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# Characterization of major phytocannabinoids, cannabidiol and cannabinol, as isoform-selective and potent inhibitors of human CYP1 enzymes

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ABSTRACT

Inhibitory effects of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), cannabidiol (CBD), and cannabinol (CBN), the three major constituents in marijuana, on catalytic activities of human cytochrome P450 (CYP) 1 enzymes were investigated. These cannabinoids inhibited 7-ethoxyresorufin *O*-deethylase activity of recombinant CYP1A1, CYP1A2, and CYP1B1 in a competitive manner. CBD most potently inhibited the CYP1A1 activity; the apparent $K_i$ value (0.155 μM) was at least one-seventeenth of the values for other CYP1 isoforms. On the other hand, CBN more effectively decreased the activity of CYP1A2 and CYP1B1 ($K_i$ = 0.0790 and 0.148 μM, respectively) compared with CYP1A1 ($K_i$ = 0.541 μM). $\Delta^9$-THC less potently inhibited the CYP1 activity than CBD and CBN, and showed low selectivity against the CYP1 inhibition ($K_i$ = 2.47–7.54 μM). The preincubation of CBD resulted in a time- and concentration-dependent decrease in catalytic activity of all the recombinant CYP1 enzymes and human liver microsomes. Similarly, the preincubation of $\Delta^9$-THC or CBN caused a time- and concentration-dependent inhibition of recombinant CYP1A1. The inactivation of CYP1A1 by CBD indicated the highest $k_{inact}/K_I$ value (540 l/mmol/min) among the CYP1 enzyme sources tested. The inactivation of recombinant CYP1A1 and human liver microsomes by CBD required NADPH, was not influenced by dialysis and by glutathione, N-acetylcysteine, and superoxide dismutase as trapping agents. These results indicated that CBD and CBN showed CYP1 isoform-selective direct inhibition and that CBD was characterized as a potent mechanism-based inhibitor of human CYP1 enzymes, especially CYP1A1.



## 1. Introduction

Marijuana is one of the most widely abused drugs, and its use is legally regulated in most countries of the world. Marijuana leaves contain at least 70 cannabinoids [1]. Among them, $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), cannabidiol (CBD), and cannabinol (CBN) are the three major constituents (Fig. 1). $\Delta^9$-THC is a primary psychoactive substance inducing hallucination in humans. In addition, this substance possesses several pharmacological effects including catalepsy, hypothermia, antiinflammation, and antinociception [2]. CBD has more potent anticonvulsant and drug-induced sleep prolongation effects as compared with $\Delta^9$-THC [3,4]. Unlike $\Delta^9$-THC, CBN does not have substantial pharmacological effects in the central nervous system.

A synthetic cannabinoid and a marijuana extract have been used as clinical drugs in North America. Marinol®, a synthetic $\Delta^9$-THC (dronabinol), is a drug used for the treatment of nausea and vomiting associated with cancer chemotherapy and for suppressing loss of appetite and weight loss related to AIDS. Sativex®, marijuana extract containing both $\Delta^9$-THC and CBD, is prescribed for the symptomatic relief for neuropathic pain in multiple sclerosis.

Due to high lipophilicity, $\Delta^9$-THC, CBD, and CBN are known to be extensively metabolized by humans and experimental animals [5–7]. Cytochrome P450 (CYP) is mainly responsible for the primary metabolism of the cannabinoids in liver microsomes [8]. Previous studies by Bornheim et al. and us demonstrated that the major CYP enzymes involved in the hepatic metabolism of $\Delta^9$-THC, CBD, and CBN are CYP2C and CYP3A [7,9–11]. Thus, it is possible that the administration of cannabinoids or marijuana leads to drug interactions with drugs or toxicants metabolized by these CYP enzymes. Benowitz et al. [12] have reported that CBD decreases the systemic clearance of hexobarbital, which is metabolized by

*Abbreviations:* BSA, bovine serum albumin; CBD, cannabidiol; CBN, cannabinol; CYP, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylase; GSH, glutathione; HLMs, human liver microsomes; NAC, *N*-acetylcysteine; SOD, superoxide dismutase; $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol.

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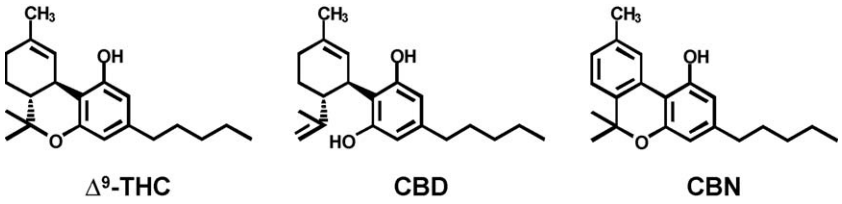


**Fig. 1.** Structures of three major cannabinoids.

CYP2C9, in human subjects. A previous study with human liver microsomes (HLMs) has shown that CBD inhibits CYP3A-mediated oxidations [13]. Interestingly, it has been reported that $\Delta^9$-THC inhibits an enzyme activity of recombinant human CYP1A1 [14]. CYP1 enzymes have an ability to metabolize cannabinoids such as THC [15,16]. However, the CYP1 family is not a major enzyme group catalyzing cannabinoid metabolism in the human liver. Therefore, metabolic interactions of various cannabinoids with human CYP1 enzymes have not been investigated extensively.

The human CYP1 family consists of CYP1A1, CYP1A2, and CYP1B1. CYP1A1 is expressed in various tissues including liver and lung [17–19], although the constitutive expression level is very low. In many cases, the hepatic and pulmonary CYP1A1 is induced by exposure to tobacco smoke [20,21]. CYP1A2 is predominantly expressed in the liver and is the most abundant form of CYP1 family (~13% of total CYP) expressed in adult human livers [19,22]. CYP1B1 is distributed in a variety of tissues including the liver [23]. In particular, the high expression of CYP1B1 has been detected in hormone-related tissues, such as prostate, mammary, ovary, and uterus [19,23]. These CYP1 isoforms are important in the bioactivation of procarcinogens, such as polycyclic aromatic hydrocarbons and heterocyclic amines [19,24]. Furthermore, these enzymes play a role in the metabolism of various drugs including phenacetin (CYP1A1 and CYP1A2) [25,26], theophylline (CYP1A2 and CYP1B1) [27–29], granisetron (CYP1A1) [30], dacarbazine (CYP1A1 and CYP1A2) [31], and flutamide (CYP1A2 and CYP1B1) [32,33].

In the present study, we investigated inhibitory effects of the three major cannabinoids ($\Delta^9$-THC, CBD, and CBN) on catalytic activities of human CYP1 enzymes. We report herein that CBD and CBN cause CYP1 isoform-selective direct inhibition and that CBD is a potent mechanism-based inhibitor of human CYP1 enzymes.

## 2. Materials and methods

### 2.1. Materials

$\Delta^9$-THC, CBD, and CBN were isolated from cannabis leaves using the method previously reported [34]. Microsomes from baculovirus-infected insect cells expressing CYP1A1, CYP1A2, and CYP1B1 each with NADPH-CYP reductase (Supersomes™) were purchased from BD Gentest (Woburn, MA). Pooled HLMs for the Reaction Phenotyping Kit Ver. 6 were obtained from XenoTech (Kansas, KS). NADPH, 7-ethoxyresorufin, and superoxide dismutase (SOD) were purchased from Sigma Chemical Co. (St. Louis, MO). Resorufin and reduced glutathione (GSH) were obtained from Wako Pure Chemical Ind. (Osaka, Japan). Fatty acid-free bovine serum albumin (BSA) and *N*-acetylcysteine (NAC) were obtained from Nakalai Tesque (Kyoto, Japan). Slide-A-Lyzer MINI Dialysis Unit was purchased from Pierce (Rockford, IL). Other chemicals and solvents used were of the highest quality commercially available.

### 2.2. Enzyme assay

The activity of 7-ethoxyresorufin *O*-deethylase (EROD), which has been widely used as a marker for CYP1-specific activity [35], was determined by using 96-well microtiter plates as reported previously [14] with minor modifications. Recombinant CYP1A1 (10 fmol), CYP1A2 (60 fmol), CYP1B1 (20 fmol), and HLMs (5 μg protein) were used as enzyme sources. An incubation mixture consisted of an enzyme source, 7-ethoxyresorufin, 1.67 mM NADPH, and 50 mM Tris–HCl buffer (pH 7.4) containing 1% BSA in a final volume of 200 μl. After pre-warming at 37 °C for 5 min, reactions were initiated by the addition of NADPH. Fluorescence derived from resorufin formation was recorded every 5 min for 30 min using FLUOstar OPTIMA® (BMG Labtech, Offenburg, Germany) with excitation and emission filters at 544 and 590 nm, respectively.

To determine kinetic parameters for the EROD reaction in recombinant CYP1 isoforms and HLMs, 7-ethoxyresorufin at 14–1000 nM was incubated with either of these enzyme sources under the same conditions as mentioned above. In preliminary experiments, these reaction conditions were confirmed to ensure linear initial rates for the formation of resorufin. Data points were fitted to the Michaelis–Menten equation by nonlinear least-squares regression analysis with Origin 7.5J Software (OriginLab, Northampton, MA).

### 2.3. Direct inhibition studies

Recombinant CYP1 isoforms and HLMs were incubated with 7-ethoxyresorufin (150 nM) in the presence of $\Delta^9$-THC, CBD, or CBN (0.025–25 μM) under the same manner as described in the enzyme assay. All compounds were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of 0.5%. The $IC_{50}$ value was calculated by nonlinear least-squares regression analysis with Origin 7.5J Software (OriginLab).

The effects of three different concentrations of each cannabinoid on the formation of resorufin were examined at five substrate concentrations to characterize the enzyme kinetics for the inhibition of human CYP1 enzymes by $\Delta^9$-THC, CBD, and CBN. The apparent $K_i$ value (inhibition constant) was determined from the *x*-intercept of a plot of apparent $K_m/V_{max}$ (obtained from the slope of the Lineweaver–Burk plots) versus inhibitor concentration. The *x*-intercept, which is equal to $-K_i$, was calculated by linear regression using the Origin 7.5J Software (OriginLab). Lineweaver–Burk plots of the enzyme kinetic data were generated to determine the mode of inhibition.

### 2.4. Inactivation studies

To identify potential mechanism-based inactivation, preliminary screening experiments were conducted. All reactions were carried out at 37 °C in a shaking water bath. The preincubation tubes contained an enzyme source, each cannabinoid (0.031–50 μM), 1.67 mM NADPH, and 50 mM Tris–HCl buffer (pH 7.4) containing 1% BSA in a final volume of 180 μl. After pre-warming at 37 °C for 5 min, reactions were initiated by the addition of NADPH. Following 20-min preincubation, 20 μl of 7-ethoxyresorufin solution was added to the preincubation mixture (final concentration 150 nM). Incubations were carried out at 37 °C for

**Table 1**
Kinetic parameters for EROD reaction catalyzed by recombinant human CYP1 isoforms and HLMs.

| Enzymes | $V_{max}$ (nmol/min/nmol P450) | $K_m$ (nM) | $V_{max}/K_m$ (ml/min/nmol P450) |
|---|---|---|---|
| CYP1A1 | 51.1 | 110 | 465 |
| CYP1A2 | 0.537 | 226 | 2.38 |
| CYP1B1 | 3.07 | 148 | 20.7 |
| Enzyme | $V_{max}$ (pmol/min/mg protein) | $K_m$ (nM) | $V_{max}/K_m$ (μl/min/mg protein) |
| HLMs | 7.54 | 223 | 33.8 |

20 min and terminated by adding 200 μl of ice-cold methanol. For the background sample, NADPH was added following the termination of the reaction. After being kept on ice for 15 min, 300 μl of the reaction mixture was transferred to a 96-well microtiter plate. The fluorescence was measured using FLUOstar OPTIMA® (BMG Labtech) with the same filters as described in the enzyme assay. Coincubations in which each cannabinoid was added together with 7-ethoxyresorufin at the end of 5-min prewarming were also carried out.

Kinetics of human CYP1 inactivation by major cannabinoids was determined as described below. The preincubation tubes contained an enzyme source, each cannabinoid (0.033–16 μM), 1.67 mM NADPH, and 50 mM Tris–HCl buffer (pH 7.4) containing 1% BSA in a final volume of 100 μl. Recombinant CYP1A1 (25 fmol), CYP1A2 (150 fmol), CYP1B1 (50 fmol), and HLMs (12.5 μg protein) were used as enzyme sources. Preincubations were carried out at 37 °C for various amounts of time up to 15 min. An aliquot (40 μl) of the preincubation mixture was transferred to a 160 μl enzyme assay mixture containing 150 nM 7-ethoxyresorufin and 1.67 mM NADPH, and the reaction was initiated. After 20-min incubation, the reaction was terminated by adding 200 μl methanol. The residual EROD activity was measured as described above. The observed rates of human CYP1 inactivation ($k_{obs}$) were calculated from the initial slopes of the linear regression lines of semilogarithmic plots (remaining EROD activity versus preincubation time). The obtained $k_{obs}$ was plotted against the cannabinoid concentration. The maximal inactivation constant ($k_{inact}$) and the half-maximal inhibitory concentration ($K_I$) were calculated by nonlinear least-squares regression analysis using the Origin 7.5J Software (OriginLab).

To determine the effect of radical trapping agents on human CYP1 inactivation by CBD, the preincubation mixture was prepared as the inactivation kinetics. Preincubations were performed with or without GSH (2 mM or 5 mM), NAC (2 mM or 5 mM), and SOD (500 U) both in the absence and presence of CBD. The preincubation time was 6 min for recombinant CYP1A1 and 9 min for HLMs.

Recombinant CYP1A1 and HLMs were preincubated with or without CBD for 6 and 9 min, respectively, to evaluate the effect of dialysis on inhibitory effects of CBD. Subsequently, the samples were transferred to a Slide-A-Lyzer MINI Dialysis Unit. Dialysis was carried out at 4 °C for 4 h in 1 l of 50 mM Tris–HCl buffer (pH 7.4) containing 1% BSA. The dialysis buffer was changed after every hour. The dialyzed samples were assayed for the EROD activity as described above.

### 2.5. Statistical analyses

The statistical significance of differences between the means of the two groups and between the means of the various groups was evaluated by means of the unpaired *t*-test and one-way analysis of variance, respectively. All statistical analyses were carried out with a program InStat (GraphPad Software, San Diego, CA).

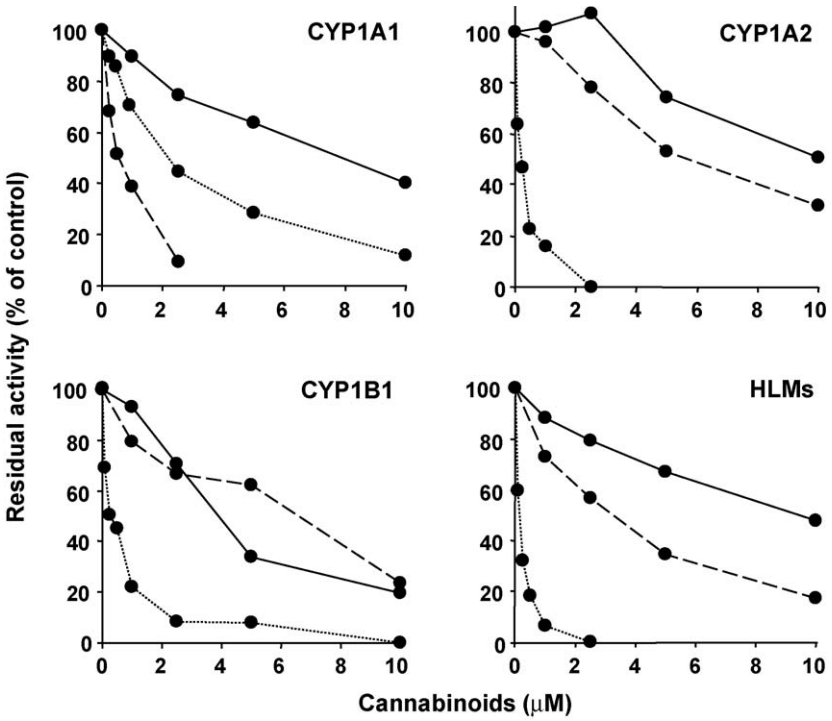


**Fig. 2.** Effects of major cannabinoids on EROD activities of human CYP1 isoforms and liver microsomes. Recombinant CYP1A1, CYP1A2, CYP1B1, and HLMs were incubated with 7-ethoxyresorufin (150 nM) in the presence of various amounts of $\Delta^9$-THC (solid lines), CBD (dashed lines), and CBN (dotted lines). Each point is the mean of two determinations.

## 3. Results

### 3.1. Direct inhibition of human CYP1 enzymes and liver microsomes by major cannabinoids

To clarify enzymatic characteristics of recombinant human CYP1A1, CYP1A2, CYP1B1, and pooled HLMs used in this study toward EROD activity, kinetic analysis was carried out with these enzyme sources. All the reactions tested followed the Michaelis–Menten kinetics based on the Eadie–Hofstee plots (data not shown). The $K_m$ values were 110–226 nM (Table 1). Thus, effects of $\Delta^9$-THC, CBD, and CBN on EROD activity were investigated at a substrate concentration of 150 nM. All the cannabinoids tested inhibited the EROD activity of recombinant CYP1A1, CYP1A2, and CYP1B1 in a concentration-dependent manner (Fig. 2). CYP1A1 was most potently inhibited by CBD with the $IC_{50}$ value of 0.537 μM, although CYP1A2 and CYP1B1 were most effectively suppressed by CBN with the $IC_{50}$ values of 0.188 and 0.278 μM, respectively. When HLMs were used as an enzyme source, the inhibitory effects of the cannabinoids were similar to those on the activity of recombinant CYP1A2, suggesting that the liver microsomal EROD activity is mainly a reflection of CYP1A2.

Kinetic analysis for the inhibition was conducted with four CYP1 enzyme sources including HLMs to characterize the mode of inhibition of these CYP1 enzymes by $\Delta^9$-THC, CBD, and CBN. Fig. 3 shows representative Lineweaver–Burk plots for inhibition by these major cannabinoids. $\Delta^9$-THC, CBD, and CBN competitively inhibited the EROD activity of all the recombinant CYP1 enzymes (Fig. 3 and Table 2). For HLMs, the CBD inhibition indicated a competitive manner whereas the inhibition by $\Delta^9$-THC and CBN indicated a mixed fashion (Table 2). The apparent $K_i$ values of $\Delta^9$-THC were 2.47–7.54 μM, showing a relatively weak inhibitory effect and low inhibition selectivity against CYP1 isoforms. The $K_i$ value of CBD for CYP1A1 was the lowest among the CYP1 isoforms tested. On the other hand, the $K_i$ values of CBN were lower for CYP1A2 and CYP1B1 than for CYP1A1.

### 3.2. Metabolism-dependent inhibition of human CYP1 enzymes and liver microsomes by major cannabinoids

An effect of preincubation on inhibition by $\Delta^9$-THC, CBD, and CBN was investigated to determine whether these cannabinoids inhibit the CYP1-mediated EROD reaction in a metabolism-dependent manner. A 20-min preincubation of $\Delta^9$-THC and CBN in the presence of NADPH increased the inhibition of the CYP1A1 activity (Table 3). The preincubation of CBD potentiated the inhibition for the EROD activity of all the enzyme sources used. No increased inhibition was seen in any other incubation conditions.

**Table 2**
Kinetic parameters for inhibition of EROD activity by major cannabinoids.

| Cannabinoids | Enzymes | $K_i$ (μM) | Mode of inhibition |
|---|---|---|---|
| $\Delta^9$-THC | CYP1A1 | 4.78 | Competitive |
| | CYP1A2 | 7.54 | Competitive |
| | CYP1B1 | 2.47 | Competitive |
| | HLMs | 4.72 | Mixed |
| CBD | CYP1A1 | 0.155 | Competitive |
| | CYP1A2 | 2.69 | Competitive |
| | CYP1B1 | 3.63 | Competitive |
| | HLMs | 1.75 | Competitive |
| CBN | CYP1A1 | 0.541 | Competitive |
| | CYP1A2 | 0.0790 | Competitive |
| | CYP1B1 | 0.148 | Competitive |
| | HLMs | 0.0808 | Mixed |

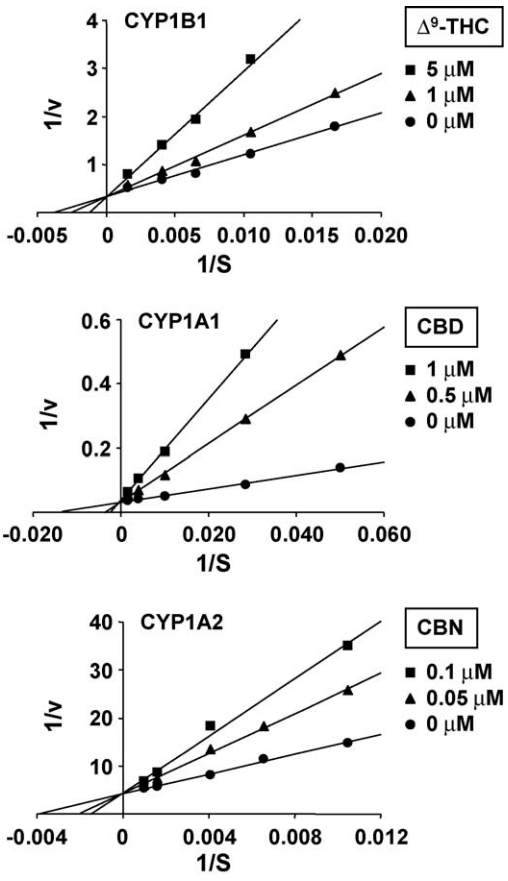


**Fig. 3.** Representative Lineweaver–Burk plots for inhibition of human CYP1 enzymes by major cannabinoids. Recombinant CYP1B1, CYP1A1, and CYP1A2 were incubated with 7-ethoxyresorufin in the presence or absence of $\Delta^9$-THC, CBD, and CBN, respectively. *S* and *v* indicate substrate concentration (nM) and EROD activity (nmol/min/nmol P450), respectively. Each point is the mean of duplicate determinations.

### 3.3. Inactivation of human CYP1 enzymes and liver microsomes by major cannabinoids

To characterize parameters for inactivation of human CYP1 enzymes by $\Delta^9$-THC, CBD, and CBN, the kinetics were analyzed under the six incubation conditions exhibiting decreased $IC_{50}$ values by the preincubation as shown in Table 3. The preincubation

**Table 3**
Effects of preincubation on inhibition of human CYP1 enzymes by major cannabinoids.

| Cannabinoids | Enzymes | $IC_{50}$ (μM) | | B/A |
|---|---|---|---|---|
| | | Preincubation time | | |
| | | 0 min (A) | 20 min (B) | |
| $\Delta^9$-THC | CYP1A1 | 7.82 | 4.16 | 0.532 |
| | CYP1A2 | 5.10 | 23.4 | 4.59 |
| | CYP1B1 | 2.10 | 2.92 | 1.39 |
| | HLMs | 7.28 | 9.30 | 1.28 |
| CBD | CYP1A1 | 0.411 | 0.0767 | 0.187 |
| | CYP1A2 | 3.81 | 1.46 | 0.383 |
| | CYP1B1 | 5.96 | 4.72 | 0.792 |
| | HLMs | 2.69 | 1.02 | 0.379 |
| CBN | CYP1A1 | 1.08 | 0.740 | 0.685 |
| | CYP1A2 | 0.131 | 0.514 | 3.92 |
| | CYP1B1 | 0.312 | 0.468 | 1.50 |
| | HLMs | 0.0985 | 0.129 | 1.31 |

All determinations were performed in duplicate.

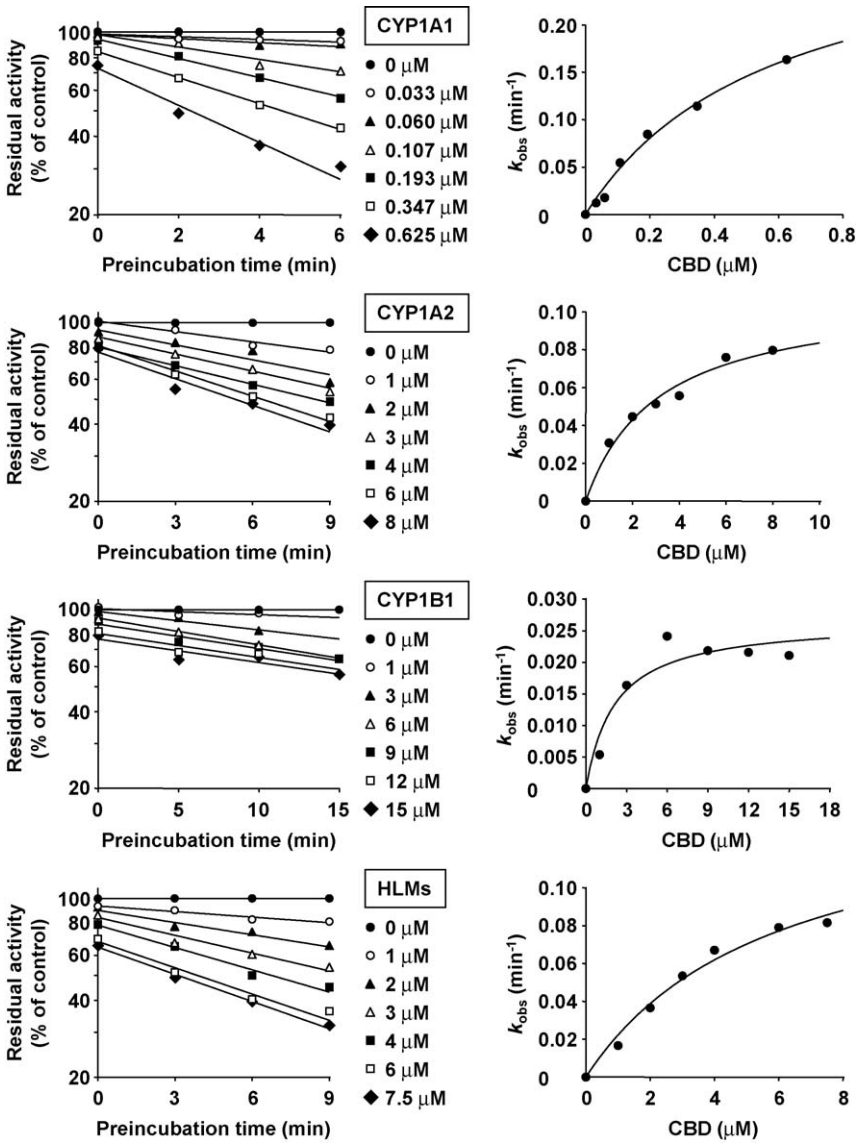


**Fig. 4.** Inactivation of human CYP1 isoforms and liver microsomes by CBD. Recombinant CYP1A1, CYP1A2, CYP1B1, and HLMs were preincubated with CBD (0–15 μM) in the presence of NADPH for 0–15 min. Aliquots were removed from the preincubation mixtures at the indicated time points and diluted for measurement of the residual activity. In the left graphs, each point is the mean of two determinations. The right graphs indicate the plot of the $k_{obs}$ against CBD concentration.

of CBD in the presence of NADPH resulted in a time- and concentration-dependent decrease in the EROD activity of all the enzyme sources used (Fig. 4). Similarly, the inhibition of CYP1A1 activity by $\Delta^9$-THC and CBN was preincubation time- and concentration-dependent (data not shown). High $k_{inact}$ values were observed in the inactivation by CBD (Table 4). In particular, the inactivation of CYP1A1 by CBD indicated the highest $k_{inact}$ value (0.316 min$^{-1}$) among the CYP1 isoforms investigated. For the $K_I$ values, the lowest value was seen in the inactivation of CYP1A1 by CBN (0.0856 μM) (Table 4). The ratio of $k_{inact}$ to $K_I$, the efficiency of enzyme inactivation, showed the highest ratio in the inactivation of CYP1A1 by CBD (540 l/mmol/min), followed by the inactivation of CYP1A1 by CBN (223 l/mmol/min) (Table 4).

**Table 4**
Kinetic parameters for mechanism-based inhibition of human CYP1 enzymes by major cannabinoids.

| Cannabinoids | Enzymes | $k_{inact}$ (/min) | $K_I$ (μM) | $k_{inact}/K_I$ (l/mmol/min) |
|---|---|---|---|---|
| $\Delta^9$-THC | CYP1A1 | 0.0239 | 1.22 | 19.6 |
| CBD | CYP1A1 | 0.316 | 0.585 | 540 |
| | CYP1A2 | 0.109 | 3.09 | 35.3 |
| | CYP1B1 | 0.0267 | 2.12 | 12.6 |
| | HLMs | 0.152 | 5.85 | 26.0 |
| CBN | CYP1A1 | 0.0191 | 0.0856 | 223 |

### 3.4. Effects of NAPDH, trapping agents, and dialysis on inactivation of CYP1A-mediated EROD reactions by CBD

The preincubation of CBD was carried out in the presence and absence of NADPH to determine whether the observed inactivation of human CYP1A1 and liver microsomes by CBD ($k_{inact} > 0.15$ min$^{-1}$) required metabolism of the cannabinoid. The preincubation of recombinant CYP1A1 and HLMs with CBD or NADPH alone did not cause a reduction in the EROD activity (Fig. 5). However, the presence of both CBD and NADPH led to a significant decrease in these enzyme activities.

To determine whether the inactivation of CYP1 enzymes by CBD was confined to the active site, effects of trapping agents on the CBD inactivation were investigated. GSH and NAC, nucleophilic

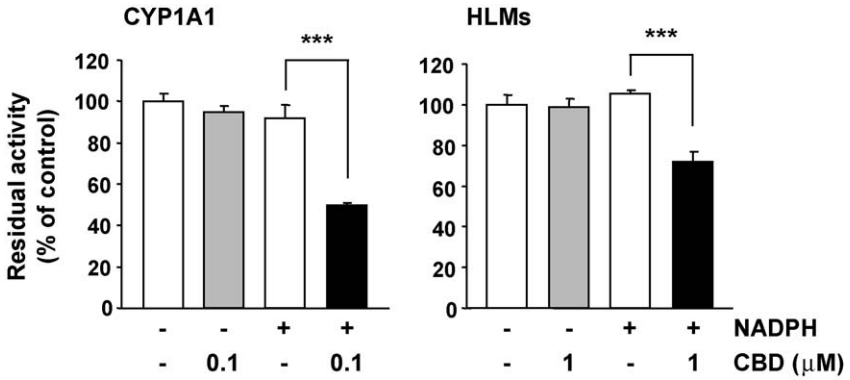


**Fig. 5.** Requirement for NADPH in inactivation of human CYP1A1 and liver microsomes by CBD. Recombinant CYP1A1 and HLMs were preincubated with CBD in the presence or absence of NADPH for 6 and 9 min, respectively. Data are expressed as the mean ± S.D. ($n = 3$). $^{***}p < 0.001$.

**Table 5**
Effects of trapping agents on inactivation of recombinant human CYP1A1 and HLMs by CBD.

| Experiments | Inactivation mixtures | Residual activity (% of control) |
|---|---|---|
| Experiment 1 | CYP1A1 | 100 ± 3 |
| | +0.1 μM CBD | 50.8 ± 4.0[a] |
| | +0.1 μM CBD + 2 mM GSH | 49.6 ± 0.3[a] |
| | +0.1 μM CBD + 5 mM GSH | 50.5 ± 1.9[a] |
| | +0.1 μM CBD + 2 mM NAC | 53.9 ± 2.9[a] |
| | +0.1 μM CBD + 5 mM NAC | 57.2 ± 4.1[a] |
| Experiment 2 | CYP1A1 | 100 ± 9 |
| | +0.1 μM CBD | 59.9 ± 0.6[a] |
| | +0.1 μM CBD + 500 U of SOD | 59.7 ± 2.5[a] |
| Experiment 3 | HLMs | 100 ± 2 |
| | +1 μM CBD | 67.9 ± 3.5[b] |
| | +1 μM CBD + 2 mM GSH | 71.2 ± 3.6[b] |
| | +1 μM CBD + 5 mM GSH | 73.1 ± 1.6[b] |
| | +1 μM CBD + 2 mM NAC | 72.8 ± 4.0[b] |
| | +1 μM CBD + 5 mM NAC | 74.3 ± 1.5[b] |
| Experiment 4 | HLMs | 100 ± 4 |
| | +1 μM CBD | 72.9 ± 4.1[b] |
| | +1 μM CBD + 500 U of SOD | 71.1 ± 3.9[b] |

Data are expressed as the mean ± S.D. ($n = 3$).
[a] Significantly different from the corresponding controls, $p < 0.01$.
[b] Significantly different from the corresponding controls, $p < 0.05$.

trapping agents, did not protect CYP1 enzymes from the CBD inactivation (Table 5). SOD, a scavenger of reactive oxygen species, also did not have a significant effect on the inactivation of CYP1A1 and HLMs by CBD.

Furthermore, the irreversibility of CBD inactivation was examined by dialysis experiments. As shown in Fig. 6, dialysis could not restore EROD activities of recombinant CYP1A1 and HLMs.

## 4. Discussion

In this study, we demonstrated that $\Delta^9$-THC, CBD, and CBN, the three major cannabinoids in marijuana, inhibited catalytic activity of human CYP1 enzymes. Roth et al. [14] first reported that $\Delta^9$-THC suppressed the EROD activity of recombinant human CYP1A1. However, CBD and CBN had more potent inhibitory effects on CYP1-mediated oxidation compared with $\Delta^9$-THC. Interestingly, CBD showed a highly selective inhibition of CYP1A1 activity, whereas CBN more effectively suppressed the activity of CYP1A2 and CYP1B1 compared with CYP1A1.

Recombinant human CYP1A1 was competitively inhibited by $\Delta^9$-THC with the $K_i$ value of approximately 4.8 μM, which was comparable to the data previously reported [14]. The type of inhibition by $\Delta^9$-THC and CBN indicated a mixed fashion for HLMs in contrast to recombinant CYP1A2, which was competitively inhibited by these cannabinoids. One possibility for the different type of inhibition by the cannabinoids is that $\Delta^9$-THC and CBN may interact with certain CYPs other than CYP1A2 in HLMs.

In general, marijuana is consumed by smoking. A peak level of plasma concentration of $\Delta^9$-THC in human subjects has been reported to be 84.3 ng/ml (268 nM) after smoking a marijuana cigarette containing $\Delta^9$-THC at 15.8 mg [36]. It has been previously reported that the plasma levels of CBD and CBN were 114 ng/ml (363 nM) and 126 ng/ml (406 nM), respectively, after smoking a placebo marijuana cigarette spiked each with 20 mg of the corresponding cannabinoid [37,38]. When a capsule containing 10 mg dronabinol (synthetic $\Delta^9$-THC) was orally administered to

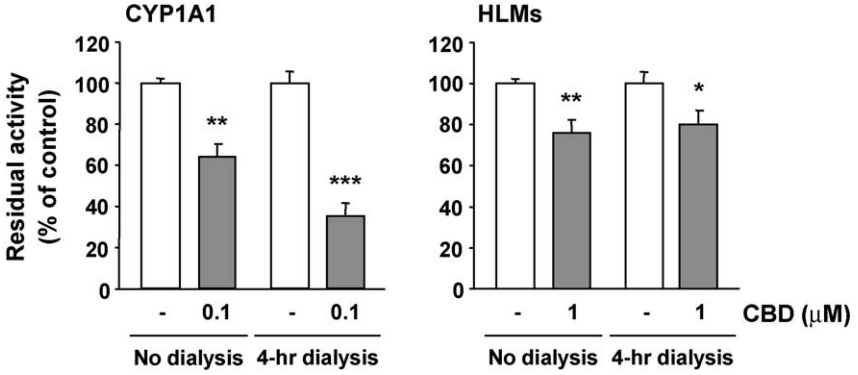


**Fig. 6.** Effect of dialysis on inactivation of human CYP1A1 and liver microsomes by CBD. Recombinant CYP1A1 and HLMs were preincubated with CBD in the presence of NADPH for 6 and 9 min, respectively. The preincubation mixtures were subjected to no dialysis or 4-h dialysis at 4 °C. Data are expressed as the mean ± S.D. ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the corresponding controls without CBD.

healthy human subjects twice a day, the $C_{max}$ of $\Delta^9$-THC was 7.88 ng/ml (25.1 nM) [39]. After four buccal sprays of Sativex®, which was estimated to contain 10.8 mg $\Delta^9$-THC and 10 mg CBD, the $C_{max}$ values of $\Delta^9$-THC and CBD reached 6.14 ng/ml (19.6 nM) and 3.02 ng/ml (9.62 nM), respectively [40]. The $K_i$ values of $\Delta^9$-THC obtained in this study are much higher than the blood concentrations described above. Thus, $\Delta^9$-THC may not significantly affect metabolic interactions with substrates for CYP1 enzymes. On the other hand, the $K_i$ values of CBD for CYP1A1 and CBN for CYP1A2 and CYP1B1 are lower than the blood levels after marijuana smoking but are higher than the blood concentrations when administering medicinal cannabis described above. Since cannabinoids, such as CBD and CBN, are readily distributed in various tissues due to a high lipophilicity [41], tissue concentrations of cannabinoids may be even higher than the blood concentration in humans. Therefore, it is suggested that the inhibition of CYP1 enzymes by CBD and CBN might be caused not only after marijuana smoking but also after the administration of medicinal cannabis.

The lowest $K_i$ values of CBD reported so far were 4.9 and 4.7 μM for aminopyrine and morphine demethylations, respectively, catalyzed by rat liver microsomes [42]. In the case of CBN, the lowest $K_i$ value (4.5 μM) was shown in the inhibition of progesterone 17α-hydroxylase activity of rat testis microsomes [43]. Thus, our study indicates that the inhibitory effects of CBD and CBN on human CYP1 activity are much stronger than the inhibitory effects of cannabinoids previously reported. When compared with other known CYP1 inhibitors, the inhibitory effect of CBD on CYP1A1 is comparable with those of 2,4,3′,5′-tetramethoxystilbene ($K_i$ = 0.13 μM) [44] and rhapontigenin ($K_i$ = 0.21 μM) [45]. CBN is a potent inhibitor of CYP1A2 next to fluvoxamine ($K_i$ = 0.040 μM) [46]. In addition, CBN is also a potent inhibitor of CYP1B1 next to kaempferol ($K_i$ = 0.014 μM) and quercetin ($K_i$ = 0.023 μM) [47]. These results indicate that both CBD and CBN are potent inhibitors for CYP1 enzymes.

Bornheim et al. have previously reported that CBD and unsaturated side-chain analogs of THC inactivate CYP3A and CYP2C enzymes [13,48,49]. The present study first demonstrated that CBD is a potent mechanism-based inhibitor of CYP1 enzymes. Furthermore, our study provides novel evidence that $\Delta^9$-THC and CBN also inactivate CYP1A1.

We further investigated to clarify a mechanism of inactivation by CBD, which had the highest ability to inactivate human CYP1 enzymes among the cannabinoids examined. The inactivation of recombinant CYP1A1 and the liver microsomal CYP1A2 was dependent on preincubation time and CBD concentration, and required the presence of NADPH in the preincubation, indicating that the inactivation proceeded via catalytic step(s). The fact that this inactivation was not affected by any trapping agents and by dialysis suggests that reactive intermediate(s) is formed at active sites of CYP1 enzymes and does not leave the active sites prior to the inactivation step. These findings characterize CBD as a mechanism-based inhibitor of human CYP1 enzymes. Bornheim and Grillo [50] have previously reported that CBD-hydroxyquinone produced by mouse CYP3A11 is a reactive intermediate causing the CYP3A inactivation. At present, however, the reactive intermediate leading to the inactivation of CYP1 enzymes by CBD is unclear.

When recombinant human CYP1A2 was used in the inactivation experiments, the present study could not confirm trapping effects of GSH and NAC on the CBD inactivation because GSH and NAC themselves stimulated the EROD activity of recombinant CYP1A2. In addition, we failed to investigate an effect of dialysis on the inactivation of recombinant CYP1A2 due to marked decrease in the CYP1A2 activity by dialysis. However, the inactivation of recombinant CYP1A2 was preincubation time- and concentration-dependent (Fig. 4), required NADPH, and was not attenuated by SOD (data not shown). These results would partly support mechanism-based inhibition of recombinant CYP1A2 by CBD.

When compared with other known mechanism-based inhibitors of CYP1 enzymes, the potency of the CBD inactivation of CYP1A1, as assessed by the $k_{inact}/K_I$ value, is similar to that of rhapontigenin inactivation ($k_{inact}/K_I$ = 667 l/mmol/min) [45]. The inactivation of CYP1A2 by CBD is more effective than those by furafylline ($k_{inact}/K_I$ = 10.1 l/mmol/min) [51] and oltipraz ($k_{inact}/K_I$ = 21.1 l/mmol/min) [52]. The inactivation of CYP1B1 by CBD is as effective as that by 4-(1-propynyl)biphenyl ($k_{inact}/K_I$ = 17.4 l/mmol/min) [53]. For HLMs, the inactivating effect of CBD is comparable with that of *trans*-resveratrol ($k_{inact}/K_I$ = 32.9 l/mmol/min) [54]. These results indicate that CBD is a potent mechanism-based inhibitor of human CYP1 enzymes.

In conclusion, we demonstrated that CBD and CBN show CYP1 isoform-selective direct inhibition and that CBD is characterized as a potent mechanism-based inhibitor of human CYP1 enzymes. CBD and CBN are both nonpsychoactive cannabinoids unlike $\Delta^9$-THC, but might lead to undesired metabolic interactions with drugs or toxicants metabolized by CYP1 enzymes.

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