Pharmacology and Toxicology of Major Constituents of Marijuana—On the Metabolic Activation of Cannabinoids and Its Mechanism

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DOI: 10.1081/TXR-120026915

0731-3837 (Print); 1525-6057 (Online)

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ABSTRACT

Many oxidative metabolites of tetrahydrocannabinols (THCs), active components of Cannabis sativa L. (Cannabinaceae), were pharmacologically potent, and 11-hydroxy-THCs, 11-oxo-Δ8-THC, 7-oxo-Δ9-THC, 8β,9β-epoxyhexahydrocannabinol (EHHC), 9α,10α-EHHC and 3'-hydroxy-Δ9-THC were more active than THC in pharmacological effects such as catalepsy, hypothermia and barbiturate synergism in mice, indicating that these metabolites are active metabolites of THCs. Cannabidiol (CBD), another major component, was biotransformed to two novel metabolites, 6-hydroxymethyl-Δ9-THC and 3-pentyl-6,7,7a,8,9,11a-hexahydro-1,7-dihydroxy-7,10-dimethyl-4H-benzo[b,d]oxepin (PHDO) through 8R,9-epoxy-CBD and 8S,9-epoxy-CBD as intermediates, respectively, identified by us. Both metabolites have some pharmacological effects comparable to Δ9-THC. Cannabinol (CBN), the other major component, was mainly metabolized to 11-hydroxy-CBN by hepatic microsomes of animals including humans. The pharmacological effects of the metabolite were higher than those of CBN demonstrating that 11-hydroxylation of CBN is an activation pathway of the cannabinoid as is the case in THCs. Tolerance developed to catalepsy, hypothermia and pentobarbital-induced sleep prolonging effects of Δ9-THC and its active metabolite, 11-hydroxy-Δ9-THC. Reciprocal cross-tolerance also developed to pharmacological effects and the magnitude of tolerance development produced by the metabolite was significantly higher than that by Δ9-THC indicating that 11-hydroxy-Δ9-THC has important role not only in the pharmacological effects but also its tolerance development of Δ9-THC. THCs and their metabolites competed with the specific binding of CP-55,940, an agonist of cannabinoid receptor, to synaptic membrane from bovine cerebral cortex.
The Ki value of THCs and their metabolites were closely parallel to their pharmacological effects in mice. A novel cytochrome P450 (cyp2c29) was purified and identified for the first time by us as a major enzyme responsible for the metabolic activation of Δ⁸-THC at the 11-position in the mouse liver. cDNA of cyp2c29 was cloned from a mouse cDNA library and its sequence was determined. All of major P450s involving the metabolic activation of Δ⁸-THC at the 11-position are belonging to CYP2C subfamily in mammalian liver.

Key Words: Tetrahydrocannabinol; Cannabidiol; Cannabinol; Cytochrome P450; Microsomal aldehyde oxygenase; Metabolic activation; Receptor binding; Tolerance development.

I. INTRODUCTION

Marijuana is generally regarded as one of the most widely abused drugs. Cannabinoids are a group of over 60 compounds containing in Cannabis sativa L., in which tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) are three major cannabinoids in the plant (Figure 1). Cannabinoids are known to have various pharmacological effects in animals and humans such as catalepsy, hypothermia, anticonvulsant, barbiturate-induced sleep prolongation, analgeia, antiemetic and antiglaucoma etc., some of which are thought to be useful for medical purpose at the present time. Δ⁹-THC is known to be an active component in marijuana and contributes to the most of pharmacological effects.

Although metabolism of THC, CBD and CBN have been studied widely, it is apparent that the cannabinoids are extensively oxidized by cytochrome P450 (CYP)-dependent system to form mono-, di-, and tri-hydroxylated metabolites together with the metabolites having keto and carboxyl groups. Cannabinoids are known to be good substrates for hepatic microsomal monooxygenase involving CYP due to their lipophilic properties. Over 80 metabolites have been identified as metabolites of THC in experimental animals and humans. The major metabolic route demonstrated by THC is allylic hydroxylation at the 11-position followed by oxidation to THC-11-oic acid as a final oxidized metabolite. Other routes of THC metabolism are shown in Figure 2. Earlier studies have shown that some of THC metabolites are pharmacologically active and have some role in the pharmacological effects of the parent cannabinoid. In this symposium, the author summarizes the metabolic activation of cannabinoids mainly carried out in our laboratory in relation to its pharmacological and toxicological point of view together with molecular biology of a novel P450 enzyme.
responsible for the metabolic activation of THC. Δ⁸-THC, and isomer of Δ⁹-THC is a minor component of marijuana, but more stable than Δ⁹-THC and has the same pharmacological profile as Δ⁹-THC.

**II. METABOLIC ACTIVATION OF THCs**

Δ⁸-THC was incubated with hepatic microsomes from various animal species including human. The metabolites formed were extracted and analyzed by GC-MS after conversion to trimethylsilyl derivatives. Relative abundance of Δ⁸-THC metabolites formed are summarized in Table 1. The data demonstrated that Δ⁸-THC was mainly metabolized at the 11-position to form 11-hydroxy-THCs (11-OH-THCs) with hepatic microsomes of

![Figure 1. Structures of major cannabinoids in marijuana.](image-url)

![Figure 2. Metabolic pathways of THCs.](image-url)
mice, rats and humans. CYPs are major enzymes responsible for the oxidation of THC in the microsomal fraction. Therefore, ratio of oxidized metabolites formed is dependent on population of CYPs present in hepatic microsomes of the experimental animals. We demonstrated that main enzymes responsible for the formation of 11-OH-THC are CYP2C in all animal species tested (Table 2).

We synthesized various THC metabolites and their pharmacological effects in mice were characterized using catalepsy, hypothermia and barbiturate synergism as indices. Pharmacological experiments demonstrated that 11-OH-THCs are active metabolites, especially the cataleptogenic effect of 11-OH-\(\Delta^8\)-THC was 5 times higher than that of \(\Delta^8\)-THC (Watanabe

### Table 1. Relative ratio of \(\Delta^8\)-THC metabolites formed with hepatic microsomes from various animal species.

<table>
<thead>
<tr>
<th>Metabolites formed</th>
<th>Animal species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
</tr>
<tr>
<td>11-OH</td>
<td>100</td>
</tr>
<tr>
<td>7-OH</td>
<td>21</td>
</tr>
<tr>
<td>7-OH</td>
<td>t</td>
</tr>
<tr>
<td>1'-OH</td>
<td>ND</td>
</tr>
<tr>
<td>3'-OH</td>
<td>ND</td>
</tr>
<tr>
<td>4'-OH</td>
<td>ND</td>
</tr>
<tr>
<td>DiOH-HHC</td>
<td>t</td>
</tr>
</tbody>
</table>

- t = Trace amount; ND = Not detected.

### Table 2. Cytochrome P450 enzymes mainly responsible for metabolic activation of \(\Delta^8\)-THC at the 11-position in Mammalian Liver.

<table>
<thead>
<tr>
<th>Animals</th>
<th>P450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Cyp2c29</td>
</tr>
<tr>
<td>Rat</td>
<td>CYP2C11</td>
</tr>
<tr>
<td>male</td>
<td>CYP2C6</td>
</tr>
<tr>
<td>female</td>
<td>CYP2C37</td>
</tr>
<tr>
<td>Monkey</td>
<td>CYP2C9</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
</tbody>
</table>
et al., 1980; Yamamoto, 1986). Other active metabolites are 11-oxo-Δ⁸-THC, 7-oxo-Δ⁸-THC, 8β,9β-epoxyhexahydrocannabinol (EHHC), 9α,10α-EHHC and 3'-OH-Δ⁸-THC, and their pharmacological effects were more potent than the parent cannabinoid.

III. NOVEL METABOLITES OF CBD

CBD has anticonvulsant and barbiturate-induced sleep prolonging effects, although it is said that the cannabinoid is devoid of psychoactivity. Metabolism of CBD has extensively been studied in various animal species. We demonstrated for the first time that three new CBD metabolites were formed from epoxy metabolites as intermediates (Figure 3). Cannabielsoin (CBE) was identified by BC-MS as a new metabolite of CBD with guinea pig hepatic microsomes. CBE did not show any significant effects on hypothermia and pentobarbital-induced sleeping time in mice.

![Figure 3. Novel metabolic pathways of CBD.](image-url)
8R,9- and 8S,9-epoxy-CBD were also identified as novel metabolites of CBD with guinea pig hepatic microsomes, which were further metabolized to 6β-hydroxymethyl-Δ⁹-THC and 3-pentyl-6,7a,8,9,11a-hexahydro-1,7-dimethylidibenzo[b,d]oxepin (PHDO), respectively (Figure 3). This is the first observation that CBD is biotransformed to a Δ⁹-THC derivative with hepatic microsomes of animals. These metabolites were also pharmacologically active, while their pharmacological effects were 2/3 to 1/7 of Δ⁹-THC in mice (Yamamoto et al., 1995a).

IV. METABOLIC ACTIVATION OF CBN

CBN is known to be formed from THC and CBD by chemical oxidation. It has been reported that THC is converted to CBN in aging conditions. Therefore, aged marijuana samples contain much higher amount of CBN than fresh samples. The evidence suggests that CBN may play some role in the pharmacological and toxicological effects of aged marijuana samples. CBN was also mainly metabolized to 11-OH-CBN in experimental animals and humans (Figure 4). 11-Hydroxylation of CBN was catalyzed by CYP2C in mice (Watanabe et al., 1993) and humans (Watanabe et al., 1995b), as is the case of THC.

CBN has been reported to be inactive or marginal active on the basis of animal and human experiments. However, our pharmacological study (Yamamoto et al., 1987) demonstrated that CBN itself exhibited significant effects on catalepsy, hypothermia and pentobarbital-induced sleep prolongation, although the effects of CBN were much less than those of THC. 11-OH-CBN was twice as active as CBN in three pharmacological indices, indicating that the 11-hydroxylation is metabolic activation pathway as is the case in THC.

![CBN and 11-Hydroxy-CBN](image-url)  
*Figure 4.* Major metabolic pathway of CBN by hepatic microsomes of animals and human.
V. ROLE OF METABOLITE FOR TOLERANCE DEVELOPMENT OF THC

11-OH-Δ⁸-THC is known to be an active metabolite of Δ⁸-THC as described above. Tolerance and reciprocal cross-tolerance developed to pharmacological effects of Δ⁸-THC and 11-OH-Δ⁸-THC. As shown in Figure 5, repeated administration of Δ⁸-THC and its active metabolites, 11-OH-Δ⁸-THC and 11-oxo-Δ⁸-THC, caused in tolerance development to their hypothermic effect in mice (Watanabe et al., 1983). After the 3rd administration (5 mg/kg/day, i.v.) of both cannabinoids, no significant hypothermia was induced by the cannabinoids. Reciprocal cross-tolerance was also demonstrated between these cannabinoids. The higher magnitude of tolerance was developed by the repeated administration of 11-OH-Δ⁸-THC compared with that by Δ⁸-THC. Tolerance was also developed to cataleptogenic effect of Δ⁸-THC and 11-OH-Δ⁸-THC. However, tolerance development to the cataleptogenic effects of these cannabinoids was incomplete. Thus, significant cataleptogenic effects of Δ⁸-THC and 11-OH-Δ⁸-THC was observed even after 7 daily administrations of these cannabinoids, although the cataleptogenic effects of both cannabinoids were highly attenuated. The magnitude of tolerance development produced by 11-OH-Δ⁸-THC was

Figure 5. Tolerance development in the hypothermic effects of Δ⁸-THC and its metabolites in mice.
greater than that by Δ⁸-THC in this index. Tolerance developed to pentobarbital-induced sleep prolonging effects of Δ⁸-THC and 11-OH-Δ⁸-THC.

The manner of tolerance development to barbiturate synergism is the same tendency to that of catalepsy. Tolerance development to barbiturate synergism is incomplete as is the case of catalepsy.

It is conclusively demonstrated that 11-OH-Δ⁸-THC has an important role in the tolerance development in the pharmacological effects of Δ⁸-THC as well as the pharmacological effects of the parent cannabinoid.

VI. CANNABINOID RECEPTOR BINDING OF THC METABOLITES

Several lines of evidence indicate that cannabinoid receptors are present in the CNS and peripheral tissues, and THC exerts some of its effects through the receptors. Cannabinoid receptor has been cloned and characterized in relation to structure and activity (Howlett et al., 1998; Matsuda et al., 1990). [³H]CP-55,940, an agonist of the cannabinoid receptor, has been used as a ligand for the receptor assay with synaptic membrane from bovine cerebral cortex. THC metabolites competed the specific CP-55,940 binding to the synaptic membrane. In Δ⁸-THC metabolites oxidized at the 11-position, potency ratio to compete the specific binding of CP-55,940 to the synaptic membrane was in the following order; 11-OH-Δ⁸-THC > 11-oxo-Δ⁸-THC > Δ⁸-THC > Δ⁸-THC-11-oic acid (Table 3) (Yamamoto et al., 1998). These results closely paralleled to the pharmacological effects (catalepsy, hypothermia and pentobarbital-induced sleep prolongation) in mice.

The binding affinities of other metabolites were also closely related to their pharmacological effects.

Table 3. Relationship between cannabinoid receptor affinity and pharmacological effects of Δ⁸-THC and its metabolites oxidized at the 11-position.

<table>
<thead>
<tr>
<th>Cannabinoids</th>
<th>CB₁ (Ki, nM)</th>
<th>Relative pharmacological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ⁸-THC</td>
<td>197</td>
<td>100</td>
</tr>
<tr>
<td>11-OH-Δ⁸-THC</td>
<td>52</td>
<td>500</td>
</tr>
<tr>
<td>11-Oxo-Δ⁸-THC</td>
<td>148</td>
<td>147</td>
</tr>
<tr>
<td>Δ⁸-THC-11-oic acid</td>
<td>917</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

*CP-55,940 as a ligand.
Nucleotide and deduced amino acid sequences of a mouse liver CYP2C29 cDNA.

![Figure 6](https://via.placeholder.com/150)

**Figure 6.** Nucleotide and deduced amino acid sequences of a mouse liver CYP2C29 cDNA.
VII. A NOVEL CYTOCHROME P450 INVOLVING METABOLIC ACTIVATION OF Δ⁸-THC

In our laboratory it was demonstrated that a novel P450 (cyp2c29) is the major enzyme responsible for the 11-hydroxylation of Δ⁸-THC in the mouse liver (Yamamoto et al., 1995b). cDNA of cyp2c29 was cloned from a mouse cDNA library (Matsunaga et al., 1994). Figure 6 shows nucleotide and deduced sequences of CYP2C29 cDNA. Table 4 also summarizes sequence identity of CYP2C29 to Rat CYP2C subfamily members (Matsunaga et al., 1994). The role of cyp2c29 in the metabolic activation of Δ⁸.

![Chemical structures](image)

**Figure 7.** Oxidation of Δ⁸-THC at the 11-position by Cyp2c29.
The cysteine residue in the heme binding region and polyadenylation signal (AATAAAA) are double underlined. ATG: Initiation codon; TAA: Termination codon THC at the 11-position was significantly characterized (Yamamoto, 1999). 11-OH-Δ8-THC was further oxidized to Δ8-THC-11-oic acid (carboxylic acid) through 11-oxo-Δ8-THC (aldehyde) as an intermediate. We clearly demonstrated that the final step of the oxidation (the oxidation of an aldehyde to a carboxylic acid) was also catalyzed by cyp2c29 indicating a novel role of P450 as microsomal aldehyde oxygenase (Watanabe et al., 1991; Yamamoto et al., 1988). Thus, cyp2c29 catalyzed three consecutive oxidation steps from Δ8-THC to Δ8-THC-11-oic acid at the 11-position. The oxygenation mechanism in the oxidation of 11-oxo-Δ8-THC to Δ8-THC-11-oic acid by cyp2c29 was proved (Figure 7).

ACKNOWLEDGMENTS

The authors are grateful to a member of Faculty staffs of Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hoku-riku University.

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