

1',1'-Dimethylheptyl- Δ -8-tetrahydrocannabinol-11-oic Acid: A Novel, Orally Effective Cannabinoid with Analgesic and Anti-inflammatory Properties¹

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ABSTRACT

1',1'-Dimethylheptyl- Δ -8-tetrahydrocannabinol-11-oic acid (CT-3) is a novel cannabinoid that is under development by Atlantic Pharmaceuticals as an anti-inflammatory and analgesic drug. The objective of the study was to investigate the effects of CT-3 on overt symptom complex (Irwin's test), nociception, gastrointestinal (GI) ulceration, and pharmacological availability after intragastric (i.g.) and intraperitoneal (i.p.) administration. Analgesic studies were assessed in the hot-plate (55°C) and the tail clip tests in mice and in the tail clip test in rats. In addition, pharmacological interaction of CT-3 with the solvent dimethyl sulfoxide (DMSO) was investigated in rats. In mice, CT-3 decreased spontaneous motor activity and induced dose-dependent, analgesic activity in the tail clip and hot-plate tests, with potency similar to morphine sulfate after i.g. and i.p. adminis-

tration. However CT-3 showed more prolonged duration of analgesic action than morphine. In rats, CT-3 showed marked analgesia in the tail clip test and had similar i.p. and i.g. median effective dose (ED₅₀ values; 5 mg/kg). CT-3 was devoid of GI ulceration when administered with DMSO either acutely at doses below 100 mg/kg or chronically at a dosage of 30 mg/kg/day for 5 days. In contrast, indomethacin induced GI ulceration and deaths. The concurrent use of DMSO with CT-3 decreased its analgesic action, increased its adverse central nervous system effects, and induced GI ulceration. The evidence indicates that CT-3 exhibits a large dissociation between its anti-inflammatory/analgesic effects and its ulcerogenic actions. CT-3 warrants clinical development as a novel anti-inflammatory and analgesic drug.

1',1'-Dimethylheptyl- Δ -8-tetrahydrocannabinol-11-oic acid (CT-3) is a novel cannabinoid with analgesic and anti-inflammatory activities (Burstein et al., 1992). CT-3 is chemically similar to Δ -9-tetrahydrocannabinol (THC, dronabilone) and nabilone (Eli Lilly, Indianapolis, IN), differing only in the substitutions made on C3 and C9 positions (Fig. 1). The analgesic activity of CT-3 was demonstrated in the mouse hot-plate (48°C) and phenylquinone writhing tests when administered in various oil-based vehicles (Burstein et al., 1998) or with the solvent dimethyl sulfoxide (DMSO) (unpublished study; Atlantic Pharmaceuticals, Raleigh, NC). The anti-inflammatory effects of CT-3 were well established in the mouse air pouch and the rat adjuvant arthritis tests with an approximate intragastric (i.g.) ED₅₀ value of 0.1 mg/kg (Zurier et al., 1998).

The possibility that unpredictable bioavailability could have occurred when CT-3 was administered in nonoptimum vehicles precluded defining desirable pharmacological actions (e.g., analgesic and anti-inflammatory activities) from undesirable actions (e.g., ulcerogenic and central nervous system activities), if any. In addition, little is known about high-dose pharmacology, toxicity, pharmacological availability of CT-3 after oral and parenteral administration, or possible interaction with DMSO. To address these issues, the objectives of the present study were: 1) to investigate the analgesic action of CT-3 in the mouse hot-plate and tail clip tests in comparison with the reference standard, morphine sulfate; 2) to investigate the acute effects of CT-3 on symptom complex, analgesia, gastrointestinal (GI) ulcerogenicity, and toxicity in rats when administered in an aqueous suspension that contained DMSO; 3) to investigate the effects of CT-3 administered without DMSO, either orally or intraperitoneally (i.p.), on symptom complex, analgesia, GI ulcerogenicity, and toxicity in rats; and 4) to investigate the chronic (5 days)

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ABBREVIATIONS: CT-3, 1',1'-dimethylheptyl- Δ -8-tetrahydrocannabinol-11-oic acid; i.g., intragastric; i.p., intraperitoneal; THC, Δ -9-tetrahydrocannabinol; DMSO, dimethyl sulfoxide; MC, methylcellulose; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug.

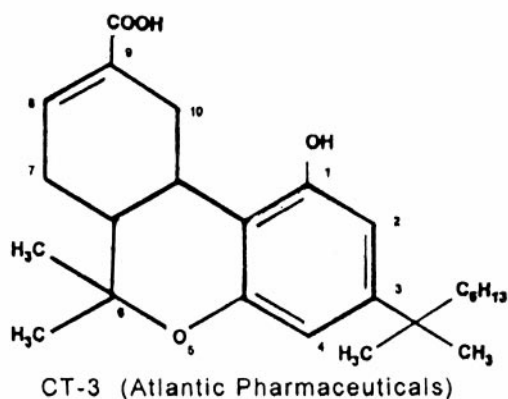
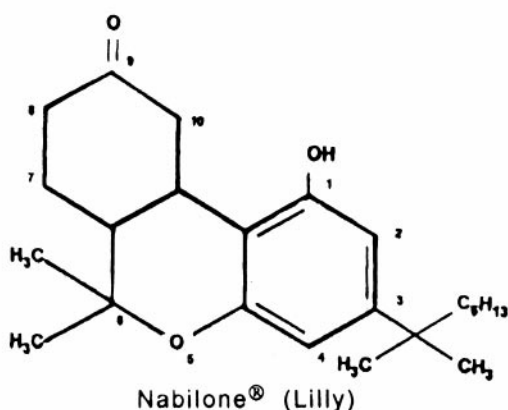
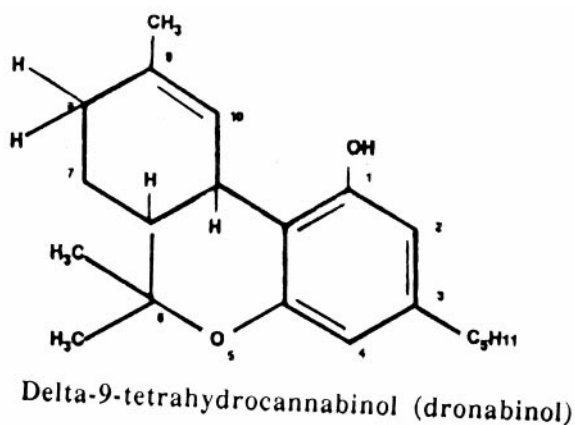


Fig. 1. Chemical structures of Δ -9-THC (dronabinol), nabilone (Eli Lilly), and CT-3 (Atlantic Pharmaceuticals).

GI ulcerogenic effects of CT-3 in comparison with the reference standard indomethacin in rats.

Materials and Methods

Test Animals. Male Wistar rats and male albino mice were obtained from the Charles River Laboratories (Wilmington, MA). The study complied with the protection guaranteed to animals in accordance with the *Guide for the Care of Laboratory Animals* as adopted

and promulgated by the National Institutes of Health and the regulations implementing the Animal Welfare Act, as amended. The animals were allowed a quarantine period of 7 days, and only healthy animals were selected for the studies. The animals were housed in community cages with approved bedding materials. The animals were kept under standard laboratory conditions (approximately 22°C, 12-h dark/light cycle). The animals had free access to standard rodent diet (Purina Chow) and clean drinking water. In the symptom complex studies, the animals were screened for response to pain, which was induced by the placement of an artery clip on the tail as described below. Animals not displaying pain on the placement of the tail clip were rejected.

Test Drugs. CT-3 was obtained from Atlantic Pharmaceuticals, Inc. Indomethacin was purchased from Sigma Chemical Co. (lot 67H1609; St. Louis, MO). Morphine sulfate was purchased from Butler Company (Dublin, OH).

CT-3 is soluble in organic solvents but not in water. Given the lack of solubility of CT-3 in water, two pharmaceutical formulations were studied: 1) the initial formulation was made by dissolving CT-3 in DMSO, and the subsequent solution was diluted with 0.25% methylcellulose (MC) solution, resulting in the formation of a 25% DMSO suspension. An initial pilot study in rats using the Irwin (1964) test (5-day observation), which was conducted with the i.g. administration of a vehicle containing 50 and 75% of DMSO administered at 10 ml/kg, did not show any obvious gross toxicity. Therefore, a vehicle containing only 25% of DMSO was provisionally considered safe for the subsequent investigation of CT-3 on symptom complex, acute toxicity, acute ulcerogenicity, and analgesic effects in rats and mice. This DMSO vehicle was also used for the chronic GI ulcerogenicity study in rats, and 2) second formulation containing 0.25% MC without DMSO was used to investigate symptom complex and acute toxicity of CT-3 after i.g. and i.p. administration in rats. The CT-3 suspensions used in both sets of formulations were always prepared immediately before each experiment. The volume of the test suspension used was 10 ml/kg i.g. or i.p.

Symptom Complex, Analgesia, and Acute Toxicity Studies.

The tests were conducted in accordance to the method of Irwin (1964) and the subsequent Abrams (1964) adaptation as described below. The Irwin test is a comprehensive procedure that makes it possible to quantify and collate, in each animal, a wide variety of grossly observable changes produced by drugs (e.g., behavioral, neurological, autonomic, and toxic). After treatment with the test drugs, the animals are systematically observed and manipulated to measure the onset, peak, duration, character, and intensity of drug action. Thirty-two different observations were assessed on an all-or-none basis. The proper use of this test allows qualitative and quantitative estimation of the selected pharmacological actions of test drugs.

In the mouse studies, only a formulation of CT-3 with DMSO was used. In the rat studies, two formulations of CT-3 suspension, with and without 25% DMSO, were used. The animals were initially screened for response to pain induced by placing an artery clip approximately 1 inch from the base of the tail. The Bianchi and Franceschini (1954) adaptation of the Haffner (1929) method was used for the assessment of analgesia. Animals not displaying pain (by turning or biting the clip within 15 s) were rejected. Healthy adult rats (200–250 g, $n = 3$) and healthy adult mice (18–22 g, $n = 3$) were fasted for 18 h before the test. Groups of rats and mice were administered i.g. or i.p. graded doses of CT-3 (0, 3, 10, 30, 100, 300, and 1000 mg/kg) suspended in 25% DMSO and 0.25% MC solution.

The i.g. and i.p. treated animals were carefully observed for overt behavioral signs in accordance with the details given by Abrams (1964). Observations were recorded during the first 5 h after the treatment and on a daily basis for 5 days. Observations were made on an all-or-none basis to avoid investigator bias and to permit the determination of ED₅₀ values at specified time intervals, whenever possible. On the fifth day, the surviving animals were weighed and sacrificed, and the internal organs were inspected for gross evidence

of GI ulcerations, if any. A 10× stereomicroscope was used for the assessment of GI mucosal ulceration, if any.

Mouse Hot-Plate Test. A modification of the test previously described by Eddy and Leimbach (1953) was used. The apparatus was obtained from IITC Life Sciences Co. (Hot Plate Analgesia Meter, model 39D; Woodland Hills, CA). Each mouse was placed in the center of the hot plate, which was 11 × 11 inches and surrounded by 12-inch-high plexiglas walls. The hot-plate temperature was 55 ± 0.2°C. Mice ($n = 6$) were administered graded doses (0, 3, 5, 10, 20, and 30 mg/kg i.g.) of either CT-3 or morphine sulfate. The reaction time of the mouse to lick a foot or jump was measured at 90, 60, and 30 min before and 30, 60, 90, and 120 min after the administration of CT-3 or the reference standard morphine sulfate. Mice not responding within 15 s on any predrug trial were discarded. For each mouse, the "normal" reaction time was defined as the mean of the three pretreatment times. A positive drug response was defined as a reaction time occurring in one or more post-treatment trials that was greater than twice the normal time. Analgesia was evaluated on an all-or-none basis.

Mouse Tail Clip Test. This analgesic test was based on a modification of Haffner's (1929) method as described by Bianchi and Franceschini (1954). Mice ($n = 6$) were administered graded doses (0, 1, 3, 10, and 30 mg/kg) of either CT-3 or morphine sulfate either i.g. or i.p. A pressure-standardized artery clip was placed approximately 1 inch from the base of tail, and only the mice that responded to the clip placement by turning or biting at the clip within 15 s were used in this test. The presence or absence of analgesia was determined at 1, 3, and 5 h after i.g. or i.p. administration of the test drugs. An analgesic effect was considered positive in each mouse if the mouse reaction time obtained after drug administration was at least double the predrug (control) value. Analgesia was evaluated on an all-or-none basis.

Chronic GI Ulcerogenicity Study in Rats. Healthy adult male rats (240–280 g, $n = 6$), previously fasted for 18 h, received logarithmically graded dosages (0, 3, 10, and 30 mg/kg/day) of either CT-3 or indomethacin. The vehicle used for both drugs contained 25% DMSO in 0.25% MC solution. The test procedure was based on the method described by Young and Yee (1994). The test drugs were administered i.g. twice daily for 4 days and on the morning of day 5. Five hours after the first i.g. dose on day 1, the animals were provided with free access to food and water for the next 4 days. Food and water were withheld from the animals 18 h before the morning dose of day 5. Two hours after the administration of the test drugs on day 5, the animals were sacrificed and posted, and the stomachs and intestines were carefully removed. The stomachs were cut along the greater curvature, gently washed with water, and examined for the presence of ulcers using a 10× stereomicroscope. Ulcer was defined as a break in the GI mucosa with a perceptible depth. Erosion was defined as a superficial break in the mucosa with no depth. The entire intestine was also carefully examined for evidence of ulceration, if any. The incidence of ulceration in each group was recorded, on an all-or-none basis. The median ulcerogenic doses (ED₅₀ values) of CT-3 and indomethacin were determined.

Statistical Analyses. Differences between mean values in body weight gains were evaluated for statistical significance by Student's *t* test at $P < .05$. In the mouse study, the ED₅₀ values were calculated by a regression analysis method described by Dixon and Massey (1969). In the rat studies, the median lethal dose (LD₅₀) and ED₅₀ values were calculated using the quantal methods of either Miller and Tainter (1944) or Litchfield and Wilcoxon (1949). Natural logarithms of doses corresponding with probit equivalence of the percentage of animals manifesting a particular sign or response at each dose were plotted with a Cricket Graph Program using a Power Macintosh 7500 desktop computer. Least-squares linear regression analyses were performed for each parameter. Statistical ($P < .05$) differences between ED₅₀ values, if any, were calculated according to the method of Litchfield and Wilcoxon (1949).

Results

Mouse Studies

Effects of CT-3 Administered i.g. with DMSO on Gross Symptom Complex, Analgesia, Ulcerogenicity, and Acute Toxicity. The i.g. administration of CT-3 induced analgesia and decreased spontaneous motor activity at doses of 3 to 30 mg/kg. Mice receiving the high doses (300 and 1000 mg/kg) of CT-3 exhibited catalepsy and diuresis. None of the mice receiving CT-3 exhibited Straub tail, miosis, loss of righting reflex, or anesthesia. At the end of the 5-day observation period, all mice in the 100 mg/kg group were alive, but their body weight gain was 30% less than that of the control mice. All mice in the 300 and 1000 mg/kg treatment groups died before the end of the 5-day observation period. The i.g. LD₅₀ value of CT-3 was estimated to be 200 mg/kg. Autopsy on day 6 did not reveal any gross pathology of the GI tract.

The i.p. administration of CT-3 induced analgesia and decreased motor activity at doses of 3 to 10 mg/kg. One of three mice assigned to the 10 mg/kg treatment group showed evidence of diuresis (wet abdomen). Mice in the 100 mg/kg treatment group showed hyperexcitability when handled by the investigator. At the end of the 5-day observation period, mice in the 100 mg/kg treatment group showed 30% less body weight than did the control animals. The i.p. LD₅₀ value of CT-3 was estimated to be 136 mg/kg.

Hot-Plate Analgesia Test. CT-3 induced profound analgesia in mice (Table 1). The i.g. analgesic ED₅₀ values for CT-3 and morphine were 6.7 and 6.1 mg/kg, respectively, indicating that CT-3 and morphine have equal analgesic potency in the hot-plate test.

Tail Clip Analgesia Test

Intragastric administration. CT-3 induced marked analgesia in the mouse tail clip test (Table 2). The peak analgesic actions of CT-3 and morphine, as determined from their lowest ED₅₀ values, were similar and occurred at 1 h after treatment. The i.g. analgesic ED₅₀ values for CT-3 and morphine, at 1 h after treatment, were 4.4 and 6.6 mg/kg, respectively, indicating that CT-3 and morphine have comparable analgesic potency in the tail clip test. However, CT-3 appeared to be longer acting than morphine. At 5 h after treatment, the i.g. analgesic ED₅₀ values for CT-3 and morphine were 16.4 and 900 mg/kg, respectively, suggesting that morphine had a much shorter duration of action than CT-3.

Intraperitoneal administration. Both CT-3 and morphine showed similar analgesic ED₅₀ values at 1, 3, and 5 h after treatment, indicating equal parenteral potency in the mouse tail clip test (Table 2). The i.g. and i.p. analgesic ED₅₀ values of CT-3 were similar, suggesting good GI absorption and/or bioavailability for CT-3 in mice.

TABLE 1

Evaluation of analgesic actions of CT-3 and morphine sulfate in mouse hot-plate (55°C) test

Groups of mice ($n = 6$) received graded doses of CT-3 or morphine sulfate i.g. Animals were examined for analgesia on an all-or-none basis using the method of Eddy and Leimbach (1953).

Test Drug	ED ₅₀ Values (95% CL)
	mg/kg i.g.
CT-3	6.7 (4.6–9.7)
Morphine	6.1 (4.8–7.8)

TABLE 2

Evaluation of analgesic actions of CT-3 and morphine sulfate in mouse tail clip test

Groups of mice ($n = 6$) received graded doses of CT-3 or morphine sulfate either i.g. or i.p. Animals were examined for analgesia on an all-or-none basis using the method of Bianchi and Franceschini (1954).

Test Drug	ED ₅₀ Values at Specified Post-treatment Intervals		
	1.0 h	3.0 h	5.0 h
	mg/kg		
Intragastric administration			
CT-3	4.4	11.6	16.4
Morphine	6.6	17.3	900.0
Intraperitoneal administration			
CT-3	5.5	15.4	29.9
Morphine	6.6	13.1	28.9

Studies in Rats

Effects of CT-3, Administered i.g. with DMSO, on Gross Symptom Complex, Analgesia, Ulcerogenicity, and Acute Toxicity. CT-3 administered i.g. at low doses of 3 to 30 mg/kg induced a dose-dependent analgesia and decreased spontaneous motor activity. The analgesic ED₅₀ values, at specified time intervals after CT-3 administration, are displayed in Fig. 2. CT-3 showed potent analgesic activity (ED₅₀ values of about 10 mg/kg), which persisted for longer than 10 h. Unlike the control rats, which always attempted to bite or remove the clip, the CT-3-treated animals responded to the clip placement by only displaying urination and vocalization during the first few seconds; however, the animals had total disregard to the pain inflicted by pinching the tail. The analgesic activity of CT-3 was not associated with Straub tail response, skeletal muscle rigidity, or miosis, all of which are common with narcotic analgesics. At i.g. doses of 100 and 300 mg/kg, ataxia and respiratory depression were noted. At an i.g. dose of 1000 mg/kg, ptosis, tremors, piloerection, and diarrhea were observed, but none of the animals displayed a loss of righting reflex or anesthesia, and all animals survived the treatment. The administration of CT-3 at doses of 100 to 1000 mg/kg showed a statistically significant decrease ($P < .05$) in body weight gains at the end of the 5-day observation period (Table 3). Post-mortem autopsies performed on day 5

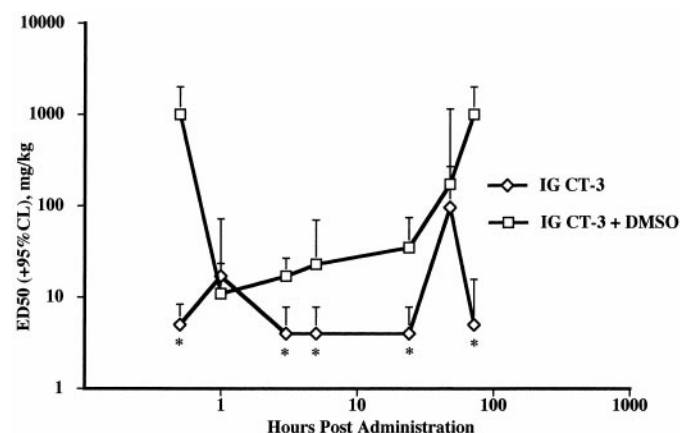


Fig. 2. Investigation of the i.g. analgesic action of CT-3, administered with and without DMSO, in the rat tail clip test. Pharmaceutical formulations of CT-3 were made using 0.25% MC suspension in the presence and absence of the solvent DMSO. Values represent ED₅₀ (95% CL) determined at various time intervals after CT-3 administration. * $P < .05$ with versus without DMSO; Statistically significant difference between the two groups receiving CT-3.

TABLE 3

Effect of acute i.g. administration of CT-3 suspension (25% DMSO and 0.25% MC) on body weight gains in rats

Groups ($n = 3$) of adult male rats were treated with logarithmically graded dosages of CT-3 (0–1000 mg/kg i.g. twice daily) for 5 consecutive days. Values represent means and S.E.M.

CT-3 Group	Start Weight	End Weight	Gain or Loss	P Values versus Control
mg/kg	g			
0	258 (8.3)	263 (5.4)	5.7 (2.9)	
10	255 (7.2)	264 (5.3)	9.0 (2.8)	.164
30	247 (0.4)	246 (2.1)	−1.0 (2.6)	.092
100	248 (3.5)	245 (3.2)	−2.7 (4.0)	.044
300	283 (9.1)	264 (4.2)	−3.7 (1.8)	.023
1000	290 (7.0)	263 (7.2)	−27.0 (0.7)	.002

after the administration of CT-3 with DMSO showed evidence of acute ulcerations in stomach, ileum, and cecum at a dose range of 100 to 1000 mg/kg. The median acute GI ulcerogenic (ED₅₀) values of CT-3 are presented in Table 4.

Effects of CT-3, Administered i.g. without DMSO, on Gross Symptom Complex, Analgesia, Ulcerogenicity, and Acute Toxicity. The i.g. administration of CT-3 in a vehicle not containing DMSO elicited qualitatively similar overt central nervous system effects as those observed with a vehicle containing DMSO. CT-3 displayed potent and long-lasting analgesic action (ED₅₀ = 4 mg/kg) persisting for about 24 h (Fig. 2). The administration of CT-3 at the dose of 1000 mg/kg showed statistically significant ($P < .05$) reduced body weight gains at the end of the 5-day observation period. There were no deaths observed during the 5-day observation period, and none of the animals exhibited any evidence of GI ulcerations.

Effects of CT-3, Administered i.p. without DMSO, on Gross Symptom Complex, Analgesia, Ulcerogenicity, and Acute Toxicity. The i.p. administration of CT-3 resulted in a qualitatively similar overt behavioral effects as those noted after i.g. administration. The lowest analgesic ED₅₀ value for CT-3 was calculated to be 5 mg/kg, which was noted at 4 and 5 h after i.p. or i.g. administration (Fig. 3). Such results suggest that CT-3 is well absorbed after oral administration.

Death occurred in one of three rats administered CT-3 at 300 mg/kg and in all three rats receiving 1000 mg/kg. The i.p. LD₅₀ value was calculated to be 494 mg/kg. None of the animals displayed any evidence of GI ulcerations at necropsy on day 5. No significant changes in body weight gains were noted up to a dose of 300 mg/kg.

Interaction of DMSO with CT-3. As previously stated in *Materials and Methods*, the administration of DMSO vehicle alone to rats showed no overt behavioral or toxicological actions. Therefore, to determine the possible interaction of

TABLE 4

Influence of DMSO vehicle on acute GI ulcerations induced by CT-3 in rats

Organ	CT-3 ED ₅₀		Ratio of ED ₅₀ without DMSO to ED ₅₀ with DMSO ^a
	With DMSO ^a	No DMSO ^b	
	mg/kg (S.E.M.)		
Stomach	173 (40)	>1000	>5.78
Ileum	545 (139)	>1000	>1.83
Colon	746 (301)	>1000	>1.34

^a CT-3 suspension contained 25% DMSO and 0.25% MC.

^b CT-3 suspension contained only 0.25% MC.

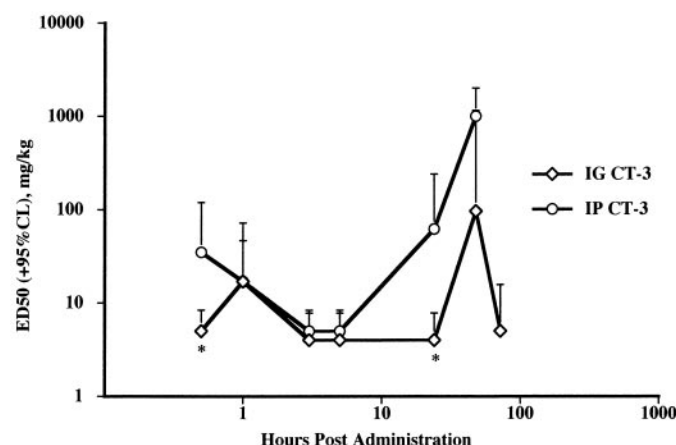


Fig. 3. Investigation of the analgesic action of CT-3, administered without DMSO and administered either i.g. or i.p., in the rat tail clip test. CT-3 was suspended in 0.25% MC solution. Values represent ED₅₀ (95% CL) determined at various time intervals after CT-3 administration. **P* < .05 i.p. versus i.g.; Statistically significant difference between the two groups receiving CT-3.

DMSO with CT-3, we compared selected pharmacological observations obtained in the presence and absence of DMSO. These comparisons allowed the examination of a broad dose range for CT-3 on a desirable pharmacological endpoint such as analgesia and undesirable endpoints (e.g., decreased motor activity, catalepsy, and ulcerogenesis). Figure 2 presents the analgesic effects of CT-3 determined in the presence and absence of DMSO. As may be evident from Fig. 2, the analgesic ED₅₀ values of CT-3 administered without DMSO were slightly lower than ED₅₀ values obtained in the presence of DMSO. However, the differences between these values were statistically significant at several time points after acute oral administration, suggesting that DMSO had reduced the analgesic effects of CT-3.

Figures 4 and 5 present the interaction of CT-3 with DMSO on decreased spontaneous motor activity and respiratory depression. DMSO significantly prolonged the CT-3-induced depressant effects on spontaneous motor activity (Fig. 4) and respiratory depression (Fig. 5). Furthermore, DMSO potentiated and prolonged the cataleptic response induced by CT-3 (Table 5).

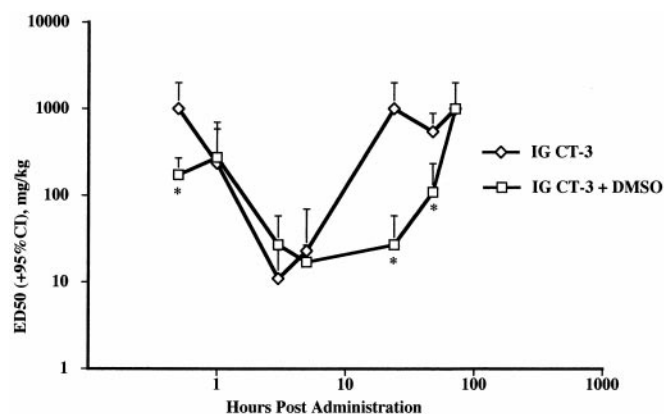


Fig. 4. Effects of CT-3 pharmaceutical formulations on spontaneous motor activity in rats. CT-3 was suspended in 0.25% MC solution with or without DMSO. Values represent ED₅₀ (95% CL) determined at various time intervals after CT-3 administration. **P* < .05 with versus without DMSO; statistically significant difference between the two groups receiving CT-3.

TABLE 5

Influence of DMSO when used as a vehicle on CT-3-induced catalepsy in rats

Interval	CT-3 ED ₅₀		Ratio of ED ₅₀ without DMSO ^b to ED ₅₀ with DMSO ^a
	With DMSO ^a	No DMSO ^b	
	mg/kg (S.E.M.)		
1.0 h	349 (150)	>1000	>2.87
3.0 h	173 (40)	172 (159)	0.99
5.0 h	109 (47)	545 (139)	5.00
1.0 day	349 (150)	>1000	>2.87
2.0 days	142 (63)	>1000	>7.04

^a CT-3 suspension contained 25% DMSO and 0.25% MC.

^b CT-3 suspension contained only 0.25% MC.

As previously noted, CT-3 administered without DMSO did not induce acute GI ulcerogenesis in rats. However, CT-3 administered with DMSO was associated with acute development of GI ulcerations noted in the stomach, ileum, and colon (Table 4). These collective observations indicated that DMSO positively interacted with CT-3.

Effect of Chronic Administration of CT-3 and Indomethacin on GI Ulceration. All dosages of CT-3 were well tolerated and were not associated with any changes in overt behavior. However, the high-dose group (30 mg/kg) was associated with a statistically significant (*P* = .005) decreased body weight gain. Post-mortem necropsy examination of all CT-3 treated animals did not reveal any ulceration in any segments of the GI tract (Table 6).

In contrast, indomethacin administered at the same dosage range as CT-3 induced decreased motor activity, sedation, ptosis, piloerection, diarrhea, and death. In addition, indomethacin significantly (*P* = .01) decreased body weight gain at doses of 10 and 30 mg/kg. Post-mortem examination of all indomethacin-treated animals revealed ulcerations and adhesions throughout the GI tract. As is evident from Table 6, the ulcerogenic ED₅₀ values of indomethacin ranged from 3 to 10 mg/kg in various segments of the GI tract. The indomethacin-induced GI ulceration was also associated with deaths (Table 6). The fact that the ulcerogenic ED₅₀ value of indomethacin was similar to its LD₅₀ value suggests the deaths were a consequence of ulcer-related complications (e.g., perforation and/or bleeding).

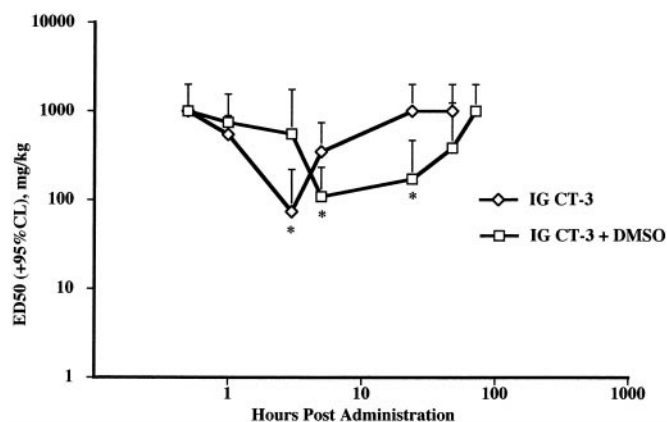


Fig. 5. Effects of CT-3 pharmaceutical formulations on respiratory depression in the rat. CT-3 was suspended in 0.25% MC solution with or without DMSO. Values represent ED₅₀ (95% CL) determined at various time intervals after CT-3 administration. **P* < .05 with versus without DMSO; statistically significant difference between the two groups receiving CT-3.

TABLE 6

Investigation of the chronic ulcerogenic effects of CT-3 and indomethacin in rats

Test drugs CT-3 and indomethacin were administered in a suspension containing 25% DMSO and 0.25% MC solution. Drugs were administered for 5 days.

Parameter	ED ₅₀	
	CT-3	Indomethacin
	<i>mg/kg/day (S.E.M.)^a</i>	
Ulcers		
Corpus	>30.0	7.8 (1.4)
Antrum	>30.0	10.6 (3.4)
Duodenum	>30.0	10.1 (5.5)
Jejunum	>30.0	6.1 (2.5)
Ileum	>30.0	3.1 (1.1)
Colon	>30.0	10.1 (4.8)
Adhesions		
Duodenum	>30.0	10.1 (6.7)
Jejunum	>30.0	4.2 (1.4)
Ileum	>30.0	19.0 (14.5)
LD ₅₀ (S.E.M.)		
Day 5		7.8 (1.8)

^a CT-3 at the highest tested dose of 30 mg/kg/day did not induce any ulcerations, adhesions, or deaths in the rats.

Comparative Ulcerogenic and Anti-inflammatory Effects of CT-3 with Selected Nonsteroidal Anti-inflammatory Drugs. Young and Yee (1994) reported their studies on the anti-inflammatory and ulcerogenic effects of selected nonsteroidal anti-inflammatory drugs (NSAIDs; cyclooxygenase-1 inhibitors) in rats. In these studies, the anti-inflammatory actions of these NSAIDs were investigated in the rat adjuvant arthritis test, a model that was essentially similar to the study reported for CT-3 by Zurier et al. (1998). The chronic (5-day) ulcerogenic test model performed on these NSAIDs by Young and Yee (1994) was also similar to the test method used in the present report as demonstrated from the observation that the ulcerogenic ED₅₀ value of indomethacin obtained by Young and Yee was similar to our present results. The fact that the animal test models used for CT-3 were similar to the test models reported by Young and Yee permits an estimation of the therapeutic indices of CT-3 and selected NSAIDs. The therapeutic index is calculated by dividing the ulcerogenic ED₅₀ by the anti-inflammatory ED₅₀ values. A higher therapeutic index indicates better GI tolerance for the test drug. As is evident from Table 7, the ther-

TABLE 7

Comparative chronic ulcerogenic and anti-inflammatory effects of CT-3 and selected NSAIDs in rats

Test Drug	ED ₅₀ (95% CL)		Therapeutic Index of ED ₅₀ for Ulcer to ED ₅₀ for Anti-inflammatory
	Ulcerogenic ^a	Anti-inflammatory ^b	
	<i>mg/kg/day</i>		
CT-3	>30 ^c	0.1 ^c	>300
Ibuprofen	115 (97–135)	32 (3–302)	3.6
Diclofenac	8.3 (6.6–10)	0.6 (0.5–0.8)	13.8
Indomethacin	4.0 (2.9–5.5)	0.3 (0.2–0.4)	13.3
Ketoprofen	8.2 (6.2–11)	0.2 (0.1–0.6)	41.0
Ketolac	10.2 (6.8–15)	1.4 (0.7–3.0)	7.3

Adapted from Young and Yee (1994).

^a Gastrointestinal ulceration of the test drugs was determined after 5 days of chronic administration.

^b The anti-inflammatory evaluation of the test drugs was performed in the rat adjuvant arthritis test.

^c The ED₅₀ values for CT-3 was only an estimate because a dosage of up to 30 mg/kg/day administered for 5 days did not produce any ulceration. In addition, the anti-inflammatory ED₅₀ dosage of CT-3 in the rat adjuvant arthritis test was estimated to be equal to 0.1 mg/kg (Zurier et al., 1998).

apeutic indices for the reference NSAIDs studied by Young and Yee ranged from 3 to 41. In contrast, the therapeutic index for CT-3 exceeded 300, suggesting that CT-3 has a very large dissociation between its anti-inflammatory and ulcerogenic action.

Discussion

Analgesia is one of the most profound effects of THC in most species after its parenteral administration, and THC had shown equivalent potency to morphine in rats and mice in a variety of analgesic tests, including the tail-flick latency measurements (Buxbaum, 1972; Sofia et al., 1975). Several synthetic cannabinoids have also shown analgesic activities in animal models selective for detecting opiate analgesics (Johnson et al., 1981). These models included the tail clip and hot-plate tests in mice and rats. The analgesic potency of a prototype synthetic cannabinoid after its subcutaneous administration was also similar to that of morphine (Johnson et al., 1982).

CT-3 showed marked analgesic activity in the hot-plate and tail clip tests in mice after i.g. and i.p. administration with potency similar to that of morphine sulfate. The analgesic ED₅₀ values for morphine sulfate that were obtained in the present tests were very similar to the previously reported ED₅₀ values in similar tests (Dajani et al., 1977). In addition, CT-3 showed marked analgesic activity in the rat tail clip test. Unlike narcotic analgesics, CT-3 did not induce Straub tail reaction, miosis, or muscular rigidity (Dajani et al., 1977). However, like opiates, the analgesic activity of CT-3 was also accompanied by decreased spontaneous motor activity. As previously observed with other cannabinoids, CT-3 induced catalepsy that occurred at high multiples of its analgesic doses (Abood and Martin, 1992; Fride and Mechoulam, 1993). These analgesic studies clearly indicate that CT-3 has an analgesic action with potency similar to that of morphine.

The oral and i.p. analgesic ED₅₀ values of CT-3 in mice and rats were essentially similar, suggesting that this drug has good oral absorption and bioavailability. Cannabinoids, however, have erratic oral absorption (Agurell et al., 1986). For example, the oral absorption of THC was reported to be slow and erratic with low bioavailability (Ohlsson et al., 1980). Due to the combined effect of first-pass hepatic metabolism and high lipid solubility, only 10 to 20% of the orally administered dose of THC reaches the systemic circulation. In addition, the absorption of THC from the GI tract was influenced by fasting or food deprivation. Fasting was found to decrease the rate of absorption of Δ-9-THC when administered in a sesame oil vehicle (Pryor et al., 1977). In contrast, the synthetic cannabinoid nabilone was readily absorbed in humans when orally administered as a coprecipitate with polyvinylpyrrolidone (Rubin et al., 1977). These observations suggest that the oral bioavailability of cannabinoids depends not only on their chemical structures but also on the type of pharmaceutical formulation used.

In the present study, we explored the relative pharmacological availability of CT-3 after oral and parenteral administration. To permit CT-3 to be dissolved, DMSO was used for the preparation of acceptable pharmaceutical formulation because CT-3 is completely soluble in this solvent. The dose of DMSO used in such formulation was 5 ml/kg, which was

well below its LD₅₀ value of 20 ml/kg (Gosseline et al., 1984). However, it is recognized that DMSO not only enhances the solubility of lipophilic drugs but also may affect their transport across many organs (e.g., blood-brain barrier). The presumably enhanced transport of CT-3 by DMSO could result in the augmentation of not only desirable but also undesirable pharmacological actions. Thus, a comparison of several pharmacological actions of CT-3 in the presence and absence of DMSO in rats would establish whether DMSO had interacted with CT-3.

The coadministration of CT-3, administered at low doses, with DMSO slightly but significantly reduced its analgesic actions. In addition, when CT-3 was administered at very high doses, DMSO enhanced and prolonged its depressant effects on spontaneous motor activity, respiratory depression, and catalepsy. Furthermore, the acute i.g. administration of high doses of CT-3 with DMSO was associated with GI ulcerogenicity, whereas acute administration of CT-3 without DMSO, either i.g. or i.p., was not associated with any GI ulcerations. The fact that DMSO administered alone without CT-3 was not associated with the induction of ulcer formation indicates that the ulcer associated with the combined administration of CT-3 with DMSO was due to a synergistic response. These observations indicate that the use of DMSO as solvent for the preparation of pharmaceutical formulations of CT-3 should be completely avoided.

The chronic administration of CT-3 at a large multiple (>300) of its effective anti-inflammatory dosage was not associated with GI ulceration, whereas the reference standard indomethacin was clearly ulcerogenic even when administered at small multiples of its effective anti-inflammatory dosage. The model used in the present study for the assessment of the drug-induced ulcer in rat has good predictive value with drug-induced ulcer in humans (Lanza et al., 1986; Dajani and Agrawal, 1990). The mechanisms for the induction of ulcer associated with the combined use of CT-3 with DMSO are unknown. However, it is well known that ulcerogenic drugs not only disrupt GI mucosal barrier but also reduce several protective factors affecting GI mucosal defense (Larsen et al., 1992).

The present results indicate that there is a species-dependent toxicity for CT-3. In rats, the i.g. LD₅₀ value of CT-3 exceeded 1000 mg/kg in the presence or absence of DMSO, whereas its i.p. LD₅₀ value was estimated to be 494 mg/kg. In mice, the i.g. and i.p. LD₅₀ values were 200 and 136 mg/kg, respectively. The i.g.-to-i.p. LD₅₀ ratio for CT-3 was approximately equal to 2 in both species, which suggests that this drug has a good bioavailability in both species. The basis for the increased toxicity of CT-3 in mice compared with that in rats is unknown but may reflect differences in the metabolic biotransformation of this drug.

The ED₅₀ analysis of the time course of major pharmacological observations noted with CT-3 in rats provided useful information about its peak and duration of actions. Because analgesia is a desirable pharmacological action for CT-3, analysis of this parameter indicates that CT-3 has a peak effect of 1 h and a duration of 5 to 24 h in rats. In mice, the duration of the analgesic action of CT-3 was maintained for about 5 h.

The mechanism of the analgesic action of CT-3 is unknown at the present time; however, cannabinoid-induced analgesia is considered multifactorial, affecting both central and pe-

ripheral receptors and involving an interaction with the cannabinoid receptors (Richardson et al., 1998), prostaglandins (Peres-Reyes et al., 1991), and opiate receptors (Smith et al., 1993, 1998). Clearly, additional studies are required to establish the mechanism of the analgesic actions of CT-3.

Narcotic analgesics are well established for inducing physical dependence in animals and in humans, whereas cannabinoid dependence has been controversial. Some investigators were unable to demonstrate physical dependence on cannabis (Harris et al., 1974; Leite and Carlini, 1974), whereas other investigators were successful in showing physical dependence (Jones et al., 1976; Dewey, 1986; Pertwee, 1991). However, the discovery of the specific cannabinoid receptor antagonist SR 141716A permitted definitive investigation of the dependence liability of cannabinoids (Rinaldi-Carmona et al., 1994). Studies using high doses of THC followed by the antagonist SR 141716A demonstrated withdrawal signs in rats (Aceto et al., 1996) and in mice (Cook et al., 1998) that are consistent with animal studies of other addictive drugs. Furthermore, it has been established that cannabinoid dependence is related to the dose and frequency of its exposure (Martin, 1995). At this time, it is unknown whether CT-3 would have the capacity for the induction of physical dependence, and additional studies are required to address this issue.

The analgesic action of CT-3 is well confirmed in rats and in mice. Available evidence indicates that CT-3 exhibits two distinct pharmacological properties: an anti-inflammatory property occurring at a very low dose (ED₅₀ = ~0.1 mg/kg i.g.; Zurier et al., 1998) and an analgesic property occurring at a higher dose (ED₅₀ = ~5 mg/kg i.g. and i.p.). The present results indicate that CT-3 is an orally effective analgesic drug, and acceptable pharmaceutical formulation of CT-3 would not require the adjuvant use of permeability enhancers to promote its bioavailability. CT-3 clearly warrants clinical development as an analgesic and anti-inflammatory drug.

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