Involvement of CYP2C in the Metabolism of Cannabinoids by Human Hepatic Microsomes from an Old Woman

Kazuhito Watanabe,† Tamihide Matsunaga,† Ikuo Yamamoto,‡,* Yoshihiko Funae,‡ and Hidetoshi Yoshimura†

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, 3-8-16 Kanagawa-machi, Kanazawa 920-11, Japan, Laboratory of Chemistry, Osaka City University Medical School, 1-4-54 Asahi-machi, Abeno-ku, Osaka 545, Japan and Department of Food and Nutrition, Nakamura Gakuen University, 5-7-1 Befu, Johnan-ku, Fukuoka 814-01, Japan. Received January 9, 1995; accepted March 27, 1995

The hepatic microsomal metabolism of cannabinoids was studied using the liver from an old woman. Δ⁹-Tetrahydrocannabinol, Δ⁹-tetrahydrocanabinol and cannabinol were biotransformed to their respective 11-hydroxy metabolites by a microsomal fraction with specific activities (nmol/min/mg protein) of 29.1, 47.1 and 27.9, respectively. In addition, both 11-oxo-Δ⁹-tetrahydrocannabinol and 11-oxo-Δ⁹-tetrahydrocannabinol were metabolized to the corresponding carboxylic acids with the microsomes. An antibody against mouse CYP2C29 almost completely inhibited 11-hydroxylation of the cannabinoids and microsomal aldehyde oxygenase (MALDO) activity for 11-oxo-Δ⁹-tetrahydrocannabinol and 11-oxo-Δ⁹-tetrahydrocannabinol, used as substrates, whereas an antibody against rat CYP3A2 conversely stimulated the 11-hydroxylation of Δ⁹-tetrahydrocannabinol and MALDO activity for 11-oxo-Δ⁹-tetrahydrocannabinol. The results indicate that a member of CYP2C is primarily responsible for the metabolism of the above cannabinoids in the human hepatic microsomes.

Key words cytochrome P450; tetrahydrocannabinol; cannabinoid; metabolism; human hepatic microsome; microsomal aldehyde oxygenase

Extensive studies have shown that cannabinoids, constituents of marijuana, are metabolized to a number of metabolites in mammals.† Relatively limited information is available on the metabolism of cannabinoids in humans, especially in an in vitro system.

Halldin et al.⁵ reported that Δ⁹-tetrahydrocannabinol (Δ⁹-THC) was metabolized mainly to 11-hydroxy-Δ⁹-THC (11-OH-Δ⁹-THC) together with 8α- and 8β-OH-metabolites by 10000 × g supernatant fraction of human livers. Our previous studies⁶ have shown that Δ⁹-THC and Δ⁹-THC are metabolized to 11-OH-THCs and other minor metabolites oxidized at the allylic positions and on the pentyl side chain. Recent studies have shown that specific forms of cytochrome P450 (P450) catalyze the formation of certain THC metabolites in animals. Several members of the CYP2C subfamily have been reported to be major isozymes responsible for the 11-hydroxylation of THC; these are mouse CYP2C29,⁴ male rat CYP2C11⁵ and female rat CYP2C6.⁶ In humans, Bornheim et al.⁷ reported that CYP2C9 catalyzed the formation of 11-OH-Δ⁹-THC, and a member of the CYP3A subfamily was responsible for the 8β-hydroxylation of Δ⁹-THC. Our recent studies have demonstrated that P450 isoforms catalyze microsomal aldehyde oxygenase (MALDO) activity for 11-oxo-metabolites of THCs,⁶ although there is no report concerning MALDO in human liver at present. The present study describes that P450 isozyme(s) belonging to CYP2C are mainly involved in the 11-hydroxylation of cannabinoids, as well as the MALDO activity for 11-oxo-Δ⁹-THC and 11-oxo-Δ⁹-THC with hepatic microsomes from the liver of an old woman.

MATERIALS AND METHODS

Chemicals NADP and glucose 6-phosphate were purchased from Boehringer Mannheim Biochem. (Tokyo, Japan). Glucose 6-phosphate dehydrogenase (type V) was obtained from Sigma Chem. Co. (St. Louis, U.S.A.). NADPH was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Δ⁹-THC and cannabinol (CBN) were isolated from cannabis leaves as described previously.⁹ Δ⁹-THC,¹⁰ 11-OH-Δ⁹-THC,¹¹ 11-OH-Δ⁹-THC,¹² 11-OH-CBN,¹³ 11-oxo-Δ⁹-THC,¹¹ 11-oxo-Δ⁹-THC,¹² Δ⁹-THC-11-oic acid,¹⁴ and Δ⁹-THC-11-oic acid were prepared by the previous methods. All other chemicals used were of the highest purity commercially available.

Preparation of Microsomes Liver was obtained from an 80-year-old Japanese woman who died of carbon monoxide poisoning. The liver was homogenized in 4 volume of 5 mm potassium phosphate buffer (pH 7.4) containing 1 mm EDTA and 1.15% KCl and centrifuged at 9000 × g for 20 min. The supernatant fraction was centrifuged at 105000 × g for 60 min. After resuspension of the resultant pellet with the same solvent, it was again centrifuged at 105000 × g for 60 min and the pellet obtained was stored at −80°C until use.

Metabolism of Cannabinoids Cannabinoids (20 μg each) were incubated with the hepatic microsomes (0.1 g liver equivalent), 0.5 mm NADP, 10 mm glucose 6-phosphate, 10 mm MgCl₂, 1 unit glucose 6-phosphate dehydrogenase and 100 mm sodium-potassium phosphate buffer (pH 7.4) to make a final volume of 1 ml. The mixture was incubated at 37°C for 20 min and then extracted with 5 ml of ethyl acetate after the addition of 5'-nor-Δ⁹-THC-4'-oic acid as the internal standard. After evaporation of the organic layer, the residue was subjected to GC-MS, after methylation of the carboxylic groups with diazomethane and trimethylsilylation of the hydroxyl groups as described previously.⁸⁸ GC-MS was carried out at 70 eV with a JEOL JMS-GCG-06 gas chromatograph coupled with a JEOL JMS-DX 300 mass data system and a JEOL-DA 5000 mass data system. The conditions were
c © 1995 Pharmaceutical Society of Japan
as follows: column, 2% OV-17 on Chromosorb W (60—
80 mesh, 3 mm × 2 m); column temperature, 270 °C; ioniz-
ing current, 300 μA; carrier gas, He 40 ml/min. Under
these conditions, the retention times of methyl esters
and/or trimethylsilyl (TMS) derivatives of 11-OH-Δ⁴-
THC, 11-OH-Δ⁹-THC, 11-OH-CBN, Δ⁴-THC-11-oic acid
and Δ⁹-THC-11-oic acid were 4.7, 4.4, 5.9, 7.0 and 6.9
min, respectively.

Polyclonal antibodies against mouse P450 MUT-2
(CYP2C29)¹⁵ and rat CYP3A2 were raised in New
Zealand white rabbits as described previously.¹⁶ The
inhibitory effect of the antibodies was determined in
the presence of 0.30 to 5.85 mg of antibody/mg of micro-
somal protein. Western immunoblot analysis was
performed by a general method using alkaline phosphatase
conjugated goat IgG after being transferred onto
nitrocellulose sheets.¹⁷ Protein content was determined
by the method of Lowry et al.¹⁸ with bovine serum
albumin as the standard. Microsomal P450 was determined
by the method of Omura and Sato¹⁹ using a molecular
coefficient of 91 mm⁻¹ cm⁻¹, and the content in the
microsomes was 0.154 nmol/mg protein.

RESULTS AND DISCUSSION

The present study demonstrated that CBN was me-
tabolized exclusively to 11-OH-CBN by human hepatic
microsomes as were Δ⁴- and Δ⁹-THC. As shown in Fig.
1, 11-OH-CBN was the only metabolite detected in the
extract of the incubation mixture of CBN with the
microsomes. A peak (M-1) with a retention time of 5.9 min
showed a mass spectrum identical to that of 2TMS-11-
OH-CBN. Metabolites formed from Δ⁴-THC and Δ⁹-THC
showed retention times of 4.7 and 4.4 min, respectively,
and shared identical mass spectra with those of synthetic
11-OH-metabolites.

The metabolism of THC in humans has been extensively
studied in vivo,²⁰ although limited data are available
concerning the in vitro metabolism of THC and other
cannabinoids. Halldin et al.²¹ reported that 11-OH-Δ⁴-
THC was a major metabolite formed with the 10000 × g
supernatant fraction from human livers. Our previous
study also demonstrated that 11-OH-THC was the most
abundant metabolite of Δ⁴- and Δ⁹-THC by human
hepatic microsomes, although the cannabinoids were also
metabolized by allylic hydroxylation at the 7- and 8-po-
sitions, respectively. The present study, however, show-
ed that Δ⁴- and Δ⁹-THC were metabolized exclusively
to 11-OH-THC by the microsomes, with specific activities
(pmoles/min/mg protein) of 29.1 and 47.1, respectively.
The 11-hydroxylation activity for Δ⁹-THC is less than
1/10 of the data reported by Bornheim et al.⁷ It is not
clear whether the lower 11-hydroxylation and lack of
8β-hydroxylation of Δ⁹-THC is related to the cause of
death of the liver donor.

The microsomal hydroxylation of these cannabinoids
appears to be catalyzed by a particular isozyme of P450.
A member of the CYP2C subfamily has accounted for
most of the 11-hydroxylation catalyzed by hepatic micro-
somes of animals and humans.⁴—⁷ Bornheim et al.⁷
reported that CYP2C9 was found to contribute mainly
to the 11-hydroxylation of Δ⁹-THC with human hepatic
microsomes, whereas P450 belonging to the 3A subfamily
catalyzed the 8β-hydroxylation of the cannabinoid. The
present study demonstrated that the antibody against
mouse CYP2C29 almost completely inhibited the 11-hy-
droxylation of all cannabinoids examined (Fig. 2 and
Table 1). These results suggest that a member of CYP2C
is responsible for most of the 11-hydroxylation activity
of the cannabinoids in the microsomes. In contrast, an
antibody against CYP3A2 stimulated the 11-hydroxy-
lation of Δ⁴-THC (Table 1). The interaction of the anti-
body with CYP3A may cause an increase in the 11-
hydroxylation of Δ⁹-THC by CYP2C, although the cata-
lytic activity of CYP3A was lost in the microsomes.
Western immunoblot analysis indicated that an immuno-
reactive protein to the antibody against mouse CYP2C29
or rat CYP3A2, having a molecular weight of 50 kDa or

Fig. 1. Mass Chromatograms and Mass Spectra of CBN Metabolites Formed with Human Hepatic Microsomes
52 kDa, respectively, was present in the microsomes (data not shown).

The human hepatic microsomes exhibited MALDO activity for 11-oxo-Δ^8-THC and 11-oxo-Δ^2-THC. In mouse hepatic microsomes, the MALDO activity for 11-oxo-Δ^8-THC was 17 times higher than that for 11-oxo-Δ^2-THC, whereas the activity for the Δ^2-isomer in the human hepatic microsomes was twice that for the Δ^8-isomer (Table 1). The MALDO activity appears to also be catalyzed by CYP2C since the activity was significantly inhibited by the antibody against CYP2C29. Incomplete inhibition by the antibody, however, suggests that other isozyme(s) may be also involved in the MALDO activity to some extent.

The present study indicates that a member of CYP2C is a major isozyme in metabolizing the cannabinoids in an old woman liver, and that CYP2C is more stable than CYP3A in the liver.

Acknowledgments We thank Mrs. R. Igarashi for carrying out GC-MS analyses. A part of the present study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by the Special Research Fund of Hokuriku University.

REFERENCES