XENOBIOTICA, 1995, VOL. 25, NO. 6, 575–584

Effects of bioflavonoids on hepatic P450 activities

M. T. OBERMEIER†*, R. E. WHITE† and C. S. YANG‡

† Metabolism and Pharmacokinetics Department, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543, USA
‡ Laboratory for Cancer Research, Department of Chemical Biology and Pharmacognosy, College of Pharmacy, Rutgers University, PO Box 789, Piscataway, NJ 08855, USA

Received 28 January 1995

1. The effects of tangeretin, green tea flavonoids, and other flavonoids on 7-ethoxyresorufin-*O*-deethylase (EROD; 450 1A), 7-pentoxyresorufin-*O*-dealkylase (PROD; P450 2B), *p*-nitrophenol hydroxylase (PNPH, P450 2E1), and erythromycin-*N*-demethylase (ERDM; P450 3A) were examined in induced rat liver microsomes. EROD, PNPH, ERDM, and nifedipine oxidase (NIFO; P450 3A4) were examined in human liver microsomes.

2. All flavonoids tested inhibited EROD activity at higher concentrations in liver microsomes. Flavone and tangeretin were potent inhibitors of EROD, with IC_{50}'s of 0·7 and 0·8 μM respectively in rat liver microsomes and 0·15 and 16 μM respectively in human liver microsomes. The green tea flavonoid (−)-epicatechin-3-gallate (ECG) was the most potent inhibitor of EROD in human liver microsomes ($IC_{50} = 75\ \mu$M). The effect of the green tea flavonoids on EROD was complex; in addition to inhibition at high concentrations of flavonoid, moderate activation was seen at lower concentrations.

3. 450 2B-, 2E1- and 3A-dependent activities in rat and human liver microsomes were only moderately inhibited by any of the flavonoids tested, and, in general, ECG was the most potent inhibitor for these activities with IC_{50}'s ranging from 75 to 300 μM.

4. Tangeretin inhibited EROD activity (P450 1A2) in human liver microsomes in a competitive manner with a $K_i = 68$ nM. Tangeretin inhibited NIFO activity (P450 3A4) in human liver microsomes in an uncompetitive manner with $K_i = 72\ \mu$M.

Introduction

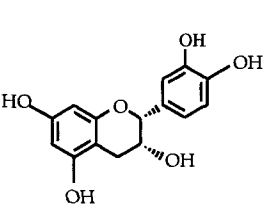
Some naturally occurring components of fruits and vegetable have been found to be potent inhibitors of P450 enzymes. One family of compounds, flavonoids, are found in fruits, vegetables, tea, seeds, bark, leaves, nuts, and flowers of many plants. Many early studies on the effects of flavonoids on benzo(*a*)pyrene metabolism (Kapitulnik *et al.* 1977, Buening *et al.* 1981, Huang *et al.* 1981, Lasker *et al.* 1984), zoxazolamine metabolism (Kapitulnik *et al.* 1977, Lasker *et al.* 1984), and related enzyme activities have been conducted (Sousa and Marletta 1985, Siess *et al.* 1989, 1990). Certain flavonoids have been shown to inhibit the mutagenesis of diol-epoxides of polycyclic aromatic hydrocarbons by inhibiting cutaneous monoxygenases and metabolism in the SENCAR mouse (Das *et al.* 1987).

Green tea contains several flavonoids, including catechins (or cianidanols) and their gallic acid derivatives (figure 1). These flavanols comprise about 16% of the dry weight of the green tea leaf (Vuataz *et al.* 1959). Green, tea, black tea, and related preparations have been shown to inhibit chemical and UV light-induced carcinogenesis in many animal models (Yang and Wang 1993).

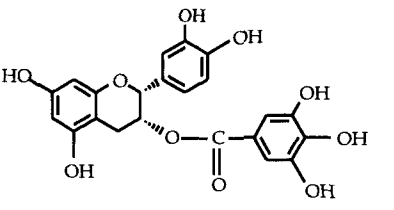
One of the mechanisms by which flavonoids might exert their anticancer and other effects is through interaction with the P450 system, either by inhibition or

* Author for correspondence.

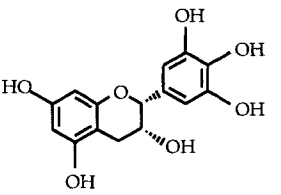
0049–8254/95 $10·00



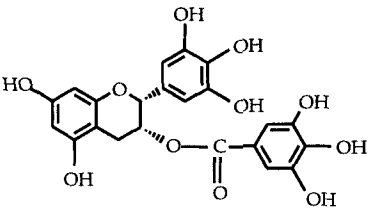
(-)Epicatechin (EC)



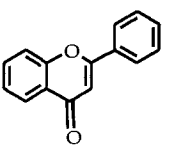
(-)Epicatechin-3-gallate (ECG)



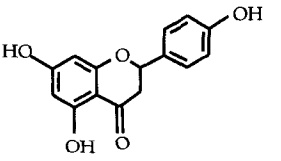
(-)Epigallocatechin (EGC)



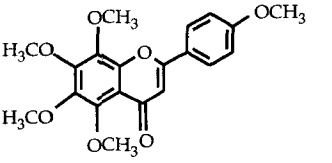
(-)Epigallocatechin-3-gallate (EGCG)



Flavone



Naringenin



Tangeretin

Figure 1. Chemical structures of green tea flavanols and other flavonoids used.

activation of certain forms of this enzyme, leading to reduced production of the ultimate carcinogen. In fact, certain P450 activities have been shown to be affected by various flavonoids (Buening *et al.* 1981, Lasker *et al.* 1984, Siess *et al.* 1989). In the present work we examined whether tangeretin, flavone, naringenin, and green tea flavanols differentially affected the activity of specific P450 enzymes.

Materials and methods

Chemicals

EORR†; PORR, and RR were obtained from Pierce Chemical Co. (Rockford, IL, USA). NADPH (tetrasodium salt, 95%), $NADP^+$ (sodium salt, 98%), glucose 6-phosphate dehydrogenase (Type IX, from Baker's yeast), glucose 6-phosphate, erythromycin, formaldehyde, *p*-nitrophenol, 4-nitrocatechol, acetone, β-naphthoflavone, dexamethosone, phenobarbital sodium, EC, flavone, and naringenin were obtained from the Sigma Chemical Co. (St Louis, MO, USA). Tangeretin was obtained from the Florida Department of Citrus (Lake Alfred, FL, USA). Green tea flavanols (EGCG, EGC, ECG and GTPE) were obtained from Dr Z. Y. Wang (Rutgers University), and were at least of 95% purity. Nifedipine and nitrendipine were obtained from Dr James Powell (Bristol-Myers Squibb Pharmaceutical Research Institute). The pyridine metabolite of nifedipine was synthesized by the method of Loev and Snader (1965). All other chemicals used were at least of reagent grade.

† Abbreviations used are: EORR, 7-ethoxyresorufin; PORR, 7-pentoxyresorufin; RR, resorufin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin-3-gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin-3-gallate; GTPE, green tea polyphenol extract; DMSO, dimethylsulphonoxide; ACN, acetonitrile; and TFA, trifluoroacetic acid.

Table 1. Enzymological characterization of liver microsomes.

Treatment	P450 enzymes induced	Protein (mg/ml)	P450 (nmol/mg protein)	Activity (mmol/min/mg protein) EROD (1A1/2)	PROD (2B1/2)	PNPH (2E1)	ERDM (3A)	NIFO (3A)
Untreated	—	13·5	0·52	0·13	0·03	0·74	1·8	—
β-Napthoflavone	1A1/2	19·2	0·76	3·8	0·13	—	—	—
vehicle (corn oil/DMSO)	—	16·5	0·50	0·18	—	—	—	—
Phenobarbital	2B1/2	14·9	1·80	—	2·4	—	—	—
vehicle (HBss)[1]	—	14·9	0·62	—	0·02	—	—	—
Acetone[b]	2E1	13·4	0·76	—	—	4·4	—	—
Dexamethasone	3A	15·3	1·13	—	—	—	9·8	—
vehicle (water/tween 80)	—	13·5	0·65	—	—	—	1·5	—
Control human pool 1[c]	—	20·0	0·38	0·028	—	1·3	0·45	—
Control human pool 2	—	20·0	0·79	—	—	—	—	4·3

[a] HBSS, Hanks; balanced salt solution.
[b] The control for the acetone-induced microsomes were microsomes from the untreated rats.
[c] EROD, PNPH, and ERDM assays were performed with human microsome pool 1, while NIFO was performed with pool 2.

Animals, treatments, and preparation of microsomes

Male Sprague-Dawley rats, weighing approximately 90–100 g, were obtained from Harlan Sprague-Dawley (Indianapolis, IN, USA). They were maintained in air-conditioned quarters with a 12-h light/dark cycle, in stainless steel cages, in groups of three to four and were allowed free access to food (Lab Rodent Diet #5001, PMI Feeds, St Louis, MO, USA) and water. Rats were treated with xenobiotics as follows: phenobarbital sodium (50 mg/kg in Hank's balanced salt solution, i.p. daily for 5 days), β-naphthoflavone (20 mg/kg in corn oil containing 5% (v/v) DMSO, i.p. daily for 5 days), dexamethasone (300 mg/kg in water containing 2% (v/v) Tween-80, p.o. daily for 4 days), or acetone (10 ml of 50% (v/v) aqueous solution/kg, p.o. by gavage, 20 h prior to killing). Animals were allowed free access to food and water throughout treatments. Rats were killed by cervical dislocation approximately 24 h after the last treatment. Livers from each group were pooled and hepatic microsomes were prepared by differential centrifugation according to a procedure by Ryan *et al.* (1978). Microsomes were stored at − 70°C until used.

Human liver microsome pools were made by combining equal volumes of separate lots of microsomes. Microsomes were obtained either from Human Biologics (Phoenix, AZ, USA) (pool 1, consisting of lots HB2, HB3, HB7, HB10, and HB11), or from Keystone Skin Bank (Exton, PA, USA) (pool 2, consisting of microsomes from donors 0525921, 0611921, 0913921, 0611922, and 0722921). Table 1 summarizes the enzymological characterization of the rat and human liver microsomes used.

Enzyme assays

Total microsomal P450 was determined from the carbon monoxide-reduced difference spectrum according to Omura and Sato (1964). Protein concentration was determined by the method of Lowry *et al.* (1951), EROD and PROD activities were measured by the method of Poul and Fouts (15) and PNPH activity was measured by the method of Reinke and Moyer (1985). ERDM was measured by the method of Wrighton *et al.* (1985) with the modification that semicarbazide was added to the incubations, as in the aminopyrine *N*-demethylase assay from Beyeler *et al.* (1983). NIFO was measured by the method of Guengerich *et al.* (1986) with the following modifications to the hplc method. The dried residues were dissolved in 50 μl methanol plus 100 μl water. The reconstitutes (100 μl) were injected on to a Perkin-Elmer hplc system consisting of a Model 410 pump, LC-235 diode array detector, ISS-100 autosampler, and 2600 data system (Perkin-Elmer Corp., Norwalk, CT, USA). A μBondapak C_{18} column (10 μm, 3·9 × 300 mm; Waters Chromatography, Milford, MA, USA) was eluted with a mobile phase consisting of a solvent A (H_2O:ACN:TFA, 95:5:0·1) and solvent B (H_2O:ACN:TFA, 20:80:0·1), programmed in a linear gradient from 90 to 0% A over 40 min at a flow rate of 1·3 ml/min. Samples were monitored at 255 nm. In this system, nifedipine was eluted at 31 min, the pyridine metabolite was eluted at 27 min, and nitrendipine (internal standard) was eluted at 35 min. The amount of the pyridine metabolite formed was calculated by comparing the ratio of area units (pyridine metabolite/nitrendipine) in the sample to that of a standard carried through the same extraction conditions.

Statistics

Mean ± SEM for the rates for the various activities were calculated. The Student's *t*-test was performed on the individual sets of data. Differences were considered significant when $p < 0.05$.

Calculation of IC_{50}

Up to five different concentrations of flavonoids were used for inhibition studies. The compounds were dissolved in DMSO, and added to the incubation mixture immediately after the microsomes were added. The final concentration of DMSO in the mixture did not exceed 0·5% (v/v) in any of the incubations. In the PNPH assay, no DMSO was used because of the sensitivity of P450 2E1 to inhibition by many organic solvents. The flavonoids were dissolved in acetone, added to the incubation tubes, acetone evaporated, and flavonoid was resuspended in the incubation medium. IC_{50}'s (i.e. inhibitor concentration that produces 50% inhibition) were calculated as below (Segel 1975):

$$i\ (\text{fractional inhibition}) = \frac{\text{inhibited rate}}{\text{control rate}} = \frac{[I]}{[I] + IC_{50}}. \quad (1)$$

This calculation of IC_{50} is the same for the three classic types of inhibition (Segel 1975). IC_{50}'s were calculated from data near 50% inhibition.

Inhibition kinetics

For the kinetics of inhibition of tangeretin towards EROD and NIFO, care was taken to insure that the true initial rate (nmol/min/mg protein) was measured at each substrate concentration. Also, substrate concentrations were selected to be in the vicinity of K_m on the basis of preliminary experiments.

Results

Effects of flavonoids on P450 activities in rat and human liver microsomes

EROD. All the flavonoids investigated were inhibitory toward EROD activity at higher concentrations (figure 2). However, in both species the green tea flavonoids were much less potent than flavone, naringenin, or tangeretin. In rat liver microsomes, the IC_{50}'s for the green tea flavonoids ranged from 75 to 400 μM, ECG being the most potent (table 2). The value for flavone, naringenin, and tangeretin were approximately 1 μM. In human liver microsomes, the IC_{50}'s for the green tea flavonoids ranged from 530 to 2600 μM (table 3), ECG again the most potent. The IC_{50}'s for flavone and tangeretin were 0·15 and 16 μM respectively. The difference in potencies of ECG for EROD in the two species may be due to the fact that EROD is primarily catalysed by P450 1A1 in rat liver (especially after treatment with β-NF or 3-MC), whereas it is catalysed by 1A2 in human liver.

PROD. In rat liver microsomes, all flavonoids except for EC were inhibitory toward PROD activity. No significant stimulation was seen. The IC_{50}'s for the green tea flavonoids ranged from 119 to 217 μM (table 2), with ECG being the most potent. Flavone was the most potent of all the flavonoids, with an $IC_{50} = 14\ \mu$M.

PNPH. With respect to PNPH activity. The flavonoids tested were inhibitory only at high concentrations. The IC_{50}'s for all the flavonoids ranged from 250 to 2333 μM, ECG being the most potent inhibitor in rat liver microsomes (table 2). In human liver microsomes, three out of six flavonoids tested had no effect on PNPH activity (table 3). ECG and naringenin were moderately inhibitory, with IC_{50}'s = 200 and 300 μM respectively (table 3). A statistically significant activation of PNPH was seen with flavone (81% at 1000 μM).

ERDM. For ERDM activity, the flavonoids tested were inhibitory at higher concentrations (figure 3). The IC_{50}'s for all the flavonoids ranged from 163 to 4000 μM in rat liver microsomes (table 2), ECG being the most potent. EC and EGC

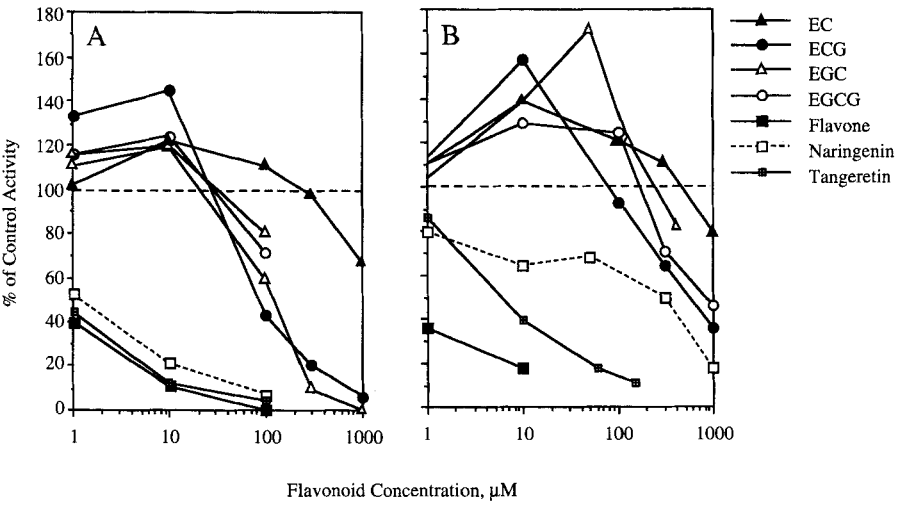


Figure 2. Effects of various flavonoids on EROD activity in rat and human liver microsomes. Control activity was 3·82 nmol/min/mg protein for rat (A) and 0·028 for man (B). A weighted average molecular weight for GTPE was calculated based on the molecular weights of the various flavanols and their % contribution to the green tea extract. Weighted quantities of green tea extract were corrected for the total per cent of flavonols (about 80%) (10). Each point represents a mean of 2–5 values. Error bars were left out for clarity, but mean SEs were 5·7 and 8·1% of the mean activities for the human data respectively.

Table 2. IC_{50}'s (μM) for inhibition of specific rat liver microsomal P450 enzyme activities by various flavonoids.

	Activity			
Flavonoid	EROD (1A1/1A2)	PROD (2B1/2B2)	PNPH (2E1)	ERDM (3A1/3A2)
GTPE	144[a]	217	887	211
EGCG	245[a]	143	317	262
EGC	400[a]	154	733	567
ECG	75[a]	119	250	163
EC	—[b]	—[b]	—[b]	4000
Naringenin	1·1	20	2333	226
Flavone	0·7	14	—[b]	222
Tangeretin	0·8	nd	nd	nd

[a] These are apparent IC_{50}'s. The effect of green tea flavonoids was complex. At lower concentrations activity is stimulated, but at higher concentrations it is inhibited.
[b] No significant changes in control enzyme activities were seen.
nd, not determined.

affected ERDM activity only weakly. Data from inhibition of ERDM by tangeretin was not included because the production of formaldehyde from tangeretin (Canivenc-Lavier *et al.* 1993) interfered with that produced from the demethylation of erythromycin. In human liver microsomes, all flavonoids investigated were inhibitory at high concentrations towards ERDM activity (figure 3B). The IC_{50}'s ranged from 60 to 1000 μM (table 3). ECG was the most potent inhibitor, closely followed by EGCG.

Table 3. IC_{50}'s (μM) for inhibition of specific human liver microsomal P450 enzyme activities by various flavonoids.

	Activity[a]		
Flavonoid	EROD (1A2)	PNPH (2E1)	ERDM (3A4)
EGCG	1175[b]	nd	100
EGC	1000[b]		1000
ECG	530[b]	200	60
EC	2600[b]	—[c]	1000
Naringenin	300	300	300
Flavone	0·15	moderate stimulation	1000
Tangeretin	16	—[c]	nd

[a] PROD was not assayed in the human liver microsomes.
[b] These are apparent IC_{50}'s. The effect of green tea flavonoids was complex. At lower concentrations activity is stimulated, but at higher concentrations it is inhibited.
[c] No significant changes in control enzyme activities were seen.
nd, not determined.

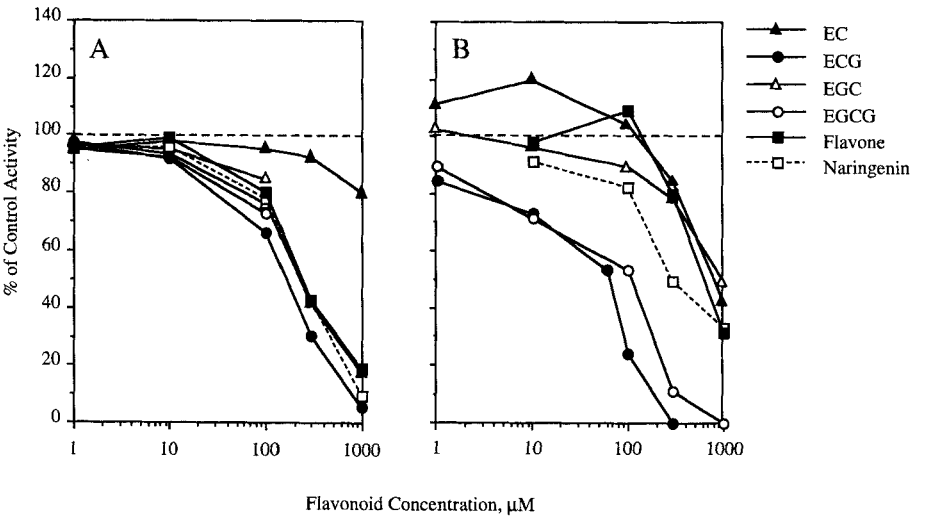


Figure 3. Effect of various flavonoids on ERDM activity in rat and human liver microsomes. Control activity was 11·8 nmol/min/mg protein for rat (A) and 0·45 for man (B). each point represents a mean of 2–5 values. Error bars were left out for clarity, but mean SEs were 2·5 and 6·5% of the mean activities for the rat and human data respectively.

Inhibition of human liver microsomal P450 1A2 by tangeretin

The kinetics of EROD were evaluated at various concentrations of the substrate EORR. From the Lineweaver–Burk plot (figure 4), K_m for EROD was 71 nM, and V_{max} was 24 pmol/min/mg protein. The inhibition of EROD activity by three concentrations of tangeretin was then determined at the same EORR concentrations and under the same conditions. The pattern of lines was consistent with tangeretin being a pure competitive inhibitor (Segel 1975) with $K_i = 68$ nM (figure 4, inset).

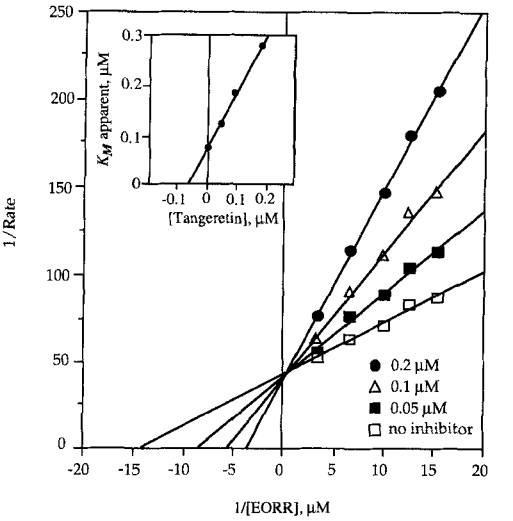


Figure 4. Lineweaver–Burk plot of tangeretin inhibition of EROD in human liver microsomes. Tangeretin (0·05, 0·1 and 0·2 μM) was added to incubations containing EORR (0·065, 0·08, 0·1, 0·15 and 0·3 μM) microsomes (200-μg protein). On the basis of preliminary experiments, these tangeretin concentrations were selected to be in the vicinity of K_i. The rate was calculated as nmol RR/min/mg protein. Each point represents a mean of three values. Error bars were left out for clarity, but mean SEs were 1·5% of the mean activities. The inset shows a replot of apparent K_m's Tangeretin concentrations were plotted against apparent K_m's (μM). The x-intercept corresponds to the K_i for tangeretin towards EROD. The linear regression equation and correlation coefficient for the line is $1{\cdot}05x + 0{\cdot}071$ ($r^2 = 0{\cdot}998$).

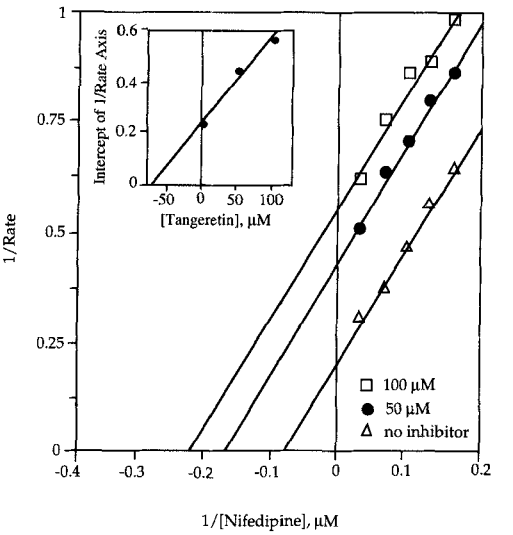


Figure 5. Lineweaver–Burk plot of tangeretin inhibition of NIFO in human liver microsomes. Tangeretin (50 and 100 μM) was added to incubations containing nifedipine (6, 7·56, 10, 15 and 33 μM) and microsomes (200-μg protein). On the basis of preliminary experiments these tangeretin concentrations were selected to be in the vicinity of K_i. The rate was calculated as nmol pyridine metabolite/min/mg protein. Each point represents a mean of three values. Error bars were left out for clarity, but mean SEs were 3·4% of the mean activities. The inset shows a replot of apparent $1/V_{max}$. Tangeretin concentrations were plotted against apparent $1/V_{max}$. The x-intercept corresponds to the K_i for tangeretin towards nifedipine oxidase. The linear regression equation for the line is $0{\cdot}0035x + 0{\cdot}250$ ($r^2 = 0{\cdot}974$).

Inhibition of human liver microsomal P450 3A4 by tangeretin

The kinetics of NIFO were evaluated at various substrate concentrations. From the Lineweaver–Burk plot (figure 5), K_m for NIFO was 10·7 μM, and V_{max} = 4·3 nmol/min/mg protein. The inhibition of NIFO activity by two concentrations of tangeretin was then determined at the same nifedipine concentrations and under the same conditions . The pattern of parallel lines is diagnostic of a purely uncompetitive inhibitor (Segel 1975). A replot (figure 5, inset) of the apparent $1/V_{max}$ as a function of inhibitor concentration gave a straight line, with an x-intercept equal to the $-K_i$ for the inhibitor (72 μM).

Discussion

Inhibition of P450 by flavonoids

From this survey of the interactions of flavonoids with various human P450 activities, one of the most potent effects was elicited by tangeretin on 1A2. Since tangeretin had not been extensively studied in this regard, the kinetics of inhibition of P450 1A2 and 3A4 were investigated more extensively. Tangeretin was found to be a potent competitive inhibitor of P450 1A2 in human liver microsomes, whereas it was a moderately potent uncompetitive inhibitor of 3A4.

For tangeretin to be a competitive inhibitor of P450 1A2, it must occupy the substrate binding site of the enzyme. However, in the case of the uncompetitive inhibition of P450 3A4 by tangeretin, the tangeretin binding site must be distinct from the substrate binding site since it binds only to the enzyme–substrate complex. It is theoretically possible for the inhibitor to bind to a site on the P450 enzyme, on NADPH P450 reductase, or on cytochrome b_5; b_5 may be involved in the catalysis by P450 3A4 (Guengerich *et al.* 1986). The present experiments, in which only the concentrations of tangeretin and nifedipine were varied, cannot distinguish whether the uncompetitive binding site is on P450, the P450 reductase, or cytochrome b_5.

Previously tangeretin has been shown to induce EROD, a P450 1A2 activity, in rat liver microsomes after dietary administration (Siess *et al.* 1989). It has also been found to enhance P450 3A4-dependent activities such as aflatoxin B_1 activation and acetaminophen oxidation in liver microsomes (Buening *et al.* 1981, Li *et al.* 1994). Since tangeretin itself is demethylated by P450, perhaps this metabolite also affects various P450 enzymes, but differently than the patent compound, resulting in the spectrum of modulation that has been seen.

Flavone was found to be one of the most potent inhibitors of EROD. This finding agrees with the work of Siess *et al.* (1989) where flavone was found to inhibit EROD and PROD. Siess *et al.* (1990) reported that human microsomal EROD activity was more sensitive to the flavonoids tested than was rat microsomal EROD activity. In this paper different flavonoids were examined, and no clear relationship between rat and human microsomes was seen in the inhibitory potencies of the compounds.

We observed a dose-dependent inhibitory effect of green tea flavanols for EROD, as did Wang *et al.* (1988). In our experiments ECG was the most potent inhibitor, whereas in the studies of Wang *et al.* (1988) EGCG was the most potent. This difference may be the result of the difference pretreatments used in the two studies, and possibly slight differences in the way the assays were conducted.

Naringenin was found to inhibit P450 3A4-dependent nifedipine oxidase, with an IC_{50} = 300 μM, which is in agreement with previous work by Guengerich and Kim (1990).

Activation of P450 1A2 by green tea flavonoids

The effect of the green tea flavonoids was complex. At lower concentrations 91–50 μM), moderate but statistically significant activation of EROD was seen, namely 16–45% in rat liver microsomes and 57–71% in human liver microsomes (figure 2). At higher concentrations EROD activity was inhibited. In the experiments of Wang *et al.* (1988) no activation at lower concentrations of green tea flavonoïds was noted, although only GTPE was evaluated with EROD activity, and the lowest concentration used was 40 μg/ml ($\approx$ 100 μM, molecular weight calculated based only on the percentage of epicatechin derivatives). With these compounds the apparent IC_{50}'s, which results from a combination of activation and inhibition, are overestimates of the concentrations that are actually required to perturb the enzyme.

There appears to be two binding sites for the green tea flavonoids associated with the P450 1A enzyme system: a high-affinity site, the occupancy of which results in the stimulation of the enzyme; and a low-affinity site, the occupancy of which results in the inhibition of the enzyme. The low-affinity site would be the catalytic site if the inhibition is competitive. However, if the inhibition at high concentrations is non-competitive or uncompetitive then there may be three sites of interaction. For ECG, the most potent activator of EROD, half-maximal activation can be extrapolated to occur at about 2 μM in human liver microsomes, about 250 times less than that of the IC_{50}, Thus, the green tea flavonoids are much more potent stimulators than they are inhibitors.

These compounds could either be binding to and altering the P450 system in such a way that substrate binding is facilitated, or binding the reductase and either facilitating the transfer of electrons to P450 or facilitating the binding of P450 to the reductase. Huang *et al.* (1981) showed that 7,8-benzoflavone and flavone stimulated the hydroxylation of benzo(*a*)pyrene in liver microsomes at least in part by enhancing the interaction between P450 and reductase. It is possible that green tea flavonoids could also facilitate this interaction.

Both tangeretin and green tea flavanols are readily available in the diet. Tangeretin is one of the major components of peel oils from oranges and mandarin oranges, found at concentrations ranging from 500 to 2800 μg/ml respectively (Gaydou *et al.* 1987), and is also found in tangerine juice (3·5 μg/g) (Veldius *et al.* 1970) by ingesting foods and beverages containing orange peel, such as orange marmalade and orange and spice herb tea, one can be exposed to tangeretin. These dietary flavonoids were found to inhibit the activities of P450 enzymes. Tangeretin in particular was found to be a potent inhibitor P450 1A2. Quercetin, another flavonoid that has been shown to affect P450 (Buening *et al.* 1981, Sousa and Marletta 1985, Siess *et al.* 1989), is also found in tea infusions at concentrations of 10–25 mg/l (Hertog *et al.* 1993). Some of the effects seen with whole green tea extract may be due to quercetin. Some of the flavonoids, with $IC_{50} \leqslant 200\,\mu$M, may be sufficiently potent to be biologically significant. However, additional studies will be needed to determine whether these flavonoids can influence these enzymes *in vivo*.

References

BEYELER, S., TESTA, B., and PERRISSOUD, D., 1983, The effect of ciandanol on rat hepatic monooxygenase activities. *Arzneim.-Forsch./Drug Research*, **33,** 564–567.

BUENING, M. K., CHANG, R., L., HUANG, M.-T., FORTNER, J. G., WOOD, A. W., and CONNEY, A. H., 1981, Activation and inhibition of benzo(*a*)pyrene and aflatoxin B_1 metabolism in human liver microsomes by naturally occurring flavonoids. *Cancer Research*, **41,** 67–72.

CANIVENC-LAVIER, M.-C., BRUNOLD, C., SIESS, M.-H., and SUSCHETET, M., 1993, Evidence for tangeretin-*O*-demethylation by rat and human liver microsomes. *Xenobiotica*, **23,** 259–266.

DAS, M., MUKHTAR, H., BIK, D. P., and BICKERS, D. R., 1987, Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occurring plant phenols. *Cancer Research*, **47,** 760–766.

GAYDOU, E. M., BIANCHINI, J.-P., and RANDRIAMIHARISOA, R. P., 1987, Orange and mandarin peel oils differentiation using polymethoxylated flavone composition. *Journal of Agriculture and Food Chemistry*, **35,** 525–529.

GUENGERICH, F. P., and KIM, D.-H., 1990, *In vitro* inhibition of dihydropyridine oxidation and AFB_1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis*, **11,** 2275–2279.

GUENGERICH, F. P., MARTIN, M. V., BEUNE, P. H., KREMERS, P., WOLFF, T., and WAXMAN, D. J., 1986, Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *Journal of Biological Chemistry*, **261,** 5051–5060.

HERTOG, M. G. L., HOLLMAN, P. C. H., and VAN DE PUTTE, B., 1993, Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agriculture and Food Chemistry*, **412,** 1242–1246.

HUANG, M. T., CHANG, R. L., FORTNER, J. G., and CONNEY, A. H., 1981, Studies on the mechanism of activation of microsomal benzo(*a*)pyrene hydroxylation by flavonoids. *Journal of Biological Chemistry*, **256,** 6829–6836.

KAPITULNIK, J., POPPERS, P. J., BUENING, M. K., FORTNER, J. G., and CONNEY, A. H., 1977, Activation of monooxygenases in human liver by 7,8-benzoflavone. *Clinical Pharmacy and Therapeutics*, **22,** 475–484.

LASKER, J. M., HUANG, M.-T., and CONNEY, A. H., 1984, *In vitro* and *in vivo* activation of oxidative drug metabolism by flavonoids. *Journal of Pharmacology and Experimental Therapeutics*, **229,** 162–170.

LI, Y., WANG, E., PATTEN, C. J., CHEN, L., and YANG, C. S., 1994, Effects of flavonoids on cytochrome P450-dependent acetaminophen metabolism in rats and human liver microsomes. *Drug Metabolism and Disposition*, **22,** 566–571.

LOEV, B., and SNADER, K., 1965, The Hantzsch reaction: oxidative dealkylation of certain dihydropyridines. *Journal of Organic Chemistry*, **30,** 1914–1916.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J., 1951, Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193,** 265–275.

OMURA, T., and SATO, R., 1964, The carbon monoxide-binding pigment of liver microsomes. *Journal of Biological Chemistry*, **239,** 2370–2378.

POUL, R. J., and FOUTS, J. R., 1980, A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Analytical Biochemistry*, **107,** 150–155.

REINKE, L. A., and MOYER, M. J., 1985, *p*-Nitrophenol hydroxylation: a microsomal oxidation which is highly inducible by ethanol. *Drug Metabolism and Disposition*, **13,** 548–552.

RYAN, D. E., LU, A. H., and LEVIN, W., 1978, Purification of cytochrome P-450 and P-448 from rat liver microsomes. In *Methods in Enzymology*, vol. LII, edited by S. Fleischer and L. Packer (New York: Academic), p. 118.

SEGEL, I. H., 1975, *Enzyme Kinetics* (New York: Wiley).

SIESS, M.-H., GUILLERMIC, M., LE BON, A. M., and SUSCHETET, M., 1989a, Induction of monooxygenase and transferase activities in rat by dietary administration of flavonoids. *Xenobiotica*, **19,** 1379–1386.

SIESS, M.-H., LE BON, A.-M., SUSCHETET, M., and RAT, P., 1990, Inhibition of ethoxyresorufin deethylase activity by natural flavonoids in human and rat liver microsomes. *Food Additives and Contaminants*, **7,** S178–181.

SIESS, M.-H., PENNEC, A., and GAUDOU, E., 1989b, Inhibition of ethoxy- and pentoxy-resorufin dealkylases of rat liver by flavones and flavonols: structure–activity relationship. *European Journal of Drug Metabolism and Pharmacokinetics*, **14,** 235–239.

SOUSA, R. L., and MARLETTA, M. A., 1985, Inhibition of cytochrome P450 activity in rat liver microsomes by the naturally occurring flavonoid, quercetin. *Archives of Biochemistry and Biophysics*, **240,** 345–357.

VELDIUS, M. K., SWIFT, L. J., and SCOTT, W. C., 1970, Fully methoxylated flavones in Florida orange juices. *Journal of Agriculture and Food Chemistry*, **18,** 590–592.

VUATAZ, L., BRANDENBERGER, H., and EGLI, R. H., 1959, Plant phenols. I. Separation of the tea leaf polyphenols by cellulose column chromatograhy. *Journal of Chromatography*, **2,** 173–187.

WANG, Z. Y., DAS, M., BICKERS, D. R., and MUKHTAR, H., 1988, Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450. *Drug Metabolism and Disposition*, **16,** 98–103.

WRIGHTON, S. A., MAUREL, P., SCHUETZ, E. G., WATKINS, P. B., YOUNG, B., and GUZELIAN, P. S., 1985, Identification of the cytochrome P-450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry*, **24,** 2171–2178.

YANG, C. S., and WANG, Z. Y., 1993, Tea and cancer. *Journal of the National Cancer Institute*, **85,** 1038–1048.