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CYP1A1 genotype-selective inhibition of benzo[*a*]pyrene activation by quercetin

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Abstract

Epidemiological studies suggest that food rich in quercetin and naringin may protect against certain types of lung cancer, and that genotype dependent inhibition of cytochrome P450 1A1 (CYP1A1)-mediated bioactivation of procarcinogens could be the underlying mechanism. We studied the inhibitory effects of quercetin and naringin on the terminal bioactivation step of benzo[*a*]-pyrene (B[*a*]P), a member of the major class of lung carcinogens. This reaction (epoxidation of (±)-*trans*-7,8-dihydro-7,8-dihydroxy-B[*a*]P to the ultimate carcinogenic product, (±)-B[*a*]P-r-7,*t*-8-dihydrodiol-*t*-9,10-epoxide (diolepoxide 2)) was examined using three of the most common allelic variants of human CYP1A1, namely wild-type CYP1A1.1, CYP1A1.2, and CYP1A1.4. Quercetin potently inhibited diolepoxide 2 formation by all CYP1A1 types with IC_{50} values between 1.6 and 7.0 μM. The differences between the wild-type enzyme and the variants were statistically highly significant ($P < 0.01$). Enzyme kinetics revealed quercetin as a mixed-type inhibitor of CYP1A1.1, CYP1A1.2, and CYP1A1.4 with K_i values of 2.0, 6.4, and 9.3 μM, respectively. Naringin inhibited diolepoxide 2 formation only slightly. Our data support the hypothesis that quercetin may have a stronger chemopreventive effect in individuals carrying wild-type compared with variant *CYP1A1* genes. Future studies should consider the influence of P450 polymorphisms on both procarcinogen activation and its inhibition to facilitate the development of genotype-specific chemoprevention regimes.

Keywords: Human cytochrome P450 1A1; CYP1A1; Benzo[*a*]pyrene activation; Genotype-dependent inhibition; Epoxidation; Diolepoxide; Quercetin; Naringin; Genetic variability; Chemoprevention of cancer

1. Introduction

Dietary intervention will be an important cancer prevention strategy [1,2]. Among potential chemopreventing agents are the natural polyphenols, such as flavonoids, which exhibit antioxidative and anticarcinogenic effects *in vitro* and *in vivo* [3]. Recently, a case-control study in Hawaii demonstrated an inverse correlation between lung cancer risk and a diet rich in the flavonoids quercetin (found in onions and apples) and naringin (found in white grapefruits) [4]. The protective effect of quercetin and onions was strongest against squamous cell carcinoma (a cell type specifically associated with

Abbreviations: P450, Cytochrome P450; CYP1A1, Human cytochrome P450 1A1; P450 reductase, Human NADPH-cytochrome P450 reductase; B[*a*]P, Benzo[*a*]pyrene; 7,8-diol-B[*a*]P, (±)-*trans*-7,8-dihydro-7,8-dihydroxy-B[*a*]P; Diolepoxide 1, (±)-B[*a*]P-r-7,*t*-8-dihydrodiol-*c*-9,10-epoxide; diolepoxide 2, (±)-B[*a*]P-r-7,*t*-8-dihydrodiol-*t*-9,10-epoxide; tetraol 1, r-7,*t*-8,*t*-9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydro-B[*a*]P; tetraol 2, r-7,*t*-8,*t*-9,*t*-10-tetrahydroxy-7,8,9,10-tetrahydro-B[*a*]P; tetraol 3, r-7,*t*-8,*c*-9,*t*-10-tetrahydroxy-7,8,9,10-tetrahydro-B[*a*]P; tetraol 4, r-7,*t*-8,*c*-9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydro-B[*a*]P; EROD, 7-ethoxyresorufin *O*-deethylation.

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subjects carrying the *CYP1A1*2* allele) and was dependent on the *CYP1A1* genotype, suggesting that CYP1A1 may play a role in this association. It was suggested that foods rich in certain flavonoids may protect against certain forms of lung cancer and that decreased activation of carcinogens by inhibition of CYP1A1 should be the underlying mechanism [4].

Quercetin is one of the most abundant of the naturally occuring flavonoids. The chemopreventive effect of quercetin on dietary carcinogen has been intensely studied in animal models [3]; however, knowledge regarding the molecular mechanism, e.g. specific inhibition of CYP1A1, is still limited. Earlier studies showed that flavonoids, including quercetin, inhibit CYP1A-mediated activities in liver microsomes [5–8]. More recent work, using cDNA-expressed CYP enzymes, explored the effects of a series of flavonoids (but not quercetin) on human CYP1A1 and CYP1A2 enzymes [9]. Several papers reported that quercetin suppresses benzo[*a*]pyrene (B[*a*]P)-induced DNA damage by altering CYP1A1 gene expression and inhibiting genetic toxicity in cells expressing CYP1A2 and CYP1A1 [10–12]. A more specific and extensive evaluation of quercetin and other flavonoids on different activities of human CYP1A1 has recently been performed by us [13].

The above mentioned epidemiological study adds to the increasing evidence that individual susceptibility to lung cancer is modulated by factors which affect the metabolism of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAH), particularly B[*a*]P. In human tissues, B[*a*]P can be activated in a multi-step process including epoxidation of (±)-*trans*-7,8-dihydro-7,8-dihydroxy-B[*a*]P (7,8-diol-B[*a*]P) to the ultimate genotoxic (±)-r-7,*t*-8-dihydrodiol-*t*-9,10-epoxy-B[*a*]P (B[*a*]P-diolepoxide 2) as the terminal reaction. Diolepoxide 2 directly binds to DNA forming adducts which can lead to cancer initiation [14–17]. The principal catalysts in B[*a*]P activation have been shown to be CYP1A1 and CYP1B1, at least in extrahepatic tissues [18,19]. Thus, the inhibition of CYP1A1-mediated diolepoxide 2 formation may be a critical step in cancer prevention.

The human *CYP1A1* gene is polymorphic and significant ethnic differences in the frequency of *CYP1A1* alleles have been observed. At present, 11 alleles have been reported; however, several are very rare and of unknown functional significance (a complete description and the systematic nomenclature of *CYP1A1* polymorphisms can be found at http://www.imm.ki.se/CYPalleles/cyp1A1.htm). Beside (wild-type) *CYP1A1*1*, the most common alleles are *CYP1A1*2B* (*Msp* I in complete linkage disequilibrium with Ile462Val mutation) and *CYP1A1*4* (Thr461Asn mutation). Distribution and allele frequency depends highly on ethnicity [20], e.g. the allele frequency of *CYP1A1*2* is observed in 2–10% of Caucasians compared with approximately 18–33% of Japanese and Chinese populations. By contrast, the *CYP1A1*4* genotype occurs in approximately 2–5% of Caucasians, and up to now has not been found in Japanese and Chinese populations.

Several studies have examined the relationship between allelic variants of *CYP1A1* and lung cancer risk in various ethnic populations. Supported by recent pooled and multi-analyses, there is increasing evidence that the *CYP1A1*2B* genotype, alone or in combination, is observed in subjects at higher risk, at least for certain types of lung cancer (squamous cell carcinoma) (reviewed in Ref. [21]). The underlying mechanisms of this genetic predisposition may include a higher prevalence of *p53* mutations and B[*a*]P-diolepoxide-DNA adduct levels in smokers [22].

Overall, the above discussed data from epidemiological studies suggest that food rich in certain flavonoids may protect against specific forms of lung cancer and that inhibition of CYP1A1-mediated procarcinogen bioactivation could be a mechanism contributing to this protection. Herein, we present an *in vitro* study that tests this hypothesis directly by measuring the CYP1A1 genotype-specific inhibition of ultimate carcinogen formation from 7,8-diol-B[*a*]P, the final and decisive step in carcinogen activation of B[*a*]P, which belongs to the major class of tobacco carcinogens causing lung cancer. We characterise the inhibitory potencies of quercetin and naringin on those common human CYP1A1 allelic variants that result from amino acid substitutions, namely CYP1A1.1 (wild-type), CYP1A1.2 (Ile462Val), and CYP1A1.4 (Thr461Asn), by means of the epoxidation of 7,8-diol-B[*a*]P. Rates of diolepoxide 2 formation were determined using an *in vitro* reconstituted system consisting of the respective purified human CYP1A1 variant, purified human NADPH-cytochrome P450 reductase (P450 reductase), and dilaurylphosphatidylcholine as the lipid component.

2. Materials and methods

2.1. Materials

Quercetin, naringin, 7-ethoxyresorufin, B[*a*]P, and dilaurylphosphatidylcholine were purchased from Sigma (Deisenhofen, Germany). 7,8-diol-B[*a*]P and the tetraols 1–4 were purchased from the National Cancer Institute (NCI) Chemical Carcinogen Repository, Midwest Research Institute (Kansas City, MI, USA). The tetraols were kindly provided by Prof. K.-L. Platt (Institute of Toxicology, University of Mainz, Mainz, Germany).

2.2. Expression and purification of recombinant enzymes and reconstitution of enzymatic activity

Methods have been described in detail elsewhere [13,23,24]. Briefly, CYP1A1 cDNAs with His-extended

C-termini and carrying the mutations were cloned into pAcMP3 baculovirus transfer vectors (Pharmingen, San Diego, CA, USA) under the control of the late basic protein promoter. Recombinant baculovirus were prepared as described in Ref. [23]. The three variants were expressed in *Spodoptera frugiperda* (Sf9) insect cells as C-terminal His-tag proteins to facilitate purification by Ni-affinity chromatography, and purified to electrophoretically homogeneous CYP1A1 with a specific P450 content of 11.2 nmol/mg protein [23]. Reduced CO-difference spectroscopy proved the expressed enzymes to be free of any inactive cytochrome P420. Human P450 reductase was also expressed in Sf9 cells and purified to an electrophoretically homogenous protein with a specific activity of 18.2 U/mg protein as described in Ref. [25]. P450 content was measured by reduced CO-difference spectroscopy [26]. Protein concentration was determined with the Coomassie Plus protein assay (Pierce, Rockford, IL, USA). P450 reductase concentration was determined spectrally using an extinction coefficient of 21.2 $mM^{-1}cm^{-1}$ (at 455 nm). Its activity was determined as NADPH-cytochrome *c* reductase activity; one unit is defined as the amount of reductase reducing 1 μmol of cytochrome *c* per min at 25 °C [27]. Reconstitution of enzymatic activity, based on the purified enzymes, CYP1A1 variant and P450 reductase, was achieved by incubation with dilaurylphosphatidylcholine. In a previous study, it was proved that the His-extension at the C terminus of CYP1A1 affected the enzymatic activity only slightly [23].

2.3. Epoxidation assay and HPLC

CYP1A1-mediated epoxidation of 7,8-diol-B[*a*]P produced the diolepoxides 1 and 2, which were separated and quantified by reversed-phase high-performance liquid chromatography (HPLC) based on previously described methods [28,29], with the exception that the following reconstituted CYP1A1 system was used. A mixture of 10 pmol purified CYP1A1, 45 pmol purified P450 reductase, and 25 μg dilaurylphosphatidylcholine was incubated for 10 min on ice followed by dilution to 200 μl with assay buffer (50 mM Tris, 100 mM NaCl, pH 7.5) and incubation with the substrate 7,8-diol-B[*a*]P (final concentration: 2 μM) and the inhibitor. Following dilution to 990 μl with buffer and preincubation for 1 min at 37 °C, the reactions were initiated by the addition of 10 μl NADPH (16.6 mg/200 μl) and conducted at 37 °C for 15 min. Hydrolysis of the diolepoxides to the tetraols, extraction of the metabolites and analysis by reversed-phase HPLC was subsequently performed as described in Refs. [24,29]. The final dimethyl sulphoxide (DMSO) content was <0.5% (v/v) during incubation, and inhibition was assessed by comparison with controls containing the corresponding amount of DMSO.

2.4. Hydroxylation of B[a]P and ethoxyresorufin O-deethylation (EROD) assay

Hydroxylation of B[*a*]P to 3-OH-B[*a*]P was detected and quantitated with fluorescence spectroscopy according to the classical AHH test (aryl hydrocarbon hydroxylation) [30]. Preparation of the reconstituted system, preincubation, and reactions were performed as described above for the epoxidation assay, except that 20 pmol of CYP1A1 were taken per assay. De-ethylation of ethoxyresorufin to resorufin was detected with fluorescence spectroscopy according to the standard EROD assay for CYP1A1 activity [31].

2.5. Data analysis

IC_{50} values (concentration causing 50% inhibition) represent the mean ± standard deviation (SD) of three separate determinations. Modes of inhibition and kinetic constants were determined by fitting the data with non-linear regression using Sigma Plot 2001 with the Enzyme Kinetics Module (Statistical Package for the Social Sciences (SPSS) Science Software, Erkrath, Germany). K_i values represent the mean ± standard error of the mean (SE) as determined by non-linear fits. Statistical significance of results was assessed using one-way ANOVA (analysis of variance) software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Inhibition of diolepoxide 2 formation by quercetin and naringin

Separation of all 7,8-diol-B[*a*]P oxidation products could easily be achieved under the conditions described. All CYP1A1 variants produced the diolepoxides 1 and 2 from 7,8-diol-B[*a*]P with the diolepoxide 2 formed at a rate that was clearly higher than that of diolepoxide 1. The ratio was approximately 2–3 and was found to be independent of the inhibitor concentration (data not shown). Though rates of formation of both products were determined, all analyses in this paper were performed only with the diolepoxide 2 product because it is both the major and ultimate carcinogenic product. In preliminary experiments, the apparent K_m value for the formation of diolepoxide 2 from 7,8-diol-B[*a*]P was determined to be 1 μM. Hence, all inhibition experiments were performed with a substrate concentration of 2 μM, two times higher than the K_m.

IC_{50} values on epoxidation of 7,8-diol-B[*a*]P were determined for all three CYP1A1 variants with quercetin and naringin. Fig. 1 shows the effect of quercetin on diolepoxide 2 formation. Quercetin markedly inhibited procarcinogen activation by all three CYP1A1

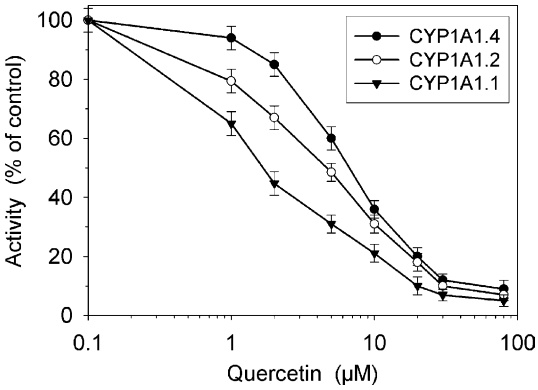

Fig. 1. Inhibition by quercetin of (±)-*trans*-7,8-dihydro-7,8-dihydroxy-B[*a*]P (7,8-diol-B[*a*]P) epoxidation activity of human CYP1A1 allelic variants in a reconstituted system consisting of purified human CYP1A1, purified human NADPH-cytochrome P450 reductase, and dilaurylphosphatidylcholine. The three allelic variants of CYP1A1 were CYP1A1.1 (wild-type), CYP1A1.2 (Ile462Val), and CYP1A1.4 (Thr461Asn). Epoxidation activities represent percent rates (control activity = 100%) of formation of (±)-B[*a*]P-r-7,*t*-8-dihydrodiol-*t*-9,10-epoxide (diolepoxide 2). Inhibition studies were performed at a substrate concentration of 2 μM, as described in the Section 2. Each point represents the average of triplicate determinations; error bars represent standard deviation (SD).

variants, but to a different extent. The action of quercetin was most effective on the wild-type enzyme CYP1A1.1 (IC_{50} = 1.6 ± 0.3 μM) and up to approximately 2–4-fold less effective on the two rarer allelic variants (Table 1). The differences in IC_{50} values between the wild-type and both CYP1A1.2 and CYP1A1.4 were statistically highly significant (P = 0.001 for CYP1A1.1 vs CYP1A1.2 and CYP1A1.1 vs CYP1A1.4), whereas the values for CYP1A1.2 and CYP1A1.4 differed less significantly (P = 0.016). By contrast, naringin inhibited diolepoxide 2 formation by the three CYP1A1 variants only slightly and at relatively high concentrations (IC_{50} values > 100 μM) (data not shown).

3.2. Inhibition kinetics

To examine the mechanism of inhibition, we performed enzyme kinetic studies with quercetin (Fig. 2(a)–(c)). Inhibition parameters are shown in Table 1. Quercetin showed mixed-type inhibition of all variants with K_i values of 2.0, 6.4, and 9.3 μM for CYP1A1.1, CYP1A1.2, and CYP1A1.4, respectively. As for the IC_{50} values, K_i values for CYP1A1.1 and CYP1A1.2 inhibition were statistically different (P = 0.004), and also for CYP1A1.1 and CYP1A1.4 (P = 0.039), whereas those for CYP1A1.2 and CYP1A1.4 were not significantly different (P = 0.306).

3.3. B[a]P hydroxylation and EROD assay

In accordance with our earlier findings for wild-type CYP1A1.1 [13], quercetin did not significantly inhibit

Table 1
IC_{50} values and inhibition parameters for quercetin on (±)-*trans*-7,8-dihydro-7,8-dihydroxy-B[*a*]P (7,8-diol-B[*a*]P) epoxidation and ethoxyresorufin deethylation (EROD) activity of human CYP1A1 allelic variants

	7,8-diol-B[*a*]P epoxidation[a]			EROD IC_{50} (μM)[e]
	IC_{50} (μM)[b]	K_i (μM)[c]	Type of inhibition[d]	
CYP1A1.1	1.6 ± 0.3	2.0 ± 0.4	Mixed-type	0.2 ± 0.1
CYP1A1.2	4.4 ± 0.5	6.4 ± 0.6	Mixed-type	0.3 ± 0.1
CYP1A1.4	7.0 ± 1.0	9.3 ± 2.4	Mixed-type	0.2 ± 0.1

[a] Epoxidation activities (formation of (±)-B[*a*]P-r-7,*t*-8-dihydrodiol-*t*-9,10-epoxide, diolepoxide 2) were determined in reconstituted CYP1A1 systems consisting of the respective purified CYP1A1 variant, purified NADPH-cytochrome P450 reductase, and dilaurylphosphatidylcholine. The cytochrome P450 concentration for determination of activities was 10 nM in all cases. Formation rates of diolepoxide 2 were determined using high performance liquid chromotography (HPLC), as described in the Section 2.

[b] Inhibition experiments to determine IC_{50} (concentration causing 50% inhibition) values were performed at a substrate concentration of 2 μM; data represent the mean ± standard deviation (SD) of three separate determinations.

[c] Enzyme kinetics studies were performed for quercetin concentrations between 0 and 10 μM and substrate concentrations of 0, 0.5, 1, 2, 4, and 5 μM; K_i data represent the mean ± standard error of the mean (SE) as determined by non-linear fits using Sigma Plot-Enzyme Kinetics software.

[d] For all variants, the fits based on non-linear regression analysis for mixed-type inhibition were better than those for non-competitive, although only slightly. However, they were significantly better than those based on both competitive or uncompetitive inhibition. Therefore, based on the present data an unambiguous decision cannot be made as to whether inhibition is of the mixed-type or non-competitive. Nevertheless, the K_i values determined for both models were not significantly different.

[e] Inhibition experiments of EROD activity to determine IC_{50} values were performed at a substrate concentration of 0.5 μM; data represent the mean ± SD of three separate determinations.

B[*a*]P 3-hydroxylation mediated by the two rare allelic variants in the standard AHH assay ($IC_{50} \geqslant 100$ μM). On the other hand, quercetin exhibited potent inhibition of all three CYP1A1 variants in the EROD assay with IC_{50} values of approximately 0.2 μM (Table 1), supporting our previous report on wild-type CYP1A1 [13]. However, contrary to data for inhibition of 7,8-diol-B[*a*]P, IC_{50} values for inhibition of EROD activity by CYP1A1.1, CYP1A1.2, and CYP1A1.4 were not statistically different ($P > 0.05$) and therefore an inhibition kinetic study was not performed.

4. Discussion

We used the 7,8-diol-B[*a*]P epoxidation reaction to assess CYP1A1 inhibition of procarcinogen activation because B[*a*]P belongs to the major class of tobacco carcinogens, the PAHs, with tobacco smoking being the major cause of lung cancer. Moreover, B[*a*]P is an ubiquitous environmental pollutant to which non-smoking

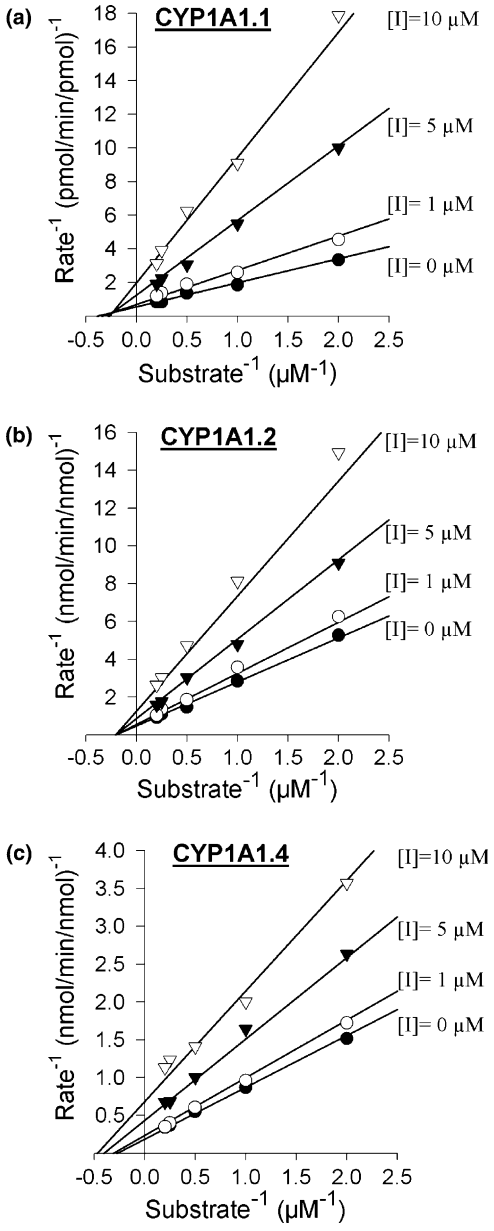

Fig. 2. Lineweaver–Burk plots of inhibition of 7,8-diol-B[*a*] P epoxidation activity (formation of diolepoxide 2) by quercetin. Enzyme kinetics were performed for quercetin concentrations between 0 and 10 µM and substrate concentrations of 0, 0.5, 1, 2, 4, and 5 µM. Each point represents the average of two separate determinations. Conditions and analysis were the same as described in the legend of Table 1: (a) CYP1A.1; (b) CYP1A1.2; (c) CYP1A1.4.

humans are also exposed (present in car exhaust, charcoaled meat, coke oven plants etc.). The epoxidation reaction used is the terminal step in the process of bioactivation of PAHs, particularly B[*a*]P, to the ultimate carcinogenic product, the diolepoxides 2, which can bind to DNA and initiate tumorigenesis [32]. Moreover, B[*a*]P diolepoxide 2 is the most frequently studied carcinogenic PAH diolepoxide and therefore it is a relevant substrate to obtain insights into the mechanisms of lung-cancer-related inhibition of carcinogen activation.

We demonstrated that inhibition by quercetin of CYP1A1-mediated activation of the procarcinogen B[*a*]P is dependent on the CYP1A1 genotype. The 7,8-diol-B[*a*]P epoxidation activity of wild-type CYP1A1 is significantly more potently inhibited than that of the variants CYP1A1.2 and CYP1A1.4. Because these inherited differences in CYP1A1 inhibition hold the potential to dictate carcinogen exposure levels for life, we suggest that this mechanism could mean that carriers of the alleles *CYP1A1*2* and *CYP1A1*4* may be at a higher risk of developing lung cancer. Though the concept of differential sensitivity of P450 mutants to inhibitors is not a new one [33], to our knowledge, this is the first study reporting a selective inhibition of naturally occuring allelic variants of a human P450 enzyme with possible relevance to individual disease risk. Our data support the recent hypothesis that intake of food rich in quercetin may protect against certain types of lung cancer specifically for subjects carrying the wild-type *CYP1A1* allele (*CYP1A1*1*), i.e. via a genotype-dependent inhibition of the mechanism of carcinogen activation [4]. They also may contribute to our understanding of the association between the *CYP1A1*2* genotype and an increased risk of lung cancer, based on several recent pooled analyses of case-control studies e.g. [34,35]. Future studies of individual susceptibility to lung cancer should consider the influence of genetic P450 enzyme variants on both procarcinogen activation and its inhibition to facilitate the development of genotype-specific chemoprevention regimes.

Inhibition kinetics revealed mixed-type inhibition by quercetin for all of the CYP1A1 variants indicating that this compound can compete for substrate binding at the active site and may also bind to a region that does not participate directly in substrate binding. Quercetin was a potent inhibitor exhibiting 3.2-fold (4.7-fold) greater selectivity for CYP1A1.1 over CYP1A1.2 (CYP1A1.4). Because the inhibition of CYP1A1 activity is partly competitive, the binding environment of the CYP1A1.1 active site might have a preference for quercetin compared with that of the mutant CYP1A1s. However, while alteration of the active site structure as a consequence of mutations cannot be excluded, we speculate that quercetin differentially affects the interaction between the CYP1A1 variants and P450 reductase. Such a hypothesis is supported by the putative location of the mutations within the 3-dimensional (3 d) structure of CYP1A1. A homology model of human CYP1A1 [36], based on the crystal structure of CYP2C5, locates the mutations Ile462Val and Thr461Asn on the proximal face of the haem group, a region generally assumed to participate in the interaction with P450 reductase [37]. However, to more precisely characterise the inhibition mechanism of quercetin, further studies, e.g. of certain partial processes of the catalytic cycle such as substrate

binding and electron transfer from P450 reductase to CYP1A1, are necessary.

P450 reductase transfers electrons from NADPH to CYP1A1 and, in some cases, inhibition by chemicals may be caused by the blocking of electron transfer by P450 reductase inhibition. Indeed, in a previous study, we found that quercetin affected the cytochrome *c* reduction activity of P450 reductase; quercetin demonstrated 50%-inhibition at approximately 20 μM [24]. Hence, quercetin probably suppresses monooxygenase activity, at least in part, by inhibiting the reduction of CYP1A1. However, at any given concentration of quercetin, epoxidation activity is inhibited to a much greater extent than is cytochrome *c* reductase activity, and therefore the general conclusions of the present paper are maintained.

A good inhibitor of CYP1A1-mediated carcinogen activation should effectively inhibit epoxidation of 7,8-diol-B[*a*]P, but should not affect the hydroxylation of B[*a*]P leading to the detoxification pathway. We demonstrated that quercetin exhibited only a negligible effect towards CYP1A1-mediated B[*a*]P hydroxylation activity in the lower μM-range. Moreover, this observation is important because 3-OH-B[*a*]P, among the 12 isomeric phenols of B[*a*]P, was previously found to be the most potent antagonist of diolepoxide 2 mutagenity, possibly by inhibiting 7,8-diol-B[*a*]P activation [38].

Are the concentrations of quercetin and naringin achieved in human diets high enough to be effective *in vivo*? Quercetin is one of the most abundant flavonoids in fruits, vegetables, tea and wine, with principal sources being onions, apples, tea, and therefore readily available in the daily diet [39]. Although pharmacokinetic data for human subjects are scarce, several recent studies showed that quercetin could be readily absorbed in human subjects and can reach micromolar concentrations in the plasma and urine [40–42]. Moreover, quercetin was eliminated slowly from the blood (elimination half-life of approximately 24 h), suggesting that repeated intake would lead to a build-up of quercetin to even higher levels [43]. Thus, these plasma concentrations are roughly in the range of the IC_{50} values of quercetin for inhibition of diolepoxide formation by CYP1A1 reported herein.

When discussing biological activities, the absorption and metabolism of quercetin in humans is an important aspect that must be taken into account. Previous work has indicated that quercetin can be absorbed as pure quercetin and as glycoside – the form in which quercetin is mainly present in the diet [40,42]. The first step in the metabolism of quercetin glycosides is deglycosidation by the small intestine. Although some studies suggested that different metabolic routes compete for further quercetin metabolism, including methylation, sulphation, glucuronidation and oxidative degradation, glucuronide conjugation seems to be a major reaction (reviewed in Ref. [44]). Formation of conjugates can dramatically alter the biological activities of metabolites; however, there are only few studies reporting such effects and/or further hepatic metabolism of the conjugates [45]. While it was reported that quercetin glucuronide may possess a preventive effect for cardiovascular diseases [46], to date, the question of whether quercetin metabolites might inhibit CYP1 enzymes and in this way, contribute to anticarcinogenic effects has not been addressed.

Taken together, oral dietary quercetin can be absorbed and reach tissues and plasma where inhibiting concentrations could be reached, supporting our hypothesis that quercetin may be involved in the prevention of lung cancer, by reducing the formation of carcinogens through inhibition of enzymes, such as CYP1A1. Our *in vitro* data indicate reduction by quercetin of CYP1A1-mediated carcinogen activation as a genotype-dependent process that is particularly favourable for subjects carrying the wild-type *CYP1A1* allele. However, additional studies will be needed to determine whether quercetin and its metabolites – or other natural polyphenols – can influence carcinogen-activating CYP1A1 activity *in vivo*.

Conflict of Interest Statement

None declared.

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