Life Sciences 136 (2015) 87–93

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Cannabidiol induces expression of human cytochrome P450 1A1 that is possibly mediated through aryl hydrocarbon receptor signaling in HepG2 cells

Satoshi Yamaori [a], Yuka Kinugasa [b], Rongrong Jiang [b], Shuso Takeda [c,1], Ikuo Yamamoto [d], Kazuhito Watanabe [b,c,*]

[a] Department of Pharmacy, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto 390-8621, Japan
[b] Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan
[c] Organization for Frontier Research in Preventive Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan
[d] Department of Hygienic Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino-machi, Nobeoka 882-8508, Japan

ARTICLE INFO

Article history:
Received 3 January 2015
Received in revised form 4 June 2015
Accepted 8 July 2015
Available online 15 July 2015

Keywords:
Marijuana
Cannabidiol
CYP1A1
Induction
AhR
Protein tyrosine kinase

ABSTRACT

Aims: We herein investigated the inducibility of cytochrome P450 1A1 (CYP1A1) by Δ^9-tetrahydrocannabinol, cannabidiol (CBD), and cannabinol, three major phytocannabinoids, using human hepatoma HepG2 cells.
Main methods: The expression of CYP1A1 and the aryl hydrocarbon receptor (AhR) was measured by a quantitative real-time polymerase chain reaction and/or Western blotting.
Key findings: Δ^9-Tetrahydrocannabinol and CBD concentration-dependently induced the expression of CYP1A1 mRNA, whereas cannabinol showed little or no induction. Among the phytocannabinoids tested, CBD was the most potent inducer of CYP1A1 expression. The induction of CYP1A1 expression by CBD was significantly attenuated by the knockdown of AhR expression with AhR small interfering RNAs. The role of protein tyrosine kinases (PTKs) in the CBD-mediated induction of CYP1A1 was then examined using herbimycin A, a PTK inhibitor. The upregulation of CYP1A1 by CBD was significantly suppressed by herbimycin A as was the induction by omeprazole but not 3-methylcholanthrene. The inducibility of CYP1A1 by CBD-related compounds was examined to clarify the structural requirements for CBD-mediated CYP1A1 induction. Olivetol, which corresponds to the pentylresorcinol moiety of CBD, significantly induced the expression of CYP1A1, whereas *d*-limonene, CBD-2′-monomethyl ether, and CBD-2′,6′-dimethyl ether did not.
Significance: These results showed that CBD may have induced human CYP1A1 expression through the activation of PTK-dependent AhR signaling, in which two phenolic hydroxyl groups in the pentylresorcinol moiety of CBD may play structurally important roles.

1. Introduction

Marijuana is the most widely used illicit drug in the world. Its use is a growing public health concern due to potential adverse effects such as dependence, association with polysubstance use, increased risk of motor vehicle crashes, impaired respiratory function, cardiovascular disease, and various health consequences [1]. Marijuana leaves contain at least 70 cannabinoids [2], with Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) being the three main constituents (Fig. 1). Δ^9-THC is the principal psychoactive component of marijuana and has various pharmacological effects such as catalepsy, hypothermia, antiinflammation, and antinociception [3]. CBD is not psychoactive, but has several pharmacological effects such as antiepileptic, anxiolytic, and antiemetic actions [4]. CBN is believed to exert minimal pharmacological effects on the central nervous system.

Marijuana is commonly consumed by smoking. Previous studies reported that habitual smokers of marijuana exhibited molecular and histopathological changes that were similar to precancerous lesions observed in the bronchial epithelium of tobacco smokers [5,6]. Furthermore, an epidemiological study revealed a correlation between marijuana use and head and neck cancer [7]. These findings suggest that a history of marijuana use may increase the risk of developing cancer. Marijuana smoke includes various procarcinogenic polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene (B[*a*]P) and benz[*a*]anthracene [8–10]. These PAHs are metabolically activated by cytochrome P450s (CYPs) to exert genotoxicity and carcinogenicity [11]. For example, B[*a*]P is metabolized by CYP1A1 and epoxide

* Corresponding author at: Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan.
E-mail address: k-watanabe@hokuriku-u.ac.jp (K. Watanabe).
[1] Present address: Laboratory of Xenobiotic Metabolism and Environmental Toxicology, Faculty of Pharmaceutical Sciences, Hiroshima International University (HIU), 5-1-1 Hirokoshingai, Kure, Hiroshima 737-0112, Japan.

http://dx.doi.org/10.1016/j.lfs.2015.07.007
0024-3205/

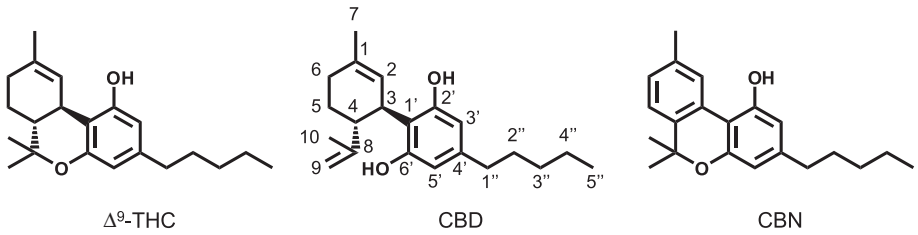

Fig. 1. Structures of three major phytocannabinoids.

hydrolase to a diol-epoxide, the ultimate carcinogen, the formation of DNA adduct by which plays a critical role in tumor initiation [12]. Some PAHs are also known to potently induce the expression of CYP1A1 [11]. These findings indicated that the potency of the catalytic activity of CYP1A1 and its expression levels are important risk factors for determining cancer induced by marijuana use. Witschi and Saint-François [13] demonstrated that B[*a*]P hydroxylase activity, an index of CYP1 activity, was increased in the lung homogenates of rats administered Δ^9-THC. Furthermore, Δ^9-THC has been shown to induce the expression of CYP1A1 in mouse hepatoma Hepa-1 cells, primary human airway epithelial cells, and human breast cancer MDA-MB-231 cells [14–16]. Phytocannabinoids are present in marijuana smoke at markedly higher concentrations than PAHs [8,9,14]. A previous study estimated that the content of Δ^9-THC was approximately 9.3 mg per marijuana cigarette whereas the contents of B[*a*]P and benz[*a*]anthracene were 22 and 56 ng per marijuana cigarette, respectively [14]. Thus, phytocannabinoids may also contribute to the induction of CYP1A1 by marijuana components. However, it currently remains unclear whether major phytocannabinoids other than Δ^9-THC, i.e. CBD and CBN, induce the expression of CYP1A1.

In the present study, we examined the inducibility of human CYP1A1 by the three major phytocannabinoids (Δ^9-THC, CBD, and CBN). We showed that CBD was the most potent inducer of the expression of CYP1A1 in human hepatoma HepG2 cells. Furthermore, the results of our study suggest that the induction of CYP1A1 by CBD was mediated through aryl hydrocarbon receptor (AhR) signaling via the activation of protein tyrosine kinases (PTKs).

2. Materials and methods

2.1. Materials

Δ^9-THC, CBD, and CBN were isolated from cannabis leaves using a previously reported method [17]. CBD-2′-monomethyl ether (CBDM) and CBD-2′,6′-dimethyl ether (CBDD) were prepared as described previously [18]. The purities of these cannabinoids were determined to be above 97% by gas chromatography, except for CBDD, the purity of which was 93% [19]. Other chemicals and materials were obtained from the following sources: olivetol, *d*-limonene, and an anti-actin (20–33) antibody produced in rabbits from Sigma-Aldrich (St. Louis, MO); 3-methylcholanthrene (3-MC) and omeprazole from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); herbimycin A from Funakoshi (Tokyo, Japan); a rabbit polyclonal antibody against human AhR (H-211) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other chemicals and solvents used were of the highest quality commercially available.

2.2. Cell culture and drug treatments

The human hepatoma HepG2 cell line was obtained from the RIKEN cell bank (Tsukuba, Japan). These cells were maintained in minimum essential medium Eagle containing nonessential amino acids (Sigma-Aldrich) supplemented with 10% fetal bovine serum (BioWest, Nuaillé, France) and penicillin/streptomycin (Gibco, Grand Island, NY) in a humidified atmosphere containing 5% CO_2 at 37 °C. Cultures of approximately 80% confluence in a 100-mm culture dish were used to seed for the following experiments. Cells were seeded into a 6-well plate (1×10^6 cells/well) and 35-mm culture dish (0.2×10^6 cells/dish) and cultured for 24 h. Cells were then treated with phytocannabinoids, CBD-related compounds, 3-MC, or omeprazole in serum-free medium for up to 12 h. Inhibition experiments were performed as described below. Cells were pretreated with herbimycin A for 12 h before treatment with the test compounds including CBD. Phytocannabinoids and other test chemicals were prepared in ethanol and/or dimethyl sulfoxide (DMSO). Control incubations had equivalent additions of ethanol and/or DMSO. Ethanol and/or DMSO did not markedly influence cell viability at the final volume used.

2.3. RNA interference

Reverse transfections were carried out in 35-mm culture dishes (0.2×10^6 cells/dish) using *Silencer*® Select Validated small interfering RNAs (siRNAs) for human AhR (ID# s1199 and s1200), *Silencer*® Negative Control #1 siRNA, and siPORT™ *NeoFX*™ transfection agent (Ambion, Austin, TX) according to the manufacturer's instructions. Forty-eight hours after transfection with siRNA, HepG2 cells were treated with CBD and 3-MC for 6 and 3 h, respectively.

2.4. RNA analysis

Total RNA was extracted from HepG2 cells using ISOGEN reagent (Nippon Gene, Toyama, Japan). Reverse transcriptase reactions were performed with the SuperScript™ III First-strand Synthesis SuperMix for qRT-PCR (Invitrogen, Carlsbad, CA). A quantitative real-time polymerase chain reaction (qPCR) was conducted using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA) with Platinum® SYBR® Green qPCR SuperMix-UDG qPCR (Invitrogen) according to the manufacturer's instructions. The primers used for mRNA measurements were as follows: CYP1A1, 5′-GTCATCTGTGCCATTTGCTTTG-3′ and 5′-CAACCACCTCCCCGAAATTATT-3′; AhR, 5′-TGGACAAGGAATTGAAGAAGC-3′ and 5′-AAAGGAGAGTTTTCTGGAGGAA-3′. Values were quantified by the comparative Ct method, and samples were normalized to β-actin; 5′-ATTGCCGACAGGATGCAGA-3′ and 5′-GCTCAGGAGGAGCAATGATCTT-3′.

2.5. Western blot analysis

Whole cell lysates were prepared from HepG2 cells 48 h after transfection with siRNA as described previously [20]. SDS-PAGE was performed using 7.5% acrylamide gels as described previously [21]. Total cellular protein (10 μg protein) was separated and transferred onto a PVDF membrane. Immunodetection was performed using anti-human AhR and anti-actin antibodies as primary antibodies and horseradish peroxidase-conjugated anti-rabbit immunoglobulin (Amersham Biosciences Inc., Piscataway, NJ) as a secondary antibody. Conjugated horseradish peroxidase was detected using Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA), and bands were scanned with ChemiDoc XRS (Bio-Rad Laboratories, Richmond, CA).