11-Hydroxy-Δ^9-tetrahydrocannabinol: Pharmacology, Disposition, and Metabolism of a Major Metabolite of Marihuana in Man

Abstract. *11-Hydroxy-Δ^9-tetrahydrocannabinol, administered intravenously to man, produces psychologic and pharmacologic effects that persist for several hours. The drug and its metabolites are excreted in urine and feces for more than 1 week. The pharmacology, disposition, and metabolism of 11-hydroxy-Δ^9-tetrahydrocannabinol mimic that of Δ^9-tetrahydrocannabinol, thus providing evidence that Δ^9-tetrahydrocannabinol (the major active component of marihuana) is converted to the 11-hydroxy compound in man, the latter compound being responsible for the effects.*

The in vitro and in vivo metabolism and disposition of Δ^9-tetrahydrocannabinol (Δ^9THC), the major active constituent of marihuana and hashish (*1*), have been studied in animals and man (*2*). Studies reveal that in vitro Δ^9THC is converted to 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9THC). This compound is also formed in vivo after the administration of Δ^9THC to animals and man. Studies in mice (*3*) demonstrated 11-OH-Δ^9THC to be twice as potent as Δ^9THC after intravenous administration and, based on indirect evidence, it has been proposed that 11-OH-Δ^9THC may be responsible for the psychologic effects of Δ^9THC in man (*4*). We now report direct evidence for the pharmacologic activity of 11-OH-Δ^9THC in man and its disposition and metabolism after intravenous administration.

Three normal male volunteers (22 to 24 years of age) were studied on two separate occasions (*5*). After being given complete medical examinations, and evaluated for emotional stability, they were told the purpose of the experiment, and informed consent was obtained. The subjects had smoked marihuana in the past from 6 to 12 times, but none had smoked during the preceding year. Chronic smokers were excluded since they apparently metabolize Δ^9THC faster; however, subjects were selected who had previously experienced a "high" on marihuana. None had a history of habitual usage of drugs or medications. Tritiated 11-OH-Δ^9-THC (1 mg; 24.6 μc) (*6*) dissolved in 1 ml of absolute alcohol (for parenteral administration) was administered intravenously into the tubing of a rapidly flowing infusion of 5 percent dextrose and water (*7*). Along with the metabolic studies, psychologic and pharmacologic effects were continuously evaluated during and after the administration of the drug or vehicle. Signs and symptoms were determined and quantified by means of a questionnaire modified after the Cornell Medical Index. Psychologic effects were evaluated by a rating scale (*4*). The subjects were instructed to rate the quantity of their psychologic "high" from 0 (no effect) to 10 (maximum effect experienced in the past while smoking marihuana). Subjects were also observed for objective signs. The intravenous administration of the vehicle (1 ml of absolute alcohol) had only minimal effects on the symptom-signs questionnaire and psychologic-"high" rating, which were quantitatively and qualitatively similar to those reported before injection. The vehicle alone was without any effect on the heart rate in these three subjects.

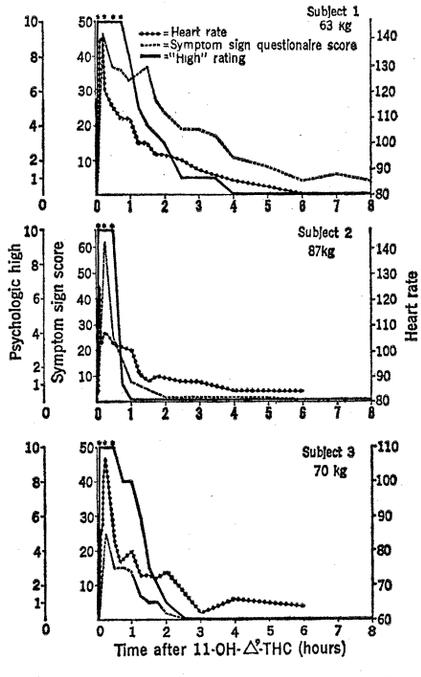

Fig. 1. Pharmacologic effects of 11-OH-Δ^9THC. 11-OH-Δ^9THC (1 mg in 1 ml of ethanol) was administered intravenously to three subjects. The heart rate was determined by continuous monitoring of a lead II electrocardiograph during the first 45 minutes of administration and followed by periodic recordings for a total of 8 hours. The symptom-signs questionnaire (modified Cornell Medical Index) and psychologic-"high" evaluation (self-rating) were administered just before the injection of the drug, at 15 minutes after the drug was administered, and at 15-minute intervals for the first 2 hours, then at 30-minute intervals for a total of 8 hours. The asterisk denotes values greater than 10.

Before the administration of 11-OH-Δ^9-THC, minimal symptoms were reported. Within 2 minutes after the infusion, symptoms and signs rapidly increased in all subjects (Fig. 1), the quantity and intensity being marked initially and gradually decreasing. The effects in subject 1 lasted for several hours; the effects in subject 2 appeared initially to be of much greater intensity, but they subsided within 1 hour. In both subjects the initial effects were described as so intense as to be unpleasant and uncomfortable. In contrast, subject 3, although he reported similar signs and symptoms, did not find the overall experience unpleasant.

Between 2 to 3 minutes (Fig. 1) after the intravenous administration of 11-OH-Δ^9THC, the psychologic "high" reached its peak and was of greater intensity than any they had previously experienced after taking marihuana. Subjects 1 and 2, who found the initial effects unpleasant, later (15 to 30 minutes) described the psychologic "high" as attaining a more pleasant quality. The symptoms most commonly reported by these subjects included numbness and funny feelings all over the body, as well as dizziness, unsteadiness, and weakness.

Heart rate increased in subject 1 from a preinfusion rate of 80 beats per minute to 130 beats per minute during the first minute, increasing to a maximum of 140 beats per minute within 3 minutes. The tachycardia persisted at this rate for more than 10 minutes and gradually returned to the preinfusion rate by 4 hours. In this subject, the T wave of the electrocardiogram became isoelectric within 1 minute and did not return to its normal configuration until 45 minutes later. In subject 2 the heart rate increased from a preinjection rate of 104 beats per minute (his usual resting rate was 80 beats per minute 30 minutes prior to infusion) to a peak rate of 124 beats per minute after 1 minute. This subject's heart rate returned to preinfusion levels within 5 minutes, but did not return to his usual rate until 2 hours after the administra-

tion of 11-OH-Δ^9THC. In subject 3 the control heart rate was 66 beats per minute and increased by 20 beats per minute within 4 minutes, and by 10 to 20 minutes the rate was 106 beats per minute, an overall increase of 40 beats per minute. The rate returned to baseline within 4 hours.

After the intravenous administration of tritiated 11-OH-Δ^9THC, plasma levels of total radioactivity and unchanged tritiated 11-OH-Δ^9THC declined rapidly during the first few hours (Fig. 2). Eight hours after the administration, total radioactivity in the plasma and unchanged 11-OH-Δ^9THC declined more slowly, with half-lives of 40 hours (range 35 to 46 hours) and 22 hours (range 19 to 24 hours), respectively. Radioactive metabolites were present in plasma for more than 72 hours.

The apparent 11-OH-Δ^9THC measured in the heptane : toluene (1 : 1) extract of plasma obtained at 10 minutes, 30 minutes, 1 hour, and 24 hours were shown to have properties identical to that of authentic 11-OH-Δ^9THC when they were cochromatographed on Eastman silica-gel chromatogram sheets and developed in a chloroform : acetone (2 : 1) system.

About 5 percent of the administered radioactivity was excreted in urine during the first 2 hours after the intravenous injection of tritiated 11-OH-Δ^9THC, increasing to 12 percent within 12 hours and 16 percent by the end of the first day. The rate of urinary excretion of radioactivity declined during the next few days with a total of 22 percent (range 19 to 26 percent) being excreted during the first week. In the urine, 11-OH-Δ^9THC accounted for a small percentage of the radioactivity while most was attributable to an unidentified polar acidic compound. The urinary radioactivity displayed extraction and chromatographic properties identical to those described for the major urinary metabolite of Δ^9THC in man (*7*) (that is, the radioactive material was extractable in organic solvents to a much greater extent at *p*H 3 than at *p*H 6.5, and had similar chromatographic properties in three chromatographic solvent systems). After administration of 11-OH-Δ^9THC, more than 80 percent of the urinary radioactivity appears to be present as this acidic, polar metabolite. In rabbits after the administration of 11-OH-Δ^8-THC, an acidic metabolite appeared in urine (*8*) having properties identical to

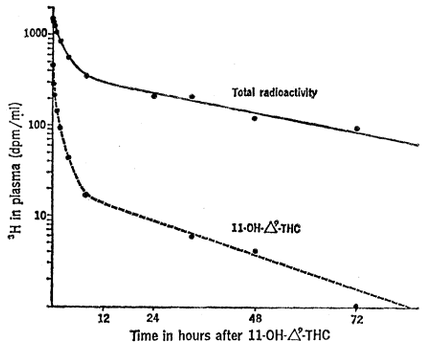

Fig. 2. Concentration of 11-OH-Δ^9THC and total radioactivity in the plasma after intravenous injection of tritiated 11-OH-Δ^9THC. Unchanged 11-OH-Δ^9THC was measured by extracting the plasma (two times) with four volumes of a mixture of heptane and toluene (1 : 1). The recovery from plasma of authentic 11-OH-Δ^9THC under similar conditions is greater than 90 $\pm$ 5 percent. Radioactivity was assayed by means of liquid-scintillation spectrometry.

that found after Δ^9THC administration (*9*), an indication that the metabolic pathways for these compounds were similar in these species.

About 50 percent of the administered dose of tritium was excreted in the feces during the 7-day collection period. Thus, 73 percent (range 67 to 78 percent) of the administered radioactivity was excreted in urine and feces during this interval. Most of the radioactivity in a methanol extract of feces, prepared as described (*7*), was extractable at *p*H 6.5 into heptane containing 1.5 percent isoamyl alcohol, although the amount extracted was independent of *p*H. Chromatography of the radioactive material in the heptane–isoamyl alcohol extract of the fecal solution revealed it to be mainly unchanged 11-OH-Δ^9THC and 8,11-di-OH-Δ^9THC, which accounted for about 20 and 16 percent of the injected tritiated 11-OH-Δ^9THC, respectively. These results are similar to the metabolic findings obtained after Δ^9THC administration (*7*).

Our studies show that 11-OH-Δ^9 THC is a very active compound in man and may be of importance, since it is a considerable metabolite of Δ^9THC (*7*), the active component of marihuana and hashish. 11-OH-Δ^9THC was capable of producing a marked tachycardia, an intense psychologic "high," and marked psychologic and pharmacologic effects atfer an intravenous dose of 14 μg per kilogram of body weight for a 70-kg man. The intravenous administration of 7 μg/kg of Δ^9THC (approximately 0.5 mg) had no effect in naive subjects and only minimum effects in chronic marihuana smokers (*7*). Most of the pharmacologic effects attributable to Δ^9THC or marihuana can be produced by 11-OH-Δ^9THC in man.

The variation in the duration of responses seen in our subjects may be related to differences in their body weights. For example, the effects produced in subject 1 (63 kg) were of longest duration, those in subject 2 (87 kg) were of shortest duration, while the effects exhibited by subject 3 (70 kg) were intermediate (Fig. 1). This would suggest that redistribution to fat or other stores is responsible for dissipating the effects of 11-OH-Δ^9THC.

The disappearance of 11-OH-Δ^9THC from plasma resembles that seen after Δ^9THC administration. In addition, the rate of excretion of the administered radioactivity after 11-OH-Δ^9THC resembles that seen after administration of ^{14}C-labeled Δ^9THC administration. Furthermore, the finding that the same metabolic profile exists for 11-OH-Δ^9-THC and Δ^9THC in urine and feces suggests that Δ^9THC is first converted to 11-OH-Δ^9THC after its administration to man. Although it appears that 11-OH-Δ^9THC is the active compound, a possibility exists that the polar, acidic metabolite present in urine is active. However, to date, there is no evidence to support this hypothesis.

From our results it can be concluded that 11-OH-Δ^9THC is a potent pharmacologic agent and that its disposition and metabolism are similar to those of Δ^9THC in man. These observations suggest that after the administration of marihuana or hashish, the Δ^9THC is rapidly converted in man to 11-OH-Δ^9-THC, which is responsible for the majority of the pharmacologic effects.

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5. Subjects were admitted to the Lilly Ward at Marion County General Hospital and were

under constant medical and nursing supervision throughout the experiment.

6. Tritiated 11-OH-Δ^9THC (specific activity, 24.6 μc/mg) labeled in the 2, 4, 8, and 10 positions was enzymatically synthesized at the Research Triangle Institute, Research Triangle Park, N.C., and supplied by Dr. M. C. Braude, National Institute of Mental Health. The material (7 mg) initially contained 80 percent 11-OH-Δ^9THC. After purification by extraction and thin-layer chromatography, 4 mg of 11-OH-Δ^9THC were obtained. On thin-layer chromatography the material contained one radioactive peak having the same chromatographic behavior as authenic 11-OH-Δ^9THC. The purified 11-OH-Δ^9THC was prepared for intravenous administration by dissolving 1 mg of the drug in absolute alcohol (parenteral administration grade). Each dose was prepared under aseptic conditions and placed in an ampoule. A portion of the 11-OH-Δ^9THC solution was analyzed by mass spectrometry and shown to contain essentially compounds having the same molecular weight and fragmentation characteristics as 11-OH-Δ^9THC. The possibility exists that minor percentages of other monohydroxylated tetrahydrocannabinols were present in the solution.

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Collagenase: Localization in Polymorphonuclear Leukocyte Granules in the Rabbit

Abstract. *Polymorphonuclear leukocyte granules were submitted to zonal fractionation through a discontinuous sucrose gradient. Azurophilic and specific granules were enzymatically characterized by peroxidase and alkaline phosphatase activity, respectively. The enzymes formed modal distributions like those reported by others. Collagenase activity was consistently associated with the specific granules containing alkaline phosphatase.*

A collagenase, active against undenatured collagen fibrils at neutral and alkaline *p*H, has been extracted from the granule fraction of human polymorphonuclear (PMN) leukocytes (*1*, *2*). The leukocyte collagenase cleaved collagen into distinctive products characteristic of other animal collagenases. The enzyme differed from other tissue collagenases in that activity was demonstrated after extraction from PMN leukocyte granules and did not require tissue culture for detection. Collagenase from PMN leukocytes was inhibited by ethylenediaminetetraacetic acid, cysteine, and reduced glutathione but not by human serum.

Cytochemical and electron microscopic studies of human marrow (*3*, *4*) indicated that neutrophilic leukocytes contained at least two granule types, azurophils and specifics, which differed in morphology, time of formation, and enzymatic activity. Baggiolini *et al.* (*5*) and Zeya and Spitznagel (*6*) isolated azurophils, specifics, and a third, less well-defined group of particles from rabbit PMN leukocytes by sucrose gradient centrifugation. Large, relatively dense azurophilic or primary granules contained essentially all of the myeloperoxidase activity, a third of the lysozyme activity, and high relative concentrations of characteristic lysosomal acid hydrolases. The smaller, less dense group of specific or secondary granules contained the majority of alkaline phosphatase activity. The third group of granules were morphologically ambiguous, and their nature and composition is at present unresolved (*4*, *7*).

Two enzymatically heterogenous populations of rabbit PMN leukocyte granules, azurophils (A) and specifics (B), have been described, the former characterized by peroxidase and the latter by alkaline phosphatase. We sought to determine whether collagenase was also localized in a particular granule fraction of rabbit PMN leukocytes.

Polymorphonuclear leukocytes were obtained from rabbit peritoneal exudates induced aseptically with 0.5 percent glycogen in saline essentially as described by Hirsch (*8*). The exudates were submitted to differential cell count in stained smears and averaged 96 to 98 percent PMN leukocytes. Exudates with less than 5×10^6 PMN cells per milliliter or with gross erythrocyte contamination or abnormally high mononuclear leukocyte concentrations were discarded. The cells were exposed to brief hemolysis with hypotonic saline, washed in 0.34*M* sucrose, and resuspended in 20 ml of 0.34*M* sucrose for homogenization. Cells were lightly homogenized for 2 minutes with a Teflon pestle. Granules were isolated from nuclei, residual erythrocytes, debris, and intact cells by centrifugation for 10 minutes at 400*g*. These initial preparation and homogenization procedures were critical for reproducible distribution of enzymes in the gradient. All steps were followed by phase microscopy.

Table 1. Relative enzyme concentration of granule pellets after centrifugation of fractions pooled as shown in Fig. 1a. Alkaline phosphatase and collagenase were found predominantly in the region containing B particles.

Fraction	Enzyme (percent recovery)		
	Alkaline phosphatase	Collagenase	Peroxidase
Soluble phase	9.6	9.8	12.2
C	8.2	14.1	2.6
CB	4.5	0	3.2
B	41.7	59.0	11.0
BA	12.0	7.2	16.1
A	5.0	9.2	42.3

The 20-ml 400*g* supernatant represented the starting sample for zonal centrifugation procedures. Fractionation was accomplished by procedures outlined by Cline and Ryel (*9*). A discontinuous sucrose gradient consisting of 17, 28, 42 and 55 percent sucrose was loaded into a spinning B-XIV Spinco zonal rotor (2000 rev/min) in order of increasing density. The sample was layered over the gradient and centrifuged at 20,000 rev/min for 18 minutes. Following centrifugation, the gradient containing the fractionated components was displaced from the spinning rotor (2000 rev/min) with 60 percent sucrose. The effluent was continuously monitored with a Beckman DB-GT spectrophotometer at 280 nm, and collected in 20-ml fractions. The sucrose concentration of individual fractions was determined with a Bausch and Lomb 3-L refractometer. All granule preparation and centrifugation operations were conducted at 4°C.

Alkaline phosphatase was assayed by a modification of the method of Babson *et al.* (*10*) with phenolphthalein monophosphate as substrate. Myeloperoxidase activity was assayed as the rate of hydrogen peroxide decomposition with *O*-dianisidine as hydrogen donor. Protein content was estimated by the method of Lowry *et al.* (*11*). Specific collagenolytic activity was measured with the [^{14}C]glycine-labeled fibril assay described by Lazarus *et al.* (*2*) with modifications described by Robertson *et al.* (*12*). All assays were conducted at 37°C in the presence of 0.005*M* $CaCl_2$, 0.02*M* NaCl, and 0.3*M* tris(hydroxymethyl)aminomethane, *p*H 7.5 (*13*). Collagen substrates were highly resistant to nonspecific protease diges-