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Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes

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ABSTRACT

Aims: Cannabidiol (CBD), one of the major constituents in marijuana, has been shown to be extensively metabolized by experimental animals and humans. However, human hepatic enzymes responsible for the CBD metabolism remain to be elucidated. In this study, we examined in vitro metabolism of CBD with human liver microsomes (HLMs) to clarify cytochrome P450 (CYP) isoforms involved in the CBD oxidations.
Main methods: Oxidations of CBD in HLMs and recombinant human CYP enzymes were analyzed by gas chromatography/mass spectrometry.
Key findings: CBD was metabolized by pooled HLMs to eight monohydroxylated metabolites (6α-OH-, 6β-OH-, 7-OH-, 1″-OH-, 2″-OH-, 3″-OH-, 4″-OH-, and 5″-OH-CBDs). Among these metabolites, 6α-OH-, 6β-OH-, 7-OH-, and 4″-OH-CBDs were the major ones as estimated from the relative abundance of m/z 478, which was a predominant fragment ion of trimethylsilyl derivatives of the metabolites. Seven of 14 recombinant human CYP enzymes examined (CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) were capable of metabolizing CBD. The correlations between CYP isoform-specific activities and CBD oxidative activities in 16 individual HLMs indicated that 6β-OH- and 4″-OH-CBDs were mainly formed by CYP3A4, which was supported by inhibition studies using ketoconazole and an anti-CYP3A4 antibody. The correlation and inhibition studies also showed that CBD 6α-hydroxylation was mainly catalyzed by CYP3A4 and CYP2C19, whereas CBD 7-hydroxylation was predominantly catalyzed by CYP2C19.
Significance: This study indicated that CBD was extensively metabolized by HLMs. These results suggest that CYP3A4 and CYP2C19 may be major isoforms responsible for 6α-, 6β-, 7-, and/or 4″-hydroxylations of CBD in HLMs.

Introduction

Cannabidiol (CBD), one of the major constituents in marijuana (Fig. 1), is not psychoactive but has several pharmacological effects such as drug-induced sleep prolongation, antiepileptic, anxiolytic, and antiemetic actions (Mechoulam et al., 2002). Some of these pharmacological effects may be of therapeutic importance. Recently, Sativex®, a marijuana extract containing CBD, has been clinically used for the symptomatic relief for neuropathic pain and spasticity in multiple sclerosis (Collin et al., 2010; Kavia et al., 2010; Selvarajah et al., 2010; Wade et al., 2010).

CBD is extensively metabolized by experimental animals and humans (Huestis, 2005). Martin et al. (1976) reported that CBD was oxidized by a 10,000×*g* supernatant from rat livers to form 7-hydroxy-CBD (7-OH-CBD) as a major metabolite together with seven other metabolites; 6α-OH-, 6β-OH-, 1″-OH-, 2″-OH-, 3″-OH-, 4″-OH-, and 5″-OH-CBDs. A comparative study using liver microsomes from seven experimental animals has shown that CBD is metabolized to these eight metabolites and 10-OH-CBD, and the metabolic profiles of CBD are considerably different among the animal species (Harvey and Brown, 1990). In an in vivo metabolism study in mice, 22 metabolites including CBD-7-oic acids and glucuronide conjugates were characterized in the liver following intraperitoneal administration of CBD (Martin et al., 1977). In addition, 33 metabolites were identified in urine from a dystonic patient treated chronically with CBD (Harvey and Mechoulam, 1990). From these findings, the main metabolic pathway of CBD in humans is suggested to be oxidation of C-atom at the 7-position followed by further hydroxylation in the pentyl side chain and the terpene moiety (Harvey and Mechoulam, 1990). The formation of these hydroxylated metabolites of CBD is thought to be catalyzed by cytochrome P450 (CYP), because it has been previously reported that a purified mouse CYP2C enzyme metabolizes CBD to form 6″-OH-, 7-OH-, and 4″-OH-CBDs (Bornheim and Correia, 1991).

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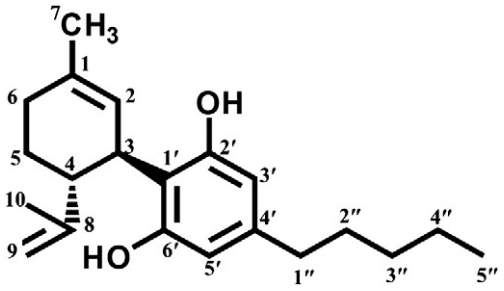

Fig. 1. Structure and numbering system of CBD.

However, overall metabolism of CBD with human liver microsomes (HLMs) and CYP isoforms responsible for CBD oxidations in human livers remain unclear.

In the present study, we investigated in vitro metabolism of CBD with HLMs. We report herein that CBD is metabolized by HLMs to produce four major metabolites, 6α-OH-, 6β-OH-, 7-OH-, and 4″-OH-CBDs together with four minor metabolites 1″-OH-, 2″-OH-, 3″-OH-, and 5″-OH-CBDs. Furthermore, our study suggests that CYP3A4 and CYP2C19 may play important roles in the 6α-, 6β-, 7-, and 4″-hydroxylations of CBD in HLMs.

Materials and methods

Materials

CBD was isolated from cannabis leaves using the method of Aramaki et al. (1968). 6α-OH-CBD, 6β-OH-CBD, and 5′-nor-Δ^8-tetrahydrocannabinol-4′-oic acid methyl ester (5′-nor-Δ^8-THC-4′-oic acid methyl ester) were prepared by the previous methods (Lander et al., 1976; Ohlsson et al., 1979). The purities of these cannabinoids were determined to be at least 98% by gas chromatography (GC). NADP and glucose 6-phosphate were purchased from Böehringer-Mannheim GmbH (Darmstadt, Germany). Glucose 6-phosphate dehydrogenase was obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan); sulfaphenazole was from Sigma Chemical Co. (St. Louis, MO); omeprazole, quinidine sulfate dihydrate, and ketoconazole were from Wako Pure Chemicals Ind. (Osaka, Japan). Other chemicals and solvents used were of the highest quality commercially available.

Enzyme sources

Individual HLMs for the Reaction Phenotyping Kit version 6 were obtained from XenoTech (Kansas, KS). CYP enzyme activities for the individual HLMs were generated by XenoTech (Pearce et al., 1996; Robertson et al., 2000). Pooled HLMs and the microsomes from baculovirus-infected insect cells expressing human CYPs (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, and CYP4A11) each with NADPH-CYP reductase and CYPs (CYP2E1, CYP3A4, and CYP3A5) each with the reductase and cytochrome b_5 were purchased from BD Gentest (Woburn, MA). The catalytic activities (nmol/min/nmol P450) of CYP enzymes measured by XenoTech are as follows: 7-ethoxyresorufin *O*-deethylation by CYP1A1, 39; phenacetin *O*-deethylation by CYP1A2, 26; 7-ethoxyresorufin *O*-deethylation by CYP1B1, 11; coumarin 7-hydroxylation by CYP2A6, 9.6; 7-ethoxy-4-trifluoromethylcoumarin *O*-deethylation by CYP2B6, 9.7; paclitaxel 6α-hydroxylation by CYP2C8, 3.9; diclofenac 4′-hydroxylation by CYP2C9, 37; diclofenac 4′-hydroxylation by CYP2C18, 0.64; *S*-mephenytoin 4′-hydroxylation by CYP2C19, 5.4; (±)-bufuralol 4′-hydroxylation by CYP2D6, 42; *p*-nitrophenol hydroxylation by CYP2E1, 9.5; testosterone 6β-hydroxylation by CYP3A4, 170; testosterone 6β-hydroxylation by CYP3A5, 66; lauric acid ω-hydroxylation by CYP4A11, 36.

Enzyme assay

The activity of CBD oxidation was measured as described below. HLMs (20 μg protein) and recombinant CYP isoforms (10 pmol P450) were used as enzyme sources. An incubation mixture consisted of 6.4 μM CBD, an enzyme source, an NADPH-generating system (0.5 mM NADP, 10 mM glucose 6-phosphate, 10 mM magnesium chloride, and 1 unit of glucose 6-phosphate dehydrogenase), and 100 mM potassium phosphate buffer (pH 7.4) to make a final volume of 0.5 ml. The mixture was incubated at 37 °C for 20 min (60 min for recombinant CYP isoforms) and then extracted with 4 ml of ethyl acetate after addition of 0.5 ml of 1 M monopotassium phosphate and 5′-nor-Δ^8-THC-4′-oic acid methyl ester as an internal standard (I.S.). A portion of the extract was evaporated to dryness, and CBD metabolites formed were derivatized to their trimethylsilyl (TMS) derivatives. The metabolites were determined by gas chromatography/mass spectrometry (GC/MS) under following conditions: A Shimadzu GCMS-QP2010 with a column DB-1 (0.25 mm × 30 m), ion source temperature 250 °C, interface temperature 280 °C, ionization energy 70 eV, emission current 60 μA, and carrier gas He (2.04 ml/min). The column oven temperature kept at 50 °C for 1 min, ramped at 25 °C/min to 200 °C, followed by 10 °C/min till 300 °C with a 5 min hold. The identification of CBD metabolites was based on the comparison of their mass spectra and retention times with those of the synthetic standards, 6α-OH- and 6β-OH-CBDs, or data reported by Binder et al. (1974) and Martin et al. (1976). Typical diagnostic ions (m/z) and retention times (min) of TMS derivatives of CBD metabolites under the above conditions were summarized in Table 1. The quantification of CBD metabolites was based on the relative abundance of their base ions in GC/MS summarized in Table 1 to that of I.S. (m/z 333).

Inhibition studies with CYP isoform-selective inhibitors

Pooled HLMs were incubated with CBD in the presence of CYP isoform selective inhibitors sulfaphenazole (CYP2C9), omeprazole (CYP2C19), quinidine (CYP2D6), or ketoconazole (CYP3A4) under the same manner as described in the enzyme assay. With the exception of quinidine that was dissolved in water, all inhibitors were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of 0.5%.

Inhibition study with anti-CYP3A4 antibody

Pooled HLMs (20 μg protein) were preincubated with a monoclonal antibody against CYP3A4 (BD Gentest) (0–20 μl/100 μg protein in HLMs) on ice for 20 min. CBD and 100 mM potassium phosphate buffer (pH 7.4) were added to the mixture, and the reaction was initiated by adding an NADPH-generating system. The enzymatic activities were determined as described above.

Table 1
Typical diagnostic ions and retention times of CBD metabolites on GC chromatograms.

Peak no.	CBD metabolites	Typical diagnostic ions (m/z)	Retention times (min)
1	1″-OH	546, 425, 421[a]	13.02
2	2″-OH	546, 425, 145[a]	13.98
3	6α-OH	546, 478[a]	14.05
4	6β-OH	546, 478[a]	14.09
5	3″-OH	546, 478[a], 425, 334	14.36
6	7-OH	546, 478, 443[a]	14.41
7	4″-OH	546, 478[a], 425, 117	14.47
8	5″-OH	546, 478[a], 425	15.02

[a] Base ion.

Statistical analyses

The correlations between catalytic activities of HLMs were assessed by linear regression analysis. All statistical analyses were carried out with a program InStat (GraphPad Software, San Diego, CA).

Results

Metabolism of CBD by pooled HLMs

Fig. 2 shows representative mass chromatograms of the TMS derivatives of CBD metabolites formed with pooled HLMs. HLMs produced eight monohydroxylated metabolites of CBD, 6α-OH-, 6β-OH-, 7-OH-, 1″-OH-, 2″-OH-, 3″-OH-, 4″-OH-, and 5″-OH-CBDs, in the presence of NADPH (Fig. 2). The typical fragment ions of these eight metabolites were in good agreement with a previous report (Martin et al., 1976). CBD metabolites formed with HLMs were analyzed by GC/MS after trimethylsilylation. All of metabolites identified showed a molecular ion at m/z 546 and a fragment ion at m/z 478. Metabolites 1, 2, 5, 7, and 8 showed a fragment ion at m/z 425, which is a diagnostic ion of the hydroxylated metabolites on the pentyl side chain (Martin et al., 1976). The metabolites 1, 2, 5, and 7 were identified as 1″-OH-, 2″-OH-, 3″-OH-, and 4″-OH-CBDs, respectively (Table 1). In addition, a metabolite 8 was identified as 5″-OH-CBD. The metabolite 6 was identified as 7-OH-CBD from its diagnostic ion at m/z 443, which was a base ion of the metabolite. The metabolites of 3 and 4 were identified as 6α-OH- and 6β-OH-CBDs, respectively, which showed a typical diagnostic ion at m/z 478 exclusively and the same retention times as those of the synthetic standards in GC/MS analysis. As shown in Fig. 2, the relative abundance at m/z 478, which is a predominant fragment ion of TMS derivatives of CBD metabolites, indicated that 6α-OH-, 6β-OH-, 7-OH-, and 4″-OH-CBDs were major metabolites of CBD in HLMs. The formation of the eight metabolites of CBD increased linearly with an incubation time up to 20 min in the presence of 40 μg/ml microsomal protein (Fig. 3A) and with a protein concentration up to 80 μg/ml for 20 min (Fig. 3B). The amounts of 6α-OH- and 6β-OH-CBDs produced from CBD were calculated from the calibration curves of synthetic standards for these CBD metabolites. The rates of 6α- and 6β-hydroxylations of CBD in pooled HLMs were 116 and 122 pmol/min/mg protein, respectively.

CBD oxidations by recombinant human CYP isoforms

The ability of individual CYP isoforms to oxidize CBD was examined with 14 recombinant human CYP enzymes (Table 2). 6α- and 6β-Hydroxylase activities of CBD were detected in the incubations with seven of 14 CYP isoforms (CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5). The formation of 7-OH- and 4″-OH-CBDs was confirmed in the incubation systems which contained six CYP isoforms (CYP1A1 or CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5). 2″-OH-, 3″-OH-, and 5″-OH-CBDs were detected in the incubations with CYP1A2, CYP2C9, CYP2D6, and/or CYP3A. CBD 1″-hydroxylation was confirmed exclusively in the incubations with CYP1A. CBD hydroxylase activities were not detected in the incubations

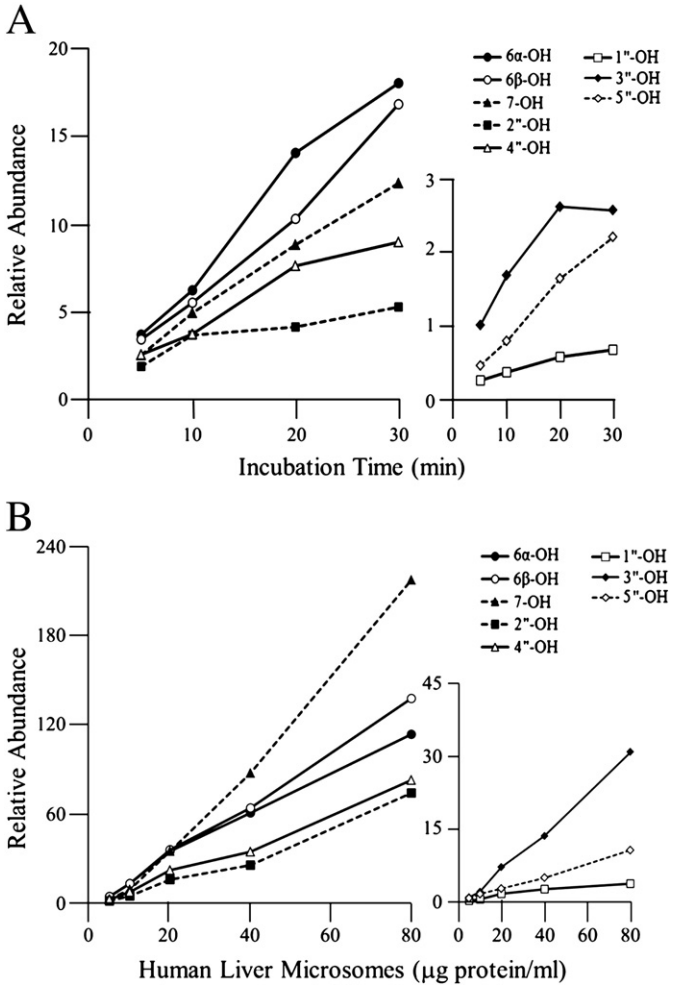

Fig. 3. The formation of eight hydroxylated metabolites of CBD as function of incubation time and liver microsomal protein concentration. (A) Pooled HLMs (40 μg protein/ml) were incubated with 6.4 μM CBD for up to 30 min. (B) Pooled HLMs (5–80 μg protein/ml) were incubated with 6.4 μM CBD for 20 min. The relative abundance is the peak area ratio of test ion peaks; m/z 478 for 6α-OH-, 6β-OH-, 3″-OH-, 4″-OH-, and 5″-OH-CBDs, m/z 443 for 7-OH-CBD, m/z 421 for 1″-OH-CBD, or m/z 145 for 2″-OH-CBD against the m/z 333 for the I.S.

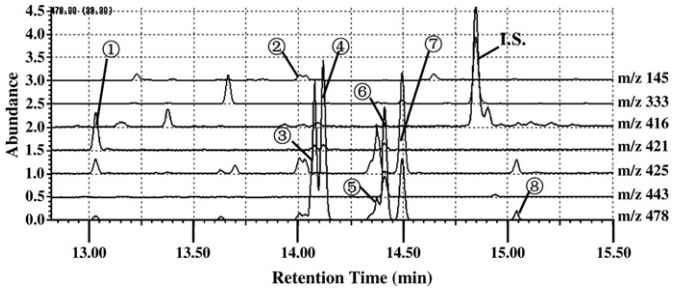

Fig. 2. Representative mass chromatograms of CBD metabolites formed by HLMs. Pooled HLMs (40 μg protein/ml) were incubated with 6.4 μM CBD for 20 min. The data, retention times, and typical fragment ions of CBD metabolites were listed along with their matching peak number in Table 1.

Table 2
Catalytic activities of CBD oxidations by recombinant human CYP enzymes.

Isoforms	6α-OH[a]	6β-OH[a]	Other CBD metabolites formed[b]
CYP1A1	2.55	3.45	7-OH, 1″-OH
CYP1A2	1.61	14.6	1″-OH, 2″-OH, 3″-OH, 4″-OH
CYP2C9	0.546	0.255	7-OH, 4″-OH, 5″-OH
CYP2C19	7.75	0.963	7-OH, 4″-OH
CYP2D6	10.4	75.7	7-OH, 4″-OH, 5″-OH
CYP3A4	12.7	12.5	7-OH, 2″-OH, 4″-OH, 5″-OH
CYP3A5	7.87	14.9	7-OH, 2″-OH, 3″-OH, 4″-OH

[a] Values are represented as the means of duplicate determinations (nmol/min/nmol P450).
[b] CBD metabolites were identified by their typical diagnostic ions on mass spectra and retention times in GC/MS analysis as shown in Table 1.

Table 3
Correlation of the CBD metabolite formation with CYP isoform specific activities in microsomes from 16 human livers.

CBD metabolites	Correlation coefficient (r)				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4/5
6α-OH-CBD	0.0551	0.244	0.522*	−0.215	0.815**
6β-OH-CBD	−0.0281	0.607*	0.224	0.0207	0.532*
7-OH-CBD[a]	−0.0727	0.152	0.835***	−0.181	0.495
4″-OH-CBD[b]	0.00704	0.448	0.452	−0.150	0.698**

CYP1A2: 7-Ethoxyresorufin *O*-deethylation.
CYP2C9: Diclofenac 4′-hydroxylation.
CYP2C19: *S*-Mephenytoin 4′-hydroxylation.
CYP2D6: Dextromethorphan *O*-demethylation.
CYP3A4/5: Testosterone 6β-hydroxylation.
[a] Relative abundance calculated from the peak area ratio of m/z 443 for 7-OH-CBD against m/z 333 of I.S.
[b] Relative abundance calculated from the peak area ratio of m/z 478 for 4″-OH-CBD against m/z 333 of I.S.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

with seven other CYP isoforms, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C18, CYP2E1, and CYP4A11, under the present conditions.

Correlation between CYP isoform-specific activities and CBD oxidative activities in HLMs

The rates of 6α-, 6β-, 7-, and 4″-hydroxylations of CBD were measured with 16 individual HLMs and compared with CYP isoforms selective enzyme activities measured by XenoTech (Table 3). CBD 6α-hydroxylation correlated well with *S*-mephenytoin 4′-hydroxylation ($r=0.522$, $p<0.05$), an index of CYP2C19 activity, and testosterone 6β-hydroxylation ($r=0.815$, $p<0.01$), an index of CYP3A activity. CBD 6β-hydroxylation correlated significantly with diclofenac 4′-hydroxylation ($r=0.607$, $p<0.05$), an index of CYP2C9 activity, and testosterone 6β-hydroxylation ($r=0.532$, $p<0.05$). CBD 7-hydroxylation highly correlated with *S*-mephenytoin 4′-hydroxylation ($r=0.835$, $p<0.001$). CBD 4″-hydroxylation correlated significantly with testosterone 6β-hydroxylation ($r=0.698$, $p<0.01$).

Effects of various CYP inhibitors and anti-CYP3A4 antibody on CBD oxidations by HLMs

To clarify the involvement of CYP2C9, CYP2C19, CYP2D6, and CYP3A in 6α-, 6β-, 7-, and 4″-hydroxylations of CBD by HLMs, effects of sulfaphenazole, omeprazole, quinidine, and ketoconazole on these oxidative activities were examined with pooled HLMs (Table 4). CBD 6α-hydroxylase activity was markedly inhibited by omeprazole at 5 μM (68% inhibition), an inhibitor for CYP2C19, and ketoconazole at 1 μM (73% inhibition), a CYP3A-selective inhibitor. CBD 6β-hydroxylase activity was inhibited by ketoconazole but not by sulfaphenazole, a CYP2C9-selective inhibitor. CBD 7-hydroxylase activity was decreased exclusively by omeprazole (64% inhibition). CBD 4″-hydroxylase activity was suppressed by omeprazole (77% inhibition) and ketoconazole (53% inhibition). None of these hydroxylations of CBD was inhibited by quinidine.

Table 4
Effects of CYP selective inhibitors on formation of CBD metabolites with HLMs.

Inhibitors (concn.)	Residual activity (% of control)			
	6α-OH-CBD	6β-OH-CBD	7-OH-CBD	4″-OH-CBD
Sulfaphenazole (5 μM)	94.2	91.0	102	103
Omeprazole (5 μM)	31.7	36.8	35.8	22.9
Ketoconazole (1 μM)	26.6	29.6	91.4	46.9
Quinidine (1 μM)	127	132	121	111

The effect of anti-CYP3A4 antibody on CBD 6α-, 6β-, and 4″-hydroxylase activities of HLMs was investigated (Fig. 4). All activities measured were inhibited to approximately 50% of the control level when the antibody was added.

Discussion

In the present study, we demonstrated that CBD is extensively metabolized by HLMs. Under the conditions of this study, eight monohydroxylated metabolites of CBD were detected in the incubation with HLMs. Based on the relative abundance of these metabolites formed, it is assumed that 6α-, 6β-, 7-, and 4″-hydroxylations are predominant metabolic pathways of CBD in HLMs. Harvey and Mechoulam (1990) have previously reported that the main metabolic pathway of CBD in humans may be 7-hydroxylation although their study was examined with only one urine sample from a dystonic patient treated chronically with CBD. These findings suggest that 7-OH-CBD may be one of the most abundant primary metabolites formed from CBD in humans. A previous study with liver microsomes from seven experimental animals has shown that CBD 7-hydroxylation is the most predominant pathway in mouse, rat, and rabbit whereas 6α-OH- and 6β-OH-CBDs are not major metabolites (Harvey and Brown, 1990). Thus, the metabolic profile of CBD in HLMs is different from those in these animal enzyme sources.

Correlation analyses and inhibition studies indicated that CBD 7-hydroxylation is predominantly catalyzed by CYP2C19. In addition, CBD 6α-hydroxylation is suggested to be mainly catalyzed by CYP2C19 and CYP3A4. For the 6β- and 4″-hydroxylations, it is suggested that CYP3A4 may be a major enzyme. In this study, we are not able to conclude that CYP2C9 significantly contributes to the 6β-hydroxylation because sulfaphenazole failed to inhibit the activity of HLMs. On the other hand, omeprazole inhibited the 6β- and 4″-hydroxylase activities of HLMs. It has been previously reported that omeprazole inhibits not only CYP2C19 activity but also CYP3A4 activity under the particular conditions (Hirani et al., 2004). Therefore, the inhibitory effect of omeprazole on the 6β- and 4″-hydroxylations of CBD by HLMs is thought to be due to the inhibition of CYP3A4 but not the inhibition of CYP2C19. Recombinant CYP2D6 efficiently catalyzed the 6α- and 6β-hydroxylations of CBD in the present study. It is suggested, however, that CYP2D6 has a minor role for the hydroxylation of CBD at the 6-position in HLMs, since none of these hydroxylations was inhibited by a

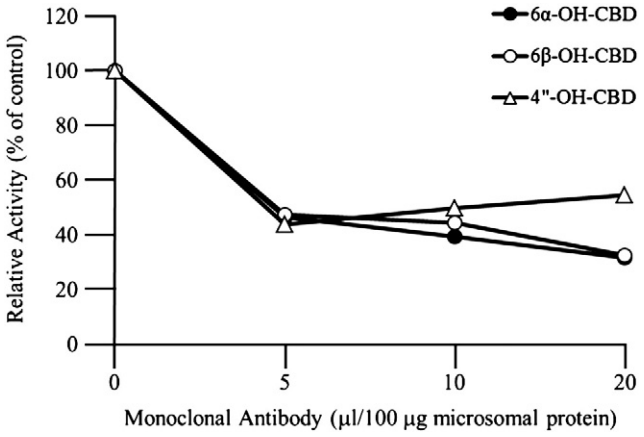

Fig. 4. Effects of anti-CYP3A4 antibody on formation of 6α-OH-, 6β-OH-, and 4″-OH-CBDs from CBD by HLMs. Pooled HLMs were preincubated with various amounts of a monoclonal antibody against CYP3A4 (0–20 μl/100 μg protein in HLMs) on ice for 20 min and then incubated with 6.4 μM CBD in the presence of an NADPH-generating system. The activities of 6α- (closed circles), 6β- (open circles), and 4″- (triangles) hydroxylations of CBD were measured by GC/MS. The control activities for 6α-OH- and 6β-OH-CBD formation without the antibody (100% as the control) were 84.3 and 60.3 pmol/min/mg protein, respectively. Each point is the mean of duplicate determinations.

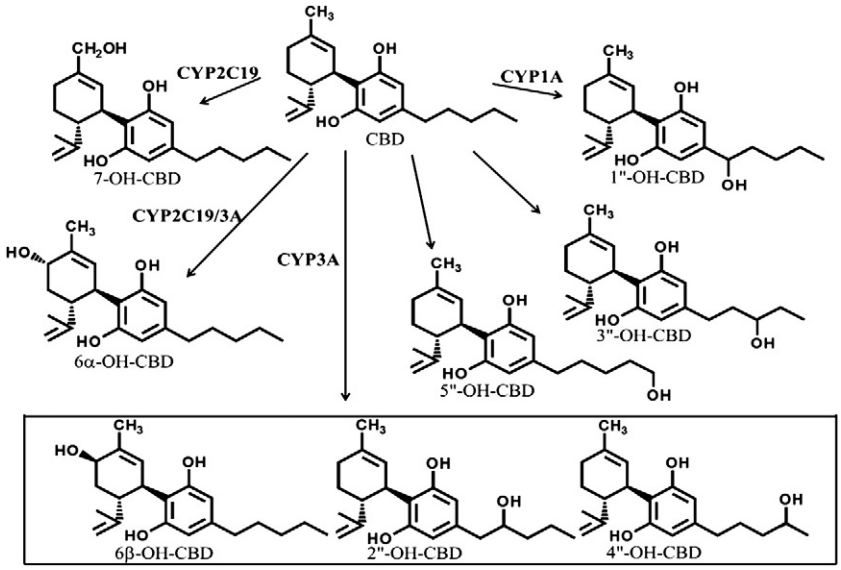

Fig. 5. Metabolic pathways of CBD in HLMs.

selective CYP2D6 inhibitor, quinidine, and correlated with the dextromethorphan *O*-demethylation by CYP2D6 in the individual HLMs.

We have previously reported that Δ^9-THC and cannabinol (CBN), other major cannabinoids in marijuana, are mainly oxidized at the 8- and 11-positions by HLMs (Watanabe et al., 1995, 2006, 2007). Furthermore, the 8- and 11-hydroxylated metabolites of these two cannabinoids are formed primarily by CYP3A4 and CYP2C9, respectively (Watanabe et al., 2006, 2007). The 8-position of Δ^9-THC and CBN corresponds to the 6-position of CBD, which was predominantly oxidized by CYP3A4 in the current study. Thus, the active site of CYP3A4 appears to accommodate CBD, Δ^9-THC, and CBN in the same orientation. On the other hand, the 11-position of Δ^9-THC and CBN corresponds to the 7-position of CBD, which was primarily oxidized by CYP2C19 in this study. It has been shown that *d*-limonene, which corresponds to the terpene moiety of these cannabinoids, is metabolized mainly by CYP2C9 and CYP2C19 to form perillyl alcohol via oxidation at the 7-position (Miyazawa et al., 2002). CBD has free rotatable structure between the terpene and resorcinol moieties. In contrast, Δ^9-THC and CBN are structurally constrained because these cannabinoids have the dibenzopyran ring. The difference in structural constraints between CBD and the other two major cannabinoids may lead to CYP2C19- and CYP2C9-mediated preferential oxidation of the methyl groups at the 7-position of CBD and at the 11-position of Δ^9-THC and CBN, respectively.

In addition to these major metabolites of CBD described above, HLMs also formed four minor hydroxylated metabolites of CBD, 1″-OH-, 2″-OH-, 3″-OH-, and 5″-OH-CBDs. CBD 1″-hydroxylation was catalyzed exclusively by recombinant CYP1A enzymes. Furthermore, CBD 1″-hydroxylase activity of HLMs was markedly inhibited by a CYP1A-selective inhibitor α-naphthoflavone at 1 μM (data not shown). Our previous study demonstrated that 11-oxo-Δ^8-THC was hydroxylated at the 1″-position by rat CYP1A1 and CYP1A2 (Watanabe et al., 1991). Although CBD 2″-hydroxylation was catalyzed by recombinant CYP1A2 and CYP3A enzymes, a preliminary inhibition study indicated that CBD 2″-hydroxylase activity of HLMs was suppressed by ketoconazole but not by α-naphthoflavone (data not shown). These results suggest that main CYP enzymes responsible for the formation of 1″-OH- and 2″-OH-CBDs in HLMs might be CYP1A and CYP3A, respectively. On the other hand, predominant CYP isoforms involved in the formation of the other two minor metabolites, 3″-OH- and 5″-OH-CBDs, in HLMs were not specified in the present study. Further studies are needed to identify CYP enzymes catalyzing the minor metabolic pathways of CBD in HLMs.

Δ^9-THC is a primary psychoactive substance in marijuana and possesses various pharmacological effects including catalepsy and hypothermia (Pertwee, 2008). Interestingly, several metabolites of Δ^9-THC oxidized by CYP enzymes exert more potent pharmacological effects as compared with Δ^9-THC (Razdan, 1986; Yamamoto et al., 2003). On the other hand, there is limited information about pharmacological effects of CBD oxidative metabolites. Carlini et al. (1975) have reported that 6-OH-CBD and the further oxidative metabolite, 6-oxo-CBD, prolong a pentobarbital sleeping time as long as CBD. In addition, it has been shown that these metabolites of CBD possess as potent an anticonvulsant effect as CBD. Further studies are needed to elucidate pharmacological effects of the other oxidative metabolites of CBD.

Conclusions

The present study conclusively demonstrated that CBD was extensively metabolized by HLMs. Our results suggest that CYP3A4 and CYP2C19 may play pivotal roles in the formation of 6α-OH-, 6β-OH-, 7-OH-, and 4″-OH-CBDs in HLMs (Fig. 5).

Conflict of interest statement

The authors have declared that no conflict of interest exists.

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