Inhibitory Effects of 6-Gingerol on Cytochrome P450 in Human Liver Microsomes

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=초록=

인체 간 미세소체에서 6-gingerol의 시토크롬 P450에 대한 억제 효과

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배경: 일상 식이 생활에서 흔히 쓰이는 생강(ginger)의 한 성분인 6-gingerol은 질병 치료 과정 중에 사용하는 약물과 상호 작용을 일으킬 수 있다. 그러나 인체 간 대사 효소인 시토크롬 P450 활성도에 대한 6-gingerol의 효과는 아직 알려져 있지 않다. 본 연구에서는 인체 간 미세소체에서 6-gingerol이 시토크롬 P450대사 효소에 미치는 억제 효과를 연구하고자 하였다.

방법: 인체 간 미세소체 모델을 이용하여 다양한 농도의 6-gingerol과 각각의 CYP450에 특이적인 기질을 함께 배양하였으며, 각각의 대사물을 HPLC로 측정하였다. 본 연구에서 사용된 CYP효소에 의해 촉매 되는 반응에는 CYP1A2에 의한 phenacetin O-deethylation, CYP2C9에 의한 tolbutamide 4-hydroxylation, CYP2C19에 의한 S-mephenytoin 4'-hydroxylation, CYP2D6에 의한 dextromethorphan O-demethylation, CYP2E1에 의한 chlorzoxazone 6-hydroxylation, 그리고 CYP3A4에 의한 midazolam 1-hydroxylation이었다.

결과: 6-gingerol은 IC₅₀가 100 µM을 상회할 정도로 CYP2C9, CYP2D6, CYP2E1 그리고 CYP3A4의 활성도에 큰 영향을 미치지 않았다. 그러나 6-gingerol은 CYP 2C19과 CYP 1A2에 대하여 *K_i* 수치가 각각 13 µM과 29 µM이 될 정도로 강한 억제 효과를 나타내었다. CYP1A2에 대하여 6-gingerol은 상경적인 형태의 억제를 초래하였다.

결론: 이러한 결과는 6-gingerol이 CYP1A2과 CYP2C19의 활성도를 잠재적으로 억제할 수 있음을 시사하였다.

Key words: 6-gingerol, Cytochrome P450, Inhibition, Human liver microsome

INTRODUCTION

Ginger rhizome (*Zingiber officinale*), commonly known as ginger, has been consumed worldwide as one of spices and flavoring agents.¹⁾

Ginger contains pungent phenolic substances collectively known as gingerols. 6-gingerol, one ingredient of ginger compound, has been known to exhibit various pharmacological responses such as anti-oxidant, anti-platelet,

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anti-inflammatory, and anti-cancer effects.²⁻⁷⁾

Oxidative metabolism of many drugs is mostly catalyzed by cytochrome P450 (CYP) enzymes, which consist of a large family of hemoprotein.^{8,9)} More than 15 isoforms have been identified in human liver and several forms play vital roles in the metabolism of many drugs.¹⁰⁾ It is very important that the CYP isoforms, which are responsible for the metabolism of drugs needs to be identified and further elucidated whether a drug has inhibitory effect on the catalytic activity of CYP isoforms to consider possible drug interactions. Especially ginger compound containing 6-gingerol is used so frequently in everyday life as a dietary ingredient or herbal drugs. This suggests the potential ginger-drug interactions, which might affect the treatment responses of therapeutic drugs. However, the effect of 6-gingerol on drug metabolism in humans is not elucidated yet and it is required to know the influence of 6-gingerol on human CYPs.

In this study, we investigated the effect of 6-gingerol on the CYP drug metabolizing enzymes using human liver microsomal models. It was therefore attempted first to identify whether 6-gingerol has the inhibitory potency on CYP isoforms and further to elucidate the mechanism of inhibition.

MATERIALS AND METHODS

1. Chemicals and human liver microsomes

6-gingerol, constituent of ginger compound, was purchased from Dalton Pharma Services (Toronto, Canada). Acetaminophen, chlorzoxazone, dextromethorphan, phenacetin, tolbutamide, MgCl₂, NADPH, glucose-6-phosphate (G6P) and glucose-6-phosphate dehydrogenase (G6PDH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dextrophan, 6-hydroxy-chlorzoxazone, 4'-hydroxymephenytoin, 1-hydroxymidazolam, 4-hydroxytolbutamide, S-mephenytoin were purchased from Ultrafine Chemicals (Manchester, UK). Solvents were HPLC grade (Fisher Scientific Co., Pittsburgh, PA, USA) and the other chemicals were as of the highest quality available. Pooled human liver microsomes used in experiments were obtained from BD Gentest (Woburn, MA, USA). The manufacturer supplied the information on protein concentration, P450 content, and enzyme activity.

2. Determination of human CYP activities

The inhibitory effects of 6-gingerol on each CYP isoenzyme using liver microsomal preparations were performed in duplicate. The reaction probes were phenacetin O-deethylation for CYP1A2, tolbutamide 4-hydroxylation for CYP2C9, S-mephenytoin 4'-hydroxylation for CYP2C19, dextromethorphan O-demethylation for CYP2D6, chlorzoxazone 6-hydroxylation for CYP2E1, and midazolam 1-hydroxylation for CYP3A4. All incubations were performed with 0.5 mg/ml human liver microsomes in a

final volume of 0.25 ml (50 mM phosphate buffer, pH 7.4). Then incubation mixture containing one of the CYP enzyme-specific substrates, an inhibitor, and human liver microsomes were preincubated for 5 min at 37°C. After preincubation, the reactions were initiated by adding the NADPH-generating system (including 3.3 mM G6P, 1.3 mM NADPH, 3.3 mM MgCl₂, and 4 unit/ml G6PDH). After incubation at 37°C for specific period, the reaction was stopped by placing the incubation tubes on ice and adding 80 µl of ice-cold acetonitrile. Samples for each assay were spiked with 25 µl volume of internal standard (10 µM carbamazepine for phenacetin, 15 or 50 µM chlorpropamide for tolbutamide and midazolam resp, 100 or 200 µM propranolol for chlorzoxazone and dextromethorphan, respectively, 10 µM carbamazepine for S-mephenytoin) and vortexed before centrifugation. The incubation mixtures were then centrifugated at 14,000 g for 5 min at 4°C. Aliquots of the supernatant were injected onto HPLC system. The analytical HPLC

system consisted of LC-20A series equipped with pump, autosampler, UV detector, and fluorescence detector from Shimadzu Co. (Kyoto, Japan). The respective products were analyzed as previously described.¹¹⁾

3. Data analysis

The IC₅₀ values (concentration of the inhibitor causing 50 % inhibition of the original enzyme activity) were calculated using a non-linear regression analysis program (WinNonlin v. 4.0, Scientific Consulting Inc., Apex, NC, USA). The method of Dixon was used to calculate inhibition constants (*K_i*).¹²⁾

RESULTS

Using the human liver microsomal model, the present study examined the *in vitro* effect of 6-gingerol to inhibit the metabolism of substrates for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 isoforms (Table 1 and Figure 1 to 4). Various concentrations

Table 1. IC₅₀ values of 6-gingerol for the cytochrome P450-catalyzed reactions in human liver microsomes

CYP isoforms	Substrates	Metabolites	IC ₅₀ (µM)
CYP1A2	Phenacetin	Acetaminophen	51
CYP3A4	Midazolam	1-OH-Midazolam	108
CYP2C9	Tolbutamide	4-OH-Tolbutamide	2,499
CYP2C19	S-Mephenytoin	4'-OH-Mephenytoin	36
CYP2D6	Dextromethorphan	Dextrophan	235
CYP2E1	Chlorzoxazone	6-OH-Chlorzoxazone	104

Figure 1. Inhibitory effect of 6-gingerol (0 to 500 μ M) on human hepatic CYP2C9-catalyzed tolbutamide 4-hydroxylation, CYP2D6-catalyzed dextromethorphan O-demethylation, CYP2E1-catalyzed chlorzoxazone 6-hydroxylation, and CYP3A4-catalyzed midazolam 1-hydroxylation.

Figure 2. Inhibitory effect of 6-gingerol (0 to 500 μ M) on human hepatic CYP1A2-catalyzed phenacetin O-deethylation and CYP2C19-catalyzed S-mephenytoin 4'-hydroxylation.

Figure 3. Representative Dixon plots for the inhibition of CYP2C19 mediated S-mephenytoin (MP) 4-hydroxylation in human liver microsomes. S-mephenytoin (15 to 50 μ M) was incubated with or without 6-gingerol (0 to 100 μ M) in human liver microsomes and a NADPH generating system.

over the wide range of 6-gingerol (0 to 500 μ M) were co-incubated with respective CYP isoform-specific substrates in human liver microsomes. Respective CYP isoform-catalyzed metabolites of substrates were examined by HPLC to determine the inhibitory potency of 6-gingerol on the microsomal CYP activities.

With IC_{50} over 100 μ M as shown in Table 1 and Figure 1, 6-gingerol did not greatly influence the metabolic activities of CYP2C9, CYP2D6, CYP2E1, and CYP3A4 isoenzymes. 6-gingerol had rather a weak inhibition on

Figure 4. Representative Dixon plots for the inhibition of CYP1A2 mediated phenacetin O-deethylation in human liver microsomes. Phenacetin (PA) (25 to 75 μ M) was incubated with or without 6-gingerol (0 to 100 μ M) in human liver microsomes and a NADPH generating system.

CYP3A4-mediated midazolam 1-hydroxylation (IC_{50} 108 μ M), CYP2D6-mediated dextromethorphan O-demethylation (IC_{50} 235 μ M), and CYP2E1-mediated chlorzoxazone 6-hydroxylation (IC_{50} 104 μ M).

With the concentration-dependent fashion, 6-gingerol had the strong inhibitory effects on CYP2C19-mediated S-mephenytoin 4-hydroxylation and CYP1A2-mediated phenacetin O-deethylation with IC_{50} value of 36 μ M and 51 μ M, respectively (Table 1 and Figure 2).

The inhibitory mechanism of 6-gingerol

was further studied by CYP2C19-catalyzed S-mephenytoin 4-hydroxylation with S-mephenytoin (15, 25, 50 μ M) and 6-gingerol (0 to 100 μ M) of CYP1A2-catalyzed acetaminophen with phenacetin (25, 50, 75 μ M) as represented in Figure 3 and 4, respectively. 6-gingerol was shown to inhibit CYP2C19 activity in a non-competitive manner as represented in Figure 3. The type of inhibition on CYP1A2 activity by 6-gingerol was considered to be competitive from Dixon plots (Figure 4).

DISCUSSION

Many herbal constituents, dietary ingredients, or beverages have been known to have drug interactions by affecting cytochrome P450 activities.^{13,14)} Ginger has been also frequently used as a spice and herb for a long time in a daily life. Therefore, there is a high possibility of drug-ginger interaction in disease treatment process. In addition, 6-gingerol, one constituent of ginger compound, has been shown to have anti-oxidant, anti-inflammatory, or other effects.²⁻⁷⁾ This suggests that 6-gingerol may affect the pharmacological response of therapeutic drugs. Clinically significant drug interactions are due to the inhibition of drug metabolizing enzymes, i.e. cytochrome P450 isoforms, resulting in decreased metabolic clearance and increased exposure to the inhibited drug. The inhibition of CYP enzymes need to be studied in order to

elucidate the potential drug interactions. However, the effect of 6-gingerol on the hepatic microsomal CYPs activities has not been known so far.

The present study examined the *in vitro* ability of 6-gingerol to inhibit the metabolism of substrates for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. It has been identified for the first time that 6-gingerol had the strong inhibitory effects on CYP1A2 and CYP2C19 catalytic activities. Also the type of inhibition on CYP1A2 was shown to be competitive.

When the therapeutic drugs for diseases are used together with taking ginger frequently, the low K_i value in this study could be used to estimate conservatively the potential for inhibitory drug interactions with 6-gingerol. To assess this possibility, the actual plasma level of 6-gingerol during daily consumption of ginger compound should be first known. In addition, other information needs to be clarified to extrapolate *in vitro* inhibition data to *in vivo* situations. Free concentrations of drug and inhibitor at the site of metabolism are important. The plasma protein binding of 6-gingerol is also needed to be determined.^{15,16)} Therefore, it is hard to assess the potential drug interactions at present and further studies are awaited.

In conclusion, above findings obtained from *in vitro* experiments performed with human liver microsomes indicated that 6-gingerol potentially inhibits CYP1A2 and CYP2C19-

mediated catalytic activity. Further studies are necessary to test the inhibitory effect of 6-gingerol on cDNA enzyme models which expressed these CYP isoforms. It is also needed to elucidate whether 6-gingerol might cause the clinical significant interactions with the CYP-metabolized drugs.

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