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Effects of an aqueous-ethanolic extract of ginger on cytochrome P450 enzyme-mediated drug metabolism

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Ginger has been extensively used as a herbal medicine for thousands of years in Asia; it has also been used as a seasoning agent in several foods and beverages worldwide. In this study, the effect of an aqueous-ethanolic extract of ginger on CYP450-mediated drug metabolism was investigated *in vitro* to elucidate the herb-drug interactions. A CYP450-specific substrates mixture was incubated with an aqueous-ethanolic extract of ginger in human liver microsomes fortified with an NADPH-generating system, and the metabolites generated from each of the CYP450-specific metabolic reactions were measured by liquid chromatography-tandem mass spectrometry. The ginger extracts were tested at concentrations of 0.05–5 μg/mL. The resulting data showed that the ginger extract inhibited CYP2C19-mediated drug metabolism in a concentration-dependent manner with an IC_{50} value of 3.8 μg/mL. When the ginger extract was pre-incubated and assessed, the inhibition pattern did not change, indicating that the inhibition of CYP2C19 was competitive rather than mechanism-based. The effects on other CYP isozyme activity were negligible at the concentrations tested. In conclusion, this inhibitory effect of ginger extract could affect the pharmacokinetics and lead to interactions with drugs that are metabolized by CYP2C19.

1. Introduction

Ginger, the rhizome of the plant *Zingiber officinale*, has been extensively used as a herbal medicine for thousands of years in Asian countries; it has also been used as a seasoning agent for a variety of foods and beverages worldwide. Ginger has been traditionally used for varied human ailments worldwide: digestive disorders, diarrhea, and nausea (Shukla and Singh 2007). Gingerols, paradols, zingerones, and shogaols are the main pungent or phenolic compounds in ginger roots (Zick et al. 2010). Gingerol possesses substantial antioxidant properties (Aeschbach et al. 1994), and has been shown to strongly inhibit skin tumor formation in mice (Surh 2002). Some phenolic substances present in ginger exert strong anti-inflammatory properties (Surh 1999).

Herbal medicines or herbal dietary supplements may have clinical significance in herb-drug interactions as they can affect drug absorption and disposition (Wilkinson 1997; Evans 2000). One of the most important pharmacokinetic regulators of drug metabolism is the cytochrome P450 (CYP450) superfamily, which may be inhibited or induced by herbal medicines or herbal dietary supplements. Notably, herb-drug interactions with ginger have been reported. The area under the plasma concentration time curve (AUC) values of tacrolimus, a well-known potent immunosuppressant, in rats pre-treated with ginger was significantly increased compared with those of normal rats (Egashira et al. 2012). Ginger extracts have shown inhibition or induction of some CYP isozymes *in vitro* (Engdal et al. 2009). However, the herb-drug interactions of ginger have not yet been fully characterized. In this study, we therefore investigated the effect of an aqueous-ethanolic extract of ginger, at different concentrations, on CYP450-mediated drug metabolism in human liver microsomes to characterize and predict the herb-drug interactions of ginger.

2. Investigations, results and discussion

The inhibitory effects of ginger extract on CYP-specific metabolic activity were evaluated in human liver microsomes. The assay system was first tested with well-known CYP isozyme selective inhibitors: furafylline (CYP1A2), methoxsalen (CYP2A6), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4). Each inhibitor selectively inhibited the corresponding CYP marker activity to <10% of the control activity (data not shown). The Fig. shows the inhibitory effect of ginger extract on the drug-metabolizing activity of six CYP isozymes. Ginger extract (0.05–5 μg/mL) showed concentration-dependent inhibitory effects on CYP2C19 with an IC_{50} value of 3.8 μg/mL. The inhibitory effect on other CYP isozymes was negligible. To examine the likelihood of mechanism-based inactivation of CYP enzymes, the extract was pre-incubated with human liver microsomes in the presence of NADPH. The inhibition patterns were generally similar to the result of the test without pre-incubation. The IC_{50} value for CYP2C19 inhibition was 4.8 μg/mL. These results indicated that the ginger extract inhibited the CYP2C19-mediated drug metabolism in a comparative manner, and that the inhibition may therefore be reversible.

According to the report by Foster et al. (2003) ginger aqueous extract and ginger tea extract inhibited the activity of CYP2C9,

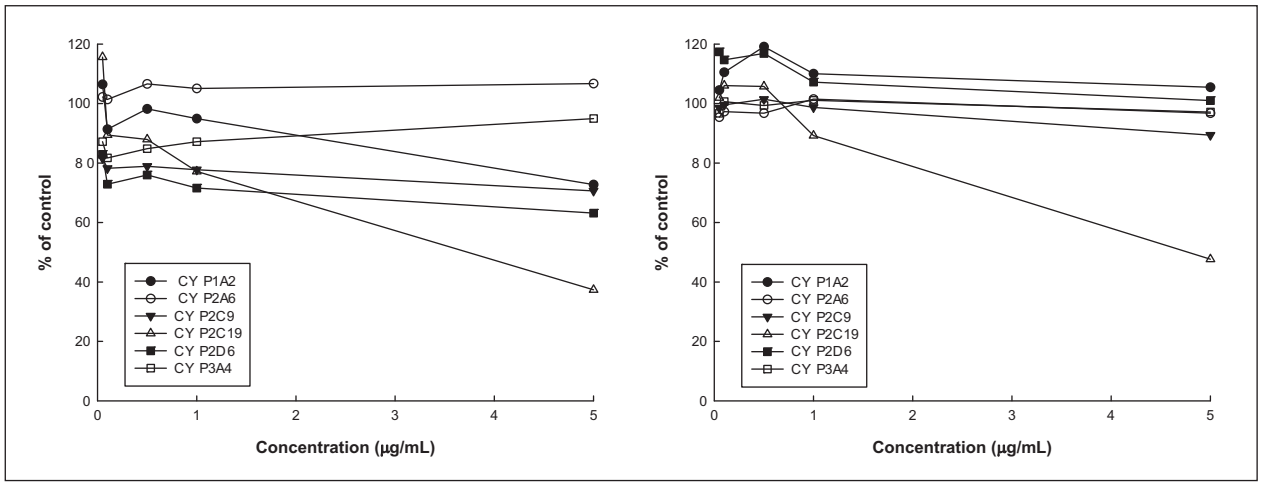

Fig: Effects of ginger extract on the activity of CYP isozymes in human liver microsomes (A) without and (B) with pre-incubation

CYP2C19, CYP2D6, and CYP3A4 isozymes by 50%–90% at a concentration of 25 mg/mL. In particular, the aqueous extract showed potent inhibitory effects on CYP2C19 and CYP3A4. However, in this study, the inhibition of CYP2C19 activity only was observed. This may be because we used a lower concentration range (0.05–5 μg/mL) of the ginger extract in an attempt to better reflect the actual concentration in the gastrointestinal tract. Furthermore, the method of extract preparation differed between the two studies, i.e., different types and percentage of organic solvents were used to prepare the extract. Moreover, the induction of CYP1A2 and CYP3A4 isozymes by ginger-containing supplements has been demonstrated (Brandin et al. 2007). Therefore, the intake of ginger-containing health supplements or herbal medicines may alter the pharmacokinetics of co-administered drugs metabolized by CYP450 enzymes and some caution may be required with respect to its clinical use.

3. Experimental

3.1. Materials

Ginger aqueous-ethanolic extract (30% ethanol) was prepared and provided by Medical Science Laboratory, Kyung Hee University, Suwon, Korea. The extract was standardized to contain 5.2% of 6-gingerol and 2.1% of 6-shogaol. Pooled human liver microsomes were purchased from BD Gentest (Woburn, MA). Glucose-6-phosphate, β-$NADP^+$, glucose-6-phosphate dehydrogenase, phenacetin, acetaminophen, coumarin, 7-hydroxy coumarin, diclofenac, 4′-hydroxy diclofenac, mephenytoin, dextromethorphan, dextrophan, midazolam, 1′-hydroxy midazolam, furafylline, methoxsalen, sulfaphenazole, ticlopidine, quinidine, ketoconazole, and terfenadine were obtained from Sigma Chemical Co. (St. Louis, MO). 4′-Hydroxy mephenytoin was purchased from BD Gentest (Woburn, MA, USA). All other chemicals were of analytical grade and were used as received.

3.2. CYP inhibition assay

The incubation mixtures consisted of 0.5 mg/mL human liver microsomes, various concentrations of the ginger extracts, a substrate mixture (Table), and an NADPH-generating system (NGS: 0.1 M glucose-6-phosphate, 10 mg/mL β-$NADP^+$, and 1 U/mL glucose-6-phosphate dehydrogenase) in a total volume of 200 μL potassium phosphate buffer (0.1 M, pH 7.4). The reaction mixture was incubated at 37 °C for 5 min prior to initiation of the reaction by the addition of NGS, and was then continuously incubated for 30 min in a water bath at 37 °C. Well-known selective CYP inhibitors were tested as positive controls (Table). After the incubation, the reaction was arrested by the addition of 400 μL of 0.1% acetic acid and 4 μL of internal standard solution (16 μM terfenadine). For the pre-incubation assay, the incubation mixture without the substrate mixture was pre-incubated for 20 min and then it was incubated for another 30 min after the substrate mixture was added. The rest of the process was as described above.

3.3. Sample preparation

The reaction solutions were passed through the activated Sep-Pak C18 cartridges (96-well type OASIS HLB extraction cartridge, Waters, Milford, MA, USA). The cartridges were washed twice with 1 mL distilled water and eluted with 1 mL methanol. The eluate was dried under nitrogen gas. The residue was dissolved in 50 μL of 0.1% formic acid:acetonitrile (85:15), and 5-μL aliquots were used for liquid chromatography-tandem mass spectrometry (LC-MS/MS).

3.4. LC-MS/MS analysis

The LC-MS/MS system consisted of a Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan) with an API4000 triple quadrupole mass spectrometer (Applied Biosystems-SCIEX, Concord, Canada) equipped with a TurboIon Spray source. Chromatographic separation was achieved on a Fortis C8 column (2.1 × 10 mm, 5.0 μm; Fortis Technologies Ltd, Cheshire, England, UK). The HPLC mobile phases consisted of 2 solvents: (A) 0.1% formic acid and (B) acetonitrile. A linear gradient program was used with a flow rate of 0.2 mL/min. The initial concentration of solvent (B) was 25%, which was gradually increased to 85% for 3 min, maintained for 1.5 min, and followed by a re-equilibration for 3.5-min. ESI was performed in a positive mode with nitrogen as the nebulizing agent, turbo spray, and curtain gas at optimal values of 50, 50, and 20 (arbitrary units), respectively. The nebulizer temperature was 450 °C. Multiple reaction monitoring (MRM) detection was employed. The precursor-product ion pairs used in MRM mode were as follows: *m/z* 151→110 for acetaminophen, 162→107 for 7-OH-coumarin, 313→187 for 4′-OH-diclofenac, 235→150 for 4′-OH-

Table: CYP-specific substrates, their metabolites, and inhibitors used in this study

P450 isozyme	Marker substrates	Concentration	Metabolites monitored	Specific inhibitors
CYP 1A2	Phenacetin	40 μM	Acetaminophen	Furafylline (10 μM)
CYP 2A6	Coumarin	2.5 μM	7-OH-coumarin	Methoxsalen (10 μM)
CYP 2C9	Diclofenac	10 μM	4′-OH-diclofenac	Sulfaphenazole (50 μM)
CYP 2C19	(±)-Mephenytoin	80 μM	4′-OH-mephenytoin	Ticlopidine (20 μM)
CYP 2D6	Dextromethorphan	5 μM	Dextrophan	Quinidine (50 μM)
CYP 3A4	Midazolam	2.5 μM	1′-OH-midazolam	Ketoconazole (5 μM)

mephenytoin, 258→157 for dextrophan, 343→325 for 1′-OH midazolam, and 472→436 for terfenadine (internal standard, IS).

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