Cross-Reactivity of Various Tetrahydrocannabinol Metabolites with a Monoclonal Antibody against Tetrahydrocannabinolic Acid

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A monoclonal antibody (MAb-4A4) against Δ^9 -tetrahydrocannabinolic acid (THCA) was prepared and its cross-reactivity analyzed with various cannabinoids including tetrahydrocannabinol metabolites by competitive enzyme-linked immunoassay (ELISA). Δ^9 -THC (1–100 $\mu\text{g/ml}$) could be determined by the ELISA. Many metabolites of Δ^8 -THC and Δ^9 -THC reacted with the antibody, and their cross-reactivities were 44–157% of Δ^8 -THC. However, the antibody did not recognize the lipophilic compounds cholesterol, testosterone, β -carotene, androstene-3,17-dione or an endogenous cannabinoid, anandamide.

Key words — cannabinoid, monoclonal antibody, cross-reactivity, tetrahydrocannabinol metabolite

INTRODUCTION

Tetrahydrocannabinol (THC), a psychoactive constituent of marijuana, is known to be extensively metabolized in humans.¹⁾ It is important to develop simple and specific methods for detection of cannabinoid metabolites together with natural cannabinoids in biological samples and marijuana preparations for forensic sciences and drug abuse programs. The analytical methods thin-layer chromatography (TLC),²⁾ gas chromatography (GC),³⁾ gas

chromatography-mass spectrometry (GC-MS)^{4,5)} and high-performance liquid chromatography⁶⁾ have been applied to detect THC metabolites in biological samples. Immunological methods have also been developed for detection of cannabinoid metabolites.^{7,8)} However, relatively limited data are available with respect to studies on a monoclonal antibody against THC.

The present communication describes the cross-reactivity of a monoclonal antibody (MAb-4A4)⁹⁾ with various THC metabolites.

MATERIALS AND METHODS

Cannabinoids and Other Chemicals — Δ^9 -THC, cannabidiol (CBD) and cannabinol (CBN) were isolated and purified from cannabis leaves by the method of Aramaki *et al.*¹⁰⁾ Δ^8 -THC,¹¹⁾ 11-hydroxy- Δ^8 -THC (11-OH- Δ^8 -THC),¹²⁾ 11-oxo- Δ^8 -THC,¹²⁾ 7-oxo- Δ^8 -THC,¹³⁾ 7 α -OH- Δ^8 -THC,¹³⁾ 7 β -OH- Δ^8 -THC,¹³⁾ 8 α ,9 α -epoxyhexahydrocannabinol (8 α ,9 α -EHHC),¹⁴⁾ 8 β ,9 β -EHHC,¹⁴⁾ 4'-OH- Δ^8 -THC,¹⁵⁾ Δ^8 -THC-11-oic acid,¹⁶⁾ 11-OH- Δ^9 -THC,¹⁷⁾ 11-oxo- Δ^9 -THC,¹⁷⁾ 8-oxo- Δ^9 -THC,¹⁷⁾ 8 α -OH- Δ^9 -THC,¹⁷⁾ 8 β -OH- Δ^9 -THC,¹⁷⁾ and Δ^9 -THC-11-oic acid¹⁷⁾ were prepared by the methods described previously. The purities of these cannabinoids were determined to be at least 95% by gas chromatography. Anandamide was synthesized by the method of Ueda *et al.*¹⁸⁾ 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and testosterone were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Peroxidase-conjugated goat IgG fraction to mouse IgG was purchased from ICN Pharm. Inc.-Cappel Products (Costa Mesa, CA, U.S.A.). Other chemicals used were obtained from Wako Pure Chem. Ind. (Osaka, Japan).

Enzyme-Linked Immunoassay (ELISA) — MAb-4A4 against Δ^9 -THCA was prepared by fusing hybridoma with mouse splenocytes as described previously.⁹⁾ Competitive ELISA was carried out basically by the previous method.⁹⁾ Δ^9 -THCA- β -alanine BSA conjugate (50–250 ng) was adsorbed on the wells of a 96 well-immunoplate (Nunc-Immuno Plate Maxi SorpTM, Roskilde, Denmark). After washing 3 times with 0.2 ml of 100 mM phosphate buffer (pH 7.4) containing 0.05% Tween 20, each well was treated with 0.3 ml of 100 mM phosphate buffer (pH 7.4) containing 0.2% gelatin for blocking. For competition, 0.025 ml of various concentrations (1–100 $\mu\text{g/ml}$) of cannabinoids and other compounds

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Table 1. Reactivity of MAb-4A4 against Δ^9 -THCA- β -Alanine BSA-Conjugate Analyzed by ELISA Using Peroxidase

Δ^9 -THCA- β -alanine-BSA (ng/well)	Absorbance at 405 nm				
	MAb-4A4 concentration (μ g/well)				
	2.6	1.3	0.7	0.4	0.2
50	0.312	0.303	0.251	0.246	0.182
125	0.539	0.372	0.260	0.197	0.179
250	0.864	0.606	0.404	0.197	0.169

The data are mean of duplicate incubations.

dissolved in 20% aqueous methanol were incubated with 0.025 ml of IgG solution. After washing 3 times with 0.3 ml of 100 mM phosphate buffer (pH 7.4) containing 0.05% Tween 20, MAb-4A4 was reacted with peroxidase-labeled anti-mouse IgG for 1 h. After washing the plate 3 times with 0.2 ml of 100 mM phosphate buffer (pH 7.4) containing 0.05% Tween 20, peroxidase activity was measured with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt as a substrate. The absorbance was measured by a microplate reader (Immuno Mini NJ-2300) at 405 nm. The cross-reactivity was represented as % of the reaction with Δ^8 -THC.

RESULTS AND DISCUSSION

Table 1 summarizes the reactivity of MAb-4A4 against Δ^9 -THCA- β -alanine BSA conjugate under the conditions described in Materials and Methods. The data indicated that MAb-4A4 concentration dependently reacted with Δ^9 -THCA- β -alanine BSA conjugate. Thus, peroxidase activity measured at 405 nm was increased with concentrations of MAb-4A4 and Δ^9 -THCA- β -alanine BSA conjugate. The concentrations of MAb-4A4 and Δ^9 -THCA- β -alanine BSA conjugate used for a typical ELISA were 2 μ g/well and 250 ng/well, respectively, in the present study. Under these conditions, Δ^9 -THC, CBD and CBN (1–100 μ g/ml) were determined by linear calibration curves as shown in Fig. 1. Among the three cannabinoids, CBD showed the highest reactivity with the antibody, and followed by CBN and Δ^9 -THC.

The present study demonstrated that MAb-4A4 cross-reacted with various THC metabolites together with natural cannabinoids (Table 2). Most of the THC metabolites used here were hydroxylated on the terpene moiety. A little stereospecificity was observed in the reaction since β -isomers of 7-hydroxy- Δ^8 -THC and 8-hydroxy- Δ^9 -THC showed relatively higher reactivity than those of α -isomers. No

Fig. 1. Calibration Curves of Δ^9 -THC, CBD and CBN

Various concentrations of cannabinoids (1–100 μ g/ml) were incubated with MAb-4A4 (2 μ g/ml) in a precoated plate with Δ^9 -THCA-BSA conjugate (250 ng/well). The reaction mixture was analyzed as described in Materials and Methods. Each point represents the mean of duplicate determinations. $\circ$ - $\circ$, Δ^9 -THC; $\diamond$ - $\diamond$, CBD; $\triangle$ - $\triangle$, CBN

significant differences in the reactivity with the antibody were observed between Δ^8 -THC metabolites and the corresponding Δ^9 -THC metabolites. The reactivity of MAb-4A4 was specific for cannabinoids, and the antibody did not react significantly with the endogenous lipophilic compounds cholesterol, testosterone, β -carotene or androstene-3,17-dione or with flavonoids in plants as described previously.⁹⁾ Anandamide, an endogenous ligand for cannabinoid receptor,¹⁹⁾ had little reactivity to MAb-4A4. The previous study demonstrated that olivetol, which corresponds to phenolic moiety of THC, had poor reactivity with the antibody.

These results suggest that MAb-4A4 recognized both the terpene and phenolic moieties of cannabinoids, although the antibody could not exactly distinguish exactly the structures of the THC metabolites.

In conclusion, Mab4A4 is a useful probe for analysis of cannabinoids and their metabolites in biological fluid and in marihuana samples.

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Table 2. Cross-Reactivity of MAb-4A4 against Cannabinoids and Other Compounds

Compound	(%)	Compound	(%)
Δ^8 -THC	(1) 100	Δ^9 -THC	(2) 147
11-OH- Δ^8 -THC	(3) 132	11-OH- Δ^9 -THC	(4) 157
11-Oxo- Δ^8 -THC	(5) 85	11-Oxo- Δ^9 -THC	(6) 141
Δ^8 -THC-11-oic acid	(7) 79	Δ^9 -THC-11-oic acid	(8) 149
7 α -OH- Δ^8 -THC	(9) 44	8 α -OH- Δ^9 -THC	(10) 60
7 β -OH- Δ^8 -THC	(11) 77	8 β -OH- Δ^9 -THC	(12) 104
7-Oxo- Δ^8 -THC	(13) 119	8-Oxo- Δ^9 -THC	(14) 113
8 α ,9 α -EHHc	(15) 84	CBN	144
8 β ,9 β -EHHc	(16) 74	CBD	199
4'-OH- Δ^8 -THC	(17) 87	Testosterone	3
Anandamide	5	Cholesterol	9
β -Carotene	0	Androstene-3,17-dione	3

The data represented are the mean of duplicate determinations.

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