Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes

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Abstract

Oleanolic acid (OA) and ursolic acid (UA), triterpene acids having numerous pharmacological activities including anti-inflammatory, anti-cancer, and hepatoprotective effects, were tested for their ability to modulate the activities of several cytochrome P450 (CYP) enzymes using human liver microsomes. OA competitively inhibited CYP1A2-catalyzed phenacetin O-deethylation and CYP3A4-catalyzed midazolam 1-hydroxylation, the major human drug metabolizing CYPs, with IC50 (K_i) values of 143.5 (74.2) μM and 78.9 (41.0) μM, respectively. UA competitively inhibited CYP2C19-catalyzed S-mephenytoin 4'-hydroxylation with an IC50 (K_i) value of 119.7 (80.3) μM. However, other CYPs tested showed no or weak inhibition by both OA and UA. The present study demonstrates that OA and UA have inhibitory effects on CYP isoforms using human liver microsomes. It is thus likely that consumption of herbal medicines containing OA or UA, or administration of OA or UA, can cause drug interactions in humans when used concomitantly with drugs that are metabolized primarily by CYP isoforms. In addition, it appears that the inhibitory effect of OA on CYP1A2 is, in part, related to its anti-inflammatory and anticancer activities.

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Keywords: Oleanolic acid; Ursolic acid; Cytochrome P450; Microsomes; Herbal medicine

Introduction

Cytochrome P450 (CYP) enzymes are involved in the metabolism of endogenous substrates such as fatty acids, cholesterol, and steroids. These enzymes also carry out an important...
function in the metabolism and ultimate clearance of many structurally distinct xenobiotics such as drugs, carcinogens, and environmental pollutants (Porter and Coon, 1991; Rendic and Di Carlo, 1997).

Oleanolic acid (OA) and ursolic acid (UA) are triterpene acids having a similar chemical structure (Fig. 1) and are found in plants worldwide. These materials are of interest as therapeutics because of their biological activities. OA has antifungal (Tang et al., 2000), anti-inflammatory (Jeong et al., 1999; Marquina et al., 2001), anti-HIV (Kashiwada et al., 2000; Ma et al., 2000), diuretic (Alvarez et al., 2002), glucose-lowering (Yoshikawa and Matsuda, 2000), and anticancer (Li et al., 1999) activities. UA also has anti-angiogenic (Sohn et al., 1995), anticancer (Es-saady et al., 1996; Li et al., 1999), and anti-inflammatory (Baricevic et al., 2001) activities.

In addition, OA shows a hepatoprotective effect against chemical-induced hepatic injury (Liu et al., 1995; Jeong, 1999). It has been suggested that the mechanism underlying this hepatoprotective effect is OA-induced inhibition of the expression of CYP2E1. CYP2E1 is a major isoenzyme involved in the bioactivation of chemicals and drugs to toxic metabolites, and its inhibition would block the formation of these metabolites (Raucy, 1995; Tanaka et al., 2000). On the other hand, UA also has been suggested to have a hepatoprotective effect against chemical-induced hepatic injury (Saraswat et al., 1996). Most xenobiotics are not reactive themselves, but exert toxicity only after metabolic activation by a variety of enzymes responsible for drug metabolism. CYPs are among the major enzymes involved in the activation of carcinogenesis (Guengerich et al., 1990; Guengerich and Shimada, 1991). Therefore, one target of the chemopreventive effect of OA and UA could be the inhibition of the metabolizing activity of CYPs.

Taking these facts into account, inhibition by OA or UA of the metabolic activation of procarcinogens and the toxic metabolite formation catalyzed by CYPs may be one of the mechanisms of carcinogenesis inhibition and hepatoprotective effects. Although reduction in the

![Fig. 1. Chemical structure of oleanolic acid (OA) and ursolic acid (UA).](image-url)
gene expression level of CYPs by these substances has been demonstrated (Liu et al., 1995; Jeong, 1999), a detailed mechanism for the inhibition by OA or UA of human CYPs has not yet been elucidated.

Thus, the purpose of the present study was to clarify whether OA and UA have inhibitory effects on CYPs in human liver microsomes.

**Methods**

**Materials**

OA, UA, phenacetin, paclitaxel, acetaminophen, chlorozoxazone, dextromethorphan, dextrophan, furafylline, NADP, NADPH, EDTA, MgCl₂, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). 1-Hydroxymidazolam, S-warfarin, 6α-hydroxypaclitaxel, 7-hydroxywarfarin, S-mephenytoin, and 4′-hydroxymephenytoin were obtained from Ultrafine Chemical Co. (Manchester, UK). Acetonitrile and methanol were acquired from Fisher Scientific Co. (Pittsburgh, PA), and midazolam was kindly provided by Bukwang Pharmaceutical Co. (Seoul, Korea). All other reagents and chemicals were of analytical or HPLC grade. Human liver microsomes (HG42, HG43, HG56, HG64, HG93) were obtained from Gentest Corp (Woburn, MA).

**Incubation studies**

All incubations were performed in duplicate, and the mean values were used for analysis. Briefly, each incubation was performed with 1 mg/ml human liver microsomes in a final incubation volume of 0.25 ml. The incubation medium contained 100 mM phosphate buffer (pH 7.4) containing an NADPH-regenerating system (including 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 3.3 mM MgCl₂, and 1.0 U/ml glucose 6-phosphate dehydrogenase). The incubation mixture with OA or UA (final concentration 1–100 µM) was preincubated for 5 min. To determine whether the inhibition of CYP isoforms by OA or UA could be mechanism-based, OA or UA was preincubated with the incubation medium at 37°C from 0 to 15 min, either in the presence or absence of the NADPH-regenerating system. After preincubation, probe substrates were added either with or without the NADPH-regenerating system. The incubation conditions, including incubation times, concentration of microsomes, probe substrate concentrations, and quenching methods, have been reported elsewhere (Shin et al., 2002; Kim and Park, 2003; Park and Kim, 2003). The time of incubation and concentration of microsomes used in each assay were determined to be in the linear range for the rate of metabolite formation. After incubation at 37°C for a specific period of time, the reaction was stopped by adding an appropriate chemical to precipitate the proteins. The incubation mixtures were then centrifuged for 5 min at 10,000 g, and an aliquot of the supernatant was analyzed using high-performance liquid chromatography (HPLC).

**HPLC analysis**

Assays for the respective products of CYP marker reactions were carried out using HPLC. The reactions investigated were phenacetin O-deethylation for CYP1A2 (Tassaneeyakul et al., 1993), S-
warfarin 7-hydroxylation for CYP2C9 (Rettie et al., 1992), S-mephenytoin 4’-hydroxylation for CYP2C19 (Wrighton et al., 1993), paclitaxel 6α-hydroxylation for CYP2C8 (Harris et al., 1994), chlorzoxazone 6-hydroxylation for CYP2E1 (Lucas et al., 1996), and midazolam 1-hydroxylation for CYP3A4 (Thummel et al., 1994). The HPLC system consisted of a Shiseido 2001 pump (Shiseido Co., Tokyo, Japan), a Shiseido model 2023 autosampler, a dsChrom2000 integrator (Donam Instruments, Suwon, Korea), a Shiseido 2002 UV detector (for analysis of CYP1A2, CYP2C9, CYP2C19, CYP2C8, CYP2E1, and CYP3A4 activity), and a Jasco FP-2020 plus fluorescence detector (Jasco Co., Tokyo, Japan; for CYP2D6 activity).

Fig. 2. Inhibitory effects of OA and UA on CYP-catalyzed reactions in human liver microsomes. OA and UA were incubated under conditions described in Methods. The enzyme reactions evaluated were CYP1A2-catalyzed phenacetin O-deethylation (△), CYP2C8-catalyzed paclitaxel 6α-hydroxylation (○), CYP2C9-catalyzed S-warfarin 7-hydroxylation (●), CYP2C19-catalyzed S-mephenytoin 4’-hydroxylation (▲), CYP2D6-catalyzed dextromethorphan O-demethylation (◇), CYP2E1-catalyzed chlorzoxazone 6-hydroxylation (▽), and CYP3A4-catalyzed midazolam 1-hydroxylation (▼). Each data point represents the mean of duplicate experiments.
Data analysis

The IC\textsubscript{50} values (concentration of inhibitor causing 50% inhibition of the original enzyme activity) were determined graphically by using curves of mean enzyme activity versus inhibitor concentration. The apparent inhibitory constant (\(K_i\)) values were calculated using graphical analysis from secondary
plots of slopes taken from double-reciprocal plots of OA or UA concentrations versus metabolite formation.

Results

A panel of CYP-substrate assays was applied to determine whether OA and UA showed CYP isoform-specific inhibition in human liver microsomal preparations, as described in Methods.

In concentrations ranging from 1 to 100 μM, OA showed a significant inhibitory effect on CYP3A4-catalyzed midazolam 1-hydroxylation with an apparent IC_{50} (K_{i}) value of 78.9 (41.0) μM, and on
CYP1A2-catalyzed phenacetin O-deethylation with an apparent IC₅₀ (Ki) value of 143.5 (74.2) μM (Figs. 2–4). The pattern of inhibition by OA on both CYP1A2 and CYP3A4 was competitive (Figs. 3 and 4). However, OA showed weak or no inhibitory effects on the other CYP isoform-catalyzed reactions tested (Fig. 2). UA showed inhibitory potential on CYP2C19-catalyzed S-mephenytoin 4’-hydroxylation with an apparent IC₅₀ (Ki) value of 119.7 (80.3) μM in a competitive manner, but very little (<20%) or no inhibition of the other CYP isoforms tested was found in this concentration range (Figs. 2 and 5). To confirm whether OA and UA show mechanism-based inhibition on the affected CYP isoforms, we preincubated OA and UA at various concentrations prior to the addition of the specific substrates. We found dose-dependent inhibition of OA and UA on CYP-catalyzed reactions but not time-dependent inhibition suggesting no mechanism-based inhibition caused by OA and UA (Fig. 6). We summarized the inhibitory effects of OA and UA on each CYP-catalyzed reaction on Table 1.

**Discussion**

The use of herbal medicines has been worldwide in Europe and Asia, and there is also a more recent trend toward using natural remedies in the United States. Since these herbal medicines are largely unregulated by the administration and have not been systematically tested for safety and efficacy, there is some concern as to the effect of simultaneous consumption of herbal and clinically used drugs (Chang, 2000). With the exceptions of grapefruit juice and St. John’s wort extract, relatively little is known about potential drug-nutrient interactions of herbal or food supplements (Schmiedlin-Ren et al., 1997; Obach, 2000).

We evaluated the effects of OA and UA on the activities of human CYPs using human liver microsomes. OA inhibited CYP1A2- and CYP3A4-catalyzed reactions, whereas UA showed an inhibition on CYP2C19-catalyzed reaction. The results of the present study demonstrate firstly that OA and UA have inhibitory effects on CYP-catalyzed reactions in human liver microsomes.

In this study, inhibitory potential of OA on CYP3A4-catalyzed midazolam 1-hydroxylation was comparable to some known CYP3A4 inhibitors including cimetidine (Ki = 36–268 μM), diltiazem (Ki = 50–75 μM), clarithromycin (Ki = 10–28 μM), and narigenin (Ki = 22–70 μM). It could be suggested that a drug interaction of OA may occur with co-administered drugs metabolized mainly by CYP3A4. CYP3A4 is responsible for the metabolism of a wide range of drugs and endogenous compounds in

<table>
<thead>
<tr>
<th>Oleanolic Acid</th>
<th>Ursolic Acid</th>
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<tbody>
<tr>
<td>IC₅₀ (Ki), μM</td>
<td>IC₅₀ (Ki), μM</td>
</tr>
<tr>
<td>Type of inhibition</td>
<td>Type of inhibition</td>
</tr>
<tr>
<td>CYP2C8 &gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>CYP2C9 &gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>CYP2C19 &gt;500</td>
<td>119.7 (80.3) Competitive</td>
</tr>
<tr>
<td>CYP3A4 78.9 (41.0) Competitive</td>
<td>&gt;500</td>
</tr>
<tr>
<td>CYP2E1 &gt;500</td>
<td>380.7</td>
</tr>
<tr>
<td>CYP1A2 143.5 (74.2) Competitive</td>
<td>352.4</td>
</tr>
<tr>
<td>CYP2D6 &gt;500</td>
<td>438.9</td>
</tr>
</tbody>
</table>

Ki and IC₅₀ values were calculated from graphical analysis (see Methods for details).
humans. In humans, CYP3A4 accounts for up to 60% of liver CYP isoforms, and over 50% of the drugs marketed are metabolized by CYP3A4 (Omiecinski et al., 1999). Food- or herb-derived compounds, such as bergamottin (from grapefruit) and glabridin (from licorice), have been also shown to have an inhibitory effect on CYP3A4-catalyzed reactions (He et al., 1998; Kent et al., 2002). The inhibition of these components may be the basis of mechanism-based inhibition of CYP3A4, but OA showed no mechanism-based inhibition in this experiment.

OA also showed inhibitory effect on CYP1A2-catalyzed reactions. Owing to the roles played by CYP1A2 and CYP2E1 in carcinogenesis and chemical toxicities (Guengerich et al., 1991; Nebert et al., 1996), modulation of CYP1A2 activity by OA is an important issue. Additionally, several in vitro and in vivo studies have shown that OA and UA have anti-inflammatory and anticancer activities related to the inhibition of CYP activity (Sohn et al., 1995; Es-saady et al., 1996; Baricevic et al., 2001). Therefore, the inhibition of CYP1A2 by OA may play, at least in part, a crucial role in these effects.

Additionally, it has been reported that OA has a protective effect against hepatotoxicity caused by carbon tetrachloride and acetaminophen (Liu et al., 1993; Liu et al., 1995; Jeong, 1999). UA has also shown a hepatoprotective effect against ethanol-induced hepatotoxicity (Saraswat et al., 2000). Considering that ethanol, acetaminophen, and carbon tetrachloride are mainly metabolized by CYP2E1, leading to the production of highly reactive toxic metabolites, the hepatoprotective effects of OA and UA may be related to inhibition or reduction of CYP2E1 isoforms, thereby reducing the formation of toxic metabolites (Raucy, 1995; Tanaka et al., 2000). Consistent with this assumption, Liu et al. (1995) reported that OA reduced the activity and expression of CYP2E1 as well as other CYP isoforms including CYP1A and CYP2A.

However, we could not find any inhibitory effects of OA as well as UA on CYP2E1-catalyzed reaction in human liver microsomal preparations. Although we observed no inhibitory effects of OA and UA on CYP2E1, we could not rule out their inhibitory effects on CYPs by reduction of CYP expression. No data on the regulation for CYP expression by OA and UA in humans was available up to recently. Since OA and/or UA are marketed in several countries as an oral drug for the treatment of several diseases (Table 2), it should be necessary as a potential therapeutics to evaluate whether OA as well as UA would cause an inhibition on CYPs by reduction of CYP expression.

As noted above, UA has shown anti-inflammatory and anticancer activities similar to OA. However, it appears that these effects are not related to inhibition of UA on CYPs. UA showed only inhibitory effect on CYP2C19-catalyzed reaction but showed weak or no inhibition on other CYPs tested. We could not review the relationship between CYP2C19 and carcinogenesis and inflammation in the literature. Given that UA has the inhibition on CYP2C19, it is possible that administration of UA alone or of herbal medicines containing UA can cause interactions with drugs that are mainly metabolized by CYP2C19 (Flockhart, 1995).

Table 2
Examples of marketed herbal medicines containing OA and/or UA

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Manufactured Country</th>
<th>Purpose of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joins™</td>
<td>Korea</td>
<td>Treatment of osteoarthritis and rheumatoid arthritis</td>
</tr>
<tr>
<td>Kaprex™</td>
<td>USA</td>
<td>Gastric mucosal protection, joint pain relief</td>
</tr>
<tr>
<td>Eakan®</td>
<td>China</td>
<td>Hepatic protection</td>
</tr>
<tr>
<td>Holy Basil</td>
<td>Worldwide</td>
<td>Support normal cortisol, blood sugar, and insulin metabolism</td>
</tr>
<tr>
<td>Anbol</td>
<td>China</td>
<td>Treatment of cancer</td>
</tr>
</tbody>
</table>
Conclusion

Our study demonstrates that OA has an inhibitory effect on CYP1A2 and CYP3A4, whereas UA inhibits CYP2C19, in human liver microsomes. It is thus likely that either consumption of herbal medicines containing OA and UA, or administration of OA or UA themselves, can cause drug interactions in humans when co-administered with drugs that are metabolized primarily by CYPs. In addition, it appears that the inhibitory effect of OA on CYP1A2 is, in part, related to its pharmacological activities including anti-inflammatory and anticancer activities.

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References


