Comparison of Pharmacological Effects of Tetrahydrocannabinols and Their 11-Hydroxy-Metabolites in Mice

Kazuhito WATANABE,[a] Takako KIJIMA,[a] Shizuo NARIMATSU,[a] Jun NISHIKAMI,[a] Ikuo YAMAMOTO,*,[a] and Hidetoshi YOSHIMURA[b]

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University,[a] 3-Ho Kanagawa-machi, Kanazawa 920–11, Japan and Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University,[b] 3–1–1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received February 16, 1990*

Pharmacological effects (catalepsy, hypothermia, pentobarbital-induced sleep prolongation, anticonvulsant and analgesic effects) of Δ^8- and Δ^9-tetrahydrocannabinols, and their 11-hydroxy-metabolites were evaluated and compared in mice. Δ^9-Tetrahydrocannabinol and 11-hydroxy-Δ^9-tetrahydrocannabinol exhibited somewhat greater effects than did Δ^8-tetrahydrocannabinol and 11-hydroxy-Δ^8-tetrahydrocannabinol, respectively, in all pharmacological indices tested. Greater effects of 11-hydroxy-metabolites than those of tetrahydrocannabinols were also demonstrated.

Keywords analgesia; anticonvulsant effect; catalepsy; 11-hydroxy-Δ^8-tetrahydrocannabinol; 11-hydroxy-Δ^9-tetrahydrocannabinol; hypothermia; pentobarbital-induced sleep; Δ^8-tetrahydrocannabinol; Δ^9-tetrahydrocannabinol

A great number of studies have been made to learn the pharmacological effects of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC), both of which are psychoactive components of marihuana.[1,2] Δ^8-THC is a minor component,[3,4] but more stable than Δ^9-THC. Δ^8- and Δ^9-THC are oxidized at the 11-position in the hepatic microsomes of mammals, including humans, to form pharmacologically active 11-hydroxy(11-OH)-metabolites.[5–7] In understanding the pharmacological effects of marihuana, it is of great importance to know the relative pharmacological activities of both isomers of the THCs and their 11-OH-metabolites. Several laboratories have reported the comparative pharmacological effects of both isomers of the THCs and/or their 11-OH-metabolites using limited pharmacological indices unsystematically.[8–12] The reported findings indicate that the relative pharmacological effects of the cannabinoids are dependent on the experimental conditions used. The present investigation was undertaken to evaluate the comparative pharmacological effects in mice of Δ^8- and Δ^9-THC, and their 11-OH-metabolites using catalepsy, hypothermia, pentobarbital-induced sleep prolongation, anticonvulsant and analgesic effects as indices.

Results and Discussion

ED_{50} values (mg/kg, i.v.) in mice for the cataleptogenic effect of Δ^8-THC, Δ^9-THC and 11-OH-Δ^8-THC have been reported to be 3.3 (1.9—5.6),[13] 2.6 (1.6—4.3)[14] and 0.66 (0.33—1.32),[15] respectively. The present study demonstrated that ED_{50} for the cataleptogenic effect of 11-OH-Δ^9-THC was 0.46 (0.30—0.71) mg/kg, i.v. The result indicates that 11-OH-Δ^9-THC is a little more potent than 11-OH-Δ^8-THC, and an active metabolite of Δ^9-THC to induce catalepsy in mice.

Table I summarizes ED_{50} values and their 95% confidence limits for the analgesic effects of the cannabinoids which were estimated by the acetic acid-induced writhing method. It is evident from Table I that Δ^8- and Δ^9-THC possess almost the same potency whereas 11-OH-Δ^9-THC is a little more active than 11-OH-Δ^8-THC, and that 11-OH-Δ^8-THC and 11-OH-Δ^9-THC are 8 and 10 times more active than Δ^8-THC and Δ^9-THC, respectively. The present results are comparable to those of Wilson and May[10] that 11-OH-Δ^8-THC and 11-OH-Δ^9-THC were 5 times more active than both isomers of the THCs in the analgesic effect measured by the hot plate method. These results suggest that 11-OH-metabolites may be active principles in the analgesic effect of THCs in mice.

The hypothermia produced by THCs and their 11-OH-metabolites are presented in Table II. All cannabinoids tested produced significant hypothermia dose-dependently. 11-OH-Metabolites showed a greater effect than did THCs at a dose of 5 mg/kg, i.v. Haavik and Hardman[16] reported that a lower dose (<4 mg/kg, i.v.) of 11-OH-Δ^9-THC exhibited a greater hypothermic effect than that of Δ^9-THC, although the effect of 11-OH-Δ^9-THC at a higher dose (32 mg/kg, i.v.) was less than that of Δ^9-THC. We previously reported that the hypothermic effect of

TABLE I. Analgesic Effects of THCs and Their 11-OH-Metabolites in Mice

Cannabinoids	ED_{50} (mg/kg, i.v.)
Δ^8-THC	1.20 (0.63—2.28)[a]
Δ^9-THC	1.05 (0.55—2.00)
11-OH-Δ^8-THC	0.15 (0.12—0.19)
11-OH-Δ^9-THC	0.10 (0.06—0.15)

a) 95% confidence limits.

TABLE II. Hypothermic Effects of THCs and Their 11-OH-Metabolites in Mice

Treatments	Dose (mg/kg, i.v.)	Change in body temperature (°C)
Control (vehicle)	—	+0.01±0.04
Δ^8-THC	5	−1.54±0.17[a]
	10	−2.45±0.24[a]
Δ^9-THC	5	−1.71±0.16[a]
	10	−2.79±0.13[a]
11-OH-Δ^8-THC	1	−1.72±0.22[a]
	5	−2.84±0.14[a]
11-OH-Δ^9-THC	1	−1.93±0.15[a]
	5	−2.60±0.12[a]

Change in body temperature (mean±S.E.) is based on difference in body temperature of 10 mice just before and 30 min after the injection of cannabinoids or the vehicle. *a*) Significantly different from control ($p<0.01$).

11-OH-Δ^8-THC was greater than that of Δ^8-THC at a dose of 5 mg/kg, i.v.[15] The effects of 11-OH-metabolites at a dose of 1 mg/kg, i.v. were almost the same magnitude as those of Δ^8- and Δ^9-THC at a dose of 5 mg/kg, i.v., although there was no significant difference in the effect between the two isomers of these cannabinoids. A greater effect in the hypothermia produced by 11-OH-Δ^9-THC rather than by Δ^9-THC may cause higher susceptibility in the tolerance development by this metabolite as is the case described previously in Δ^8-THC.[17]

The effects of cannabinoids on pentobarbital-induced sleeping time are shown in Fig. 1. Mean sleeping time in the control mice given a 50 mg/kg, i.p. dose of pentobarbital was 60±6 min. At a dose of 1 mg/kg, i.v., all cannabinoids except for Δ^8-THC significantly prolonged the sleeping time. The prolongation with Δ^9-THC (1.9-fold) was significantly longer than that with Δ^8-THC (1.3-fold), and those with 11-OH-Δ^8-THC and 11-OH-Δ^9-THC were 2.7 and 3.4-fold, respectively. At a dose of 5 mg/kg, i.v., the prolongation with THCs and their 11-OH-metabolites were increased a little to 2.9 (Δ^8-THC), 3.3 (Δ^9-THC), 4.6 (11-OH-Δ^8-THC) and 4.7-fold (11-OH-Δ^9-THC). We previously reported that 11-OH-Δ^8-THC had higher activity than Δ^8-THC in a prolonging effect on pentobarbital-induced sleeping time.[15] The present study demonstrated that 11-OH-Δ^9-THC is also more active than Δ^9-THC for prolonging pentobarbital-induced sleep.

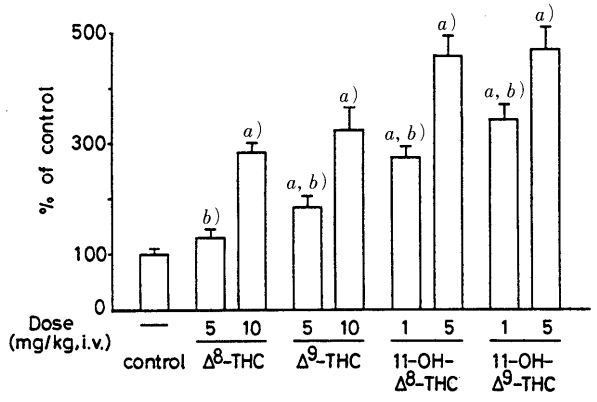

Fig. 1. Effects of THCs and Their 11-OH-Metabolites on Pentobarbital-Induced Sleeping Time

Cannabinoids were administered 20 min prior to the injection of pentobarbital (50 mg/kg, i.p.). Each bar represents the mean±S.E. of 10 mice as % of control. The mean sleeping time in control mice was 60±6 min. *a*) Significantly different from control ($p<0.01$). *b*) Significantly different from the corresponding isomer ($p<0.05$).

TABLE III. Anticonvulsant Effects of THCs and Their 11-OH-Metabolites against PTZ-Induced Seizures

Treatments	Dose (mg/kg, i.v.)	(N)[a]	Latent period (s±S.E.)	
			Clonic seizures	Tonic seizures
Control	—	(15)	103± 7	426± 8
Δ^8-THC	5	(10)	128±28	559± 5[b]
	10	(10)	126±12	546± 52[b]
Δ^9-THC	5	(13)	154±23	610± 57[b]
	10	(13)	137±12[b]	617± 59[b]
11-OH-Δ^8-THC	1	(10)	227±64	652± 32[c]
	5	(10)	186±40	690± 81[c]
11-OH-Δ^9-THC	1	(10)	259±62[b]	617± 88[b]
	5	(10)	337±85[b]	943±122[c]

a) Number in parenthesis indicates number of animals used. *b*) Significantly different from control ($p<0.05$). *c*) Significantly different from control ($p<0.01$).

An anticonvulsant effect of Δ^8-THC and 11-OH-Δ^8-THC has been reported.[18] The cannabinoids prolonged the latent periods for both clonic and tonic seizures induced by pentylenetetrazol (PTZ), although the cannabinoids did not block the seizures completely. The present study confirmed the previous finding that Δ^8-THC and 11-OH-Δ^8-THC (5 mg/kg, i.v.) significantly prolonged the latency for PTZ-induced seizures. Moreover, Δ^9-isomers of THC and the metabolite exhibited a prolonging effect on the latency for PTZ-induced seizures. The strongest effect of 11-OH-Δ^9-THC was also demonstrated among the cannabinoids tested. (Table III)

The present study demonstrates that 11-OH-metabolites of THCs are more potent than THCs in all pharmacological indices tested, especially in cataleptogenic and analgesic effects (5 to 10 times more active). The results also indicate that Δ^9-THC and its 11-OH-metabolite are slightly more active than the corresponding Δ^8-isomers, and preferable to Δ^8-THC and its 11-OH-metabolite for experimental use.

Experimental

Preparation of Cannabinoids Δ^9-THC was purified from cannabis leaves by the method of Aramaki *et al.*[19] Δ^8-THC was prepared by the isomerization of Δ^9-THC.[20] 11-OH-Δ^8-THC and 11-OH-Δ^9-THC were synthesized by the methods of Inayama *et al.*[21] and Pitt *et al.*,[22] respectively. The structures of 11-OH-THCs were confirmed by their mass (MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectra. 11-OH-Δ^8-THC; MS *m/z*: 330 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (t, 3H, 5'-CH_3), 1.06, 1.37 (s, 3H×2, *gem*-CH_3), 3.48 (dd, 1H, C_{10a}-H), 4.06 (s, 2H, C_9-CH_2OH), 5.72 (m, 1H, C_8-H), 6.14, 6.28 (s, 1H×2, aromatic-H). 11-OH-Δ^9-THC; MS *m/z*: 330 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (t, 3H, 5'-CH_3), 1.06, 1.37 (s, 3H×2, *gem*-CH_3), 3.20 (d, 1H, C_{10a}-H), 3.98 (s, 2H, C_9-CH_2OH), 6.74 (m, 1H, C_{10}-H), 6.12, 6.26 (s, 1H×2, aromatic-H). The purities of these cannabinoids were determined to be more than 95% by gas chromatography.

Animals and Drugs Male ddN mice (20—25 g of body weight) were used in the following pharmacological experiments. Cannabinoids were suspended in saline containing 1% Tween 80 and injected i.v. through the tail vein of mice (10 ml/kg body weight). Sodium pentobarbital and PTZ were purchased from Tokyo Kasei Kogyo Co., Ltd. and Mallinckrodt Chem. Works, respectively, and dissolved in saline. All animal experiments were carried out in an ambient temperature of 22—24 °C.

Catalepsy Four groups of 8 mice were injected with 11-OH-Δ^9-THC (0.3, 0.5, 0.8 and 1.0 mg/kg, i.v.). The cataleptogenic effect of the cannabinoids was assessed 15 to 20 min after the injections by a simple bar test previously described.[13]

Analgesia Each group of 6 mice was injected with Δ^8-THC, Δ^9-THC, 11-OH-Δ^8-THC or 11-OH-Δ^9-THC (0.1 to 3.0 mg/kg, i.v.). The analgesic effect was assessed by the blockade of of acetic acid (60 mg/kg, i.p.)-induced writhing previously described.[23]

Hypothermia Δ^8-THC, Δ^9-THC, 11-OH-Δ^8-THC or 11-OH-Δ^9-THC (1, 5 or 10 mg/kg, i.v.) were injected into each group of 10 mice. The control mice received the vehicle only. Maximal hypothermia produced by Δ^8-THC and 11-OH-Δ^8-THC was reported to be induced around 30 min after the i.v. injection of the cannabinoids.[15,17] The rectal temperature of mice was therefore measured just before and at 30 min after the injection by a thermistor thermometer (Natsume Seisakusho Co., Ltd.). The mean initial body temperature in the mice of each group was 38.03 to 38.53 °C.

Pentobarbital-Induced Sleep Prolongation Each group of 10 mice was injected with Δ^8-THC, Δ^9-THC, 11-OH-Δ^8-THC or 11-OH-Δ^9-THC (1 or 5 mg/kg, i.v.) or the vehicle. Sodium pentobarbital (50 mg/kg, i.p.) was injected 20 min after the injection of the cannabinoids or the vehicle. The loss of righting reflex was used as an index of sleep.

Anticonvulsant Effect Against PTZ-Induced Seizures Each group of 10 to 15 mice was used. PTZ (120 mg/kg, s.c.) was injected in the mice 20 min after the i.v. injection of the cannabinoids (1, 5 or 10 mg/kg) or the vehicle. The latency for clonic and tonic seizures was recorded.[18]

Statistical Analyses ED_{50} values with 95% confidence limits were

calculated by the method of Litchfield and Wilcoxon.[24] The statistical significance of difference was determined by Student's *t*-test.

Acknowledgments We thank Prof. I. Nishioka and Assoc. Prof. Y. Shoyama, Faculty of Pharmaceutical Sciences, Kyushu University, for their generous gift of cannabis leaves from which THCs and their 11-OH-metabolites were prepared.

References

1) R. K. Razdan, *Pharmacol. Rev.*, **38**, 75 (1986).
2) H. Edery, Y. Grunfeld, Z. Ben-Zvi and R. Mechoulam, *Ann. N.Y. Acad. Sci.*, **191**, 40 (1971).
3) R. L. Hively, W. A. Mosher and F. W. Hoffman, *J. Am. Chem. Soc.*, **88**, 1832 (1966).
4) B. I. Field and R. R. Arndt, *J. Pharm. Pharmacol.*, **32**, 21 (1980).
5) I. M. Nilsson, S. Agurell, J. L. G. Nilsson, A. Ohlsson, F. Sandberg and M. Wahlqvist, *Science*, **168**, 1228 (1970).
6) R. L. Foltz, A. F. Fentiman, E. G. Leighty, J. L. Walter, H. R. Drewes, W. E. Schwartz, T. F. Page and E. B. Truitt, *Science*, **170**, 1320 (1970).
7) M. E. Wall, D. R. Brine, B. A. Brine, C. G. Pitt, R. I. Freudenthal and H. D. Christensen, *J. Am. Chem. Soc.*, **92**, 3466 (1970).
8) Y. Grunfeld and H. Edery, *Psychopharmacologia*, **14**, 200 (1969).
9) H. D. Christensen, R. I. Freudenthal, G. T. Gidley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. Pitt and M. E. Wall, *Science*, **172**, 165 (1971).
10) R. S. Wilson and E. L. May, *J. Med. Chem.*, **18**, 700 (1975).
11) P. Consroe, A. R. Martin and B. S. Fish, *J. Med. Chem.*, **25**, 596 (1982).
12) L. E. Hollister, *Pharmacology*, **11**, 3 (1974).
13) H. Yoshimura, K. Watanabe, K. Oguri, M. Fujiwara and S. Ueki, *J. Med. Chem.*, **21**, 1079 (1978).
14) S. Narimatsu, I. Yamamoto, K. Watanabe and H. Yoshimura, *J. Pharmacobio-Dyn.*, **6**, 558 (1983).
15) K. Watanabe, I. Yamamoto, K. Oguri and H. Yoshimura, *Eur. J. Pharmacol.*, **63**, 1 (1980).
16) C. O. Haavik and F. Hardman, *Life Sci.*, **13**, 1771 (1973).
17) I. Yamamoto, K. Watanabe, S. Narimatsu and H. Yoshimura, *Eur. J. Pharmacol.*, **111**, 159 (1985).
18) I. Yamamoto, K. Watanabe, K. Oguri and H. Yoshimura, *Res. Commun. Subst. Abuse*, **1**, 287 (1980).
19) H. Aramaki, N. Tomiyasu, H. Yoshimura and H. Tsukamoto, *Chem. Pharm. Bull.*, **16**, 822 (1968).
20) Y. Gaoni and R. Mechoulam, *Tetrahedron*, **22**, 1481 (1966).
21) S. Inayama, A. Sawa and E. Hosoya, *Chem. Pharm. Bull.*, **22**, 1519 (1974).
22) C. G. Pitt, M. S. Fowler, S. Sathe, S. C. Srivastava and D. L. Williams, *J. Am. Chem. Soc.*, **97**, 3798 (1975).
23) K. Watanabe, F. Tanabe, T. Tanaka, I. Yamamoto and H. Yoshimura, *Res. Commun. Subst. Abuse*, in press (1990).
24) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).