Metabolic Activation of Marijuana Constituents, Cannabinoids, in Relation to Their Toxicity for Human and Its Oxidation Mechanism

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Abstract

Many oxidative metabolites of tetrahydrocannabinols (THCs), active components of marijuana, were pharmacologically active, and 11-hydroxy-THCs, 11-oxo-Δ^8-THC, 7-oxo-Δ^8-THC, 8β,9β-epoxyhexahydrocannabinol (EHHC), 9α,10α-EHHC and 3'-hydroxy-Δ^9-THC were more active than THC in pharmacological effects such as catalepsy, hypothermia and barbiturate synergism in mice. Cannabidiol (CBD), another major component, was biotransfomred to two novel metabolites, 6-hydroxymethyl-Δ^9-THC and 3-pentyl-6, 7, 7a, 8, 9, 11a-hexahydro-1, 7-dihydroxy-7,10-dimethyldibenzo[b,d]oxepin (PHDO) through 8R,9-epoxy-CBD and 8S, 9-epoxy-CBD, respectively. Both metabolites exhibited some pharmacological effects comparable to Δ^9-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 metabolic activation pathway of the cannabinoid as is the case in THCs. Tolerance and reciprocal cross-tolerance developed to pharmacological effects Δ^8-THC and 11-hydroxy-Δ^8-THC ,and the magnitude of tolerance development produced by the metabolite was significantly higher than that by Δ^8-THC. The results indicate that 11-hydroxy-Δ^8-THC has an important role not only in the pharmacological effects but also its tolerance development of Δ^8-THC. THCs and their metabolites competed to 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 paralleled to their pharmacological effects in mice. A novel cytochrome P450 (cyp2c29) was purified and identified as a major enzyme responsible for the metabolic activation of Δ^8-THC at the 11-position in the mouse liver. cDNA of CYP2C29 was cloned from a mouse cDNA library and its sequence was determined. The oxidation mechanism of THC by cyp2c29 was proposed.

INTORDUCTION

Cannabinoids are constituents of *Cannabis sativa* L., in which tetrahydrocannabinol

(THC), cannabidiol (CBD) and cannabinol (CBN) are three major cannabinoids in the plant. Cannabinoids are known to have various pharmacological effects in animals and humans such as catalepsy, hypothermia, anticonvulsant, barbiturate-induced sleep prolongation, analgesia, antiemetic and antiglaucoma etc., some of which are thought to be useful for medical purpose at the present time.

Cannabinoids are known to be good substrates for hepatic microsomal monooxygenase involving cytochrome P450 (P450). Over 80 metabolites have been identified as metabolites of THC in experimental animals and humans (Fig. 1). 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 the oxidation mechanism of the cannabinoids by P450.
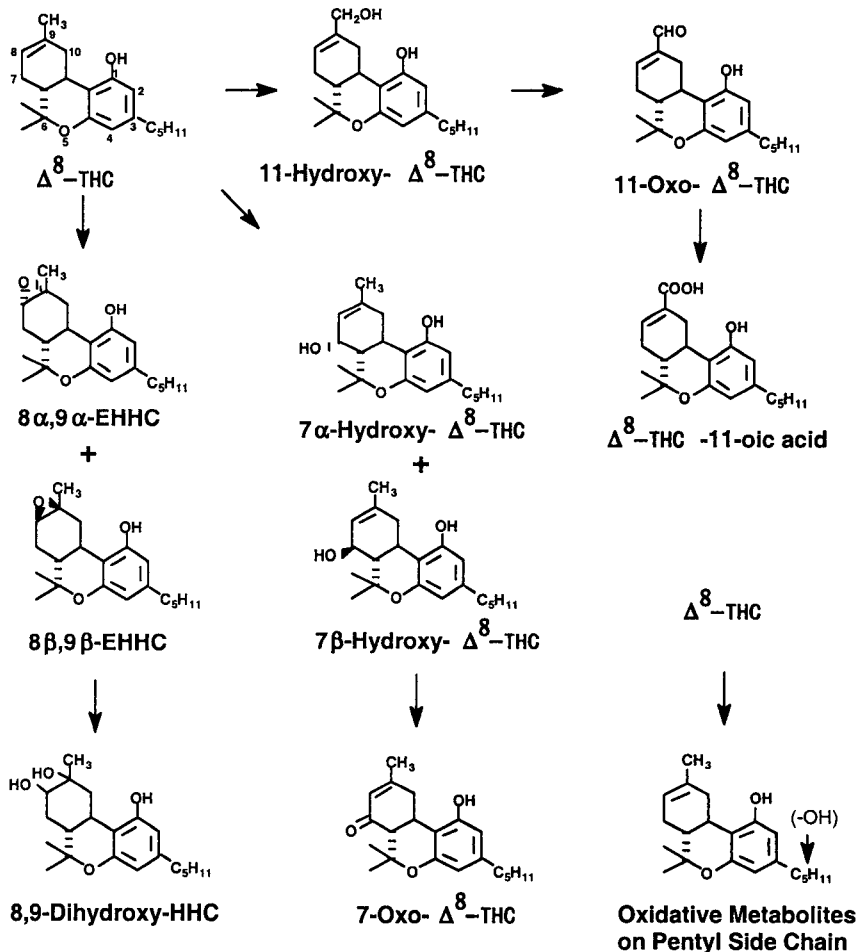

Fig. 1 Major Pathways of Δ^8-THC Metabolism

MATERIALS AND METHODS

Pharmacological experiments and metabolic studies were carried out by the methods described in the literatures cited in References (1,7,9).

RESULTS AND DISCUSSION

1. Metabolic activation of THCs

Δ^8- and Δ^9-THC were mainly metabolized at the 11-position to form to 11-hydroxy-THCs (11-OH-THCs) with hepatic microsomes of mice, rats and humans (Table I). In all animal species, main enzymes responsible for the formation of 11-OH-THC are CYP2C (Table II). Various THC metabolites were synthesized 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 cataleptogenic effect of 11-OH-Δ^8-THC was 5 times more higher than that of Δ^8-THC .[1] Other active metabolites are 11-oxo-Δ^8-THC, 8β,9β-epoxyhexahydrocannabinol (EHHC), 9α, 10α-EHHC and 3'-OH-Δ^9-THC.

Table I   Relative Ratio of Δ^8-THC Metabolites Formed with Hepatic Microsomes from Various Animal Species

Metabolites	Mouse	Rat	Guinea pig	Rabbit	Monkey	Human
11-OH	100	100	65	74	95	100
7-OH	21	17	100	100	100	44
7-OH	t	ND	21	54	9	ND
1'-OH	ND	5	t	ND	ND	t
3'-OH	ND	t	24	ND	5	t
4'-OH	ND	58	ND	71	35	ND
DiOH-HHC	t	6	33	t	ND	18

t = Trace amount   ND = Not detected

Table II   Cytochrome P450 Enzymes Mainly Responsible for Metabolic Activation of Δ^8-THC at the 11-Position in Mammalian Liver

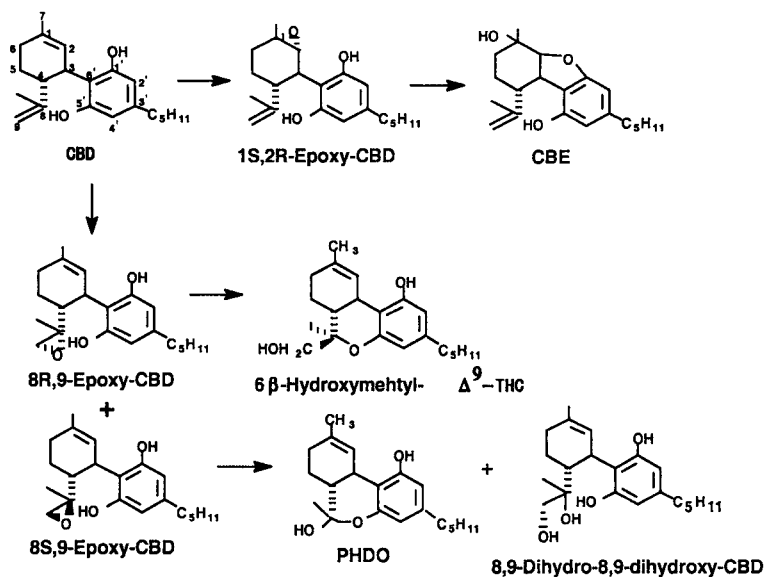| Animals	P450
Mouse	Cyp2c29
Rat male	CYP2C11
female	CYP2C6
Monkey	CYP2C37
Human	CYP2C9

Fig. 2   Novel Metabolic Pathways of CBD

2.   Novel metabolites of CBD

8R,9- and 8S,9-epoxy-CBD were identified as novel metabolites of CBD with guinea pig hepatic microsomes, which were further metabolized to 6-hydroxymethyl-
Δ^9-THC and 3-pentyl- 6, 7, 7a, 8, 9, 11a-hexahydro-1,7-dimethyldibenzo[b,d]oxepin (PHDO), respectively (Fig. 2).   This is the first observation that CBD is biotransformed to a Δ^9-THC derivative with hepatic microsomes of animals.   These metabolites were also pharmacologically active, while their pharmacological effects were 2/3 to 1/7 of Δ^9-THC in mice.[2]

3.   Metabolic activation of CBN

CBN is also mainly metabolized to 11-OH-CBN in experimental animals and humans. 11-Hydroxylation of CBN is catalyzed by CYP2C in humans.[3]   Our pharmacological study [4] demonstrated that CBN itself exhibited significant effect on catalepsy, hypothermia and pentobarbital-induced sleep prolongation.   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.

4. Role of metabolite for tolerance development of THC

Repeated administration of Δ^8-THC and its active metabolite, 11-OH-Δ^8-THC, caused in tolerance development to their cataleptogenic effect in mice.[5] Reciprocal cross-tolerance was also demonstrated between both cannabinoids. The higher magnitude of tolerance was developed by the repeated administration of 11-OH-Δ^8-THC compared with that by Δ^8-THC. Tolerance was also developed to hypothermic effect of Δ^8-THC and 11-OH-Δ^8-THC. The magnitude of tolerance development produced by 11-OH-Δ^8-THC was greater than that by Δ^8-THC in this index. Tolerance developed to pentobarbital-induced sleep prolonging effect of Δ^8-THC and 11-OH-Δ^8-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-Δ^8-THC has an important role in the tolerance development in the pharmacological effects of Δ^8-THC.

5. Cannabinoid receptor binding of THC Metabolites

THC metabolites competed the specific [^{3}H]CP-55,940 binding to the synaptic membrane from bovine cerebral cortex. In Δ^8-THC metabolites oxidized at the 11-position, potency ratio was in the following order; 11-OH-Δ^8-THC > 11-oxo-Δ^8-THC > Δ^8-THC >> Δ^8-THC-11-oic acid.[6] These results were closely paralleled to their pharmacological effects in mice.

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

6. A novel cytochrome P450 involving metabolic activation of Δ^8-THC

In our laboratory it was demonstrated that a novel P450 (CYP2C29) is the major enzyme responsible for the 11-hydroxylation of Δ^8-THC in the mouse liver.[7] cDNA of CYP2C29 was cloned from a mouse cDNA library.[8] The role of CYP2C29 in the metabolic activation of Δ^8-THC at the 11-position was significantly characterized.[9] 11-OH-Δ^8-THC was further oxidized to Δ^8-THC-11-oic acid (carboxylic acid) through 11-oxo-Δ^8-THC (aldehyde) as an intermediate. We clearly demonstrated that the final step of the oxidation was also catalyzed by CYP2C29 indicating a novel role of P450 as microsomal aldehyde oxygenase. [10] The oxygenation mechanism in the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid by CYP2C29 was proved.

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