Minireview

Herbal bioactivation: The good, the bad and the ugly

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Abstract

It has been well established that the formation of reactive metabolites of drugs is associated with drug toxicity. Similarly, there are accumulating data suggesting the role of the formation of reactive metabolites/intermediates through bioactivation in herbal toxicity and carcinogenicity. It has been hypothesized that the resultant reactive metabolites following herbal bioactivation covalently bind to cellular proteins and DNA, leading to toxicity via multiple mechanisms such as direct cytotoxicity, oncogene activation, and hypersensitivity reactions. This is exemplified by aristolochic acids present in *Aristolochia* spp, undergoing reduction of the nitro group by hepatic cytochrome P450 (CYP1A1/2) or peroxidases in extrahepatic tissues to reactive cyclic nitrenium ion. The latter was capable of reacting with DNA and proteins, resulting in activation of *H-ras* oncogene, gene mutation and finally carcinogenesis. Other examples are pulegone present in essential oils from many mint species; and teucrin A, a diterpenoid found in germander (*Teuchrium chamaedrys*) used as an adjuvant to slimming diets. Extensive pulegone metabolism generated *p*-cresol that was a glutathione depletory, and the furan ring of the diterpenoids in germander was oxidized by CYP3A4 to reactive epoxide which reacts with proteins such as CYP3A and epoxide hydrolase. On the other hand, some herbal/dietary constituents were shown to form reactive intermediates capable of irreversibly inhibiting various CYPs. The resultant metabolites lead to CYP inactivation by chemical modification of the heme, the apoprotein, or both as a result of covalent binding of modified heme to the apoprotein. Some examples include bergamottin, a furanocoumarin of grapefruit juice; capsaicin from chili peppers; glabridin, an isoflavan from licorice root; isothiocyanates found in all cruciferous vegetables; oleuropein rich in olive oil; dially sulfone found in garlic; and resveratrol, a constituent of red wine. CYPs have been known to metabolize more than 95% therapeutic drugs and activate a number of procarcinogens as well. Therefore,

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mechanism-based inhibition of CYPs may provide an explanation for some reported herb-drug interactions and chemopreventive activity of herbs. Due to the wide use and easy availability of herbal medicines, there is increasing concern about herbal toxicity. The safety and quality of herbal medicine should be ensured through greater research, pharmacovigilance, greater regulatory control and better communication between patients and health professionals.

Keywords: Herbal medicine; Toxicity; Metabolism; Bioactivation

Introduction

There is resurgence in the use of herbal medicines worldwide. An estimated one third of adults in the Western world use alternative therapies, including herbs. These herbs may be used either in their primary forms or combined into mixtures. In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines. However, problems may arise due to intrinsic toxicity, adulteration, substitution, contamination, misidentification, drug-herb interactions, and lack of standardization (de Smet, 1995; Ernst, 2002; Ernst and Pittler, 2002; Koh and Woo, 2000). Both adverse drug reactions and poisonings associated with the use of herbal medicines have increasingly been reported (Deng, 2002; Ernst and Pittler, 2002; Klepser and Klepser, 1999). Herbal use has been associated with organ toxicities of heart, liver, blood, kidneys, central nervous system, and skin and carcinogenesis (Bensoussan et al., 2002; Ernst, 2000; Greensfelder, 2000; Haller and Benowitz, 2000; Kessler, 2000; McRae et al., 2002; Stedman, 2002; Villegas et al., 2001; Zaacks et al., 1999).

The human cytochrome P450 (CYP) (EC 1.14.14.1) superfamily, containing 57 genes (Nelson, 2003), contributes to the metabolism of a variety of xenobiotics including therapeutic drugs, carcinogens, steroids and eicosanoids (Gonzalez, 1990; Nebert and Russell, 2002; Nelson et al., 1996; Rendic, 2002; Rendic and Di Carlo, 1997). The resultant increases in polarity usually facilitate excretion and are considered to be a detoxification process, but in some instances, foreign compounds are converted to products with much greater cytotoxicity, mutagenicity, or carcinogenicity (Guengerich and Liebler, 1985; Guengerich and Shimada, 1991; Zhou, 2003). Metabolic activation of procarcinogens is often catalyzed by a limited number of human CYPs including CYP1A1, 1A2, 1B1, 2A6, 2B6 and 3A4 (Guengerich, 2001; Guengerich and Liebler, 1985; Guengerich and Shimada, 1991; Zhou, 2003). Among them, CYP1A and 1B enzymes bioactivate polycyclic arylamines, polyaromatic hydrocarbons and aflatoxin B1 (Gonzalez and Gelboin, 1994). These enzymes are regarded as the targets for blocking tumor initiation and specific CYP inhibitors or inactivators could be beneficial for preventing tumor formation (Guengerich and Shimada, 1991; Shimada et al., 1996; Shimada et al., 1989). It is likely that constituents in herbal preparations may be substrates, inhibitors, or inducers of CYPs (Zhou et al., 2003), and have an impact on the pharmacokinetics of any coadministered drugs metabolized by this system.

Herbal/dietary constituents may be metabolized by CYPs to nontoxic metabolites and excreted, but the formation of toxic metabolites is possible (Yang et al., 1992). In addition, the inhibition of CYPs by herbal constituents may decrease the formation of toxic metabolites and thus inhibit carcinogenesis, as CYPs play an important role in procarcinogen activation. In some cases, the formation of a reactive intermediate by CYP may also lead to the inactivation of the enzyme. CYP substrates, which are

metabolized to reactive intermediates that inactivate the enzyme, are classified as mechanism-based inhibitors. Thus, the inhibition of CYP-mediated carcinogen activation by herbal/dietary constituents has been extensively studied.

The mechanism for herbal toxicity remains elusive, but there are accumulating data suggesting the role of the formation of reactive metabolites/intermediates through the bioactivation of major herbal constituents in herbal toxicity and carcinogenicity. It has been hypothesized that the resultant reactive metabolites following herbal bioactivation covalently bind to cellular proteins and DNA, leading to toxicity via multiple mechanisms such as direct cytotoxicity, oncogene activation, and hypersensitivity reactions. This review highlights the role of formation of reactive intermediates via bioactivation in herbal toxicity using aristolochic acids, pulegone and germander as examples. In addition, mechanism-based inhibition of CYPs by many herbal constituents and the clinical and toxicological relevance are discussed.

Herbal bioactivation: Formation of toxic metabolites

Aristolochic acid nephropathy

Aristolochic acids (AAs), a family of structurally related nitrophenanthrene carboxylic acids, are primarily from herbal medicines such as *Aristolochia* spp. (e.g. *A. fangchi* (Guang Fangji), *A. clematits*, and *A. manshuriensis*) (Ioset et al., 2003). The predominant AAs are AAI (8-methoxy-6-nitro-phenanthro-(3,4-*d*)-1,3-dioxolo-5-carboxylic acid) and AAII (6-nitro-phenanthro-(3,4-*d*)-1,3-dioxolo-5-carboxylic acid). The consumption of herbs containing AAs has been associated with severe nephropathy which is characterized by unique renal fibrosis and development of urothelial cancer (Abt et al., 1995; Arlt et al., 2002; Cosyns, 2003; Lord et al., 2001; Nortier and Vanherweghem, 2002; Schwetz, 2001; Zhu, 2002). Approximately 200 cases of AA nephropathy have been reported worldwide, with many of them needing renal replacement therapy (Arlt et al., 2002). A large cohort of these patients with AA nephropathy have developed urothelial cancer (Cosyns, 2003; Nortier and Vanherweghem, 2002).

As alkaloids, both AAI and AAII underwent reduction of the nitro group catalyzed by oxidative enzymes to reactive cyclic nitrenium ions (Fig. 1) (Schmeiser et al., 1997; Stiborova et al., 2001a,b; Stiborova et al., 2002). Hepatic microsomal CYP1A1/2, NADPH:CYP reductase, DT-diaphorase, cyclooxydase-1 and other peroxidases have been found to catalyze the reaction. Addition of inhibitors or inducers of CYP1A1/2 was found to decrease or increase the formation of DNA adducts (Stiborova et al., 2001a,b). The resultant reactive cyclic nitrenium ions are capable of reacting with DNA and/or proteins, leading to adduct formation. For example, the DNA adducts (e.g. 7-(deoxyadenosin-N^6-yl)aristolactam I or II and 7(deoxyguanosin-N^2-yl)aristolactam I or II) have been detected in kidney and ureter tissues of patients taking herbs containing AAs, several months or even years after cessation of the herbal consumption (Pfau et al., 1990; Schmeiser et al., 1996; Stiborova et al., 1999). The former was the most predominant DNA adduct in human and rat tissues (Pfau et al., 1990; Schmeiser et al., 1996; Stiborova et al., 1999).

Studies in the mouse and rat indicated that *H-ras* protooncogenes were activated with high incidence of an AT → TA transversion mutation in codon 61 of DNA from AAI-induced tumors (Schmeiser et al., 1990; Schmeiser et al., 1991). This is consistent with the identification of an N6-deoxyadenosine-AAI
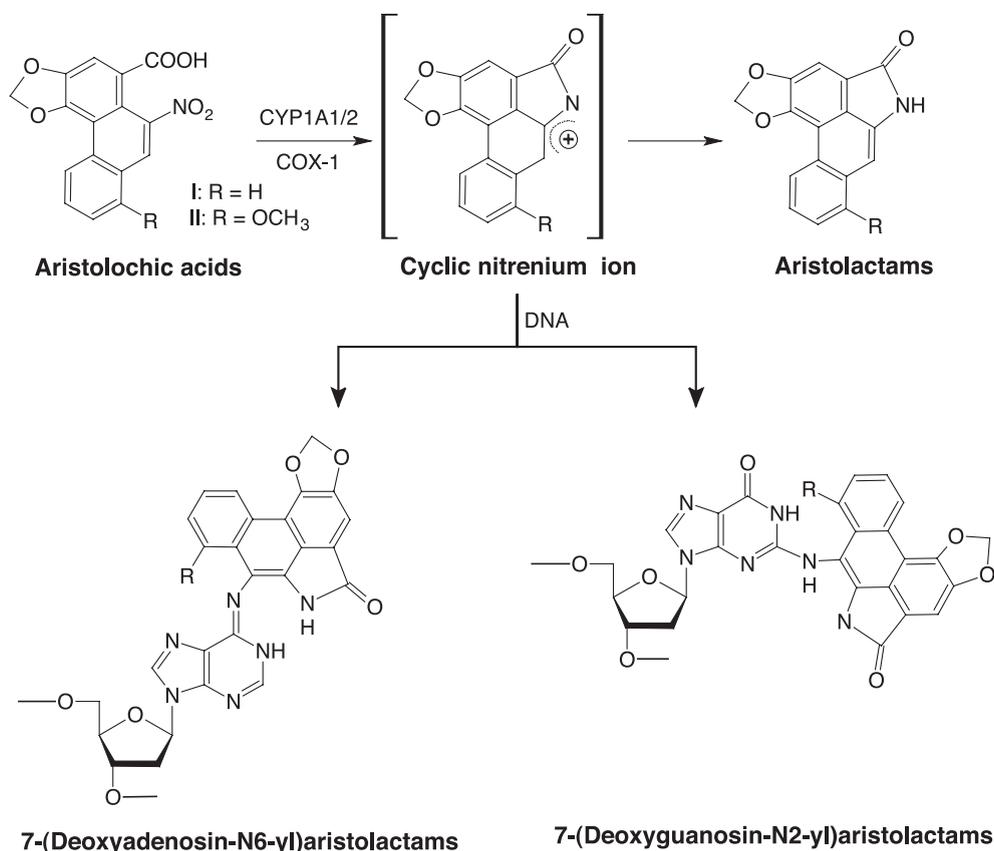
Fig. 1. Proposed bioactivation of aristolochic acids.

adduct formed in vitro and in patients. All these results indicated that deoxyadenosine adduct formation was a critical step in tumor initiation by AAs. A recent study using the λ/lacZ transgenic mice has shown that administration of AAs caused selective AT → TA mutations (Kohara et al., 2002), which is in agreement with the extensive 7-(deoxyadenosin-N^6-yl)aristolactam I adduct formation in rats and humans. In rats treated with a single dose of AAI, the 7(deoxyguanosin-N^2-yl)aristolactam I adduct was removed continuously from the DNA, whereas the 7-(deoxyadenosin-N^6-yl)aristolactam I adduct persisted without proper repair of the DNA (Fernando et al., 1993), leading to mutations and oncogene activation.

Pulegone toxicity

Pulegone is a monoterpene ketone present in essential oils from many mint species (e.g. *Hedeoma pulegoides* and *Mentha pulegium*, both called pennyroyal) (Budavari, 1996; Grundschober, 1979). Pennyroyal oil has been used as a flavoring agent in foods and beverages, a fragrant constituent and a flea repellent (Hall and Oser, 1965). Pennyroyal has also been used to induce menstruation and abortion. However, pennyroyal oil, especially at high doses, has been reported to cause hepatic failure, central nervous system toxicity, gastritis, renal and pulmonary toxicity, and death (Anderson et al., 1996).

Pulegone, constituting >80% of the terpenes in pennyroyal oils, was found to be hepatotoxic and pneumotoxic in mice (Gordon et al., 1982).

The metabolites of pulegone have been considered to be responsible for its toxicity (Gordon et al., 1987). Both menthofuran and *p*-cresol were potential candidates. Menthofuran is a naturally occurring hepatotoxin (Thomassen et al., 1992) and *p*-cresol is a known glutathione depletory and toxin (Thompson et al., 1994). In vitro studies have demonstrated CYP-mediated conversion of pulegone to menthofuran which was then bioactivated to reactive γ-ketoenal (Gordon et al., 1987; Thomassen et al., 1991). γ-Ketoenal was capable of covalently binding to cellular proteins and caused hepatic injury (Thomassen et al., 1992). The γ-ketoenal can be converted to mintlactones, as well as other reactive intermediates in CYP-mediated oxidation of menthofuran.

The in vivo metabolism of pulegone is very complicated, with about 14 Phase I and 10 Phase II metabolites being identified and characterized in rats (Chen et al., 2001; Madyastha and Raj, 1993; McClanahan et al., 1989; Moorthy et al., 1989; Thomassen et al., 1991). These metabolites accounted for only 3% of total radioactivity excreted in bile, and their structures could not be established solely by mass spectral analysis. The metabolism of pulegone in rats involves three major metabolic pathways: a) conjugation by glucuronic acid following C-5 or methyl group hydroxylation or by GSH; with the conjugates being further metabolized; b) the formation of menthofuran; and c) the formation of piperitenone beginning with 5-hydroxylation followed by dehydration (Fig. 2) (Chen et al., 2001; Madyastha and Gaikwad, 1999; Madyastha and Raj, 1992; Madyastha and Raj, 1993). The CYP system plays an important role in the metabolism of *R*-(+)-pulegone (Madyastha and Gaikwad, 1999; Madyastha and Raj, 1992). Most of the metabolites of pulegone are derived from menthofuran and piperitenone (Madyastha and Gaikwad, 1999; Madyastha and Raj, 1992; Madyastha and Raj, 1993). Studies carried out in vivo have shown 4-methyl-2-cyclohexenone as one of the metabolites of *R*-(+)-pulegone (Madyastha and Raj, 1993), and conversion of 4-methyl-2-cyclohexenone to *p*-cresol has been demonstrated in vitro (Madyastha and Raj, 1990; Madyastha and Raj, 1993; Madyastha and Raj, 2002).

Germander hepatotoxicity

Germander (*Teuchrium chamaedrys*), a diterpenoid-containing herbal medicine, was traditionally used as a folk medicine for its alleged choleretic and antiseptic effects. In 1991, germander teas or capsules were promoted as an adjuvant to slimming diets. However, more than 30 cases of hepatotoxicity (mainly cytolytic hepatitis, chronic hepatitis or cirrhosis) after consumption of germander have been reported mainly in France, including cases with positive rechallenge (Ben Yahia et al., 1993; Castot and Larrey, 1992; Dao et al., 1993; Laliberte and Villeneuve, 1996; Larrey et al., 1992; Mattei et al., 1992), and a fatality due to fulminant hepatic necrosis (Mostefa-Kara et al., 1992). This medicinal plant has been prohibited in France since 1992.

Germander caused hepatotoxicity in animals (Kouzi et al., 1994; Loeper et al., 1994) and humans (Laliberte and Villeneuve, 1996). Germander contains saponins, glycosides, flavonoids and furano neoclerodane diterpenoids (Piozzi et al., 1987). The hepatotoxicity has been ascribed to the diterpenoids including teucrin A and teuchamaedryn A. The furan ring of these diterpenoids is oxidized by CYP3A4 to reactive epoxide which reacts with proteins such as CYP3A and epoxide hydrolase (Fig. 3) (Fau et al., 1997; Lekehal et al., 1996; Loeper et al., 2001; Loeper et al., 1994). The reactive epoxide led to mitochondrial permeability transition, caspase activation, and apoptosis in rat and mouse hepatocytes (Fau et al., 1997; Lekehal et al., 1996; Loeper et al., 1994). Teucrin A covalently bound to rat hepatocyte
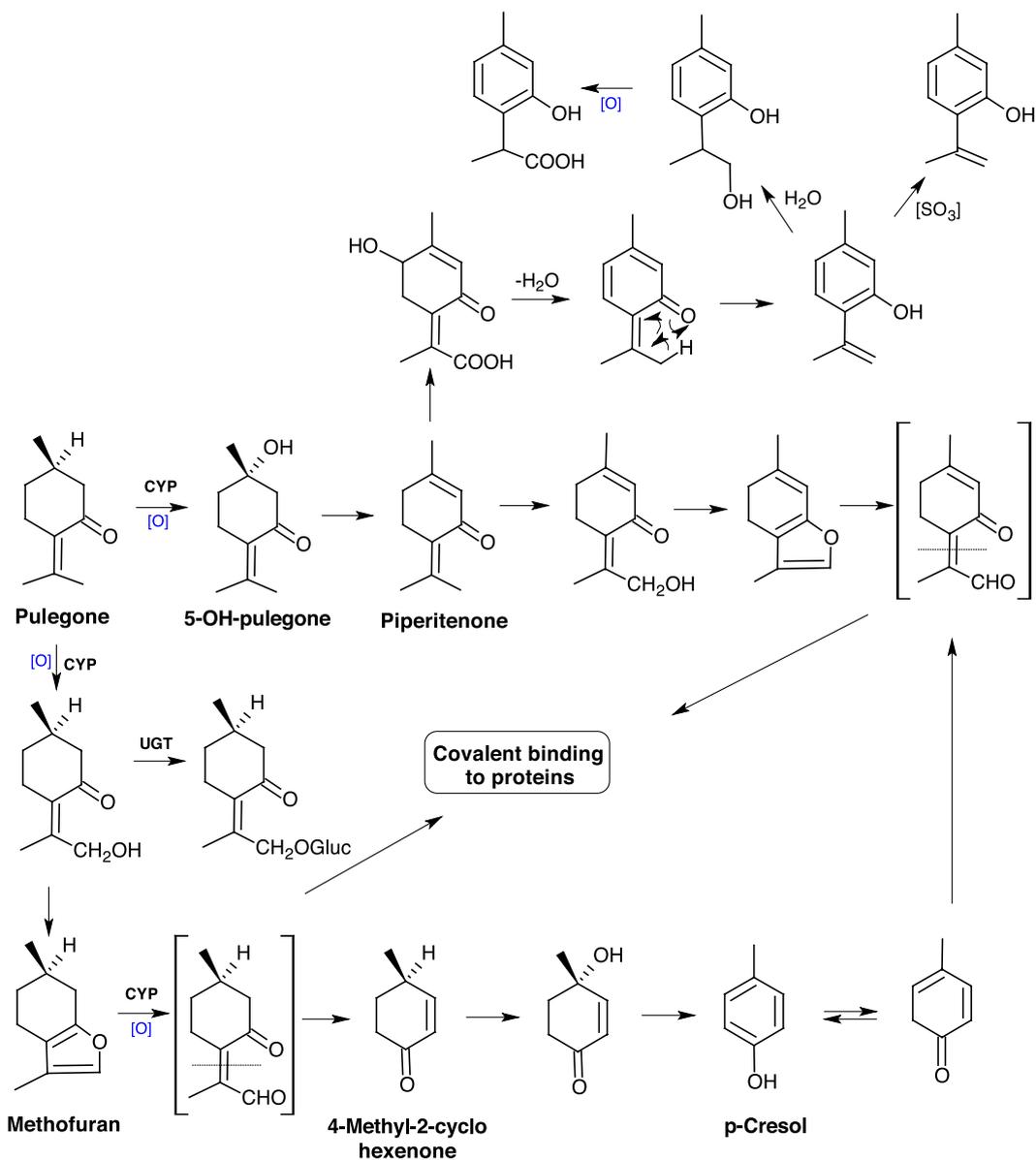
Fig. 2. Proposed bioactivation of pulegone.

proteins, and the furano diterpenoid fraction decreased cell GSH and cytoskeleton-associated protein thiols, but increased cytosolic levels of Ca^{2+} (Fau et al., 1997; Lekehal et al., 1996). All these events led to the formation of plasma membrane blebs, DNA fragmentation, and cell apoptosis.

The diterpenoid-mediated cytotoxicity was modulated by many factors such as CYP3A inducers or inhibitors. Pretreatment of male rats with troleandomycin (an inhibitor of CYP3A) reduced cellular GSH depletion and cytotoxicity, whereas dexamethasone treatment (an inducer of CYP3A) had opposite effects (Lekehal et al., 1996). Feeding male rats with a sulfur amino acid-deficient diet decreased cellular
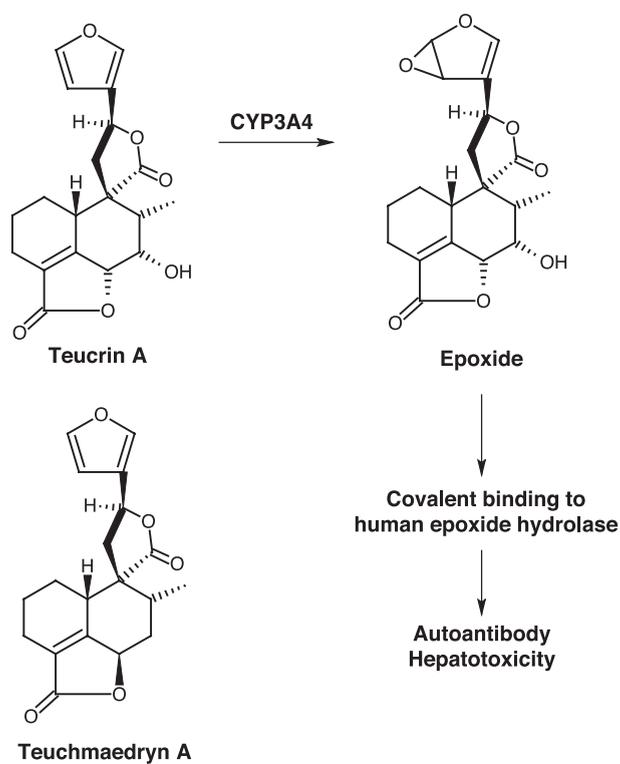
Fig. 3. Proposed bioactivation of teucrin A from germander.

GSH level and enhanced cytotoxicity, whereas supplementation of the standard diet with cystine had opposite effects (Lekehal et al., 1996). Female rat hepatocytes exhibited little toxicity as they expressed low levels of CYP3A, unless the animals were treated with dexamethasone (Lekehal et al., 1996). Furthermore, the hepatotoxicity in mice was attenuated by pretreatment with butylated hydroxyanisole or clofibrate (two inducers of microsomal epoxide hydrolase) and markedly increased by phorone-induced GSH depletion (Loeper et al., 1994). Pretreatment of mice with either 3-methylcholanthrene or phenobarbital had no effect (Loeper et al., 1994). In addition, genetic factors may be important for the development of germander-induced hepatotoxicity. For example, polymorphism of CYP3A and major histocompability complex molecule can affect the formation of toxic metabolite and antigen presenting to T helper cells, respectively.

Epoxide hydrolase is a plasma membrane-located Phase II microsomal enzyme. It is catalytically competent and may participate in the inactivation of reactive epoxides arising from teucrin A. Thus, it may act as target for reactive metabolites from teucrin A. When incubated with teucrin A, epoxide hydrolase was inactivated in a time-dependent manner, suggesting that a reactive oxide derived from teucrin A could alkylate and inactivate the enzyme (De Berardinis et al., 2000). Interestingly, anti-microsomal epoxide hydrolase autoantibodies were detected in the sera of patients taking germander teas for a long period of time (De Berardinis et al., 2000; Loeper et al., 2001; Polymeros et al., 2002). Dose-dependent immunoprecipitation of human epoxide hydrolase by these human sera confirmed human epoxide hydrolase as the autoantigenic target (De Berardinis et al., 2000). It

appeared that both direct toxicity and secondary immune reactions were involved in germander-induced heaptotoxicity. The covalent binding of epoxide hydrolase on the outer surface of human hepatocytes by teucrin A might trigger immune responses and induced the formation of autoanti-body, leading to cell dealth.

Mechanism-based herbal inhibitors of cytochrome P450s

Many herbal constituents have been found to inhibit various CYPs (Zhou et al., 2003). The nature of inhibition may be competitive, non-competitive, or mechanism-based. The latter is characterized by NADPH-, time- and concentration-dependent enzyme inactivation, occurring when some herbal constituents are converted by CYPs to reactive metabolites that are capable of irreversibly binding to CYPs (Ortiz de Montellano and Correia, 1995; Osawa and Pohl, 1989; Silverman, 1998). Mechanism-based inhibitors require at least one cycle of the CYP catalytic process to form reactive metabolites. The resultant metabolites lead to CYP inactivation by chemical modification of the heme, the apoprotein, or both as a result of covalent binding of modified heme to the apoprotein.

Capsaicin

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a major pungent phenolic constituent present in a variety of capsicum fruits such as hot peppers, and thus represents an important ingredient of the majority of spicy foods (Park et al., 1998; Surh and Lee, 1995). It has shown a wide range of pharmacological properties, including pain-killing, antigenotoxic, antimutagenic, and anticarcinogenic effects (Surh et al., 1998; Surh and Lee, 1995). Capsaicin has been used to treat various peripheral painful conditions such as rheumatoid arthritis and diabetic neuropathy (Surh and Lee, 1995).However, capsaicin has been reported to be a tumor promoter, carcinogen and potential mutagen (Agarwal et al., 1986; Lopez-Carillo et al., 1994; Toth and Gannett, 1992; Ward and Lopez-Carrillo, 1999). It caused strand scission in calf thymus and plasmid DNA in the presence of Cu(II). This breakage is mediated by reactive oxygen species arising from capsaicin, especially the hydroxyl radical (Singh et al., 2001a,b).

Capsaicin undergoes bioactivation by CYP2E1 to reactive species (Gannett et al., 1990). The major bioactivation pathways include: a) epoxidation of the vanillyl ring moiety to produce an arene oxide; b) one-electron oxidation of the ring hydroxyl group to a phenoxy radical; and c) O-demethylation at the aromatic ring and subsequent oxidation of the resulting catechol metabolite to semiquinone and quinone derivatives (Fig. 4). The resultant reactive species are capable of binding covalently to the active site of CYP2E1 as well as DNA (Surh and Lee, 1995). The interaction with target cell DNA would trigger mutagenicity and malignant transformation. However, metabolism of capsaicinoids by CYPs may also represent a detoxification process (in contrast to bioactivation), resulting in a reduction in cytotoxicity (Reilly et al., 2003). For example, a novel dehydrogenation metabolic pathway of capsaicin yields macrocyclic, diene and imide metabolites (Reilly et al., 2003).

Furanocoumarins (bergamottin and 8-methoxypsoralen)

Bergamottin is a major furanocoumarin in grapefruit juice. It reversibly inhibited the activities of CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in human liver microsomes (He et al., 1998). It also
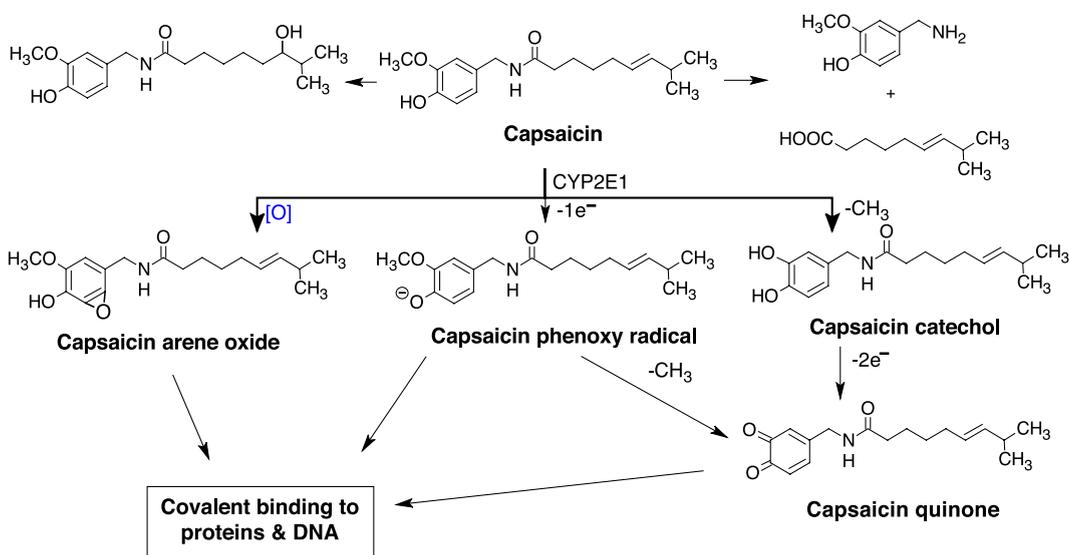
Fig. 4. Proposed bioactivation of capsaicin.

inactivated CYP3A4 following metabolic activation in a time- and concentration-dependent manner, with k_{inact} and K_I values of 0.3 min^{-1} and 7.7 μM, respectively (He et al., 1998). The loss of catalytic activity exhibited pseudo-first-order kinetics. During bergamottin-induced inactivation, CYP3A4 retained more than 90% of the heme, but 50% of the apoprotein in the inactivated CYP3A4 could not be recovered. This suggests that the inactivation may involve apoprotein modification in the active site of the enzyme instead of heme adduct formation or heme fragmentation (He et al., 1998). The reactive metabolites of bergamottin remain unknown, but it may undergo oxidation to form a reactive furanoepoxide that covalently binds to CYP3A4 (Fig. 5).

8-Methoxypsoralen is a natural furanocoumarin rich in many foodstuffs such as parsnips and parsley. It is used in combination with long wavelength ultraviolet light to treat psoriasis, vitiligo and T cell lymphoma (Anderson and Voorhees, 1980; Edelson et al., 1987; Parrish et al., 1974). The usefulness of 8-methoxypsoralen in treating these diseases resides in its ability to be photoactivated to a species capable of binding covalently to nucleic acids and lymphocytes, and thus inhibiting DNA synthesis and cellular proliferation. Early studies indicated that 8-methoxypsoralen was a potent mechanism-based inhibitor of CYPs in rodents (Fouin-Fortunet et al., 1986; Labbe et al., 1989; Letteron et al., 1986; Mays et al., 1990) and humans (Tinel et al., 1987). Further studies found that 8-methoxypsoralen was a potent mechanism-based inhibitor of CYP2A6, with K_I of 0.8– 1.9 μM and k_{inact} of 1–2 min^{-1} in a reconstituted and native liver microsomes (Koenigs et al., 1997; Koenigs and Trager, 1998a,b; Maenpaa et al., 1993). 8-Methoxypsoralen was also a potent inactivator of CYP2B1, with a K_I of 2.9 μM and k_{inact} of 0.34 min^{-1} (Koenigs and Trager, 1998a,b). However, the reactive species of 8-methoxypsoralen remains unknown, but it is suggested that 8-methoxypsoralen as a furanocoumarin undergoes CYP2A6/2B1-mediated oxidation to form a furanoepoxide (Fig. 6). The resultant epoxide is then converted to dihydrofuranocoumarin products following hydrolytic attack or direct attack by exogenous nucleophiles (Koenigs and Trager, 1998a,b).
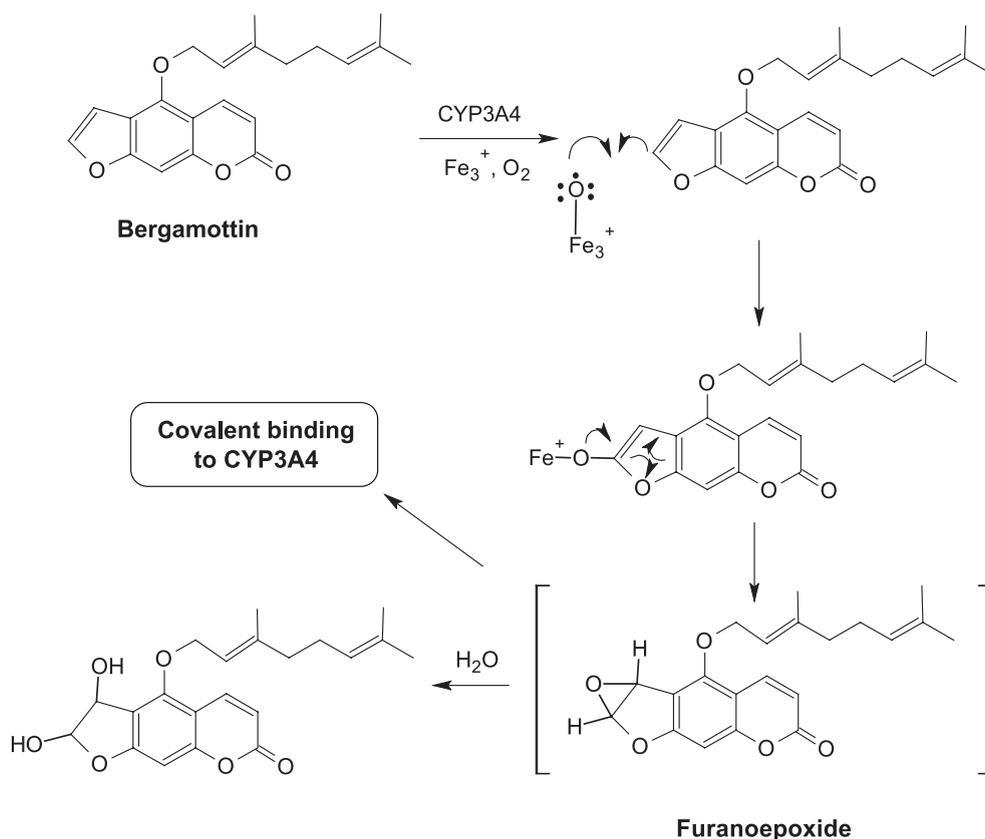
Fig. 5. Proposed bioactivation of bergamottin.

Glabridin

The dried roots of the licorice (*Glycyrrhiza glabra*) have been consumed for the past 6000 years and are used as flavoring and sweating agents, as demulcents and expectorants in the Western world and as antiallergic and antiinflammatory agents in Asian countries including Japan and China (Chandler, 1985). Licorice contains glycyrrhizin (glycyrrhizic acid, a glycoside which is 50 times sweeter than sugar), oleane triterpenoids, glucose, ammonia, polyphenols, flavonoids, and sucrose (Hatano et al., 1991a,b). Flavanoid components of licorice root have been shown to have antitumorigenic, antimicrobial, antiviral, antiinflammatory, estrogen-like, and antioxidative activity (Fujisawa et al., 2000; Fukai et al., 2002; Shibata, 2000; Tamir et al., 2001). Licorice root extract, as well as its major flavanoid, the isoflavan glabridin, are potent antioxidants against LDL oxidation in mice and humans (Rosenblat et al., 1999). Glabridin was also shown to inhibit the activity of macrophage NADPH-oxidase presumably by inhibiting protein kinase C and serotonin re-uptake (Ofir et al., 2003; Rosenblat et al., 1999). In addition, glycyrrhizin was recently found to be a potent inhibitor of the SARS-associated coronavirus in vitro (Cinatl et al., 2003). However, licorice may cause hypermineralocorticoidism (Nobata et al., 2001), arrhythmia (Bocker and Breithardt, 1991), pseudoaldosteronism (Ferrari et al., 2001), and hypertension (Astrup, 2001). These toxicities have
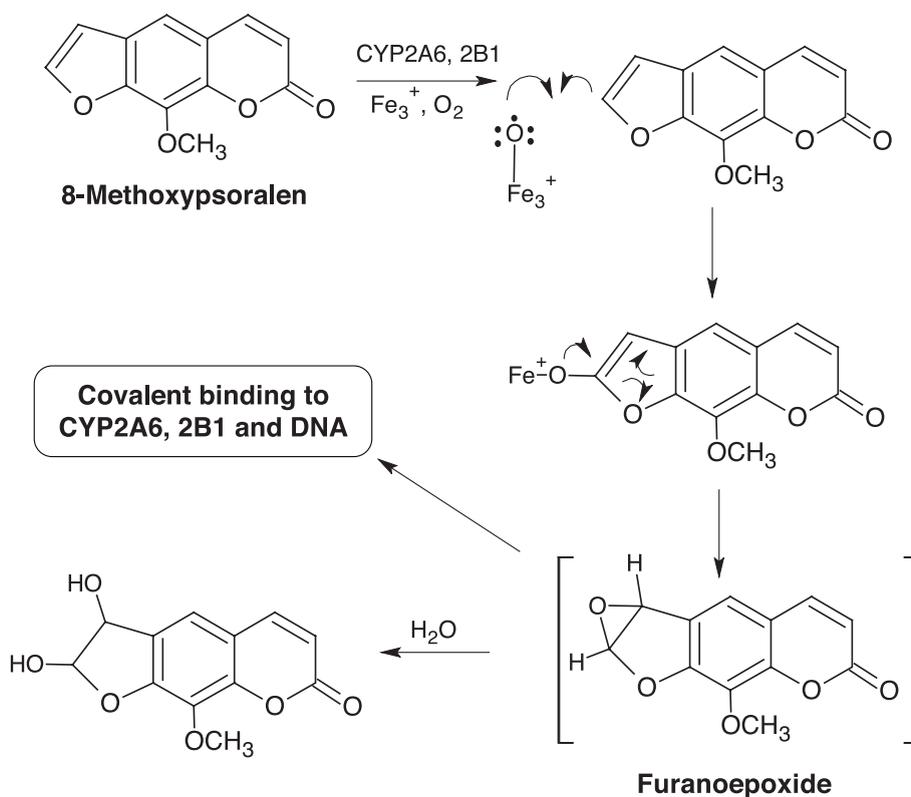
Fig. 6. Proposed bioactivation of 8-methoxypsoralen.

been ascribed to the inhibitory activity of glycyrrhizin and glycyrrhetic acid from licorice on 11-hydroxy-steroid dehydrogenase (Ferrari et al., 2001).

An in vitro study indicated that glabridin inactivated CYP3A4 and 2B6 in a time- and concentration-dependent manner, but CYP2C9 was competitively inhibited (Kent et al., 2002). Metabolism of glabridin by CYP3A4 resulted in the destruction of the heme moiety, as the loss in activity with different concentrations of glabridin correlated well with the loss in the CYP3A4-reduced carbon oxide spectrum and detectable heme at 405 nm (Kent et al., 2002). No other peaks indicative of glabridin-alkylated heme eluting after the native heme were detected at 405 nm. Presumably glabridin undergoes metabolism, forming reactive intermediates causing heme fragmentation. Incubations with 2,4-dimethylglabridin did not lead to a loss in the enzymatic activity of CYP3A4. Incubation of glabridin with CYP3A4 and NADPH resulted in a metabolites, suggesting that the two hydroxyl groups on the $2'$ and $4'$ position of the flavonoid B ring of glabridin are required for CYP3A4 inactivation (Fig. 7) (Kent et al., 2002). Indeed, these two hydroxyl groups are also believed to be essential for its antioxidative activity (Belinky et al., 1998).

Isothiocyanates

Isothiocyanates are released upon chewing or maceration of cruciferous vegetables, such as cabbage, cauliflower, and broccoli where they occur as thioglucoside conjugates called glucosinolates (Fenwick et
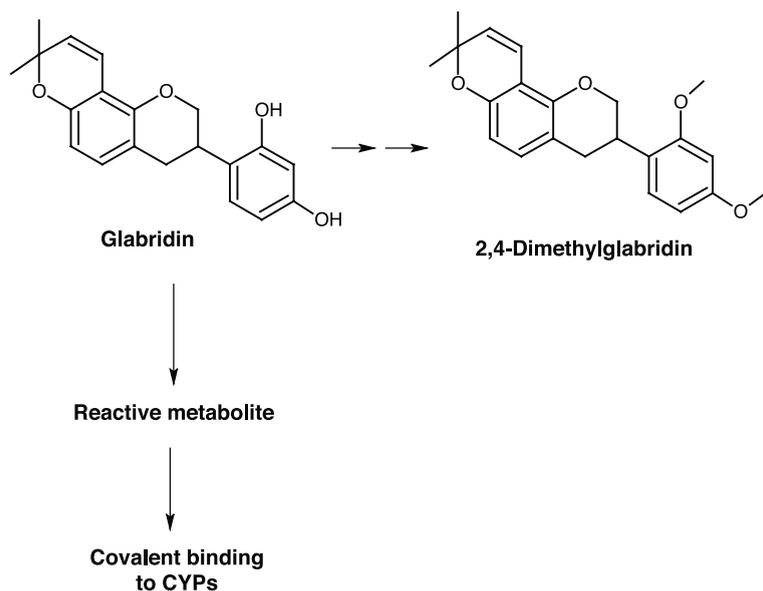
Fig. 7. Chemical structure of glabridin.

al., 1983). The enzyme myrosinase is released at the same time and hydrolyzes the glucosinolate with rearrangement of intermediates producing the isothiocyanates, hydrogen sulfate, and glucose as the major products. Typical isothiocyanates include benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC). These compounds have been shown to be potent modulators of CYPs in vitro and in vivo.

Isothiocyanates inhibited CYP1A1, 1A2, 2A1, 2B1, and 2E1 which are involved in carcinogen activation (Yang et al., 1994). BITC and PEITC have been shown to inhibit *N*-nitrosodimethylamine demethylation activity. BITC is a potent mechanism-based inactivator of rat CYP1A1, 1A2, 2B1, and 2E1, as well as human CYP2B6 and 2D6 (Goosen et al., 2000; Moreno et al., 1999). The K_I for CYP1A1, 1A2, 2B1, and 2E1 were 35, 28, 16, and 18 μM, respectively; and the corresponding k_{inact} values were 0.26, 0.09, 0.18, and 0.05 min^{-1}, respectively (Goosen et al., 2001; Moreno et al., 1999). Human CYP2C9 and rat CYP3A2 were not inactivated by BITC. BITC was also a potent inactivator of CYP2B1, as indicated by a partition ratio of approximately 11:1 (Goosen et al., 2001).

The mechanism of CYP inhibition by isothiocyanates involves both competitive and mechanims-based inhibition. The competitive inhibition involves competition with substrates at the active site of CYP (Ishizaki et al., 1990; Smith et al., 1993), while mechanism-based inhibition would involve the metabolic activation of isothiocyanates (Goosen et al., 2000; Ishizaki et al., 1990; Lee, 1996; Moreno et al., 1999; Smith et al., 1996). The metabolic activation of isothiocyanates results in reactive intermediates that could ultimately bind to the heme moiety or apoprotein, thereby inactivating the enzyme. For example, BITC was metabolized to a reactive benzyl isocyanate intermediate that is capable of binding covalently to CYP apoprotein or hydrolyzed to form benzylamine (Fig. 8) (Goosen et al., 2001). BITC may be oxidized at the α-carbon to benzaldehyde, instead of being desulfurated to benzyl isocyanate. Alternatively, benzylamine could be deaminated to give benzaldehyde and ammonia. The subsequent oxidation of benzaldehyde would yield benzoic acid. The formation of benzoic acid was also
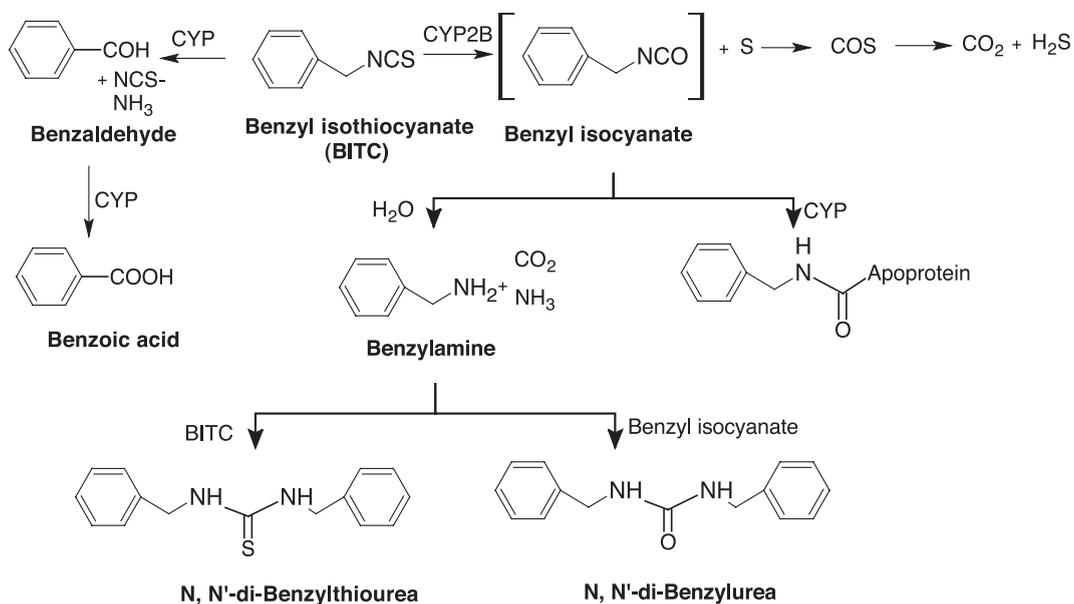
Fig. 8. Proposed bioactivation of benzyl isothiocyanate (BITC).

observed in dogs, where administration of BITC resulted in the excretion of hippuric acid, the glycine conjugate of benzoic acid (Brüsewitz et al., 1997). In humans and rats, BITC is metabolized through conjugation with GSH and finally excreted as mercapturic acid. In addition, oxidative desulfuration of the isothiocyanate could also release elemental sulfur, which could further contribute to enzymatic modification and possibly inactivation, as in the case of parathion (Butler and Murray, 1993).

The in vivo administration of isothiocyanates to rodents also modulate CYP content and activity (Guo et al., 1992; Smith et al., 1993). The effects appeared to depend on the experimental conditions, the isothiocyanate used and treatment regimen, the target tissue, and the specific CYP evaluated (Zhang and Talalay, 1994). For example, acute administration of PEITC to rats decreased liver CYP2E1 activity, whereas CYP2B1 activity and content increased approximately 10-fold, but without any significant effect on lung CYP1A2 and 2B1 activities (Guo et al., 1992). However, chronic administration of PEITC induced both CYP2B1 and 2E1 with related increases in carcinogenesis (Smith et al., 1993).

Kavalactones (methysticin and dihydromethysticin)

Kava (*Piper methysticum*, found in Polynesia, Melanesia, and Micronesia) is an effective herbal medicine for the therapy of anxiety and insomnia (Pittler and Ernst, 2000; Rouse, 1998; Singh and Singh, 2002; Volz and Kieser, 1997). Clinical studies have shown that kava and kavalactones are effective in the treatment of anxiety at subclinical and clinical levels, anxiety associated with menopause and various other medical conditions (Bilia et al., 2002; Pittler and Ernst, 2000; Stevinson et al., 2002; Volz and Kieser, 1997; Wheatley, 2001) The major constituents of kava are pharmacologically active kavalactones which are responsible for about 95% of the total activity of kava (Singh and Singh, 2002). Yangonin, desmethoxyyangonin, methysticin, 7,8-dihydromethysticin, kawain, and

7,8-dihydrokawain are present in the highest levels of kavalactones, accounting for approximately 96% of lipidic extracts (Lebot and Lévesque, 1989).

Both methysticin and dihydromethysticin (Fig. 9) were mechanism-based inhibitors of CYPs, as indicated by the unidentified metabolic intermediate complexes at 455 nm when incubation with human liver microsomes (Mathews et al., 2002). This 455-nm absorbing complex was both NADPH- and time-dependent, with absorption increasing with increasing incubation time. Methysticin analogs in kava extract have been shown to inhibit multiple CYPs after metabolic activation through the formation of metabolic intermediate complexes (Murray and Reidy, 1989; Murray et al., 1983). Preincubation of whole kava extract with human liver microsomes inhibited CYP1A2, 2C9, 2C19, 2D6, 3A4, and 4A9/ 11; whereas CYP2A6, 2C8, and 2E1 were unaffected (Mathews et al., 2002). Various kavalactones inhibited different CYPs. Kava extract and kavalactones inhibited CYP3A4 in cDNA-expressed microsomes (Unger et al., 2002).

Oleuropein

The Mediterranean diet has been associated with longevity and low rates of coronary artery disease and cancers despite its high dietary fat content (Braga et al., 1998; Owen et al., 2000; Roche et al., 1998). The Mediterranean diet includes the consumption of large amounts of olive oil. Olive oil is a source of at least 30 phenolic compounds (total: 60–1000 mg/kg) (Tuck and Hayball, 2002). The beneficial effect of olive oil has been mainly attributable to oleuropein and its two major metabolites, hydroxytyrosol and tyrosol. Oleuropein is the major phenolic compound in olive fruit, which can be as much as 14% in dried fruit. It is an ester consisting of hydroxytyrosol and elenolic acid which are potent antioxidant agents (Briante et al., 2001).

The phenolic components in olive oil are believed to play a vital role in the prevention of coronary artery disease and atherosclerosis. These compounds, in particular hydroxytyrosol, are potent antioxidants and radical scavengers (Tuck and Hayball, 2002; Visiol and Galli, 2002; Visioli and Galli, 1994; Visioli et al., 2002). They exert protective effects on cardiovascular system mainly by inhibiting platelet aggregation and endothelial activation (Carluccio et al., 2003), modulating arachidonic acid metabolism (Kohyama et al., 1997) and preventing the oxidation of low density lipids (Visiol and Galli, 2002; Visioli et al., 1995; Visioli et al., 2002). It is presumed that hydroxytyrosol as water and lipid soluble molecule can penetrate in cell membranes and thus inhibit the production of leukotriene B4 effectively from endogenous arachidonic acid (Kohyama et al., 1997).
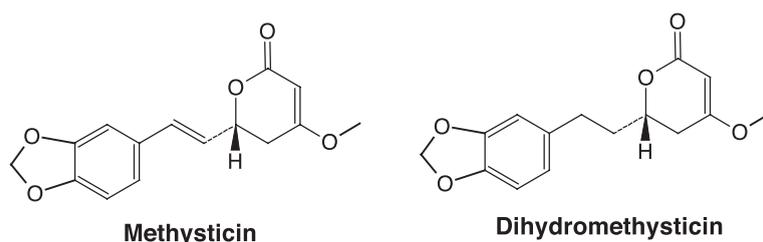

Fig. 9. Chemical structures of methysticin and dihydromethysticin from kava.

In vitro studies indicated that oleuropein inactivated androstenedione 6β-hydroxylase (CYP3A4) activity in human liver microsomes, with k_{inact} and K_I of 0.09 min^{-1} and 22.2 μM, respectively (Stupans et al., 2001; Stupans et al., 2000). The reactive metabolites of oleuropein remain undetermined, but oleuropein might undergo CYP3A-mediated oxidation to unidentified metabolite(s) capable of binding and inactivating CYP3A4 (Fig. 10). In addition, hydroxytyrosol and tyrosol and/or
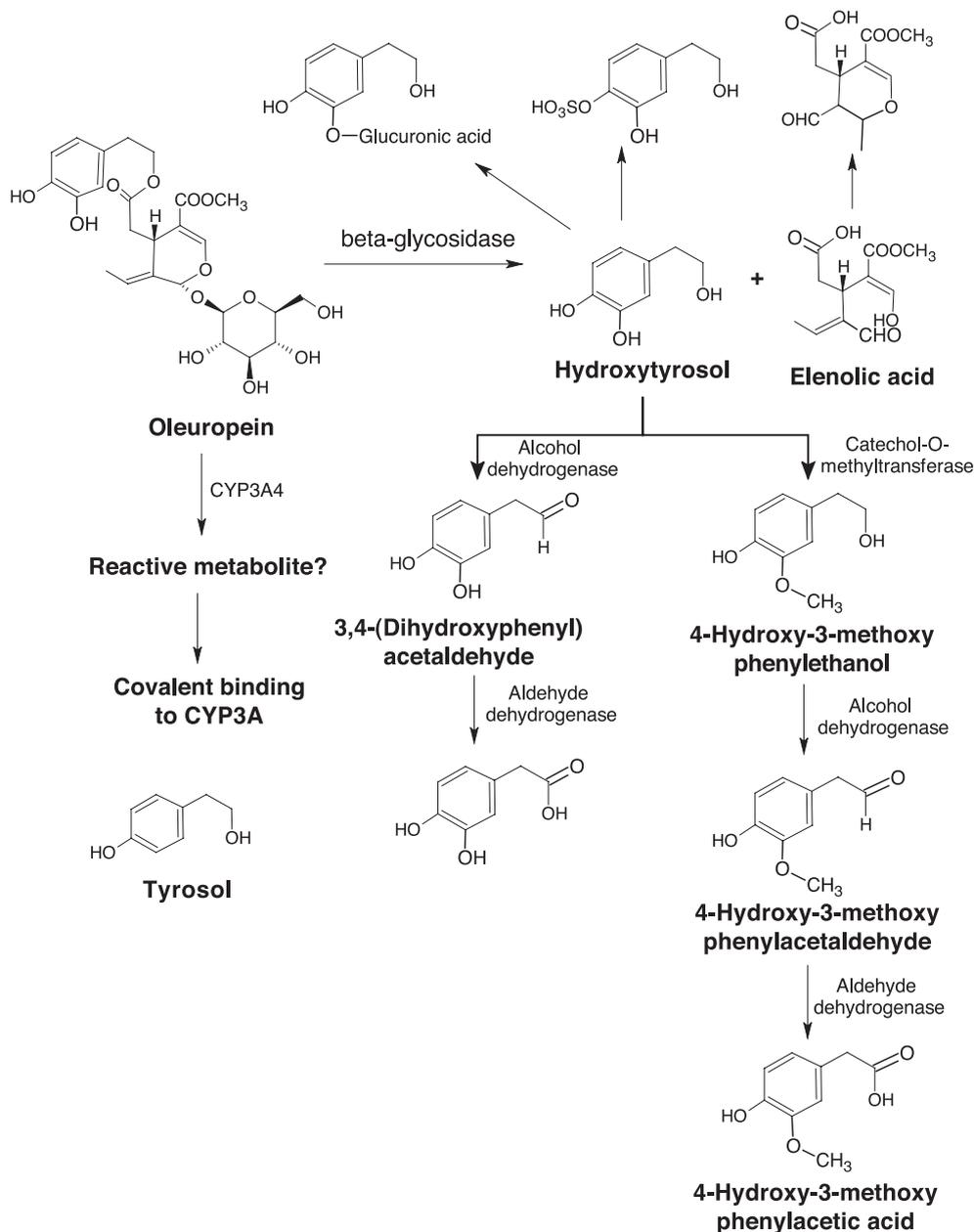

Fig. 10. Metabolism of oleuropein.

their metabolites may be involved. Metabolism studies have indicated that hydroxytyosol is excreted into the urine unchanged and as its glucuronide and sulfate conjugates (Fig. 10) (Miro-Casas et al., 2003; Tsarbopoulos et al., 2003; Tuck and Hayball, 2002). Hydroxytyrosol can also be sequentially oxidized to 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylacetaldehyde by alcohol and aldehyde dehydrogenase, or methylated by catechol methyltransferase to homovanillic acid and then oxidized to homovanillic alcohol (D'Angelo et al., 2001).

Organosulfur compounds

Garlic is frequently used as a flavor-enhancing ingredient in food preparation and healthy supplement in the practice of folk medicine. Organosulfur compounds are the major active components of garlic. Garlic also contains numerous flavonoids/isoflavonoids (such as nobiletin, quercetin, rutin, and tangeretin), polysaccharides, prostaglandins, saponins, and terpenes (such as citral, geraniol, linalool, α- and β-phellandrene) (Dausch and Nixon, 1990; Singh et al., 2001a,b). Alliin (S-allylcysteine sulfoxide) is one major component of garlic, which is converted by alliinase to allicin. Allicin is further transformed to other garlic compounds, including diallyl sulfide. Organo-sulfur compounds have been reported to have hypolipidemic, antiplatelet, immune-enhancing, anticancer, chemopreventive, hepatoprotective, antihypertension, and procirculatory effects (Rahman, 2001; Spigelski and Jones, 2001).

Organosulfar compounds modulated CYPs in vitro and in vivo (Brady et al., 1991). For example, extracts of fresh garlic, and samples of garlic oil, freeze dried garlic, and aged garlic inhibited human CYP2C9, 2C19, 3A4, 3A5 and 3A7 activity, whereas the CYP2D6 activity was unaltered (Foster et al., 2001). The organosulfur compounds 4,4′-dipyridyl disulfide, di-n-propyl disulfide and diallyl sulfide were also potent competitive inhibitors of coumarin 7-hydroxylase (CYP2A6), with a K_i value of 0.06, 1.7 and 2.1 μM respectively (Fujita and Kamataki, 2001). In vivo studies in the mouse indicated that garlic administration increased CYP2E1 and 1A2 levels (Kishimoto et al., 1999), whereas the administration of garlic constituents in the rat decreased the CYP2E1 activity and/or protein level, but increased or did not alter the CYP1A, CYP2B and CYP3A activities (Dalvi, 1992; Haber et al., 1995; Haber et al., 1994). A single dose of garlic oil in the rat resulted in a significant inhibition of hepatic CYP-catalyzed reactions including aminopyrine N-demethylase (CYP2C) and aniline hydrox-ylase (CYP2E1) activity, but administration of garlic for 5 days led to a significant increase in these hepatic CYP activities (Fitzsimmons and Collins, 1997). In addition, administration of garlic oil to healthy subjects for 28 days reduced CYP2E1 activity by 39% when chlorzoxazone was used as a probe compound (Gurley et al., 2002).

Diallyl sulfide is converted by CYP2E1 to diallyl sulfoxide and sequentially to diallyl sulfone (Fig. 11) (Teyssier et al., 1999). All these compounds are competitive inhibitors of CYP2E1 (Brady et al., 1991). A single oral dose of diallyl sulfone inactivated CYP2E1- and 1A-dependent activities in mouse liver microsomes (Lin et al., 1996). Diallyl sulfone is also a mechanism-based inhibitor of CYP2E1, involving the formation of a reactive metabolite which forms a CYP complex leading to autocatalytic destruction (Brady et al., 1991; Jin and Baillie, 1997; Premdas et al., 2000). A recent study has demonstrated that diallyl sulfone was oxidized at one of the terminal double bonds, yielding the monoallyleopoxide that inactivated CYP2E1 (Fig. 11) (Premdas et al., 2000). This monoallyleopoxide was conjugated by GSH. It appeared that the reactive intermediate from diallyl sulfone might initially affect the heme and/or apoprotein.
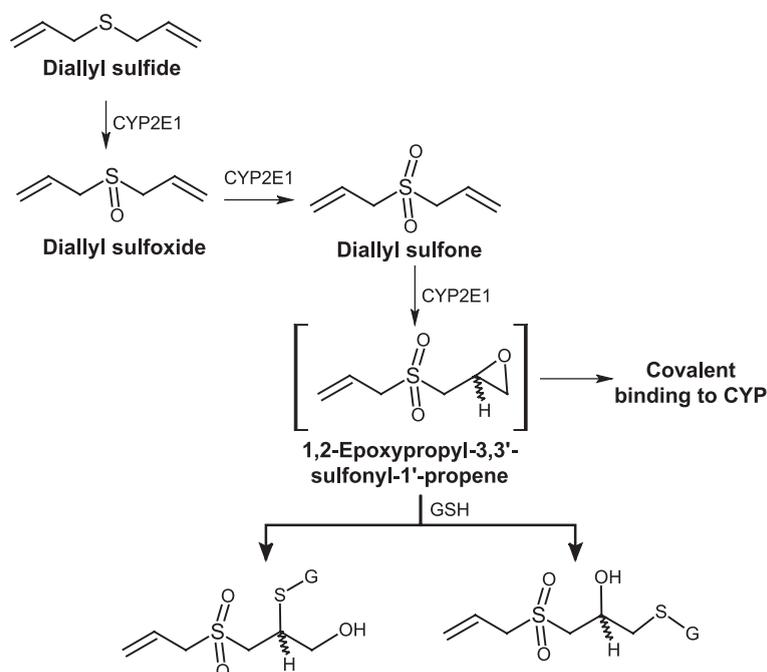
Fig. 11. Metabolism of diallyl sulfide by CYP2E1.

Resveratrol

Resveratrol is a polyphenolic phytoalexin found in red wine, grapes and peanuts. It has been reported to have antioxidant, cardioprotective, antimutagenic, antiinflammatory, chemopreventive and antiplatelet effects (Aziz et al., 2003; Cal et al., 2003; Gusman et al., 2001; Kimura, 2003; Orallo et al., 2002; Roemer and Mahyar-Roemer, 2002). Resveratrol has also been reported to possess substantial cytotoxic activities in different tumour cell lines (El-Mowafy and Alkhalaf, 2003; Ferry-Dumazet et al., 2002; Kuo et al., 2002; Roman et al., 2002). The cytotoxicity may be ascribed to suppressed activation of NF-κB and cell proliferation, inhibition of DNA synthesis (Kuwajerwala et al., 2002), and induction of G2 arrest and apoptosis of tumour cells (Estrov et al., 2003; Liang et al., 2003; Niles et al., 2003; Pozo-Guisado et al., 2002; Roman et al., 2002). In addition, resveratrol is able to activate estrogen receptor (Bhat et al., 2001; Klinge et al., 2003; Levenson et al., 2003; Mueller et al., 2003; Ratna and Simonelli, 2002), inhibit eicosanoid synthesis and cyclooxygenase-1 and 2 (Johnson and Maddipati, 1998; Martinez and Moreno, 2000; Moreno, 2000; Pace-Asciak et al., 1995), activate adenylyl and guanylyl cyclase (El-Mowafy, 2002; El-Mowafy and Alkhalaf, 2003), and enhance the expression and activity of endothelial nitric oxide synthase (Hattori et al., 2002; Wallerath et al., 2002).

Resveratrol was shown to be an irreversible (mechanism-based) inhibitor for CYP3A4 and a non-competitive reversible inhibitor for CYP2E1, with IC_{50} values of 4-150 μM in microsomes from rat liver, human liver or cells containing cDNA-expressed CYPs (Chan and Delucchi, 2000; Chan et al., 1998; Piver et al., 2001). In a study using Sf9 insect microsomes containing baculovirus-derived human CYP3A4 and NADPH-cytochrome CYP reductase, resveratrol inactivated CYP3A4 in a time- and NADPH-dependent manner, with k_{inact} and K_I of 0.20 min^{-1} and 20 μM, respectively (Chan and
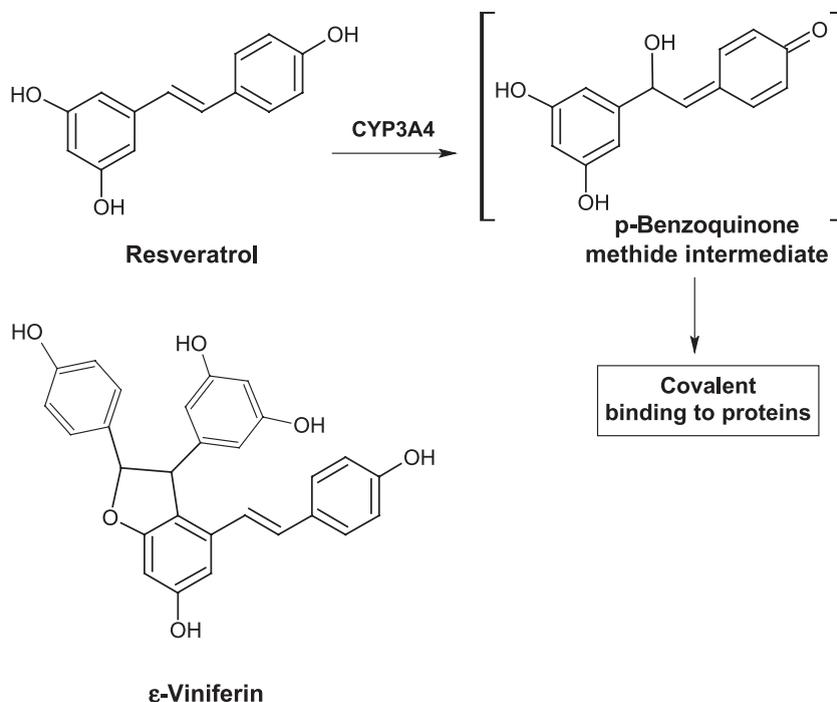
Fig. 12. Proposed bioactivation of resveratrol.

Delucchi, 2000; Chan et al., 1998). Resveratrol is an electron-rich molecule with two aromatic benzene rings linked by an ethylene bridge. CYP3A-mediated aromatic hydroxylation and epoxidation of this compound are possible, resulting in a reactive p-benzoquinone methide metabolite which is capable of binding covalently to CYP3A4 and inactivating it (Fig. 12).

Interestingly, ε-viniferin, a dimer of resveratrol, was not a mechanism-based inhibitor of human CYPs (Piver et al., 2003). It showed mixed-type inhibitions for various CYPs with K_i of 0.5–20 μM, except for CYP2E1 (non-competitive). ε-Viniferin was isolated in wine at concentration of 0.5–5 μM (Landrault et al., 2002). It is generated by oxidation from resveratrol, the parent compound and produced by grapevine in response to fungal infection. ε-Viniferin has been shown to have better antifungal and antioxidant activity than resveratrol (Baderschneider and Winterhalter, 2000; Bala et al., 2000). ε-Viniferin also had hepatoprotective activity (Oshima et al., 1995).

Mechanism-based inhibition of CYPs and herb-drug interactions

Herbs are often administered in combination with therapeutic drugs, raising the potential of pharmacokinetic and/or pharmacodynamic herb-drug interactions. A number of clinically important herb-drug interactions have been documented and many of them led to altered efficacy and/or adverse events (Fugh-Berman, 2000; Fugh-Berman and Ernst, 2001). Pharmacokinetic herb-drug interactions are due to altered absorption, metabolism, distribution and excretion of drugs. Frequently, one of the underlying mechanisms of altered drug concentrations by concomitant herbal medicines is the induction

or inhibition of hepatic and intestinal CYPs. CYP3A4 are expressed at high levels in the villus tip of enterocytes, the primary site of absorption for orally administered drugs. Thus, the modulation of intestinal CYP3A represents an important mechanism for the enhanced or reduced bioavailability of coadministered drugs. In particular, the mechanism-based inhibition of CYPs (in particular CYP3A4) by herbal constituents may have important pharmacokinetic implications.

Administration of garlic extract for 3 months in healthy volunteers did not alter the oxidative metabolism of acetaminophen, but caused a slight increase in sulfation (Gwilt et al., 1994). However, a study in mice indicated that diallyl sulfone decreased the plasma levels of oxidative acetaminophen metabolites, but not nonoxidative acetaminophen metabolites (Lin et al., 1996). This is considered to be due to the inhibition of CYP2E1 that is the major enzyme responsible for acetaminophen bioactivation (Manyike et al., 2000). In liver microsomes, diallyl sulfone significantly inhibited the acetaminophen oxidation to N-acetyl-p-benzoquinone imine (the toxic metabolite of acetamoniphen) (Lin et al., 1996). All these results provide an explanation for the protective effect of diallyl sulfone on acetaminophen-induced hepatotoxicity (Lin et al., 1996). When administered orally 1 hr prior to, immediately after, or 20 min after a toxic dose of acetaminophen, diallyl sulfone (25 mg/kg) completely protected mice from development of hepatotoxicity (Lin et al., 1996).

Case reports and pharmacologic studies have indicated a potentiation of the central nervous system effects of benzodiazepines, including alprazolam (Almeida and Grimsley, 1996), in the presence of kava extract and/or kavalactones. Since alprazolam is a substrate of CYP3A4 (Gorski et al., 1999) and kavalactones are potent inhibitors of CYP3A4 (Zou et al., 2002), decreased elimination of alprazolam by kava may contribute to the additive effects reported upon coadministration of kava and alprazolam. Coadministration of kava markedly increased hexobarbital sleep time (Meyer, 1962) and ethanol-induced hypnotic effect (Jamieson and Duffield, 1990) in mice, indicative of inhibition of CYP2C and CYP2E1.

In a crossover study in healthy human volunteers, oral ingestion of aqueous licorice extract for 7 days did not significantly alter the pharmacokinetic parameters and sedative effects of midazolam (Shon et al., 2001). Midazolam is a typical substrate of CYP3A4 (Gorski et al., 1994). The discrepancy between in vitro and animal and human studies may reflect the importance of herbal dosing and regimen in the modulation of CYPs.

Grapefruit juice has been found to significantly increase oral bioavailability of most dihydropyridines (e.g. felodipine), terfenadine, saquinavir, cyclosporine, midazolam, triazolam and verapamil (Bailey et al., 2000; Bailey et al., 1998; Bailey et al., 1991; Ducharme et al., 1995; He et al., 1998; Kane and Lipsky, 2000; Mohri and Uesawa, 2001; Yee et al., 1995). The plasma concentrations or area under the concentration-time curve (AUC) of lovastatin, cisapride and astemizole can also be markedly increased by grapefruit juice (Bailey et al., 2000; Bailey et al., 1998). As the duration of effect of grapefruit juice can last 24 hr, repeated consumption of grapejuice can lead to a cumulative increase in the AUC and C_{max} of coadministered drugs. The inhibition of CYP3A4 activity with no change of CYP3A4 mRNA and P-glycoprotein is believed to be the primary mechanism (Bailey et al., 1998; Kane and Lipsky, 2000). However, the pharmacokinetics of many other drugs were not altered by grapefruit juice. For example, grapefruit juice did not alter the bioavailability of digoxin, diltiazem and amlodipine in human volunteers, and indinavir in HIV-positive patients (Becquemont et al., 2001; Sigusch et al., 1994; Vincent et al., 2000). Although these drugs undergo presystemic metabolism, CYP3A4 is a minor contributor. These finding indicate the difficulty in predicting herb-drug interactions.

The clinical outcome of herb-drug interactions depends on factors that are related to coadministered drugs (dose, dosing regimen, administration route, pharmacokinetic and therapeutic range), herbs (species, dose, dosing regimen, and administration route) and patients (genetic polymorphism, age, gender and pathological conditions) (Dresser et al., 2000). Generally, a doubling or more in drug plasma concentration has the potential for enhanced adverse effects. However, less marked changes may still be clinically important for drugs with a steep concentration-response relationship or a narrow therapeutic index.

Mechanism-based inhibition of CYPs and herbal chemoprevention

Chemoprevention refers to the application of natural or synthetic compounds to block, reverse, or prevent the development of invasive cancers (Shureiqi et al., 2000; Sporn and Suh, 2000; Tamimi et al., 2002; Young and Wilson, 2002). Clinical evidence has indicated the feasibility of this approach in reducing the risk of major human cancers. For example, tamoxifen and raloxifene (both oestrogen receptor modulators) reduce the risk of developing breast cancer in women at increased risk (Fisher et al., 1998; Salih and Fentiman, 2001). Results with aspirin and non-steroidal anti-inflammatory agents have proved consistently encouraging in epidemiological studies in lowering the incidence of colorectal cancer (Jolly et al., 2002). However, although these compounds have shown a potential to reduce cancer incidence, several problems have been associated with them. These include severe side effects due to long-term use, high costs and non-compliance. Therefore, safer and more selective chemopreventive agents are needed.

A number of naturally occurring products from herbs have shown chemopreventive properties against carcinogenesis using in vitro and animal models (Chen et al., 1998; Fukutake et al., 2000; Fukutake et al., 1998; Lahiri-Chatterjee et al., 1999; Zheng et al., 1997). The mechanisms for the chemopreventive effects of herbal preparations are not fully elucidated, but inhibition of activating enzymes and other enzyme systems, protective effects from toxic xenobiotics, beneficial regulation of cell cycles and cellular signalling pathways have all been suggested (Wargovich et al., 2001).

Naturally occurring isothiocyanates have been shown to be potent and selective inhibitors of carcinogenesis induced by a number of chemical carcinogens (Boysen et al., 2003; Hecht et al., 2002; Yang et al., 2002). In most studies, the chemopreventative activity of isothiocyanates required administration either before or during exposure to the carcinogen. PEITC inhibits lung carcinogenesis induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, whereas BITC is ineffective in this regard (Staretz and Hecht, 1995). In turn, BITC effectively inhibits benzo(a)pyrene-induced lung tumors in A/J mice, whereas PEITC is ineffective (Lin et al., 1993). These effects might be related to the inhibitory mechanisms and potencies for CYP2B1 and 1A, respectively. Some isothiocyanates such as PEITC are able to induce CYP2B1 and 2E1 following chronic administration, with increased toxicity (Smith et al., 1993). The extensive conjugation of BITC with GSH might also be important for the in vivo effects of BITC. The conjugation with GSH has been shown to decrease potency, and could contribute to mutagenicity (Raulf-Heimsoth and Baur, 1998). It appears that local release of the conjugated product might also contribute to the toxicity of BITC (Hirose et al., 1998).

Epidemiological studies suggest that the consumption of fruit and vegetables decreases the risk for cancer development (Block et al., 1992). The combination of BITC and PEITC has recently also been

proposed for the chemoprevention of lung cancer in individuals resistant to smoking cessation (Hecht, 1997; Kelloff et al., 1996). These effects appear to be mediated through modulation of both Phase I and Phase II enzymes involved in carcinogen metabolism by isothiocyanates. In particular, the isothiocyanate-mediated inactivation of CYP1A and 2B responsible for carcinogen activation would inhibit carcinogenesis (Boysen et al., 2003; Scharf et al., 2003; Zhang and Talalay, 1994). In addition, the induction of detoxifying Phase II enzymes may also contribute to the beneficial effects of isothiocyanates.

Organosulfur compounds from garlic showed chemopreventive effects at several organ sites in rodents after administration of chemical carcinogens, perhaps by inhibiting CYP2E1-mediated carcinogen activation (Reddy et al., 1993; Yang et al., 2001). These compounds have also been shown to reduce the incidence of a multitude of chemically induced tumors in animal models. Pretreatment with aqueous garlic extract significantly reduced the frequencies of N-methyl-N′-nitro-N-nitrosoguanidine-induced micronuclei and chromosomal aberrations (Arivazhagan et al., 2001). These compounds have also been shown to reduce toxicity induced by thioacetamide, carbon tetrachloride, N-nitrosodimethyl-amine- and acetaminophen (all CYP2E1 substrates) in rodents (Ramaiah et al., 2001; Wang et al., 2001; Wang et al., 2000). The protective effect was observed when the organosulfur compounds were given before, during or soon after chemical treatment.

Diallyl sulfide also has been shown to induce other CYP and phase II enzymes as well as decrease hepatic catalase activity (Yang et al., 2001). The preventive effects of garlic extract on bromobenzene-induced hepatotoxicity in precision-cut liver slices was related to an elevation of hepatic glutathione content, and a glutathione sparing effect, possibly due to conjugation of organosulphur compounds in garlic extract with toxic bromobenzene metabolites (Guyonnet et al., 2001; Wang et al., 1999). Organosulfur compounds also inhibit the formation of DNA adducts in several target tissues. Antiproliferative activity has been described in several tumor cell lines (Hirsch et al., 2000; Nakagawa et al., 2001), and may be due to induction of apoptosis and alterations of the cell cycle (Frantz et al., 2000; Kwon et al., 2002). However, all of these effects are observed at concentrations much higher than what is normally ingested by humans and clinical trials will be needed to define the effective dose that has no toxicity in humans.

Resveratrol seems to be a promising cancer chemopreventive agent and it has been shown to afford protection against several cancer types in several bioassay systems (Aziz et al., 2003; Banerjee et al., 2002; Li et al., 2002). The mechanism for these effects is not completely understood, but multiple effects of resveratrol may be involved. Inhibition of Phase I enzymes and induction of Phase II enzymes will contribute to the chemopreventive activity of resveratrol (Dubuisson et al., 2002; Jang et al., 1997). It also has activity in the regulation of multiple cellular events associated with carcinogenesis. However, it is not known whether resveratrol inhibits the catalytic activity of CYP1A1, 1A2, and 1A2 in vivo. The potential in vivo effect of resveratrol on the bioactivation of CYP1 substrates may depend not only on the pharmacokinetics of resveratrol but also the tissue of interest (Schwedhelm et al., 2003). It is known that the expression of many CYPs such as CYP1A1, 1A2, and 1B1 is tissue-dependent (Omiecinski et al., 1999).

Conclusions

Bioactivation of herbal constituents appears a critical step for the toxicity induction of some herbs. The resultant reactive intermediates bind covalently to DNA and proteins, leading to organ toxicity and

even carcinogenicity. On the other hand, some herbal/dietary constituents were shown to form reactive intermediates capable of irreversibly inhibiting various CYPs. The resultant metabolites lead to CYP inactivation by chemical modification of the heme, the apoprotein, or both as a result of covalent binding of modified heme to the apoprotein. The mechanism-based inhibition of CYPs may provide an explanation for some reported herb-drug interactions. Naturally occurring compounds that inactivate CYPs may represent a novel type of chemopreventive agents with higher selectivity and lower toxicity compared to synthetic compounds.

Herbal medicines often contain multiple active substances and multiple cellular molecules might be the targets of herbal medicine. The identification of these targets may provide molecular evidence for the herb's pharmacological activity and toxicity. Recently, there is an increasing application of genomic approaches (e.g. high-density microarray analysis) to examine the genomic responses to herbal medicines (Afshari et al., 1999; Gohil, 2002; Gohil et al., 2000; Owuor and Kong, 2002; Watanabe et al., 2001; Yang et al., 2003). These approaches can identify a huge number (e.g. 1000–12 000) of genes which are repressed or stimulated by a herbal compound of interest. The genomic approaches may offer a powerful tool for defining and predicting pharmacological activity and toxicity of herbal medicines.

Due to the wide use and easy availability of herbal medicines, herbal toxicity has become an issue of concern. The safety and quality of herbal medicine should be ensured through greater research, pharmacovigilance, greater regulatory control and better communication between patients and health professionals.

Acknowledgements

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