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The monoterpenoids citral and geraniol are moderate inhibitors of CYP2B6 hydroxylase activity

Kyung-Ah Seo [a], Hyunmi Kim [a], Hei-Young Ku [a], Hee-Jeong Ahn [a], Soo-Jin Park [a], Soo Kyung Bae [b], Jae-Gook Shin [a,b], Kwang-Hyeon Liu [a,c,*]

[a] Department of Pharmacology, PharmacoGenomics Research Center, Inje University College of Medicine, Busan, South Korea
[b] Department of Clinical Phamacology and Clinical Trial Center, Inje University Busan Paik Hospital, Busan, South Korea
[c] Frontier Inje Research for Science and Technology, Inje University, Busan, South Korea

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ABSTRACT

Monoterpenes are found in the volatile essence of flowers, plants oils, and herbal medicines. Some are commonly used as food additives and fragrance components, and many are found in cosmetics, soaps, cleaning products, disinfectants, preservatives, and medicines. We have recently discovered a moderate inhibitory effect of borneol and isoborneol toward CYP2B6-catalyzed bupropion hydroxylase activity. Based on that result, we expanded our study to evaluate the inhibitory effects of 22 monoterpenoids on CYP2B6 activity *in vitro*. Among the monoterpenoids screened, borneol, camphor, cineole, isoborneol, menthol, and perillaldehyde showed slight inhibition of CYP2B6-catalyzed bupropion hydroxylation, displaying greater than 50% inhibition at 50 μM. Citral and geraniol strongly inhibited CYP2B6 hydroxylase activity in a competitive manner, with K_i values of 6.8 and 10.3 μM, respectively, which are higher than the K_i (1.8 μM) of the well-known CYP2B6-selective inhibitor thio-TEPA. These *in vitro* data indicate that high amounts of these two monoterpenoids might interact with drugs that are metabolized by CYP2B6. The *in vivo* pharmacokinetics of these compounds should be examined to determine whether the inhibition of CYP2B6 activity by monoterpenoids has clinical relevance.

1. Introduction

Cytochrome P450 (P450) enzymes catalyze the oxidative metabolism of a wide variety of chemicals such as drugs, environmental pollutants, and endogenous compounds [1]. One of the numerous P450 enzymes identified to date, CYP2B6, is involved in the hydroxylation of bupropion [2] and efavirenz [3]. Our studies have shown that CYP2B6 also catalyzes sibutramine *N*-demethylation [4]. It is not unusual for bupropion and efavirenz to be administered in combination with several other drugs. For example, tenofovir increases the plasma concentration of efavirenz, a substrate of CYP2B6, under conditions of limited efavirenz metabolism [5]. Therefore, it is important to characterize the inhibitory potential of chemicals on P450 activity. Several chemicals such as thio-TEPA [6], clopidogrel [7], and ticlopidine [6,7] have already been identified as CYP2B6 isoform-selective inhibitors.

Essential oils [8] isolated from plants, flowers, seeds, and spices have been investigated for decades, mainly because of their significant applications in the manufacture of perfumes, flavoring agents, paints, and other products [9,10]. Their medicinal use, however, has not been formally investigated until recently [11–13]. Among numerous natural

Abbreviations: P450, cytochrome P450; HLM, human liver microsomes; LC/MS/MS, liquid chromatography/tandem mass spectrometry.

* Corresponding author at: Department of Pharmacology, PharmacoGenomics Research Center, Inje University College of Medicine, Gaegum-2-dong 633-165, Busanjin-gu, Busan, South Korea.
Tel.: +82 51 890 6412; fax: +82 51 893 1232.
E-mail address: dstlkh@inje.ac.kr (K.-H. Liu).

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products, terpenes play an important role in medicinal therapy [8,14,15], and several terpenes have been used directly as pharmaceuticals and flavoring agents for pharmaceutical products [16]. Terpenes are also commonly used as food additives and as fragrance components in cosmetics, soaps, and cleaning products [17].

In our previous study, we found that borneol and isoborneol, which are monoterpenoids, moderately inhibit CYP2B6 activity, with a potency comparable to that of thio-TEPA, a well-known CYP2B6 inhibitor [4]. Therefore, we expanded our study to isolate other monoterpenoids that possess an inhibitory effect on CYP2B6 activity. We investigated 22 monoterpenoids for their ability to inhibit CYP2B6 activity, which was determined by bupropion hydroxylation in a human liver microsomal system.

2. Materials and methods

2.1. Chemicals and reagents

Monoterpeniods, bupropion, thio-TEPA, β-nicotinamide adenine dinucleotide phosphate (β-NADP), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma–Aldrich (St. Louis, MO). Hydroxybupropion and pooled human liver microsomes (H161, pooled from 22 individual livers) were obtained from BD Gentest Co. (Woburn, MA). The solvents were HPLC grade and were purchased from Fisher Scientific Co. (Pittsburgh, PA).

2.2. Incubation studies using human liver microsomes

CYP2B6 inhibition assays were evaluated with the probe drug bupropion in human liver microsomal incubations. Monoterpenoids were dissolved in methanol, with the final concentration of organic solvent not exceeding 1% [18]. All incubations were performed with 0.25 or 0.5 mg/mL pooled human liver microsomes (Gentest, H161) in a final volume of 0.25 mL of 100 mM phosphate buffer, pH 7.4. The incubation mixture containing bupropion (50 μM), an inhibitor (monoterpenoid, 50 μM), and human liver microsomes was pre-incubated for 5 min at 37 °C, and then the reaction was initiated by the addition of a NADPH-generating system composed of 3.3 mM glucusoe-6-phosphate, 1.3 mM β-$NADP^+$, 3.3 mM $MgCl_2$, and 1.0 unit/mL glucose-6-phosphate dehydrogenase. To determine the inhibitory potentials (K_i values) of citral, geraniol, and thio-TEPA for CYP2B6-catalyzed hydroxylation of bupropion in human liver microsomal preparations, each compound (0, 2, 5, 20, and 50 μM) was added to reaction mixtures containing different concentrations of bupropion (20, 50, and 100 μM). After pre-incubated at 37 °C, the reaction was initiated for 30 min in a shaking water bath. The reaction was terminated by the addition of 100 μL of acetonitrile on ice, and the incubation mixtures were centrifuged at 10,000 × *g* for 5 min at 4 °C. Aliquots of the supernatants were analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The reaction rates were linear with incubation time and microsomal protein content under these conditions.

2.3. LC/MS/MS analysis

The concentration of hydroxybupropion was measured by LC/MS/MS, as described previously [19], using an API 3000 LC/MS/MS system (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface to generate positive ions $[M+H]^+$. The compounds were separated on a reversed-phase column (Luna C_{18}, 2.0 mm i.d. × 30 mm, 3 μm particle size; Phenomenex, Torrance, CA) with an isocratic mobile phase consisting of acetonitrile and water (20:80, v/v) containing 0.1% formic acid. The mobile phase was eluted at 0.2 mL/min using an Agilent 1100 series pump (Agilent, Wilmington, DE). The Turboion Spray interface was operated in the positive ion mode at 5000 V and 400 °C. The operating conditions were: nebulizing gas flow, 1.46 L/min; auxiliary gas flow, 4.0 L/min; curtain gas flow, 1.44 L/min; orifice voltage, 80 V; ring voltage, 400 V; collision energy, 25 eV; and collision gas (nitrogen) pressure, 3.58×10^{-5} Torr. The mass transitions used to quantify hydroxybupropion and chlorpropamide (IS) were m/z 256 → 238 and 277 → 155, respectively. The analytical data were processed using Analyst software (Version 1.2; Applied Biosystems).

2.4. Data analysis

The apparent kinetic parameters for the inhibitory potential (K_i) were first estimated by graphical methods such as Lineweaver–Burk, Dixon, and Eadie–Hosftee plots and were finally determined by nonlinear least squares regression analysis based on the best enzyme inhibition model [20], using WinNonlin software (Version 4.0, Pharsight, Mountain View, CA). In our experiment, the inhibition data were consistently best fitted by the competitive inhibition model, via the Akaike information criteria (AIC) and Schwartz criteria (SC). The models tested included pure and partial competitive inhibition, noncompetitive inhibition, mixed-type inhibition, and uncompetitive inhibition [21].

3. Results

3.1. Screening for inhibition of CYP2B6 by 22 monoterpenoids

Based on our previous data, we explored the possibility that other monoterpenoids might inhibit CYP2B6 activity. We initially tested 22 compounds at a concentration of 50 μM for their ability to inhibit CYP2B6-mediated bupropion hydroxylation (Fig. 1). Most of the monoterpenoids did not show significant inhibition (Fig. 2). Among the monoterpenoids tested, borneol, camphor, cineole, isoborneol, menthol, and perillaldehyde displayed moderate inhibition of CYP2B6 (50–80%). Citral and geraniol showed strikingly potent inhibition (>80%) of CYP2B6 activity.

3.2. Inhibitory effects of citral and geraniol on CYP2B6 activity

As citral and geraniol displayed the greatest inhibition of CYP2B6, we sought to clarify the mechanism of inhi-