COMPARATIVE INHIBITION OF HUMAN CYTOCHROMES P450 1A1 AND 1A2 BY FLAVONOIDS

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ABSTRACT:

Flavonoids are a class of dietary phytochemicals that modulate various biological activities. The effects of flavone and five hydroxylated derivatives on the methoxyresorufin O-demethylase activity catalyzed by cDNA-expressed human cytochromes P450 (CYP1A1 and 1A2) were examined. Flavone was a less potent inhibitor of CYP1A1 (IC50 = 0.14 μM) than CYP1A2 (IC50 = 0.066 μM). Four hydroxylated flavone derivatives (3-hydroxy-, 5-hydroxy-, 7-hydroxy-, and 3,7-dihydroxyflavone) were also potent inhibitors of CYP1A1 (IC50 < 0.1 μM) and CYP1A2 (IC50 < 0.3 μM). For CYP1A1, 7-hydroxyflavone exhibited a competitive mode of inhibition, with a Kᵢ value of 0.015 μM and 6-fold selectivity for CYP1A1 over CYP1A2. 3,5,7-Trihydroxyflavone (galangin) showed the highest potency toward CYP1A2. The inhibition by galangin of the methoxyresorufin O-demethylase activity of CYP1A2 was mixed-type, with a Kᵢ value of 0.008 μM. Galangin showed 5-fold selectivity in its inhibition of CYP1A2 over CYP1A1. The results indicate that some flavonoids have high potencies and selectivities for inhibition of CYP1A1 isozymes. This may have important implications for cancer prevention, as well as other pharmacological and toxicological effects of these compounds.

Epidemiological studies have shown that frequent consumption of fruits and vegetables is associated with low risks of various cancers (Block et al., 1992; Wattenberg, 1992). This protective effect has been attributed in part to flavonoids, which are ubiquitously present in plant-derived foods and are important constituents of the human diet (Hertog et al., 1994). However, because that study showed that some flavonoids had different effects on mouse and human CYP1A2, one cannot extrapolate the findings for the mouse P450s to human P450s. Earlier studies showed that flavonoids inhibit CYP1A-mediated 7-ethoxyresorufin O-deethylase activity in rat and human liver microsomes (Siess et al., 1989, 1990, 1995). More recent work using cDNA-expressed P450s explored the effects of flavonoids on activities catalyzed by mouse CYP1A1 and CYP1A2 and human CYP1A2 (Tsyrlov et al., 1994). However, because that study showed that some flavonoids had different effects on mouse and human CYP1A2, one cannot extrapolate the findings for the mouse P450s to human P450s. Little is known regarding the relative effects of flavonoids on human CYP1A1 and CYP1A2. A recent study found small differences (<40%) in the sensitivity of these P450s to α-naphthoflavone and apigenin (Pastrakuljic et al., 1997). Besides the findings for these two flavonoids, a more extensive comparative evaluation of flavonoid effects on the activities of these P450s has not been performed. Accordingly, the objective of this study was to investigate the effects of a series of hydroxyl-substituted flavonoids on the activities of human CYP1A1 and CYP1A2 and to elucidate the structural features governing flavonoid interactions with these P450s.

Materials and Methods

Materials. Flavone (fig. 1), 3-hydroxyflavone, 5-hydroxyflavone, 7-hydroxyflavone, 3,7-dihydroxyflavone, and 3,5,7-trihydroxyflavone (galangin)
were obtained from Indofine Chemical Co. (Somerville, NJ). Resorufin, methoxyresorufin, ethoxyresorufin, NADP, G-6-P, and G-6-PD were obtained from Sigma Chemical Co. (St. Louis, MO).

P450s. Microsomes containing human CYP1A1 and CYP1A2 expressed in a human B lymphoblastoid cell line were obtained from Gentest (Woburn, MA).

MROD Activity Assay. MROD was assayed as previously described (Burke et al., 1985). The reaction mixtures contained 0.2 mg/ml expressed microsomal protein, 1 mM NADP, 10 mM G-6-P, 5 units/ml G-6-PD, 5 mM magnesium chloride, and 100 mM potassium phosphate buffer (pH 7.4), in a total volume of 2 ml. The mixtures were incubated at 37°C for 3 min, and the reaction was initiated by addition of the NADPH-generating system (G-6-P, G-6-PD, and NADP). Formation of the resorufin product was continuously measured for 2 min by monitoring its fluorescence with a Perkin-Elmer model LS-5 spectrofluorometer (Perkin-Elmer, Oak Brook, IL), with excitation and emission wavelengths of 540 and 590 nm, respectively. Enzyme activities were quantified by comparison with a resorufin standard. In preliminary experiments, we established the apparent $K_M$ values for $O$-demethylation of methoxyresorufin. These values were 0.77 and 0.39 mM for expressed CYP1A2 and expressed CYP1A1, respectively. Flavonoid inhibition studies were performed using concentrations approximately 2 times the $K_M$ values. Therefore, 0.75 mM substrate was used with CYP1A1 and 1.5 mM substrate with CYP1A2. Flavonoids were dissolved in dimethylsulfoxide. The final concentration of dimethylsulfoxide was 0.5%.

Data Analysis. The $IC_{50}$ values for the activity-concentration curves from individual experiments were calculated with GraFit software (Erithacus Software, London, UK), using a nonlinear regression equation. The mode of flavonoid inhibition of MROD activity was determined from the Lineweaver-Burk plots, and $K_i$ values were derived from replots of slope vs. inhibitor concentration. All data shown are the results from at least three separate experiments.

Results

Specific hydroxyl substitutions on the flavone nucleus were evaluated for their effects on the MROD activity of expressed CYP1A1 and CYP1A2. The derivatives were hydroxylated at the 3-, 5-, and/or 7-positions (fig. 1). They differed in their inhibitory potency and isozyme selectivity (fig. 2). For CYP1A1, all substitutions resulted in more potent inhibition, compared with flavone (fig. 2A). In contrast, the 7-hydroxy- and 3,7-dihydroxyflavones were weaker CYP1A2 inhibitors than was flavone, whereas the remaining derivatives were more potent inhibitors (fig. 2B).

In addition to comparing the effects of substitution on a single P450s, it is important to consider the differential sensitivities of these P450s to a given flavonoid. Thus, the $IC_{50}$ values for CYP1A1 and CYP1A2 were plotted inversely for the flavonoids, to facilitate comparison of their relative potencies (fig. 3). The most potent CYP1A2 inhibitor of all flavonoids examined was galangin (3,5,7-trihydroxyflavone). In addition, this compound exhibited the greatest selectivity, because it exhibited 5-fold greater inhibition of CYP1A2 than of CYP1A1. 7-Hydroxyflavone exhibited the greatest selectivity for CYP1A1, because the inhibition of CYP1A1 was 6-fold greater than that of CYP1A2.

To further explore the inhibition mechanism for the two most selective flavonoids, galangin and 7-hydroxyflavone, kinetic analyses of the MROD activities of cDNA-expressed CYP1A2 and CYP1A1 were performed. With galangin, mixed inhibition was observed for CYP1A2, with an increase in the apparent $K_M$ and an apparent $K_i$ of 0.008 mM (fig. 4). With 7-hydroxyflavone,
competitive inhibition was observed for CYP1A1, with a $K_i$ value of 0.015 μM (fig. 5).

**Discussion**

Human CYP1A2 is expressed principally in the liver, where it metabolizes many important drugs as well as carcinogens (Guengerich, 1988; Gonzalez, 1989; Rendic and Di Carlo, 1997). In contrast, CYP1A1 primarily metabolizes the latter and is poorly expressed in human liver, although its synthesis can be markedly induced in many extrhepatic tissues, notably the lungs (Guengerich, 1988; Gonzalez, 1989; Rendic and Di Carlo, 1997). The two P450s have overlapping substrate specificities, which is probably a consequence of their strong sequence similarity.

CYP1A1 is induced by polycyclic aromatic hydrocarbons, a class of ubiquitous environmental chemicals, and activates them to carcinogenic metabolites (Guengerich, 1988; Gonzalez, 1989; McLemore et al., 1990). This process is believed to contribute to pulmonary carcinogenesis, because increased lung CYP1A1 expression and activity are associated with a high risk of lung cancer (Guengerich, 1988; McLemore et al., 1990). High CYP1A1 activity is also associated with other cancers, such as colorectal cancer (Sivaraman et al., 1994). CYP1A2 also converts some procarcinogens (polycyclic aromatic hydrocarbons, nitrosamines, and arylacetamides) to carcinogens (Guengerich, 1990) and plays a role in human tobacco-related cancers (Smith et al., 1996). Therefore, factors that inhibit these P450s may have an important impact on cancer prevention.

Because of their potential effects on drug disposition and inhibition of toxicological processes, flavonoids are of much current interest, from the perspectives of both nutrition and pharmacotherapy. Their anticarcinogenic properties have been demonstrated in rodents (Mukhtar et al., 1988; Verma et al., 1988; Wei et al., 1990; Dechner et al., 1991). Galangin and several other flavonoids showed anticlastogenic effects against benzo(a)pyrene, a procarcinogen and substrate for CYP1A isozymes, and other mutagen-induced micronuclei in erythrocytes and reticulocytes of mice (Heo et al., 1992, 1996). Flavone inhibits 7-ethoxyresoruﬁn O-deethylase activity in rat and human liver, with IC$_{50}$ values for human liver microsomes that are 100 times lower than those for rat liver microsomes (Siess et al., 1995). Flavone inhibits mouse CYP1A1 and CYP1A2, with IC$_{50}$ values of 9.2 and 0.3 μM, respectively (Tsyrlov et al., 1994). In vivo studies demonstrated that grapefruit juice consumption increased the plasma half-lives of drugs such as caffeine; this effect was attributed to inhibition of CYP1A2 by naringin, a flavonoid (Fuhr et al., 1993).

To elucidate the structural features of flavonoids that are responsible for modulating P450 activities, we examined flavone and several derivatives that differ only in the number and position of hydroxyl substituents. In contrast to 7-hydroxyflavone, the IC$_{50}$ value of 7,8-ethoxyresoruﬁn O-deethylase activity in rat and human liver microsomes (Siess et al., 1995). Flavone inhibits mouse CYP1A1 and CYP1A2, with 2-fold greater potency toward the latter. Other flavonoids also show potent inhibitory effects on both CYP1A isozymes. The data show that 3- and 5-hydroxylation signiﬁcantly increases, whereas 7-hydroxylation markedly decreases, inhibition of CYP1A2 activity. In contrast to 7-hydroxyﬂavone, the IC$_{50}$ value of 7,8-benzoflavone for this activity was one half that of flavone (Tsyrlov et al., 1994). This indicates that the large hydrophobic substituent at position 7 elicits higher aﬃnity for CYP1A2 than does the hydrophilic hydroxyl substituent, whereas the hydroxyl substitutions at positions 3 and 5 increase binding aﬃnity. A molecular model of human CYP1A2 supports this interpretation of the results (Dai et al., 1998).

In the present study, 3,5,7-trihydroxylation inhibited CYP1A2 activity to a greater extent than did hydroxylation at position 3 or 5 alone. Thus, galangin was the most potent CYP1A2 inhibitor of all the tested compounds. Furthermore, galangin displayed almost 5-fold selectivity for CYP1A2 over CYP1A1. The mixed-type inhibition of CYP1A1 and CYP1A2 by galangin indicates that this compound can compete for substrate binding at the active site and also may bind to a region that does not participate directly in substrate binding. In contrast, 7-hydroxyflavone was a potent inhibitor of CYP1A1 and...
exhibited 6-fold greater selectivity for CYP1A1 over CYP1A2. Thus, among the series of flavonoids that were tested, galangin and 7-hydroxyflavone were chosen for kinetic characterization on the basis of their potencies and P450 selectivities. Although the other flavonoids exhibited varying degrees of inhibition, their mechanisms were not investigated further, because of their limited potencies or selectivities.

Regarding flavonoid inhibition of CYP1A1- and CYP1A2-mediated ethoxyresorufin deethylation activities, it has been shown that the competitive $K_i$ values of apigenin (5,7,4′-trihydroxyflavone) are 320 nM for CYP1A1 and 360 nM for CYP1A2 (Pastrukulic et al., 1997). It is interesting that additional 5- and 4′-hydroxylations dramatically reduce the selectivity between these two P450s. This suggests that the binding environment of the CYP1A1 active site has a preference for the 7-hydroxy substituent, because the corresponding inhibition of CYP1A1 activity is competitive.

Studies show that humans ingest approximately 0.6–1 g of flavonoids daily (Kuhnau, 1976). Flavones (hydroxylated or methoxylated flavones) may be present in considerable amounts in leafy vegetables (Pierpoint, 1986), whereas galangin is found in honey (Sabetier et al., 1992). Variable dietary exposure to flavonoids with different structures may contribute to some of the interindividual variation in the pharmacokinetics and pharmacological responses that is observed for drugs such as phenacetin, caffeine, and theophylline, which are substrates for CYP1A2 (Rendic and Di Carlo, 1997), as well as that observed for drugs that are substrates for other P450 isoforms. However, to more precisely evaluate the effects of dietary flavonoids, quantitative estimates of the contents of flavonoids in various foods are necessary. Nonetheless, our results support the hypothesis that flavonoids may be involved in the prevention of malignant transformation, by reducing the formation of carcinogens through inhibition of enzymes such as CYP1A1 and CYP1A2, both of which are known to be involved in carcinogen activation.

**References**


