Comparison in the In Vitro Inhibitory Effects of Major Phytocannabinoids and Polycyclic Aromatic Hydrocarbons Contained in Marijuana Smoke on Cytochrome P450 2C9 Activity

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Summary: Inhibitory effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN), the three major constituents in marijuana, and polycyclic aromatic hydrocarbons (PAHs) contained in marijuana smoke on catalytic activity of human cytochrome P450 (CYP) 2C9 were investigated. These phytocannabinoids concentration-dependently inhibited S-warfarin 7-hydroxylase and diclofenac 4'-hydroxylase activities of human liver microsomes (HLMs) and recombinant CYP2C9 (rCYP2C9). In contrast, none of the twelve PAHs including benz[a]anthracene and benzo[a]pyrene exerted substantial inhibition (IC₅₀ > 10 µM). The inhibitory potentials of Δ^9-THC (Kᵢ = 0.937–1.50 µM) and CBN (Kᵢ = 0.882–1.29 µM) were almost equivalent regardless of the enzyme sources used, whereas the inhibitory potency of CBD (Kᵢ = 0.954–9.88 µM) varied depending on the enzyme sources and substrates used. Δ^9-THC inhibited both S-warfarin 7-hydroxylase and diclofenac 4'-hydroxylase activities of HLMs and rCYP2C9 in a mixed manner. CBD and CBN competitively inhibited the activities of HLMs and rCYP2C9, with the only notable difference being that CBD and CBN exhibited mixed-type inhibitions against diclofenac 4'-hydroxylation and S-warfarin 7-hydroxylation, respectively, by rCYP2C9. None of Δ^9-THC, CBD, and CBN exerted metabolism-dependent inhibition. These results indicated that the three major phytocannabinoids but not PAHs contained in marijuana smoke potently inhibited CYP2C9 activity and that these cannabinoids can be characterized as direct inhibitors for CYP2C9.

Keywords: marijuana; cannabinoids; PAHs; CYP2C9; inhibition; drug-drug interaction

Introduction

Marijuana is the most widely abused drug in the world. There is growing concern about various health consequences due to association with polysubstance use and potential harmful effects on brain and peripheral functions. Marijuana leaves contain at least 70 cannabinoids. Among them, Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) are the three major constituents. Δ^9-THC is the major psychoactive component of marijuana causing hallucination, while CBD and CBN are thought to be nonpsychoactive. Marijuana is commonly consumed by smoking. In addition to cannabinoids, numerous chemical compounds including polycyclic aromatic hydrocarbons (PAHs) (Fig. 1), nitrogen oxides, carbon monoxide, and formaldehyde are contained in marijuana smoke. Carcinogenic PAHs such as benz[a]anthracene (B[a]A) and benzo[a]pyrene (B[a]P) are suggested to play important roles in the
Metabolic Interaction of Phytocannabinoid with CYP2C9

Fig. 1. Structures of major phytocannabinoids and PAHs contained in marijuana smoke

cytotoxicity, mutagenicity, and carcinogenicity of marijuana smoke, as well as tobacco smoke.4,5

There have been several reports on marijuana component(s)-drug interactions.6-11 Previously, Benowitz et al.8 reported that CBD reduced systemic clearance of hexobarbital in human subjects. The metabolism of hexobarbital is mainly catalyzed by cytochrome P450 (CYP) 2C9.12 Recently, Yamreudeewong et al.11 reported that marijuana smoking by a subject receiving chronic warfarin therapy caused increased international normalized ratio values and a more potent anticoagulant effect than R-warfarin.13 It has been previously shown that S-warfarin is predominantly metabolized by CYP2C9.14 Thus, it is possible that marijuana component(s) may increase the anticoagulant effect of warfarin due to the inhibition of its metabolism. Our recent in vitro studies have shown that Δ²-THC, CBD, and CBN inhibit the catalytic activities of CYP1A2, CYP2B6, CYP2D6, and CYP3A4.15-18 Many PAHs have been reported to potently inhibit CYP1A2 activity.19 However, little is known about the inhibitory effects of marijuana components including phytocannabinoids and PAHs on CYP2C9, which is one of the most important enzymes in the hepatic drug metabolism.20

In this study, we carried out in vitro inhibition studies on CYP2C9 with major phytocannabinoids and PAHs contained in marijuana smoke shown in Figure 1. We found that Δ⁹-THC, CBD, and CBN can be characterized as potent direct inhibitors for CYP2C9 whereas the twelve PAHs examined do not show substantial inhibition.

Materials and Methods

Materials: Δ⁹-THC, CBD, and CBN were isolated from cannabis leaves using the method previously reported.21 Purities of these cannabinoids were determined to be above 97% by gas chromatography.22 Other chemicals were obtained from the following sources: S-warfarin from Cayman Chemical (Ann Arbor, MI); diclofenac, acenaphthene, anthracene, fluoranthene, naphthalene, and pyrene from Sigma-Aldrich Corp. (St. Louis, MO); 7-hydroxywarfarin and 4'-hydroxydiclofenac from BD Gentest (Woburn, MA); acenaphthylene, B(a)P, 1-methyl-naphthalene, 2-methylnaphthalene, and phenanthrene from Wako Pure Chemical Ind. (Osaka, Japan); fluorene from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); NADP, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase from Oriental Yeast Co. Ltd. (Tokyo, Japan). All other chemicals and solvents used were of the highest quality commercially available.

Enzyme sources: Microsomes from baculovirus-infected insect cells expressing CYP2C9 and CYP1A2, each with NADPH-CYP reductase (Supersomes19), were purchased from BD Gentest. Pooled human liver microsomes (pHLMs) (Catalog# CPH-08-069) were purchased from XenoTech (Lenexa, KS). A human liver sample was obtained from a 57-year-old Japanese female who died in a traffic accident. The use of the human liver for these studies was approved by the Ethics Committee of Kanazawa University, Graduate School of Medical Science. The individual HLMs (iHLMs) were prepared as reported previously.23 The protein concentration was estimated by the method of Lowry et al.,24 using bovine serum albumin as a standard.

Enzyme assays: S-Warfarin 7-hydroxylase activity of CYP2C9 was determined as reported previously25 with minor modifications. pHLMs (20 µg protein), iHLMs (50 µg protein), and recombinant CYP2C9 (rCYP2C9) (6 pmol) were used as enzyme sources. An incubation mixture consisted of an enzyme source, S-warfarin, an NADPH-generating system (0.5 mM NADP, 10 mM glucose 6-phosphate, 10 mM magnesium chloride, and 1 unit/ml glucose 6-phosphate dehydrogenase), and 50 mM potassium phosphate buffer (pH 7.4) in a final volume of 200 µl. Following pre-warming at 37°C for 5 min, reactions were initiated by the addition of the NADPH-generating system. Incubations were carried out at 37°C for 20 min and terminated by adding 10 µl of 70% (v/v) perchloric acid. After the removal of protein by centrifugation, 90 µl of the supernatant was subjected to high-performance liquid chromatography (D-7500 integrator, L-7100 pump, L-7200 autosampler, and L-7485 fluorescence detector, Hitachi, Tokyo, Japan) with a Mightysil RP-18 GP column (4.6 × 150 mm, 5 µm, Kanto Chemical, Tokyo, Japan). The mobile phase was a mixture of 36% (v/v) acetonitrile containing 0.04% (v/v) phosphoric acid. Elution was performed at a flow rate of 1.5 ml/min. The formation of 7-hydroxy-warfarin was monitored at an excitation wavelength of 320 nm and emission wavelength of 415 nm.

The diclofenac 4'-hydroxylase activity of CYP2C9 was determined as reported previously26 with minor modifications. Briefly, an incubation mixture consisted of iHLMs
(50 μg protein) or rCYP2C9 (2 pmol), diclofenac, an NADPH-generating system (0.5 mM NADP, 10 mM glucose 6-phosphate, 10 mM magnesium chloride, and 1 unit/ml glucose 6-phosphate dehydrogenase), and 100 mM Tris-HCl buffer (pH 7.5) in a final volume of 250 μl. Following pre-warming at 37° C for 5 min, reactions were initiated by the addition of the NADPH-generating system. Incubations were carried out at 37° C for 20 min and terminated by adding 50 μl of ice-cold acetonitrile. After centrifugation, 90 μl of the supernatant was subjected to high-performance liquid chromatography (Hitachi L-2130 pump, L-2200 autosampler, and L-2400 UV detector, Hitachi) with a Mightysil RP-18 GP column (4.6 × 250 mm, 5 μm, Kanto Chemical). The mobile phase consisted of 12.5 mM Tris-HCl buffer (pH 7.4), methanol, and acetonitrile (80:15:5, v/v) for solvent A and methanol for solvent B. The metabolite, 4′-hydroxycyclofenac, was separated using a linear gradient of 100 to 0% solvent A, 0 to 20 min at a flow rate of 1.0 ml/min. The formation of 4′-hydroxycyclofenac was monitored at a wavelength of 280 nm.

To determine kinetic parameters for the S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation by HLMs and rCYP2C9, S-warfarin (1.25 to 100 μM) or diclofenac (0.5 to 80 μM) was incubated with the corresponding enzyme sources under the same conditions as described above. In preliminary experiments, these reaction conditions were confirmed to ensure linear initial rates for the formation of 7-hydroxywarfarin and 4′-hydroxycyclofenac. Data points were fitted to the Michaelis-Menten equation by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab, Northampton, MA).

The 7-ethoxyresorufin O-deethylase (EROD) activity of rCYP1A2 was determined as reported previously. Inhibition studies: Each enzyme source was incubated with a substrate in the presence of phytocannabinoids (0.25 to 20 μM), PAHs (0.001 to 10 μM), or sulfaphenazole (0.05 to 5 μM) in the same manner as described for the enzyme assays. All compounds were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of ≤ 0.2%. The IC₅₀ value was calculated by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab).

The effects of three different inhibitor concentrations on S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation activities were examined at four or five substrate concentrations to characterize the enzyme kinetics for the inhibition of CYP2C9 by phytocannabinoids. The apparent Kᵢ value (inhibition constant) was determined from the x-intercept of a plot of apparent Kᵢ/Vₘₐₓ (obtained from the slope of the Lineweaver-Burk plots) versus inhibitor concentration. The x-intercept, which is equal to −Kᵢ, was calculated by linear regression. Lineweaver-Burk plots of the enzyme kinetic data were generated to determine the mode of inhibition.

To identify potential metabolism-dependent inhibition of CYP2C9 by phytocannabinoids, inhibition experiments were performed as described below. The preincubation mixture contained pHLMs (20 μg protein) or rCYP2C9 (6 pmol), each major phytocannabinoid (0.25 to 10 μM), the NADPH-generating system, and 50 mM potassium phosphate buffer (pH 7.4) in a final volume of 180 μl. After pre-warming at 37° C for 5 min, reactions were initiated by the addition of the NADPH-generating system. Following 20-min preincubation, 20 μl of S-warfarin solution was added to the preincubation mixture (final substrate concentration 3 μM). Incubations were conducted in the same manner as described in the enzyme assays for S-warfarin 7-hydroxylase activity.

Results

Kinetic analyses for S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation by CYP2C9: To clarify the enzymatic characteristics of HLMs and rCYP2C9 used in this study toward S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation activities, kinetic analyses were conducted with these enzyme sources. All the reactions tested followed the Michaelis-Menten kinetics based on the Eadie-Hofstee plots (data not shown), and the kinetic parameters were therefore determined from the Michaelis-Menten equation. The Vₘₐₓ values of S-warfarin 7-hydroxylation by iHLMs, pHLMs, and rCYP2C9 were 9.90 pmol/min/mg protein, 2.97 pmol/min/mg protein, and 52.4 pmol/min/nmol P450, respectively, while the corresponding Kₘ values were 3.56, 4.38, and 2.90 μM, respectively (Table 1). For diclofenac 4′-hydroxylation, the Vₘₐₓ values for iHLMs and rCYP2C9 were 1.25 nmol/min/mg protein and 3.24 nmol/min/nmol P450, respectively; the apparent Kₘ values were 9.19 and 0.679 μM, respectively (Table 1).

Effects of major phytocannabinoids and PAHs contained in marijuana smoke on CYP2C9 activity: Effects of Δ⁹-THC, CBD, and CBN on oxidative activities of CYP2C9 were examined. All the phytocannabinoids tested inhibited CYP2C9-mediated S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation in a concentration-dependent manner (Fig. 2). Inhibitory effects of Δ⁹-THC and CBN on S-warfarin 7-hydroxylation activity of pHLMs (Fig. 2A) and iHLMs were similar, showing IC₅₀ values of 2.29 to 2.72 μM

<p>| Table 1. Kinetic parameters for S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation by HLMs and rCYP2C9 |
|---|---|---|</p>
<table>
<thead>
<tr>
<th>Reactions</th>
<th>Enzymes</th>
<th>Kᵢ (μM)</th>
<th>Vₘₐₓ (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-warfarin</td>
<td>pHLMs</td>
<td>3.56 ± 0.14</td>
<td>9.90 ± 0.10</td>
</tr>
<tr>
<td>7-hydroxylation</td>
<td>iHLMs</td>
<td>4.38 ± 0.25</td>
<td>2.97 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>rCYP2C9</td>
<td>2.90 ± 0.06</td>
<td>52.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>rCYP2C9</td>
<td>0.679 ± 0.125</td>
<td>3.24 ± 0.12</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>iHLMs</td>
<td>9.19 ± 0.47</td>
<td>1.25 ± 0.02</td>
</tr>
<tr>
<td>4′-hydroxylation</td>
<td>rCYP2C9</td>
<td>0.679 ± 0.125</td>
<td>3.24 ± 0.12</td>
</tr>
</tbody>
</table>

Values are represented as mean ± S.E. of kinetic parameters.
Table 2). The inhibitory effect of CBD on the liver microsomal activities (IC\textsubscript{50} ≈ 4.8 µM) was less potent than those of Δ\textsuperscript{9}-THC and CBN. In contrast, Δ\textsuperscript{9}-THC, CBD, and

CBN equivalently inhibited S-warfarin 7-hydroxylase activity of rCYP2C9 (Fig. 2B and Table 2). The inhibitory effects of Δ\textsuperscript{9}-THC and CBN on diclofenac 4′-hydroxylase activity of iHLMs were similar (Fig. 2C), indicating IC\textsubscript{50} values of 4.44 and 3.90 µM, respectively (Table 2). The inhibitory effect of CBD on the activity of iHLMs (IC\textsubscript{50} = 13.1 µM) was less potent than those of Δ\textsuperscript{9}-THC and CBN (Fig. 2C). The inhibitory profiles of these cannabinoids against rCYP2C9-mediated diclofenac 4′-hydroxylation were similar to those against iHLMs (Figs. 2C and 2D). The IC\textsubscript{50} values of Δ\textsuperscript{9}-THC, CBD, and CBN for rCYP2C9 were 1.02, 2.70, and 1.13 µM, respectively (Table 2).

The effects of PAHs on CYP2C9 activity were next investigated (Table 2). B[a]A at a concentration of 10 µM inhibited the S-warfarin 7-hydroxylase activity of rCYP2C9 by approximately 20%, whereas the compound did not exert any inhibitory effect on the activity of pHLMs. In addition, B[a]P inhibited the activity of pHLMs and rCYP2C9 in a concentration-dependent manner, exhibiting approximately 20% inhibition at a concentration of 10 µM. The other PAHs examined, acenaphthene, acenaphthylene, anthracene, fluoranthene, fluorene, 1-methylnaphthalene, 2-methylnaphthalene, naphthalene, phenanthrene, and pyrene, did not show any significant inhibition under the current conditions. In contrast, anthracene, B[a]A, B[a]P, fluoranthene, fluorene, phenanthrene, and pyrene strongly inhibited the EROD activity of rCYP1A2 (Table 2).

In addition, the inhibitory effect of sulfaphenazole, a CYP2C9-selective inhibitor, on CYP2C9 activities was...
Table 3. Kinetic parameters for inhibition of CYP2C9-mediated drug oxidations by major phytocannabinoids

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Enzymes</th>
<th>Cannabinoids</th>
<th>$K_i$ (µM)</th>
<th>Modes of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Warfarin 7-hydroxylation pHLMs</td>
<td>Δ9-THC</td>
<td>1.50</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>5.60</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>0.931</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iHLMs</td>
<td>Δ9-THC</td>
<td>0.937</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>3.46</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>1.25</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rCYP2C9</td>
<td>Δ9-THC</td>
<td>1.40</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>0.954</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>0.882</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td>Diclofenac 4'-hydroxylation iHLMs</td>
<td>Δ9-THC</td>
<td>1.31</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>9.88</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>1.29</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rCYP2C9</td>
<td>Δ9-THC</td>
<td>1.39</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>2.31</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>1.29</td>
<td>Competitive</td>
<td></td>
</tr>
</tbody>
</table>

Data are derived from two replicates.

examined (Table 2). S-Warfarin 7-hydroxylation activity of pHLMs, iHLMs, and rCYP2C9 was strongly inhibited by sulfaphenazole, with IC50 values of 0.442, 0.181, and 0.222 µM, respectively. Furthermore, the diclofenac 4'-hydroxylation activity of iHLMs and rCYP2C9 was efficiently inhibited by sulfaphenazole with IC50 values of 0.342 and 0.216 µM, respectively. The inhibitory effects of sulfaphenazole on CYP2C9 activities in this study were comparable to the previous findings.27,28

Kinetic analyses for phytocannabinoid-mediated inhibition of CYP2C9 activity: Kinetic analyses for the inhibition were conducted to characterize the modes of inhibition of CYP2C9 by the major phytocannabinoids (Table 3). Δ9-THC inhibited S-warfarin 7-hydroxylase activity of pHLMs in a mixed manner, while CBD and CBN competitively inhibited the activity of pHLMs. The $K_i$ values of Δ9-THC, CBD, and CBN were 1.50, 5.60, and 0.931 µM, respectively. The modes of inhibition of iHLMs by these phytocannabinoids were consistent with those of pHLMs. Δ9-THC and CBN exhibited a mixed-type inhibition against rCYP2C9-mediated S-warfarin 7-hydroxylation although CBD showed a competitive inhibition. The $K_i$ values of Δ9-THC, CBD, and CBN for rCYP2C9 were 1.40, 0.954, and 0.882 µM, respectively. Δ9-THC inhibited the diclofenac 4'-hydroxylation activity of iHLMs in a mixed manner, whereas CBD and CBN inhibited the activity in a competitive manner. The $K_i$ values of Δ9-THC, CBD, and CBN were 1.31, 9.88, and 1.29 µM, respectively. With respect to rCYP2C9, Δ9-THC and CBD showed a mixed-type inhibition, and CBN exhibited a competitive inhibition. The $K_i$ values of Δ9-THC, CBD, and CBN for rCYP2C9 were 1.39, 2.31, and 1.29 µM, respectively.

Metabolism-dependent inhibition of CYP2C9 activity by major phytocannabinoids: The effect of preincubation on inhibition by Δ9-THC, CBD, and CBN was investigated to determine whether these phytocannabinoids inhibit CYP2C9-mediated oxidation in a metabolism-dependent manner. A 20-min preincubation of Δ9-THC, CBD, and CBN in the presence of NADPH did not potentiate the inhibition of S-warfarin 7-hydroxylase activity of pHLMs or rCYP2C9 (Table 4).

Discussion

In this study, we demonstrated that Δ9-THC, CBD, and CBN themselves inhibited CYP2C9 activity. The inhibitory potentials of these major phytocannabinoids for CYP2C9 are comparable to those of amiodarone, diclofenac, and tolbutamide.29 Interestingly, the inhibitory effects of these phytocannabinoids on CYP2C9 are more potent than those of several nonsteroidal anti-inflammatory drugs, warfarin, and phenytoin.29 These results reveal that these major phytocannabinoids can be categorized as potent inhibitors for CYP2C9.

The inhibitory potencies (IC50 and $K_i$) for S-warfarin 7-hydroxylation by rCYP2C9 were similar among the phytocannabinoids tested. When diclofenac was used as a substrate, however, the inhibitory potency of CBD for rCYP2C9 was weaker than those of Δ9-THC and CBN although there was only a small difference in the $K_i$ value. These results suggest that CBD has a different susceptibility to the CYP2C9 inhibition, depending on the CYP2C9 substrates used. The $K_i$ values of Δ9-THC and CBN for HLMs were almost the same as those for rCYP2C9. In contrast, the $K_i$ values of CBD for HLMs were higher compared with those for rCYP2C9. CBD is predominantly oxidized at the 7-position by CYP2C19 and at the 6-position by CYP3A4, while CYP2C9 plays a minor role in the CBD oxidations.30 Thus, it is suggested that the metabolism of CBD by CYP2C19 and/or CYP3A4 in HLMs may attenuate the inhibitory effect of CBD on CYP2C9 activity.

It has been previously shown that CYP2C9 is capable of metabolizing B(a)P, B(a)A, phenanthrene, and pyrene.31-34 However, substantial inhibition of CYP2C9 by twelve PAHs including these compounds was not observed under the current conditions. In contrast, many of the PAHs examined...
potently inhibit human CYP1A2 activity, as reported previously.\textsuperscript{19} PAHs are present in marijuana smoke at much lower concentrations than the phytocannabinoids.\textsuperscript{3,35} For example, the contents of B[a]A and B[a]P are 56 and 22 ng per marijuana cigarette, respectively, although the content of $\Delta^9$-THC is estimated to be approximately 9.3 mg per marijuana cigarette.\textsuperscript{33} These findings suggest a minor role of PAHs in the CYP2C9 inhibition by marijuana.

Our results may partly explain the mechanisms underlying in vivo marijuana-drug interactions reported so far. A reduced systemic clearance of hexobarbital by the coadministration of CBD\textsuperscript{88} might be attributed to CBD inhibition of the hexobarbital metabolism catalyzed by CYP2C9. In addition, increased anticoagulation of warfarin by marijuana smoking\textsuperscript{11} might be due to inhibition of the CYP2C9-mediated warfarin metabolism by cannabinoids. The mean contents of $\Delta^9$-THC, CBD, and CBN in dried plant preparations of marijuana are 4.5, 0.4, and 0.3%, respectively.\textsuperscript{16} A clinical study has shown that the peak plasma concentration of $\Delta^2$-THC is 162 ng/ml (0.516 $\mu$M) after smoking a single marijuana cigarette containing 34 mg $\Delta^2$-THC, the content of which is 3.55%.\textsuperscript{37} The $K_i$ values of $\Delta^2$-THC for CYP2C9 are 2- to 3-fold higher than the plasma concentration. Since cannabinoids are readily distributed in various tissues due to their high lipophilicity,\textsuperscript{38} tissue concentrations of $\Delta^9$-THC may be even higher than the plasma concentration. The relative contents and inhibitory potentials of these phytocannabinoids suggest that $\Delta^9$-THC may be one of the factors influencing the warfarin metabolism after marijuana smoking. Further investigations are required to clarify the precise mechanisms of in vivo marijuana-drug interactions.

In conclusion, we demonstrated that the three major phytocannabinoids but not PAHs exerted potent direct inhibition against CYP2C9. This study will provide useful information about possible interactions between marijuana and drugs metabolized by CYP2C9.

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