

PRENYLFLAVONOIDS FROM HOPS INHIBIT THE METABOLIC ACTIVATION OF THE CARCINOGENIC HETEROCYCLIC AMINE 2-AMINO-3-METHYLIMIDAZO[4,5-F]QUINOLINE, MEDIATED BY CDNA-EXPRESSED HUMAN CYP1A2

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ABSTRACT:

The heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) is a potential human carcinogen found in cooked food that requires initial metabolic activation by cytochrome P450s, primarily CYP1A2. The present study was conducted to examine whether recombinant human CYP1A2 expressed in insect cells mediates the metabolic activation of IQ and whether prenylflavonoids found in hops and beer would modulate the CYP1A2-mediated activation of IQ. The cDNA-expressed human CYP1A2 was found to strongly activate IQ as measured by the Ames Salmonella assay and by the covalent binding of IQ metabolites to calf thymus DNA and protein. Inhibition studies showed that the prenylchalcone xanthohumol and the prenylflavanones 8-prenylnaringenin and isoxanthohumol

strongly inhibited the mutagenic activation of IQ mediated by cDNA-expressed human CYP1A2 in the Ames Salmonella assay. The three prenylflavonoids also markedly inhibited the human CYP1A2-mediated binding of IQ to metabolites that bind to DNA. The inhibition of the metabolic activation of IQ was paralleled by the inhibition of acetanilide 4-hydroxylase activity of human CYP1A2. Thus, xanthohumol, isoxanthohumol, and prenylflavanones 8-prenylnaringenin are potent inhibitors of the metabolic activation of IQ and may have the potential to act as chemopreventive agents against cancer induced by heterocyclic amines activated by CYP1A2.

Heterocyclic amines such as IQ¹ (2-amino-3-methylimidazo[4,5-f]quinoline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) are among the most potent mutagens found in cooked food and are potentially carcinogenic in humans (Felton and Knize, 1991; Hatch et al., 1992; Sugimura, 1997). These compounds are metabolically activated by a two-step process involving cytochrome P450 CYP1A1, CYP1A2, and CYP1B1 (Edwards et al., 1994; Hammons et al., 1997; Turesky et al., 1998), followed by acetylation or sulfation to form more reactive species (Yanagawa et al., 1994; Yamazoe et al., 1995) that bind to DNA and protein (Snyderwine et al., 1988; Lodovici et al., 1989; Wallin et al., 1992; Huber et al., 1997; Xu et al., 1997). Knowing that CYP1A2 is a key factor in the metabolic action of carcinogenic heterocyclic amines, the inhibition of the expression or activity of this enzyme may represent a logical strategy for preventing the development of human cancers induced by the heterocyclic amines.

Certain flavonoids have been shown to modulate the catalytic activity of cDNA-expressed human CYP1A2. For example, hydroxylated flavonoids inhibited the benzo[a]pyrene hydroxylase activity of

cDNA-expressed human CYP1A2 but increased its 7-methoxyresorufin *O*-demethylation and acetanilide 4-hydroxylation activities (Tsyrllov et al., 1994). In contrast, nonhydroxylated flavone inhibited 7-methoxyresorufin *O*-demethylation and acetanilide 4-hydroxylation activities but not benzo[a]pyrene hydroxylase activity of cDNA-expressed human CYP1A2. It appears that CYP1A2 activity could be inhibited or stimulated by a given flavonoid depending upon the substrate used. Therefore, if the inhibition of CYP1A2 activity is to be considered as a method for preventing heterocyclic amine-induced cancers in humans, studies should focus on the actual effect of the flavonoid on the metabolic activation of heterocyclic amines mediated by CYP1A2.

Recently, several flavanones and chalcones with prenyl or geranyl groups have been identified in hops and beer (Stevens et al., 1997, 1999a,b). Some of these prenylflavonoids such as xanthohumol (XN), isoxanthohumol (IX), and 8-prenylnaringenin (8PN) have been shown to inhibit the ethoxyresorufin *O*-deethylase activity of recombinant human CYP1A1 and CYP1B1, and acetanilide 4-hydroxylase activity of CYP1A2 (Henderson et al., 2000). The metabolic activation of aflatoxin B1 as measured by aflatoxin B1-protein binding mediated by human CYP1A2 was also markedly inhibited by 8PN and IX (Henderson et al., 2000). As inhibitors of CYP1A2, these prenylflavonoids may have the potential to prevent the mutagenicity and carcinogenicity of heterocyclic amines activated by this P450 isoform. The present study was carried out to determine whether prenylflavonoids from hops and beer inhibit the mutagenic activation of IQ mediated by the recombinant human enzyme. To accomplish this goal, it was neces-

¹ Abbreviations used are: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; XN, xanthohumol; IX, isoxanthohumol; 8PN, 8-prenylnaringenin.

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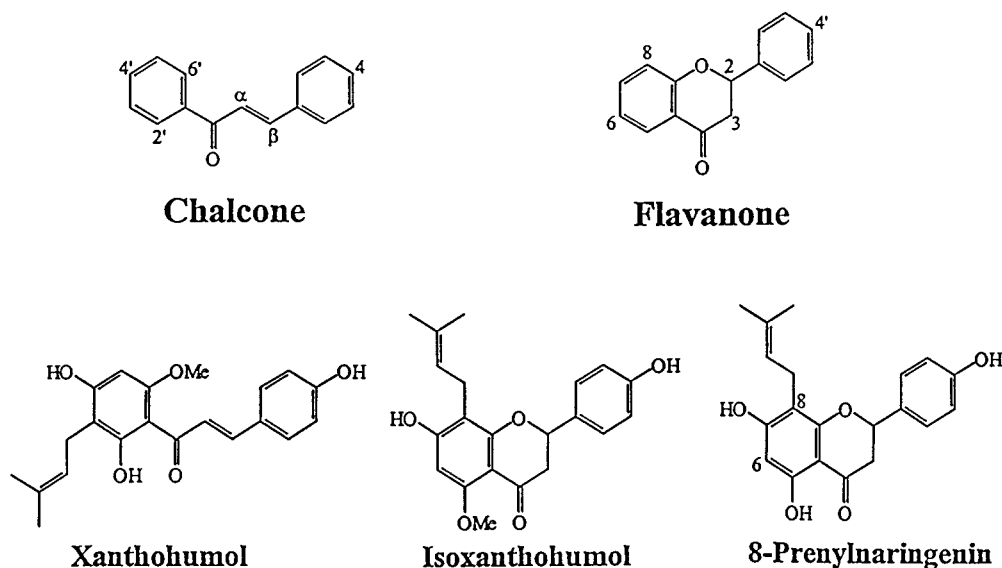


FIG. 1. Chemical structures of xanthohumol, isoxanthohumol, and 8-prenylnaringenin.

The backbone structure of a chalcone and flavanone is also depicted.

sary to first demonstrate whether cDNA-expressed human CYP1A2 can be used as a metabolic activating enzyme system in the Ames mutagenicity assay. Therefore, initial studies were conducted to compare the mutagenic activation of IQ by the recombinant human CYP1A2 with that mediated by liver S9 fraction from Aroclor 1254-treated rats or by recombinant rat CYP1A2 in the Ames test. For studies on the inhibition of IQ mutagenicity in the Salmonella assay, the human CYP1A2 enzyme was used as the metabolic activating enzyme system. The inhibitory effects of the prenylflavonoids on IQ activation were further assessed by measuring the human CYP1A2-mediated binding of IQ metabolites to calf thymus DNA and protein *in vitro*. Parallel studies were performed on the inhibition of the 4-hydroxylation of acetanilide, a known diagnostic substrate of human P450 CYP1A2.

Materials and Methods

Chemicals. IQ and the [^{14}C]IQ (>99% purity, with a specific activity of 10 mCi/mmol) were obtained from Toronto Research Chemicals Inc., North York, Ontario, Canada. Naringenin was purchased from Sigma Chemical Co., St. Louis, MO. The prenylchalcone XN was previously isolated from hops, whereas the prenylflavanone IX was prepared by chemical treatment of xanthohumol (Stevens et al., 1997). The prenylflavanone 8PN was prepared from naringenin (Stevens et al., 1999b). These products were purified by preparative HPLC on a 10- μm Econosil RP-18 column (22 \times 250 mm) using gradient elution, typically from 40 to 80% acetonitrile in 1% aqueous formic acid over 30 min, at a flow rate of 11.2 ml/min. The UV absorbance was monitored at 290 or 370 nm. The flavonoids were recovered from peak fractions by rotary evaporation and lyophilization, and were obtained as white-cream colored (flavanones) or yellow-orange (chalcones) powders. The purity of the flavonoid materials was determined to be $\geq 98\%$ by HPLC, atmospheric pressure chemical ionization-mass spectrometry, and ^1H NMR spectroscopy (data not shown). The chemical structures of the flavonoids used in these studies are shown in Fig. 1.

Ames Salmonella Assay. The inhibitory effects of the prenylflavonoids on the mutagenic activation of IQ (6 ng/plate) were examined by using the Ames assay (Maron and Ames, 1983) as modified by Tachino et al. (1994). In the initial study, three metabolic activating systems were compared for their ability to convert IQ to a mutagen in the Ames Salmonella assay. The activating enzymes were S9 fraction of liver from Aroclor 1254-treated rats (MolTox, Annapolis, MD) or microsomes (supersomes) from baculovirus-infected insect cells (BTI-TN-5B1-4) expressing rat or human CYP1A2 as well as human

NADPH cytochrome P450 reductase (Gentest Corporation, Woburn, MA). A typical incubation mixture consisted of 6 μl of 1 $\mu\text{g}/\text{ml}$ IQ stock solution in dimethyl sulfoxide, 200 μl of a suspension of *Salmonella typhimurium* TA98 (kindly provided by Dr. B. N. Ames, Dept. of Biochemistry and Molecular Biology, University of California, Berkeley), 1 μl of flavonoid (final concentration of 2 or 10 μM) stock solution in 25% ethanol, and 200 μl of standard S9 mix containing 0.28 mg (or 200 pmol of total P450) of Aroclor 1254-induced rat liver S9 (MolTox). Control incubations contained 1 μl of 25% ethanol instead of flavonoid. Some incubation mixtures contained no IQ to correct for spontaneous revertants. In other incubations, the rat liver S9 fraction in the S9 mix was replaced by 20 pmol of cDNA-expressed rat or human CYP1A2 (Gentest Corporation). The incubation mixtures (total volume of 407 μl) were preincubated for 20 min at 37°C before the addition of 2 ml of top agar. The total mixture was poured onto minimal glucose plates and then incubated at 37°C for 2 days. The number of His⁺ revertant colonies per plate was counted after 2 days of incubation at 37°C. Antimutagenic activity (percentage of inhibition) was calculated as follows: $1 - \frac{[(\text{number of revertants in the presence of flavonoid}) - (\text{spontaneous revertants})]}{[(\text{number of revertants in the presence of ethanol}) - (\text{spontaneous revertants})]} \times 100$.

The decrease in mutant colonies could be the result of inhibitory effect of the flavonoids on the activating enzyme, CYP1A2, or on cell toxicity induced by the flavonoids. To rule out the latter possibility, backgrounds were inspected under a light microscope. There was no cell toxicity seen in plates where the mutant colony counts had decreased in any of the experiments performed.

Covalent Binding of IQ to DNA. The metabolic activation of IQ was further assessed by the covalent binding of IQ metabolites to calf thymus DNA *in vitro*. [^{14}C]IQ-DNA binding was determined essentially as described by Takahashi et al. (1995) with minor modifications. In a final volume of 250 μl , reaction mixtures contained 150 μg of calf thymus DNA, 1.5 mg of protein (with 0.7 nmol of P450/mg) from Aroclor 1254-induced rat liver S9 (or 20 pmol of recombinant rat or human CYP1A2), 50 mM potassium phosphate buffer (pH 8.0), 0.1 mM EDTA, 15 mM MgCl₂, flavonoids (10 μM), and 100 μM [^{14}C]IQ (0.5 $\mu\text{Ci}/\text{incubation}$). Reactions were initiated by addition of 1 mM NADPH. After incubation for 30 min with shaking at 37°C, the reaction was terminated by the addition of 50 μl of 10% SDS. DNA was isolated by successive extractions with equal volumes of buffer-saturated phenol, chloroform, and chloroform-isoamyl alcohol (24:1, v/v). DNA was precipitated from the final aqueous phase by the addition of 1 ml of isopropanol followed by centrifugation. The resulting pellet was washed once with isopropanol, dried at room temperature, and resuspended in TE buffer [0.1 mM Tris-HCl (pH 8), 10

TABLE 1

Comparison of rat and human cytochrome P450 enzymes in the metabolic activation of IQ as measured by the Ames Salmonella assay (number of revertants/plate) and by the covalent binding of [¹⁴C]IQ to DNA and protein

Incubations contained either 200 pmol of total P450 from S9 fraction of liver of Aroclor 1254-treated rats or 20 pmol of cDNA-expressed rat or human CYP1A2. Values are means ± S.E. of triplicate determinations. Means within each row sharing a letter are not significantly different, *P* < .05 (Tukey's Studentized Range Test).

Metabolic Activating System	No. of Revertants/Plate	[¹⁴ C]IQ Bound	
		pmol/μg DNA	nmol/mg protein
Rat liver S9	980 ± 45 ^a	0.254 ± 0.021 ^a	0.270 ± 0.023 ^a
Rat CYP1A2	997 ± 9.8 ^a	0.429 ± 0.021 ^b	1.874 ± 0.092 ^b
Human CYP1A2	3059 ± 398 ^b	0.495 ± 0.086 ^c	4.516 ± 0.265 ^c

mM EDTA]. Aliquots were counted on a Packard scintillation spectrometer and DNA concentration estimated by measuring absorbance at 260 nm.

Covalent Binding of IQ to Protein. The metabolic activation of IQ to metabolites that bind to protein was examined by incubating P450 enzymes (Aroclor 1254-induced rat liver S9 fraction equivalent to 1 nmol of P450, or supersomes equivalent to 20 pmol of recombinant rat or human CYP1A2) in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM MgCl₂, 150 mM KCl, 0.2 mM EDTA, and 1 mM NADPH in a final volume of 0.25 ml. Flavonoids (XN, IX, or 8PN, in ethanol) were added to a final concentration of 10 μM. Controls contained ethanol only (0.1%, v/v, final concentration). After preincubation for 10 min at 37°C, [¹⁴C]IQ (100 μM, 0.5 μCi/incubation) was added and incubations continued for an additional 30 min at 37°C. Reactions were stopped by the addition of 0.25 ml of methanol. After protein precipitation and centrifugation, the supernatants were removed and the pellets exhaustively extracted with methanol. The protein was then redissolved in 1 N NaOH, neutralized with HCl, and an aliquot was counted in a liquid scintillation counter after the addition of scintillation fluid (Optifluor).

Acetanilide 4-Hydroxylase Activity. The 4-hydroxylation of acetanilide mediated by human CYP1A2 in supersomes was determined as described by Liu et al. (1991) with some modifications. The incubation mixture consisted of 0.5 mM acetanilide in 50 mM potassium phosphate buffer (pH 7.4) in the presence of 0.1 nmol of P450 from rat S9 (or 20 pmol of recombinant rat or human CYP1A2), 5 mM MgCl₂, 1 mM EDTA, 10 mM glucose 6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, and 1 mM NADPH in a final volume of 0.25 ml. The flavonoids (XN, IX, and 8PN, in ethanol) were added to the incubation mixture to a final concentration of 2 or 10 μM. Vehicle controls contained 0.2% ethanol. The amounts of P450 used in the incubations were based on our previous work on inhibition of acetanilide 4-hydroxylase activity by prenylflavonoids (Henderson et al., 2000). After a 1-h incubation at 37°C, the reaction was terminated by placing the samples in ice water and by adding 25 μl of H₃PO₄. The samples were vortexed and centrifuged at 16,000g for 20 min before analysis by HPLC (Henderson et al., 2000).

Statistical Analysis. Multiple mean comparisons for the mutagenicity studies were determined by the unpaired Student's *t* test. Data from the enzyme assays and covalent binding studies were analyzed by one-way ANOVA followed by the "post hoc" Tukey's Studentized Range Test using the SAS computer program. Statistical significance was set at *P* < .05.

Results

Ames Salmonella Assay. The effects of the prenylflavonoids on the mutagenic activation of IQ were assessed using three different metabolic enzyme systems in the Ames assay. Recombinant human CYP1A2 was found to be highly efficient in activating IQ to a mutagen, producing a large number (3059 ± 387) of revertants per plate (Table 1). In contrast, the number of revertants per plate obtained in the standard Ames assay using rat liver S9 and rat CYP1A2 were 997 ± 9.8 and 980 ± 45 (Table 1). The three flavonoids significantly inhibited the mutagenic activation of IQ mediated by human CYP1A2 (Table 2). At 2 μM, 8PN produced a dramatic decrease in mutant colonies (94% inhibition), followed by IX (84% inhibition) and XN (48% inhibition). At 10 μM, the three flavonoids

TABLE 2

Effects of prenylated flavonoids on the mutagenicity of IQ (6 ng/plate) activated by cDNA-expressed human CYP1A2 in *Salmonella typhimurium* TA98

Treatment	Flavonoid Concentration	Number of Revertants per plate ^a
	nmol/ml top agar	
Control (ethanol)	0	3059 ± 387
8PN	2	191 ± 30.9 ^b (93.8)
	10	4.7 ± 4.7 ^b (99.9)
XN	2	1579 ± 82.3 ^b (48.4)
	10	364 ± 43.2 ^b (88.1)
IX	2	499 ± 4.9 ^b (83.7)
	10	189 ± 9.8 ^b (93.8)

^a Values are means ± S.E. of triplicate plates and have been corrected for spontaneous revertants (58 ± 10). Values in parentheses are percent inhibition as compared with control.

^b *P* < .05 versus control (Student's *t* test).

almost completely prevented the formation of revertant colonies in the Ames assay with IQ.

Covalent Binding of IQ to DNA Catalyzed by Recombinant Rat and Human CYP1A2. In the absence of prenylflavonoids, cDNA-expressed human CYP1A2 was more active than the recombinant rat CYP1A2 or rat liver S9 in mediating the covalent binding of IQ metabolites to calf thymus DNA *in vitro* (Table 1). Figure 2A shows the effects of the flavonoids (at 10 μM) on the CYP1A2-mediated binding of IQ to calf thymus DNA *in vitro*. The metabolic activation of IQ to DNA-binding metabolites mediated by human CYP1A2 was significantly inhibited by XN, IX, and 8PN. 8PN was the most effective inhibitor of IQ-DNA binding.

Covalent Binding of IQ to Protein Catalyzed by Recombinant Rat and Human CYP1A2. Based on the studies of Wallin et al. (1992), the metabolic activation of IQ can be evaluated by measuring the covalent binding of the reactive metabolites of the mutagen to protein. As shown in Table 1, IQ was metabolized to reactive intermediates that bound covalently to proteins after incubation with rat liver S9 or with supersomes containing recombinant rat or human CYP1A2. The human CYP1A2 was more active than rat CYP1A2 or rat liver S9 in mediating the covalent binding of IQ to protein (Table 1). Addition of XN or 8PN (at 10 μM) to the incubation mixture significantly inhibited the binding of IQ metabolites to protein of supersomes containing human CYP1A2 (Fig. 2B). However, IX slightly inhibited IQ-protein binding mediated by human CYP1A2 but the decrease in protein binding was not statistically significant.

Effect of Prenylflavonoids on Acetanilide 4-Hydroxylase Activity of Recombinant Human CYP1A2. To determine the effects of XN, IX, and 8PN on the catalytic activity of human CYP1A2, the NADPH-dependent 4-hydroxylation of acetanilide was determined. At 2 μM, XN, 8PN, and IX significantly inhibited human CYP1A2-mediated acetanilide 4-hydroxylase activity (8PN > IX > XN) (Table 3). At concentrations of 10 μM, 8PN also showed the greatest inhibitory activity toward human CYP1A2-mediated activity, reducing the production of 4-hydroxyacetanilide virtually to zero.

Discussion

Human CYP1A2 has been shown to activate IQ to mutagenic metabolites but in the Ames Salmonella assay for IQ, the use of cDNA-expressed human CYP1A2 as an exogenous metabolic activating system has not been documented. Previous mutagenicity assays for IQ involved the use of human CYP1A2 expressed in *Salmonella* tester strain (Suzuki et al., 1998; Aryal et al., 1999), in *Escherichia coli* tester strain (Kranendonk et al., 1999), or in Chinese hamster CHL cells (Yanagawa et al., 1994). Human liver microsomes also

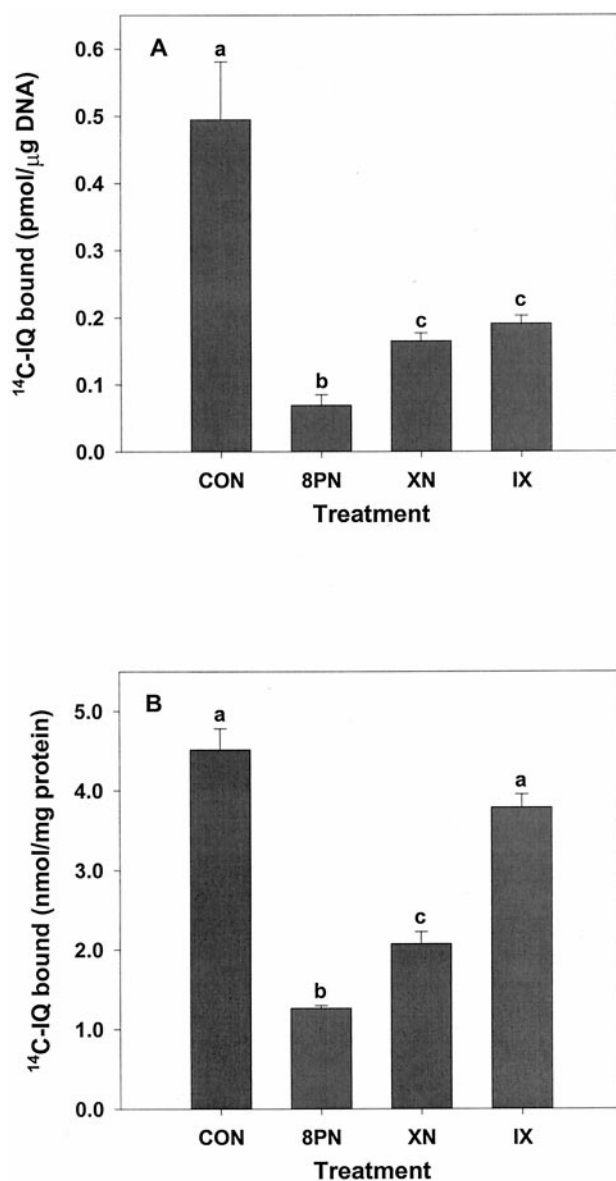


FIG. 2. Effect of prenylflavonoids (10 μ M) on covalent binding of IQ to DNA (A) and to protein (B) in vitro catalyzed by cDNA-expressed human CYP1A2. Columns represent the mean \pm S.E. of three determinations. Columns sharing a letter are not significantly different, $P < .05$ (Tukey's Studentized Range Test).

TABLE 3

Effect of prenylflavonoids on acetanilide 4-hydroxylase activity of cDNA-expressed human CYP1A2

Treatment	Flavonoid Concentration	Acetanilide 4-Hydroxylase ^a
	μ M	nmol/min/nmol P450
Control (ethanol)	0	23.0 \pm 0.31 ^b
8PN	2	7.97 \pm 0.16 ^c
	10	0.0 \pm 0.0 ^d
XN	2	16.4 \pm 0.09 ^e
	10	8.23 \pm 0.44 ^f
IX	2	10.5 \pm 0.55 ^g
	10	2.45 \pm 0.12 ^h

^a Values are mean \pm S.E. of three determinations. Means within each row sharing a letter are not significantly different, $P < .05$ (Tukey's Studentized Range Test).

have been used to activate IQ in the Ames Salmonella mutagenicity assay (McManus et al., 1988). The present study showed that insect cell microsomes containing cDNA-expressed human CYP1A2 (Gen-

test Corporation) are an excellent exogenous activating enzyme system for IQ in the Ames Salmonella assay (Table 1). The cDNA-expressed human CYP1A2 is more relevant than rat liver S9 or rat CYP1A2 as an exogenous activating enzyme system in the Ames assay for human extrapolation.

The finding that cDNA-expressed rat or human CYP1A2 can activate IQ to mutagenic metabolites or to DNA- or protein-binding metabolites (Table 1) was surprising. Unlike the rat liver S9, the microsomes or "supersomes" from the baculovirus-infected insect cells only expressed the cDNA-expressed human or rat CYP1A2 but not the sulfotransferases or *N*-acetyltransferases that are needed to further activate *N*-hydroxy-IQ to more reactive species. There is a possibility, although remote, that the supersomes may have endogenous conjugating enzymes or other unidentified enzymes that can activate IQ to more reactive species. Aside from P450s and conjugating enzymes, IQ can be activated by ATP (Agus et al., 2000), hydrogen peroxide (Anari et al., 1997), and prostaglandin H synthase (Liu and Levy, 1998; Wolz et al., 2000). The possible endogenous

expression of sulfotransferases or *N*-acetyltransferases or other potential activating systems for IQ in supersomes from baculovirus-infected insect cells merits further investigation.

All three prenylflavonoids found in hops and beer were effective in inhibiting the mutagenic activation of IQ in the Ames test. Of the three prenylflavanones tested, 8PN was the most active in inhibiting the mutagenic activation of IQ (Table 2). IX, with a methoxy group instead of a hydroxyl group (in 8PN) at position 5 (Fig. 1) was less inhibitory than 8PN. XN, the chalcone isomer of IX, was the least inhibitory. Additional prenylchalcones and prenylflavanones have to be examined before structure-activity relationships can be established for the inhibition of the mutagenic activation of IQ mediated by CYP1A2.

Several studies have indicated that inhibition of metabolic activation is an important mechanism of protection by flavonoids against mutagenic and carcinogenic heterocyclic amines (Edenharder et al., 1997; Kanazawa et al., 1998; Hammons et al., 1999). For example, Kanazawa et al. (1998) have reported that the antimutagenic activity of flavones and flavonols was due to the inhibition of the activation of Trp-P-2 by rat P4501A1 to *N*-hydroxy-Trp-P-2, the ultimate mutagenic form. Lee et al. (1996) have shown that the antimutagenicity of flavonoids against IQ correlated with the inhibition of cytochrome P450 1A1-linked 7-ethoxyresorufin *O*-deethylase and P450 1A2-linked 7-ethoxycoumarin *O*-deethylase activities in rat hepatic microsomes, and with inhibition of *N*-hydroxy-IQ formation from IQ metabolized by rat hepatic microsomes. Hammons et al. (1999) have suggested that one of the mechanisms of protection by flavonoids and other agents against the carcinogenicity of PhIP is inhibition of the *N*-hydroxylation of this heterocyclic amine mediated by rat and human liver microsomes. Based on these observations, it is likely that inhibition of human CYP1A2 is responsible for the antimutagenic activity of the prenylflavonoids against IQ in the Ames test.

N-hydroxy-IQ, the mutagenic metabolite of IQ, has been shown to bind to calf thymus DNA *in vitro* under nonenzymatic conditions at pH 7.4, forming the major adduct *N*-(deoxyguanosin-8-yl)-IQ (Snyderwine et al., 1988). The C-8 guanine adduct was detected in nuclear and mitochondrial DNA isolated from the liver of rats given IQ (Davis et al., 1994). The N-2 adenine adduct also was identified in the liver of rats dosed with IQ. Certain chemopreventive agents such as chlorophyllin and indole-3-carbinol are known to inhibit IQ-DNA binding in the liver and colon of rats (Guo and Dashwood, 1994). These findings have led us to postulate that the formation of IQ-DNA adducts may be used as another endpoint to measure the inhibitory effect of the flavonoids on the metabolic activation of IQ. IQ-DNA binding (Fig. 2A) mediated by human CYP1A2 was highly correlated with inhibition of the mutagenic activation of IQ (Table 2), further supporting the contention that the antimutagenic activity of the prenylflavonoids results from the inhibition of the metabolic activation of IQ.

The binding of IQ to microsomal protein has been used as an indicator of the metabolic activation of IQ. Binding of IQ to protein was NADPH-dependent, was increased by using microsomes from rats treated with Aroclor 1254 or β -naphthoflavone, and was inhibited by α -naphthoflavone (Wallin et al., 1992). In the present study, covalent binding of IQ to protein mediated by human CYP1A2 was significantly inhibited by 8PN and XN but not by IX (Fig. 2B). This finding was unexpected because IX inhibited the binding of IQ to DNA (Fig. 2A) and significantly inhibited the acetanilide 4-hydroxylase activity of human CYP1A2 (Table 3). In a previous study (Henderson et al., 2000), IX (IC₅₀ of 4.5 μ M) was 7 times more active than XN (IC₅₀ of 31.4 μ M) in the inhibition of acetanilide 4-hydroxylase activity of cDNA-expressed human CYP1A2 similarly obtained

from Gentest Corporation. Therefore, IQ-protein binding may not be a suitable endpoint to evaluate the effects of inhibitors on the metabolic activation of IQ mediated by human CYP1A2. The reason for the lack of a significant inhibition of CYP1A2-mediated binding of IQ to protein is not known and further work is needed to examine this question.

Human CYP1A2 is thought to be the major enzyme responsible for the activation (*N*-hydroxylation) of IQ (Hammons et al., 1997). CYP1A2 is also the major catalyst for the 4-hydroxylation of acetanilide in rodents and humans (Liu et al., 1991). In the present study, we used acetanilide 4-hydroxylase activity to measure the effects of prenylflavonoids on the enzymatic activity of recombinant human CYP1A2. All three flavonoids inhibited acetanilide 4-hydroxylase activity of human CYP1A2 (Table 3), a finding consistent with our previous inhibition study on these compounds (Henderson et al., 2000). 8PN, the most effective inhibitor of acetanilide 4-hydroxylase activity of human CYP1A2, was also the most effective inhibitor of the mutagenic activation of IQ (Table 2). Thus, it is concluded that inhibition of human CYP1A2-mediated activity might be a major mechanism for the inhibition of the mutagenic activation of IQ in the Ames assay.

Beer and hop extracts have been reported to inhibit the mutagenic activity of indirect-acting (Trp-P-1) and direct-acting [Trp-P-2(NHOH) and Glu-P-1(NHOH)] mutagens (Arimoto-Kobayashi et al., 1999). Phenolic compounds present in beer other than flavonoids were only slightly antimutagenic, suggesting that other components may be responsible for the antimutagenic activity of beer and hops. However, it is interesting to note that beer did not provide protection against the mutagenicity of other heterocyclic amines such as activated IQ, IQ(NHOH), activated PhIP, activated MeIQ, and activated MeIQx (Arimoto-Kobayashi et al., 1999). On the other hand, the results of our present study showed that prenylflavonoids such as XN, 8PN, and IX, which are found in beer (Stevens et al., 1999a,b), exhibited antimutagenic activity against IQ. If beer did not inhibit the mutagenicity of activated IQ (Arimoto-Kobayashi et al., 1999) whereas XN, IX, and 8PN were inhibitory, then it is likely that the beer used in the previous study (Arimoto-Kobayashi et al., 1999) may not have contained prenylflavonoids in amounts sufficient to inhibit the mutagenicity of IQ. Certain unidentified components other than prenylflavonoids may be responsible for the antimutagenic activity of beer toward activated Trp-P-2, Trp-P-2(NHOH), and Glu-P-1(NHOH) reported previously (Arimoto-Kobayashi et al., 1999). It remains to be established whether the prenylflavonoids used in our study are antimutagenic against other heterocyclic amines such as Trp-P-2 or Trp-P-2(NHOH).

Taken together, the results of this study suggest the prenylflavonoids from hops and beer such as 8PN, IX, and XN are potent inhibitors of the metabolic activation of IQ by human CYP1A2. These findings indicate the potential of these compounds to act as chemopreventive agents against cancer induced by chemicals activated by CYP1A2. To achieve a plasma concentration (e.g., 1 μ M or 0.354 mg/l) that could inhibit IQ activation *in vivo*, a 70-kg person would have to ingest at least 10.6 mg/day of total prenylflavonoids (equivalent to 2.6 liters of beer per day), assuming that only 10% of the ingested compounds is absorbed. Therefore, for chemoprevention programs, the prenylflavonoids would have to be used as dietary supplements, and not obtained from drinking beer.

Further studies are needed to elucidate other mechanisms by which the prenylflavonoids inhibit the mutagenic activity of IQ. In addition to inhibition of metabolic activation of IQ, there might be other possible mechanisms by which chemopreventive agents inhibit IQ

mutagenesis, such as modulation of DNA repair and modulation of transmembrane transport (De Flora, 1998; Kuroda and Hara, 1999).

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