) on the ATP binding site of a chaperone protein (GRP78) [125].

23.6.6 Cell Signaling

As alluded to above, most of the flavonoid therapeutic effects have been attributed to their antioxidant properties. However, the exact mechanisms involved in the biological actions of flavonoids are only partly understood, and the classical view of the antioxidant action of flavonoids to explain their pharmacological actions is challenged by several authors [126].

Indeed, several observations indicate that flavonoids could exert their action through other mechanisms independent of their antioxidant effect. For example, contrary to in vitro experimental systems where the aglycone is almost exclusively studied, flavonoids are extensively metabolized in vivo, and their redox potential is therefore modified. It now appears plausible that flavonoid bioactive forms may not be the initial compounds found in plants (e.g., aglycones and their glycosides), but instead their metabolites formed after intestinal absorption and hepatic metabolism [19].

For example, several flavonoids first undergo a deglycosylation in the intestine and then a phase II hepatic metabolism to glucuronide, sulfate, and *O*-methylated metabolites. In addition, modifications by the intestinal flora are known to modify further the flavonoids to phenolic acids which can also be reabsorbed and be further metabolized in the liver. All these metabolic transformations lead to a drastic decrease of their classical antioxidant potential [126]. Moreover, the concentrations of flavonoids and their

metabolites found in vivo in plasma and tissues are relatively low (in the nanomolar range) compared to other natural antioxidant molecules such as ascorbic acid and alpha-tocopherol which are found at micromolar concentrations.

The above observations on the metabolism of flavonoids have led several authors to consider that flavonoids could exert their cellular effects via their interaction with key proteins involved in the intracellular signal transduction cascade instead than by their antioxidant properties [127]. Flavonoids were shown to act on the MAP kinase (*mitogen-activated protein kinase*) signaling pathway [128], and other [129] signaling pathways such as the phosphoinositide 3-kinase (PI 3-kinase), the Akt/protein kinase B (Akt/PKB), the tyrosine kinases, and the protein kinase C (PKC) (reviewed in [126]). The inhibition or stimulation of these pathways can profoundly affect cellular functions by altering the phosphorylation status of key target molecules or modifying the expression of certain genes.

It now appears that flavonoids are biomolecules which are acting through modulation of cell signaling instead of being merely antioxidant molecules and that a better understanding of these mechanisms is needed in order (it is hoped) to improve their therapeutic effects in cancer.

23.6.7 Effect on Hormone-Dependent Cancers

As mentioned above, epidemiological studies have shown that soy isoflavones present in the diet of several Asian countries are probably playing an important role in the lower incidence of breast and prostate cancers. Genistein, the major isoflavone found in soy-based foods has been found to inhibit carcinogenesis in animal models through its antagonist action of estrogen- and androgen-mediated signaling pathways (reviewed in [130]). Other flavonoids have also been identified as chemopreventive compounds in prostate cancer including the dietary agents such as green tea, pomegranate, lupeol, fisetin, and delphinidin [131]. Fisetin has also been shown to inhibit androgen receptor signaling and human prostate tumor growth in athymic nude mice [132].

23.6.8 Antiangiogenic Properties

Since the seminal article by Folkman in 1971 [133] which contributed to identify tumor angiogenesis as a key and essential player in metastasis and tumor growth, angiogenesis has become the target of several approaches aimed at preventing the formation of new vessels in tumors or attempting to destroy existing tumor vasculature.

Flavonoids have been shown to inhibit angiogenesis in vitro at micromolar concentrations, for example, 3-hydroxyflavone, 3',4'-dihydroxyflavone, 2',3'-dihydroxyflavone, fisetin, apigenin, and luteolin [134]. SAR studies have shown that a C4 oxo and a C2–C3 double bond are needed for antiangiogenic activity. Genistein was also shown to possess antiangiogenic properties [130, 135].

The mechanism of the antiangiogenic action by flavonoids involves the inhibition of the expression of VEGF (vascular endothelial growth factor) and HIF-1 (hypoxia-inducible factor-1) [136]. In addition, it has recently been shown that EGCG could decrease the VEGF mRNA and significantly reduce the growth of gastric tumors [137]. Synthetic flavonoids have also been shown to inhibit aminopeptidase N and to inhibit angiogenesis [138, 139]. The inhibition of NO synthase has also been shown to be involved in the inhibition of angiogenesis by quercetin in vitro and in vivo [140].

Endothelial cells were shown to be particularly responsive to flavonoid action. For example, fisetin, quercetin, kaempferol, apigenin, and morin were recently shown to induce the formation of cell extensions and filopodias at noncytotoxic concentrations and that this morphological alteration was linked to a cytoskeletal stabilization [141]. These flavonoid morphological modifications may also be linked to the inhibition of tubulin polymerization [100] and also with interaction with actin polymerization [142]. Fisetin has also been recently found to inhibit angiogenesis in vitro and also in a murine lung tumor in vivo [143].

23.6.9 Vascular Disrupting Properties

Vascular disrupting agents are low molecular weight compounds that selectively destroy tumor vasculature while they leave normal

vasculature intact. This vascular disruption causes a shutdown in blood flow to solid tumors resulting in extensive tumor cell necrosis [144]. This flavonoid action is particularly important considering that most tumors are unfortunately detected when they already have developed an important vascular system. Some synthetic flavonoids have shown vascular disrupting activity, for example, flavone-8-acetic acid and its analogue DMXAA (5,6-dimethylxanthenone-4-acetic acid) which is now undergoing clinical testing ([145] and references therein). These vascular disrupting flavonoids appear to act through local cytokine production, but their exact mechanism of action is still debated [144].

23.6.10 Flavonoids Combination with Cancer Treatments

Several groups have reported the beneficial effects of combining flavonoids with anticancer drug treatments. Genistein was shown to reverse radio- and chemo-resistance in cancer chemotherapy [130], and also to increase the effect on hormone-independent human prostate cancer cells [146]. Genistein was also found to be synergistic with 5-aza-deoxycytidine, a potent DNA methylation inhibitor, in leukemia cell lines [147]. Genistein can also act synergistically with several other drugs such as tamoxifen, cisplatin, 1,3-bis 2-chloroethyl-1-nitrosourea (BCNU), dexamethasone, daunorubicin, and tiazofurin [148].

A synergistic effect of silibinin on growth inhibition, reversal of chemo-resistance, apoptosis induction, and a strong increase in G2-M checkpoint arrest was observed when given in combination with several chemotherapeutic drugs [149]. Silibinin was also reported to restore sensitivity to paclitaxel-resistant human ovarian carcinoma cells [150]. It was also recently observed that fisetin combined with cyclophosphamide can lead to a synergistic anticancer activity in Lewis lung carcinoma-bearing mice [143].

Although most data indicate that flavonoids can advantageously be combined with chemotherapeutic agents in order to increase efficacy and also with the aim to decrease toxicities, care should nonetheless be recommended. For example, an antagonistic effect was recently reported with the combination of the proteasome inhibitor

bortezomid with green tea polyphenols, where it was noted that the anticancer agent's effect was negated by the flavonoids [151].

23.7 Flavonoid Toxicity

Flavonoids are considered as safe compounds, because unwanted toxic effects in humans are not frequently encountered. Some cases of hemolytic anemia have been reported with catechin and its metabolites which can bind to erythrocytes and cause an immune reaction which disappears upon treatment discontinuation [152]. Some flavonoids can generate quinones that may be involved in contact sensibilization. However, flavonoids can be considered as weak allergens, because humans are frequently in contact with this type of compounds in their alimentation [153].

Flavonoids can be administered in humans at relatively high doses because of their low toxicity. For example, a phase I study has been conducted with quercetin administered as a rapid intravenous injection every 3 weeks in cancer patients, and the maximal tolerated dose was found to be as high as 1700 mg/m^2 where nephrotoxicity was observed, but without myelosuppression, with a phase 2 recommended dose of 1400 mg/m^2 [154]. Of interest, this study has shown an anticancer effect in a case of hepatocarcinoma and in an ovarian cancer case. Flavonoids can therefore be considered as relatively nontoxic compounds in man.

23.8 Concluding Remarks and Future Directions

Flavonoids can be regarded as compounds possessing clearcut pharmacological activities in a variety of diseases, and also in cancer prevention and treatment, as was demonstrated in various in vitro and in vivo preclinical systems. However, few flavonoids have emerged thus far in the clinical setting in relation to their potential use in cancer prevention and/or treatment. This is probably due to the fact that most clinical studies have tried to mimic the high-dose regimens usually employed in cytotoxic therapies, and that

flavonoids would perhaps need to be administered at metronomic dosages, that is, at low doses over a long period of time.

Because of the poor oral bioavailability of flavonoids and other polyphenols in their aglycone forms, much work needs to be accomplished to overcome this serious problem before the use of these agents could be recommended as nutraceuticals in humans. The design of prodrugs that could be better absorbed is currently under way [155]. The permethylation of polyphenols could also be helpful to increase their metabolic stability [156]. Pharmaceutical formulations of flavonoids as nanoemulsions or liposomes could also be helpful to improve their bioavailability and potentially increase their efficacy in vivo.

In the meantime, it is advisable to suggest that the regular consumption of fruits and vegetables is beneficial to prevent cancer as was shown by several epidemiological studies in humans. Some authors have suggested that a mixed flavonoid diet may be better than the ingestion of specific flavonoids [157]. However, concerning the combination of flavonoids with existing cancer chemotherapeutic regimens, extreme care should be the rule for the present time before more preclinical data are available, because antagonism between anticancer molecules and flavonoids is always possible, especially with new chemotherapeutic regimens [151].

Although several plants and spices containing flavonoids have been used for thousands of years in traditional Eastern medicine, and also in spite of the important preclinical data demonstrating the efficacy of this class of compounds in prevention and therapy of cancer, this class of nontoxic agents has yet to gain its place in Western medicine [24]. Based on the growing interest of the scientific community in the study of natural products use in medicine, it seems likely that the next decade will see the emergence of new improved flavonoids, because this class of compounds offers an almost unlimited resource for new cancer drug discovery.

Acknowledgments The authors would like to thank the following organizations for their support: the Institut national de la santé et de la recherche médicale (INSERM), the Centre national de la recherche scientifique (CNRS), the Institut Fédératif de Recherche (IFR-71) of the Université Paris Descartes, and the Institut National du Cancer (National Cancer Institute of France, 92513

Boulogne-Billancourt (Cedex), France). We apologize to the many authors whose work could not be cited due to space constraints.

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