Pharmacology & Therapeutics 126 (2010) 9–20

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes

Vasilis P. Androutsopoulos [a,c,*], Athanasios Papakyriakou [b], Dionisios Vourloumis [b], Aristidis M. Tsatsakis [a], Demetrios A. Spandidos [c]

[a] Laboratory of Forensic Sciences and Toxicology, University of Crete, Heraklion, Voutes, 71003, Greece
[b] Laboratory of Chemical Biology of Natural Products and Designed Molecules, Institute of Physical Chemistry, NCSR "Demokritos" 15310 Agia Paraskevi Attikis, Athens, Greece
[c] Laboratory of Clinical Virology, University of Crete, Heraklion, Voutes, 71409, Greece

ARTICLE INFO

Keywords:
Flavonoids
Chemoprevention
CYP1 enzymes
Inhibition
Metabolism

ABSTRACT

Flavonoids are polyphenolic compounds that have attracted the attention of the scientific community as the hallmark molecules responsible for cancer prevention by a plethora of different mechanisms. One of their most important characteristics, responsible for their cancer preventive properties, is their interaction with cytochrome P450 CYP1 enzymes. Flavonoids have traditionally been described as CYP1 inhibitors due to the inhibition of carcinogenic product formation and consequent blockage of the initiation stage of carcinogenesis. However, mounting evidence indicate that flavonoids are also capable of acting as CYP1 substrates, undergoing bioactivation to more antiproliferative agents within cancer cells. In this review, a comprehensive summary of the two models is presented. Structural features responsible for CYP1 inhibition or substrate turnover are discussed and limitations as well as discrepancies between procarcinogen-activating and 7-ethoxyresorufin-inhibition assay systems are further explored in vitro and in vivo. Moreover, a thorough investigation of the substrate specificity of flavonoids for the active site of CYP1 enzymes is undertaken. Finally, issues concerning the bioavailability and metabolic fate of these compounds in vivo are addressed. Ultimately, the mode of flavonoid action, in terms of CYP1 inhibition or CYP1-mediated bioactivation, is dependent on the lipophilicity or hydrophilicity of each compound. The degree of hydroxylation or methoxylation of the A and B rings is the major factor which determines the accessibility to the tumor site, in terms of hepatic and intestinal metabolism, and the introduction of the molecules to the CYP1 active site, respectively.

Contents

1. Introduction . . . 10
2. Flavonoids as CYP1 inhibitors . . . 10
3. Flavonoids as CYP1 substrates . . . 12
4. Ligand-binding interactions of flavonoids with CYP1 enzymes . . . 13
5. Bioavailablity of dietary flavonoids-CYP1 activation in cancer therapy and prevention . . . 15
6. Future considerations—Potential of methoxylated flavones in chemoprevention . . . 17
7. Conclusion . . . 17
References . . . 18

Abbreviations: CYP1, cytochrome P450 1 family enzymes; B[*a*]P, benzo[*a*]pyrene; EROD, 7-ethoxyresorufin-*O*-deethylase; MROD, 7-methoxyresorufin-*O*-demethylase; SGLT, sodium dependent glucose transporter; LPH, lactate phlorizin hydrolase; SULT, sulfotransferase enzyme; UGT, Uridine-5′-diphospho-glucoronosyl transferase enzyme; diosmin, diosmetin 7-*O*-rhamnoside.

* Corresponding author. Laboratory of Forensic Sciences and Toxicology, University of Crete, Heraklion, Voutes, 71003, Greece. Tel.: +30 2810 394870; fax: +30 2810 542098.
E-mail address: vasilis_androutsopoulos@yahoo.com (V.P. Androutsopoulos).

0163-7258/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2010.01.009

1. Introduction

Flavonoids are naturally occurring polyphenolic compounds that constitute the most abundant class of dietary natural products and are present in fruits, vegetables, beverages and various dietary supplements, including herbal remedies such as Gingo Biloba and Milk Thistle, originating from medicinal plants. Extensive investigations of their bioactivity in the past 30 years have demonstrated their potential to prevent various diseases, such as cardiovascular disease, inflammatory disorders, viral infections, diabetes and neurological conditions (Scalbert & Williamson, 2000; Vaouzour et al., 2008; Rathee et al., 2009; Andres et al., 2009). Consequently, flavonoids are considered to be the key natural products that provide the most essential link between diet and the prevention of chronic disorders. Among the wide range of biochemical and pharmacological properties, one of their most investigated activities is their contribution to cancer prevention. Flavonoids can inhibit tumor formation and proliferation of cancer cells through various biological mechanisms of action. Particularly their effects on procarcinogen-activating enzymes, notably the cytochrome P450 CYP1 family, have been the focus of attention in cancer prevention during the last decade.

CYP1A1 and CYP1B1 enzymes have been shown to be overexpressed in tumors and metabolize procarcinogens to epoxide intermediates, which are further activated to diol epoxides by the enzyme epoxide hydrolase (Murray et al., 1995; Murray et al., 1997; Shimada & Fujii-Kuriyama, 2004). The most common chemical, extensively studied for its carcinogenicity, is Benzo[*a*]pyrene or B[*a*]P. Formation of B[*a*]P-7,8-diol-9,10-epoxides, referred to as bay region epoxides, causes accelerated DNA mutations due to the high reactivity of these chemicals. Any compound that interferes with this process, by blocking the formation of reactive intermediates, can potentially prevent the initiation of carcinogenesis. The ability of flavonoids to inhibit CYP1-enzymatic activity, and as a result CYP1-mediated formation of carcinogenic products, was established by various research groups (Ciolino et al., 1998; Ciolino & Yeh, 1999; Ciolino et al., 1999; Wen & Walle, 2005). In addition, it is becoming increasingly accepted that flavonoids may themselves be substrates for CYP1 enzymes and can cause inhibition of tumor cell growth by the formation of more pharmacologically active conversion products (Arroo et al., 2008, 2009; Androutsopoulos et al., 2009c).

The present review focuses on the flavonoid inhibitor and substrate interactions of the cytochrome P450 CYP1 family enzymes. Such interactions control entry of the latter compounds in the CYP1 active site and consequently regulate the mechanism by which the flavonoid–CYP1 chemopreventive effect is exerted. The structural features of the classes of flavonoids investigated in the present review are outlined in Fig. 1. Notably flavones such as apigenin, luteolin, diosmetin and tangeretin, flavonols such as myricetin, quercetin and kaempferol and isoflavones such as genistein and daidzein, are compounds abundant in dietary nutrients that are consumed daily worldwide. Thus, these compounds have been predominantly investigated, in terms of their abilities to interact with the cytochrome P450 CYP1 family of enzymes.

2. Flavonoids as CYP1 inhibitors

The potential of dietary flavonoids to inhibit CYP1 enzymatic activity has been demonstrated by numerous studies. The majority of the information in the literature comes from in vitro experimental data. Inhibition of *O*-deethylation of 7-ethoxyresorufin has been employed as a model assay for the determination of CYP1 inhibitory capacity of certain dietary flavonoids. Studies in the mid and late 1990s have shown the ability of these compounds to inhibit EROD and MROD activities in human and rat liver microsomes (Siess et al., 1995; Zhai et al., 1998a,b). These studies were focused on the inhibition of CYP1A2 by flavonoids, since only negligible expression of the extrahepatic

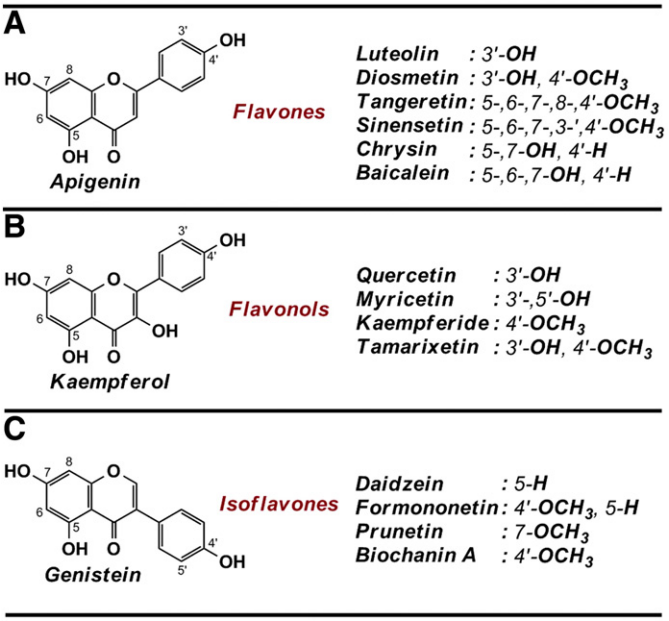

Fig. 1. Chemical structures of the three most important classes of flavonoids. **A**. Flavones. **B**. Flavonols. **C**. Isoflavones. Each flavonoid molecule is composed of three rings assigned **A**, **B** and **C** and varying number of hydroxyl or methoxy substitutions, which results in approximately eight thousand different compounds, based on the same core structure. Dietary products containing flavonoids are citrus fruits (tangeretin, sinensetin, and nobiletin), soya products (genistein and daidzein) and various other vegetables and plants (luteolin, apigenin in green peppers and parsley).

enzymes CYP1A1 and CYP1B1 is observed in liver microsomes. The structural features of the flavonoid molecule, which were initially found to be responsible for this inhibition, were the C2–C3 double bond and the number of hydroxyl substitutions in the A ring. Di- and tri-hydroxylated flavonoids, such as chrysin and galangin were highlighted as potent inhibitors of CYP1A2 (Zhai et al., 1998a). Subsequent studies in recombinant human CYP1A1 and CYP1A2 enzymes have corroborated the initial findings on flavonoid CYP1A inhibitory activity, since the flavone apigenin, which can be regarded as the 4′-hydroxylated analogue of chrysin and the flavone acacetin or 4′-methoxy chrysin, were shown to inhibit strongly CYP1A1 and CYP1A2 enzymatic activities (Pastrakuljic et al., 1997; Doostdar et al., 2000). Zhai et al. (1998a) further reported that galangin (3-hydroxy chrysin) was the most potent inhibitor of CYP1A2, whereas 7-hydroxyflavone exhibited the greatest selectivity for CYP1A1 in a small series of hydroxylated flavonoids. Flavones containing the C2–C3 double bond were more potent CYP1 inhibitors as opposed to flavonones lacking the specific functionality (Doostdar et al., 2000). Moreover, methoxy substitutions at the 4′-positions of the B ring of flavones or flavonones, in place of vacant or hydroxylated positions, enhanced further the inhibitory activity towards CYP1 enzymes (Doostdar et al., 2000).

Of note is that, compared to the CYP1A isozymes, CYP1B1 was more profoundly affected by acacetin and the structurally similar flavonoids diosmetin, naringenin, hesperitin, eriodictyol and homoeriodictyol, as shown in a study undertaken by Doostdar et al. (2000). In agreement with these results, Gingo Biloba extract containing flavonoids, was demonstrated to inhibit CYP1B1-catalyzed EROD activity and Benzo[*a*]pyrene (B[*a*]P) hydroxylation to a greater extent, compared to CYP1A1 and CYP1A2 (Chang et al., 2006). The flavonols kaempferol, quercetin and isorhamnetin were highlighted as potent CYP1B1 inhibitors, representing the 3-hydroxylated equivalents of the flavones apigenin, luteolin and diosmetin, respectively. Luteolin was also reported to be a potent inhibitor of CYP1A1 by Kim et al. (2005), whereas chrysin exhibited the most pronounced effects of CYP1A2-dependent EROD inhibition. Thus, it appears that the strongest