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FORMATION OF SIMILAR SPECIES TO CARBON MONOXIDE DURING HEPATIC MICROSOMAL METABOLISM OF CANNABIDIOL ON THE BASIS OF SPECTRAL INTERACTION WITH CYTOCHROME P-450

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Abstract—Cannabidiol induced a carbon monoxide-like complex with mouse hepatic microsomal cytochrome P-450 during NADPH-dependent metabolism *in vitro* on a spectral basis. The reduction by dithionite was required for the maximal development of a spectrum. The complex showed a peak at 450 nm which shifted to 419 or 423 nm, respectively, by further addition of hemoglobin or myoglobin. Cannabidiol-induced complex formation required molecular oxygen, and was decreased by the addition of inhibitors of cytochrome P-450-dependent monooxygenase. Pretreatment of mice with phenobarbital (80 or 100 mg/kg, i.p. for 3 days) but not 3-methylcholanthrene (80 mg/kg, i.p.) increased the complex formation. In contrast, pretreatment with cobaltous chloride (40 mg/kg, i.p. for 3 days) decreased the complex formation. 8,9-Dihydro- and 1,2,8,9-tetrahydrocannabidiols also induced the same spectrum as that of above complex, whereas cannabidiol monomethyl- and dimethylethers reduced this ability. In addition, both cannabidivarin and cannabigerol induced the complex formation, although Δ^9-tetrahydrocannabinol, cannabinol and cannabielsoin did not. Olivetol but not *d*-limonene induced the spectrum of the complex to some extent. These results indicate that cannabidiol induces a carbon monoxide-like complex with cytochrome P-450 during hepatic microsomal metabolism, and suggest that phenobarbital-inducible cytochrome P-450s mediate at least one of the metabolic steps of CBD to form the complex, as well as the importance of the resorcinol moiety of CBD for the complex formation.

Cannabidiol (CBD), one of the major cannabinoids in marihuana, exhibits a prolonging effect on barbiturate-induced sleeping time, which is mainly associated with the inhibition of hepatic microsomal metabolism of barbiturates [1–3]. CBD has a high affinity to hepatic microsomal cytochrome P-450 and produces a type I spectrum with the cytochrome, which has been considered to be a part of mechanism for the inhibitory effect of the cannabinoid on the drug metabolism. A decrease of the content of hepatic microsomal cytochrome P-450 is also suggested to be partially responsible for the effect of CBD on the drug metabolism leading to the prolongation of barbiturate-induced sleep [4, 5]. However, the mechanism of CBD to inhibit the hepatic microsomal drug metabolizing enzymes has not been fully established. Karler *et al.* [6] and Borys and Karler [7] have suggested that the inhibitory effect of CBD on drug metabolism in the liver may be attributable to the formation of inhibitory metabolites. We recently reported that CBD decreased hepatic microsomal cytochrome P-450 measured by its carbon monoxide (CO) difference spectra, when incubated in the presence of an NADPH-generating system [8, 9], suggesting the requirement of metabolic activation for the effect of CBD on cytochrome P-450. Borys *et al.* [4] suggested that CBD might generate CO during CBD metabolism with hepatic microsomes. The present study has investigated not only the nature of the enzymes of mice which catalyzed the formation of CO-like complex with cytochrome P-450 during hepatic microsomal metabolism of CBD, but also the structural requirement for producing the complex.

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MATERIALS AND METHODS

Chemicals. NADP, NADPH and glucose-6-phosphate were purchased from Boehringer Mannheim GmbH (Darmstadt, F.R.G.). NAD, NADH, catalase (bovine liver, 2890 units/mg protein EC 1.11.1.6), superoxide dismutase (bovine blood, 3200 units/mg protein, EC 1.15.1.1), myoglobin (equine skeletal muscle), glucose-6-phosphate dehydrogenase (type V, EC 1.1.1.49), metyrapone and sodium deoxycholate were obtained from Sigma Chemical Co. (St. Louis, MO), and hemoglobin (bovine blood), *d*-limonene, glutathione, cobaltous chloride ($CoCl_2$) and 3-methylcholanthrene (3-MC) were from Wako Pure Chemical Ind. (Osaka, Japan). α-Naphthoflavone and 2,5-dimethylfuran were purchased from Tokyo Chemical Ind. (Tokyo, Japan) and Aldrich Chemical Co. (Milwaukee, WI), respectively. SKF 525-A (2-diethylaminoethyl-2,2-

diphenylvalerate hydrochloride) was supplied from Smith Kline and French Lab. (Philadelphia, PA). All other chemicals and solvents used were of analytical reagent grade.

Cannabinoids. CBD, cannabidiolic acid (CBDA) and Δ^9-tetrahydrocannabinol (Δ^9-THC) were isolated and purified from cannabis leaves supplied by Prof. I. Nishioka, Kyushu University (Fukuoka, Japan) according to the method of Aramaki *et al.* [10]. Cannabidivarin (CBDV) and cannabigerol (CBG) were also supplied by Prof. I. Nishioka and National Institute on Drug Abuse (Rockville, MD), respectively. CBDA methyl ester was prepared by the methylation of CBDA with diazomethane. CBD-monomethylether, CBD-dimethylether, 8,9-dihydro-CBD and 1,2,8,9-tetrahydro-CBD were prepared by the methods described previously [9]. Cannabielsoin (CBE) and olivetol were prepared by the methods of Uliss *et al.* [11] and Lieman *et al.* [12], respectively. The purities of these cannabinoids used were determined to be at least 95% by gas chromatography.

Animal treatments and preparation of microsomes. Male ddN mice (25–35 g) were pretreated with phenobarbital (80 or 100 mg/kg, i.p. for 3 days), 3-MC (80 mg/kg, i.p.) or $CoCl_2$ (40 mg/kg, i.p. for 3 days). Mice were killed by cervical dislocation 24 hr (phenobarbital and $CoCl_2$ treatments) or 48 hr (3-MC treatment, single injection) after the last injection. Control mice were not given any drugs or vehicle. Hepatic microsomes were prepared as described previously [13]. Cytochrome P-450 and protein in the microsomes were determined by the methods of Omura and Sato [14] and Lowry *et al.* [15], respectively.

Incubation system and spectral measurement. A typical incubation mixture consisted of CBD (160 μM, 15 μl dimethylsulfoxide), microsomes from phenobarbital-treated mice unless otherwise noted (0.2 g liver equivalent), 0.5 mM NADP, 10 mM glucose-6-phosphate, 1 mM disodium ethylenediaminetetraacetic acid, 10 mM $MgCl_2$, 4 mM nicotinamide, 1.5 units glucose-6-phosphate dehydrogenase and 100 mM potassium phosphate buffer (pH 7.4) to make a final volume of 3 ml. The reaction mixture was incubated at 25° for 10 min. In all cases, a reference incubation was treated identically with the exception that the NADPH-generating system was omitted. The difference spectra (400 to 500 or 600 nm) were measured after the addition of dithionite into both incubations using a Union SM 401 spectrophotometer. For the studies on the cofactor requirement, the NADPH-generating system was replaced by NAD, NADP, NADH, NADPH (each 1 mM) or cumene hydroperoxide (0.5 mM). For the study on molecular oxygen requirement, the reaction mixture was placed on a Warburg type reaction vessel and equilibrated with an appropriate gas phase. Difference spectra produced by the interaction of cannabinoids and related compounds with cytochrome P-450 were measured by the method of an earlier report [16]. The spectral dissociation constant (K_s) was determined using a double reciprocal Lineweaver–Burk plot of the absorbance difference between a peak and a trough against the concentration of each compound.

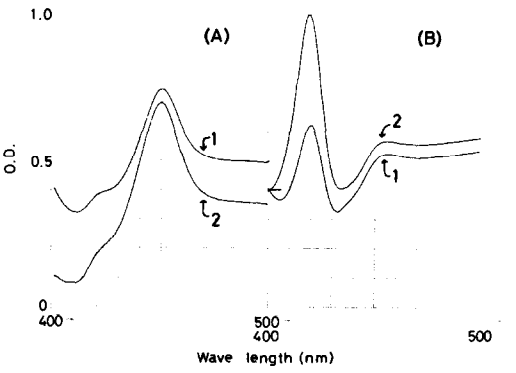


Fig. 1. Spectral measurement of incubation mixture metabolizing CBD. CBD (160 μM) was incubated with hepatic microsomes (4.47 mg protein and 10.2 nmol cytochrome P-450) in the presence of an NADPH-generating system at 25° for 10 min. (A-1) The difference spectrum was measured after the addition of dithionite. (A-2) CO-difference spectrum of the same concentration of microsomal suspension. (B-1) and (B-2) Spectra were measured after the addition of sodium deoxycholate (final concentration, 0.3%) into (A-1) and (A-2), respectively.

RESULTS

CO-*like complex formation with cytochrome P*-450

CBD induced a spectrum similar to that of CO complex with reduced form of cytochrome P-450, when incubated with mouse hepatic microsomes in the presence of the NADPH-generating system (Fig. 1A). Δ^9-THC and CBN did not show such spectral change. The addition of sodium deoxycholate (final concentration 0.3%), which is known to be a denaturant of cytochrome P-450, resulted in the shift of an absorbance peak at 450 nm to 420 nm (Fig. 1B). This is also a characteristic of CO complex with cytochrome P-450 [14]. The magnitude of the spectral change (ΔO.D. 450–490 nm) was markedly decreased by less than 10% when nitrogen gas was bubbled for 1 min into the incubation mixture or the incubation mixture was aspirated for 5 min before the addition of dithionite. The complex formed by the incubation of CBD with the microsomes was replaced by further addition of hemoglobin or myoglobin, producing a spectrum that showed a peak at 419 or 423 nm, respectively (Fig. 2). These spectra were superimposable to those of CO complex with both hemoproteins.

Cofactor and molecular oxygen requirement

As seen in Table 1, the complex formation by CBD required NADPH. NADH was much less effective as an electron donor and did not show any significant synergistic effect on the complex formation when added together with NADPH. Cumene hydroperoxide, which is known to support the oxidation by cytochrome P-450 [17, 18], could be used quite effectively for the complex formation. The complex formation by CBD required molecular oxygen and was completely inhibited 100% nitrogen atmosphere (data not shown).

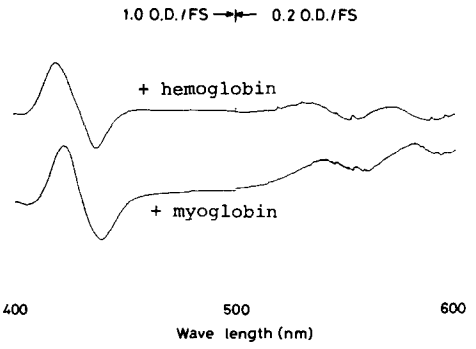


Fig. 2. Spectral change of CBD incubation mixture after addition of hemoglobin or myoglobin. CBD (160 μM) was incubated with hepatic microsomes (3.95 mg protein and 8.2 nmol cytochrome P-450) in the presence of an NADPH-generating system at 25° for 10 min. The difference spectra were measured after the addition of dithionite and then hemoglobin or myoglobin (each 1 mg) in both cuvettes.

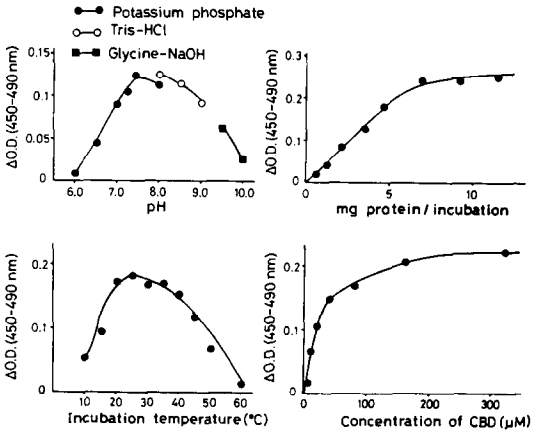


Fig. 3. Dependence of cytochrome P-450 complex formation by CBD on pH (upper left), protein concentration (upper right), incubation temperature (lower left) and CBD concentration (lower right). The incubations and analyses were carried out as described in Materials and Methods except that the parameters were varied as indicated on each figure. Cytochrome P-450 contents in hepatic microsomes used were as follows: effect on pH, 2.09 nmol/mg protein; effects of incubation temperature and protein concentration, 2.34 nmol/mg protein; effect of CBD concentration, 2.51 nmol/mg protein.

Dependence of the complex formation on pH, protein concentration, incubation temperature, substrate concentration and dithionite reduction

The complex formation by CBD was linear with protein concentration up to about 2.5 mg protein/ml (Fig. 3). The optimal complex formation was observed in the range of pH 7.4 to 8.5 and of 20 to 40° of the incubation temperature (Fig. 3). The complex formation was constant over 160 μM of the CBD concentrations. Figure 4 shows the requirement of dithionite reduction for maximal development of the spectra similar to CO complex with cytochrome P-450. NADPH alone indicated a lag time for about 20 min to induce the spectra, whereas the addition of dithionite induced spectra rapidly, which became maximum size within 10 min of incubation time. The maximal ΔO.D. (450–490 nm) observed in the NADPH-generating system was approximately 75% as compared with that of dithionite reduction.

Effects of inducers, inhibitors or scavengers on the complex formation

Pretreatment of mice with phenobarbital increased the complex formation by 400% as compared with control, whereas that with $CoCl_2$ lowered the complex formation with concomitant decrease of cytochrome P-450 content in hepatic microsomes (Table 2). 3-MC treatment did not affect significantly on the complex formation by CBD. The complex formation by CBD was not significantly affected by the addition (each 1 mM) of glutathione, *dl*-α-tocopherol, ascorbic acid or 2,5-dimethylfuran to the reaction mixture, all of which are known as scavengers of radicals or singlet oxygen (Table 3), although butylhydroxytoluene exhibited the inhibition of the complex for-

Table 1. Cofactor requirement for cytochrome P-450 complex formation by CBD

Cofactor	ΔO.D. (450–490 nm)	% of NADPH or NADPH-generating system
Experiment-1		
NAD	0	0
NADP	0	0
NADH	0.060 ± 0.007	27
NADPH	0.220 ± 0.012	100
NADH + NADPH	0.219 ± 0.013	100
Experiment-2		
NADPH-generating system	0.125 ± 0.003	100
Cumene hydroperoxide	0.095 ± 0.004	76

CBD (160 μM) was incubated with hepatic microsomes (experiment 1, 4.76 mg protein and 10.9 nmol cytochrome P-450; experiment 2, 3.95 mg protein and 7.6 nmol cytochrome P-450) as described in Materials and Methods except that various pyridine nucleotides (each 1 mM) were used instead of the NADPH-generating system in experiment 1. Cumene hydroperoxide used was 0.5 mM in experiment 2. The results represent the mean ± SE from triplicate incubations of the same pool of microsomes.

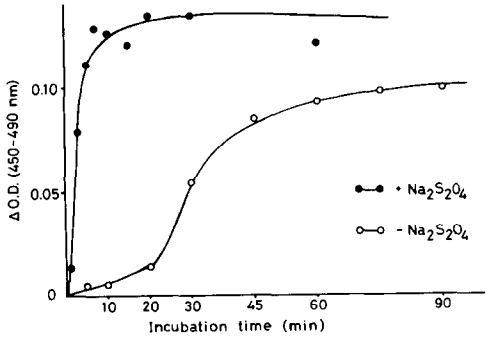


Fig. 4. Time course of spectral changes during hepatic microsomal metabolism of CBD. CBD (160 μM) was incubated with hepatic microsomes (3.53 mg protein and 7.8 nmol cytochrome P-450) as described in Materials and Methods. (●——●) ΔO.D. (450–490 nm) after addition of dithionite; (○——○) ΔO.D. (450–490 nm) observed in incubation mixture with an NADPH-generating system, but not without addition of dithionite.

mation to some extent. None of catalase, KCN and superoxide dismutase affected significantly the complex formation by CBD. The complex formation was significantly inhibited by three known inhibitors of cytochrome P-450, metyrapone (1 mM), α-naphthoflavone (0.5 mM) and SKF 525-A (1 mM), suggesting that cytochrome P-450 itself plays some role as oxygenase in the complex formation by CBD. Involvement of cytochrome P-450 in the complex formation by CBD was further demonstrated in Fig. 5, showing a high correlation ($r = 0.996$) between the complex formation by CBD and cytochrome P-450 content in hepatic microsomes. Cytochrome P-450 content in hepatic microsomes and the complex formation by CBD decreased with increasing the aging of the microsomes on ice.

The structural requirement for the complex formation

The structural requirement for the formation of CO-like complex with cytochrome P-450 is shown in Table 4. 8,9-Dihydro- and 1,2,8,9-tetrahydro-CBDs, which are reduced derivatives of CBD on the double bonds in the terpene moiety, induced the same com-

Table 2. Effects of phenobarbital-, 3-MC- and $CoCl_2$-treatments on cytochrome P-450 complex formation by CBD

Treatments	Cytochrome P-450 content (nmol/mg protein)	Complex formation [ΔO.D. (450–490 nm) /mg protein × 10^3]	Complex formation [ΔO.D. (450–490 nm /nmol cytochrome P-450 × 10^3]
Control	0.655 ± 0.064 (100)	5.6 ± 1.0 (100)	8.4 ± 1.0 (100)
Phenobarbital (80 mg/kg, i.p. for 3 days)	1.666 ± 0.085† (254)	22.7 ± 2.4† (405)	13.5 ± 0.8† (161)
3-MC (80 mg/kg, i.p.)	0.992 ± 0.062† (151)	8.5 ± 1.1 (152)	8.5 ± 0.8 (101)
$CoCl_2$ (40 mg/kg, i.p. for 3 days)	0.313 ± 0.057† (48)	1.7 ± 0.3* (30)	5.4 ± 1.2 (64)

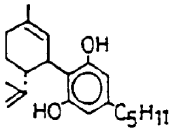
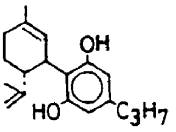
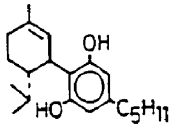
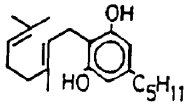
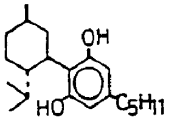
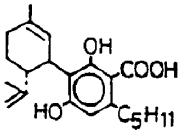
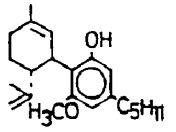
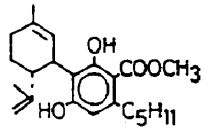
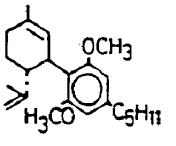
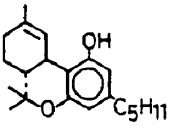
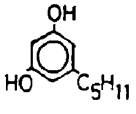
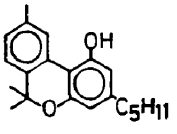
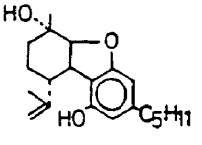
Incubations and analyses were carried out as described in Materials and Methods. The results represent the mean ± SE of four determinations. Numbers in parentheses represent percent of control.
* Significantly different from control ($P < 0.05$).
† Significantly different from control ($P < 0.01$).

Table 3. Effects of various inhibitors or scavengers on cytochrome P-450 complex formation by CBD

Inhibitors or scavengers	% of control	Inhibitors or scavengers	% of control
Control	100	Butylhydroxytoluene	75*
Ascorbic acid	91	Metyrapone	59†
Glutathione	98	α-Naphthoflavone	29†
dl-α-Tocopherol	91	SKF 525-A	22†
2,5-Dimethylfuran	95	Catalase (882 units)	85
KCN	93	Superoxide dismutase (471 units)	100

Concentration of inhibitors or scavengers used was 1 mM except for α-naphthoflavone (0.5 mM). CBD (160 μM) was incubated with hepatic microsomes (3.68 mg protein and 8.8 nmol cytochrome P-450) as described in Materials and Methods. The spectral change (ΔO.D. 450–490 nm) observed in control was 0.180 ± 0.010. The results represent the mean of triplicate determinations as percent of the control.
* Significantly different from control ($P < 0.05$).
† Significantly different from control ($P < 0.01$).

Table 4. Comparison in CBD and related compounds inducing cytochrome P-450 complex and showing K_s value for cytochrome P-450

Compounds	Complex formation*	K_s (μM)	Compounds	Complex formation*	K_s (μM)
 CBD	100	5.1	 CBDV	93	8.0
 8,9-dihydro-CBD	33	16.6	 CBG	131	9.7
 1,2,8,9-tetrahydro-CBD	71	6.7	 CBDA	0	33.4
 CBD-monomethylether	5	66.4	 CBDA methylester	19	30.6
 CBD-dimethylether	0	N.D.†	 Δ^9-THC	0	8.5
 olivetol	14	38.7	 CBN	0	6.1
 *d*-limonene	0	6.7	 CBE	0	—‡

* $\frac{\text{Spectral change (}\Delta\text{O.D. 450–490 nm) in compounds tested}}{\text{Spectral change (}\Delta\text{O.D. 450–490 nm) in CBD}} \times 100.$

† Substrate-induced binding spectrum was not detectable up to the concentration of 0.2 mM.

‡ Not determined.

In the experiment of cytochrome P-450 complex formation, all compounds (160 μM) were incubated with hepatic microsomes (3.47 mg protein and 6.8 nmol cytochrome P-450) as described in Materials and Methods. The mean spectral change (ΔO.D. 450–490 nm) in CBD incubation was 0.115. Cytochrome P-450 content in hepatic microsomes used for K_s value determination was 2.35 nmol/mg protein. The results represent the mean of triplicate determinations.

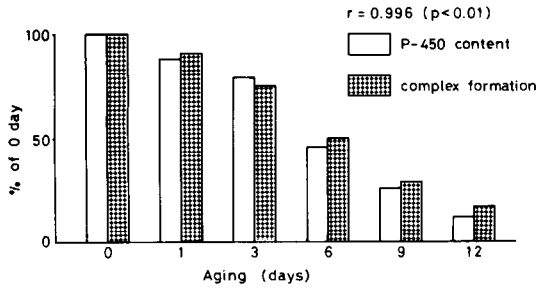


Fig. 5. Correlation between cytochrome P-450 content and cytochrome P-450 complex formation by CBD. Incubation conditions were described in Materials and Methods. Microsomal suspension (1 g liver equivalent/ml 1.15% KCl) was stocked on ice until experiment. Cytochrome P-450 content in hepatic microsomes used and the complex formation (ΔO.D. 450–490 nm) on day 0 were 2.18 nmol/mg protein and 0.150, respectively.

plex as that of CBD to some extent. On the other hand, CBD monomethyl- and dimethylethers significantly decreased or completely lost the ability to form the complex, indicating the importance of the resorcinol moiety but not the terpene moiety of CBD in the complex formation. CBDA methylester but not CBDA produced the complex. The ability of CBDV and CBG to produce the complex was comparable to that of CBD, although Δ^9-THC, CBN and CBE did not produce the complex. Olivetol but not *d*-limonene produced the complex, supporting the theory that the resorcinol moiety of CBD plays an important role in the complex formation. The complexes formed by CBD related compounds behaved similarly to that of CBD by the treatments as shown in Figs 1 and 2, indicating that the complexes formed by these compounds are the same species. Table 4 also shows K_s values of these compounds for mouse hepatic microsomal cytochrome P-450. All compounds except for CBD-dimethylether produced a type I spectrum with cytochrome P-450 (peak 385–390 nm, trough 420–425 nm). CBD ($K_s = 5.1\ \mu M$) showed the highest affinity to cytochrome P-450 among the compounds tested. In the resorcinol derivatives, the compounds having lower K_s values showed relatively higher ability to form the complex, although *d*-limonene (6.7 μM), Δ^9-THC (8.7 μM) and CBN (6.1 μM), which did not induce the complex at all, showed high affinity to cytochrome P-450 comparable to that of CBD.

DISCUSSION

We previously reported that CBD decreased hepatic microsomal cytochrome P-450 when incubated with an NADPH-generating system by measuring its CO difference spectra [8, 9]. The present study demonstrated that CBD produced CO-like complex with cytochrome P-450 under similar experimental conditions as above studies. This indicates that the complex formation resulted in a decrease of spectrally detectable cytochrome P-450. Therefore, most of the decrease in cytochrome P-450 by CBD described previously [8, 9] can be explained by the CO-like complex formation demonstrated in the present study. Assuming the 450 nm complex is a CO complex, it can be estimated that about 60% of cytochrome P-450 bound with CO under the present experimental conditions. The complex formation by CBD required NADPH and molecular oxygen, and was inhibited by metyrapone, α-naphthoflavone and SKF 525-A, which are known inhibitors of cytochrome P-450. The results indicate that the complex formation requires oxidative metabolism of CBD which is catalyzed by cytochrome P-450. Increase in the complex formation by phenobarbital-treatment and decrease in that by $CoCl_2$, which is known to decrease cytochrome P-450 content [19], also suggest that phenobarbital-inducible cytochrome P-450s mediate at least one of the metabolic steps of CBD to form the CO-like complex. Little or no change in the complex formation by 3-MC treatment suggest that 3-MC does not cause induction of cytochrome P-450s responsible for the complex formation by CBD. The lack of effect of radical scavengers, glutathione, ascorbic acid and *dl*-α-tocopherol and of KCN, catalase, 2,5-dimethylfuran and superoxide dismutase indicates that none of singlet oxygen, hydroxyl radicals, hydrogen peroxide and superoxide anion is significantly responsible for the complex formation. Some effect observed in butylhydroxytoluene, which is known to be a radical scavenger, may be due to the inhibitory effect of this compound on cytochrome P-450 as described by Yang *et al.* [20].

Borys *et al.* [4] suggested that the complex formed by CBD is a CO complex with reduced form of cytochrome P-450, although they did not show definite evidence and did not specify the source of CO. The present study provided the additional evidence supporting the finding of Borys *et al.* The complex formed by CBD was replaced by further addition of hemoglobin or myoglobin, appearing the same spectra as those of CO complexes with these hemoproteins. With respect to the complex by CBD, dithionite is required for full development of the 450 nm peak in the difference spectrum. The complex formed by CBD was stable in the presence of dithionite. The present study demonstrated a lag time in the appearance of the 450 nm peak in NADPH-reduced microsomes. Yu *et al.* [21] have reported the same phenomena in the formation of CO complex with cytochrome P-450 during the microsomal metabolism of methylene-dioxyphenyl compounds. They suggested that concentration of oxygen dissolved in the reaction medium is critical for the development of the CO complex with cytochrome P-450 in the NADPH-reduced microsomes. These findings are not conclusive but strongly suggest that the complex formed by CBD is CO complex with cytochrome P-450. However, these results could not entirely exclude the possibility that a metabolic intermediate formed during microsomal metabolism of CBD interacts with cytochrome P-450 and similar molecular configuration of cytochrome P-450 to CO complex with the cytochrome.

The source of CO, which may be generated during the microsomal metabolism of CBD, is not clear at present. Halomethanes [22, 23] and methylenedioxyphenyl compounds [24] are known to produce CO

during the microsomal metabolism. However, there is no similarity in the structure between CBD and these compounds. NADPH-dependent lipid peroxidation can induce microsomal degradation of cytochrome P-450 heme and the formation of CO [25]. Heme catabolism by heme oxygenase is also known to be a source of CO generation in hepatic microsomes [26]. In our experimental conditions, the lipid peroxidation was negligible in the presence of ethylenediamine-tetraacetic acid, which is a strong inhibitor of the lipid peroxidation. In the case of CBD, moreover, the heme breakdown has been ruled out as the possible source of CO, because of the inability of CBD to affect heme oxygenase [8] and heme content [9] in hepatic microsomes *in vitro*.

We recently reported the importance of the resorcinol moiety in the decrease of cytochrome P-450 by CBD *in vitro* and in the inhibitory effect of CBD on the hepatic microsomal drug-metabolizing enzymes [9]. The present study also demonstrates that the resorcinol moiety of CBD plays an important role in the complex formation. Thus, 8,9-dihydro- and 1,2,8,9-tetrahydro-CBDs, which are the reduced derivatives of CBD in the terpene moiety, induced the complex formation, suggesting that epoxy metabolites of CBD in the terpene moiety are not involved in the complex formation. In contrast, CBD-monomethyl- and dimethylethers almost lost the ability to produce the complex. The ability of CBDV and CBG, which are the resorcinols, to produce the complex was comparable to that of CBD, although Δ^9-THC, CBN and CBE, which are monophenols, did not produce the complex. CBDA is the only exceptional resorcinol derivative which did not induce the complex. CBDA has a carboxyl group which could associate with a phenolic hydroxyl group of the *ortho* position by hydrogen bonding. Therefore, CBDA may act as a phenol rather than a resorcinol.

These results indicate the positive correlation between the complex formation and the inhibitory effect, suggesting that the complex formation by CBD is part of the mechanism in the inhibitory effect of CBD on the hepatic microsomal drug-metabolizing enzymes *in vitro*. Further studies, however, are necessary to clarify whether the complex formation by CBD is related to the inhibitory effect of the cannabinoid on the drug metabolism *in vivo*. More recently, we have reported data suggesting that CBD decreases a specific form of cytochrome P-450 *in vivo* [27].

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