CHAPTER 18

ANALGESIC AND ANTITUMOR POTENTIAL OF THE CANNABINOIDS

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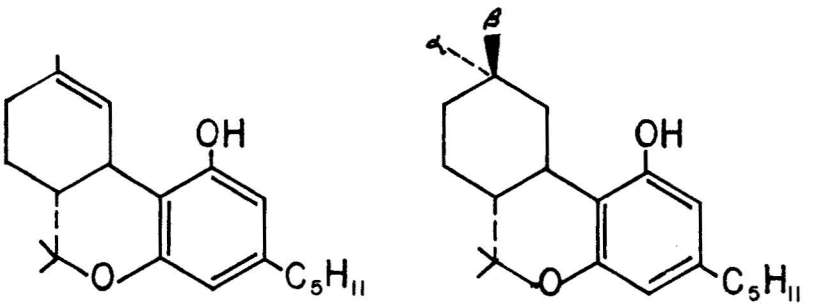
There is an extensive folklore concerning the analgesic activity of cannabis in man (Mikuriya, 1961). On careful examination, however, there was no objective evidence for the efficacy of cannabis in this regard. With the structural identity and synthesis of (-)-delta-9-tetrahydrocannabinol (delta-9-THC) (Gaoni and Mechoulam, 1964), the major active principle of cannabis, came reports of analgesic activity in laboratory animals. Indeed, potency equal to morphine was reported for delta-9-THC (Grunfield and Edery, 1968; Bauxbaum, Sanders-Bush and Efron, 1969).

Using the standard analgesic test procedures in our laboratory, we have not had the same results (Dewey, Harris and Kennedy, 1972). In our hands, delta-9-THC was inactive in the hot-plate and tail-flick tests at doses below those that produced severe behavioral and psychomotor impairment. This may be due to the fact that we run a more stringent test procedure. For instance, in the tail-flick test we use a normal reaction time of 2-4 seconds with a cutoff time of 10 seconds in mice and 20 seconds in rats

(Harris and Pierson, 1964; Harris, Dewey, Howes, Kennedy and Pars, 1969). Using these criteria, until recently only the narcotic analgesics were active. Indeed, there was such a good correlation between antinociceptive activity in these tests and analgesics in man that we could accurately predict the dose necessary to relieve pain in man. The development of the narcotic-antagonist analgesics, which are inactive in the hot-plate and tail-flick tests as we run them, caused a rethinking of our test procedures and led to the extensive use of the "writhing" or "abdominal stretching" test (Hendershot and Forsacth, 1959; Pearl and Harris, 1966; Pearl, Aceto and Harris, 1968). This test did reveal activity for the narcotic antagonists. However, delta-9-THC is again not active in this test unless doses are used which produce behavioral and motor impairment. To date there have been four reports of controlled analgesic studies with delta-9-THC in man (Noyes, Brunk, Baram and Canter, 1975; Regelson, Butler, Schulz, Kirk, Peek, Green and Zakio [in press]; Hill, Goodwin, Schwin and Powell, 1974; Roft, Gregg, Ghia and Harris, pending). One of these reported good analgesic activity (Noyes et al., 1975). Another reported no analgesic activity (Regelson et al., in press) and two reported hyperalgesia (Hill et al., 1974; Roft et al., pending). Thus, to date, the results with this compound are equivocal.

Recently, a new cannabinoid 9-hydroxy-9-nor-hexahydrocannabinol (HHC), with potent analgesic activity has been prepared by Wilson and May (1974; also Wilson, May, Martin and Dewey, in press) (see Figure 1).



Δ^9-THC

	α	β
9-nor-9α-OH-HHC	OH	H
9-nor-9β-OH-HHC	H	OH

Figure 1. Structures of hexahydrocannabinols compared to delta-9-THC

This compound exists in the α- and β-form and diasterioisomers exist for each. We have found that potent antinociceptive activity is found in the β-isomer and that the β-l-isomer is approximately twice as potent as the racemic mixture in the hot-plate test and considerably more active in the tail-flick test (Table 1). This is the first compound closely related to the natural products which has shown potent antinociceptive activity in our test procedures.

In an attempt to determine whether β-HHC resembled the narcotic analgesics, we attempted to reverse its antinociceptive activity with naloxone. The results of one such experiment is shown in Table 2. While some antagonism of β-HHC could be obtained with naloxone, we were never able to completely reverse its antinociceptive activity. Another characteristic of narcotic analgesics is the cross-tolerance which occurs between members of this class of compounds. We attempted to determine whether cross-tolerance occurred between morphine and β-HHC. For this purpose, we used mice implanted with morphine pellets (Way, Loh and Shen, 1969). The results are shown in Table 3. While there is a marked tolerance to morphine, there is no cross-tolerance to β-HHC. Indeed, if anything, there is some enhancement of

Table 1. Antinociceptive Activity of Cannabinoids

Compound	Tail Flick Test (i.p.)	ED_{50} mg/kg Hot-Plate Test (i.p.)	Abdominal Stretch Test (s.c.)
Delta-9-THC	NA[a]	NA	NA
(±)- α-HHC[b]	NA	NA	NA
(±)- β-HHC	7.1	2.9[c]	-
(-)- β-HHC	1.0	1.6[c]	0.03
Morphine SO_4	5.8	1.2[c]	0.56

[a] Not active at doses below 10 mg/kg.

[b] (±)- α-9-hydroxy-9-nor-hexahydrocannabinol.

[c] Wilson et al., J. Med. Chem.

Source: Martin, Dewey, Earnhardt, Adams, Harris, Wilson, May, and Razdan (pending).

Table 2. Reversal of the Effects of b-HHC on the Tail-Flick by Naloxone

Treatment	Tail-Flick % MPE
Vehicle	1
(±)- β-HHC, 20 mg/kg	85
(±)- β-HHC, 20 mg/kg + Naloxone 2 mg/kg	52

Source: Bloom, Dewey, Harris, and Brosins (1975).

Table 3. Tail-Flick Activity of Morphine and 9-nor-9β-OH-HHC in Placebo and Morphine Pellet Implanted Mice

	ED_{50} and 95% Confidence Limits mg/kg	
	Placebo Pellet	Morphine Pellet
Morphine	3.40 (1.20-9.64)	17.28 (9.21-32.44)
9-nor-9β-OH-HHC	7.20 (1.98-20.62)	3.83 (1.18-12.61)

Source: Bloom et al., 1975.

the antinociceptive effects. These results, coupled with data from the morphine-dependent monkey and the results with the heterocyclic derivatives presented later in this Symposium, give hope that a non-dependence-producing strong analgesic may emerge from the cannabis field.

The reports (Nahas, Suica-Foca, Armand and Marishima, 1974; Gupta, Grieco and Cushman, 1974; Munson, Levy, Harris and Dewey, in press) that marihuana and the naturally occurring cannabinoids interfered with certain immune systems prompted us to look at the effects of these compounds on some tumor systems. It was our hypothesis that since delta-9-THC to some degree inhibited the T-cell system, which is one of the body's few natural defenses against cancer, it would cause tumors to grow faster. The tumor system we first chose to study was the Lewis lung adenocarcinoma. In this system, 1×10^6 tumor cells were injected into the right hind gluteus muscle of C57BL/6 mice. Tumor size was measured weekly and converted to tumor weight by the method of Mayo (1972). The Lewis lung adenocarcinoma is a solid tumor and grows according to a Gompertzian function (Figure 2).

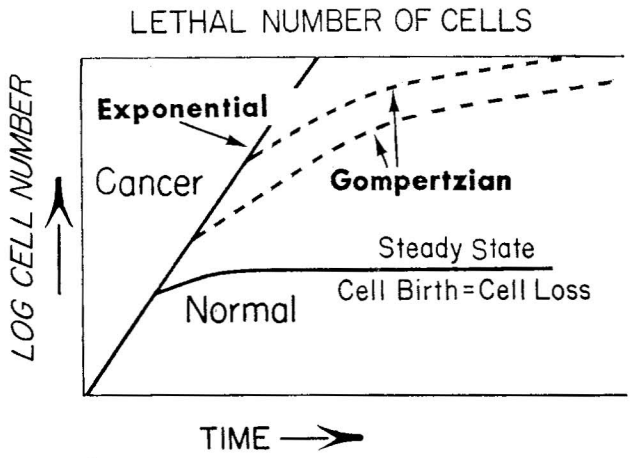


Figure 2

Although the tumor grows to a large mass, the animals do not die of the primary tumor but by metastases particularly to the lung. The results of our first experiment are shown in Figure 3. Much to our surprise, there was a marked inhibition of tumor growth and a significant increase in life span (Table 4) (Harris, Munson and Carchman, in press; Munson, Harris, Friedman, Dewey and Carchman, 1975). Of interest was the fact that the animals soon became tolerant to the behavioral effects of these rather large doses of delta-9-THC and did not lose weight, as is the case with most of the standard anti-tumor agents. This reflects the low toxicity of the cannabinoids relative to the standard anticancer drugs. Since these results were obtained with a 10-day treatment regimen, it was natural to try longer treatment schedules. When this was done, the early marked inhibition of tumor growth was overcome and there was no increase in life span. We have found that treatment every third day produces results as good as can be obtained but no better than our original regimen.

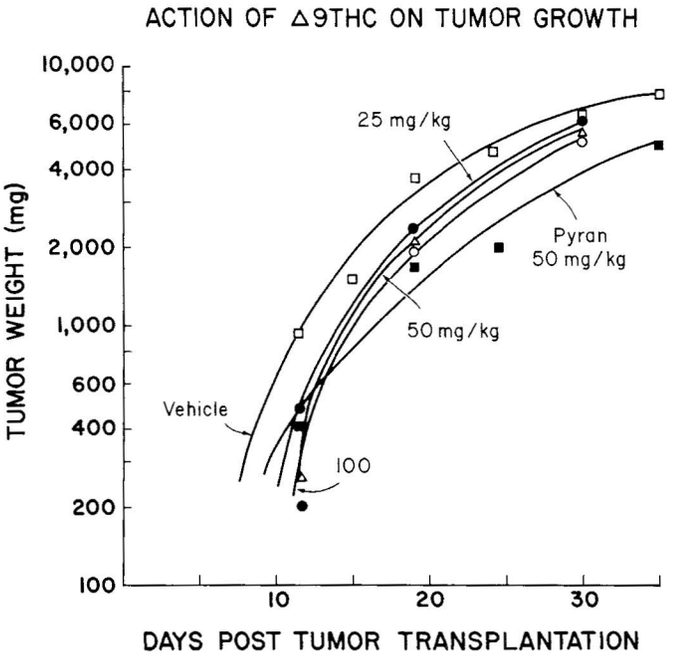


Figure 3

Table 4. Survival Time of delta-9-THC-Treated Mice (10 Days) Hosting Lewis Lung Carcinoma[a]

Treatment	Dose (mg/kg)	Wgt.(g)[b]	Survival Time (Days)	% ILS[c]
Vehicle[d]	-	+1.0	25.8 ± 1.3	-
Pyran	50	-0.5	38.2 ± 1.5	48*
Delta-9-THC	25	+0.9	30.3 ± 2.0	17
Delta-9-THC	50	-0.3	27.4 ± 0.6	6
Delta-9-THC	100	-0.1	35.5 ± 1.1	36*

a. Group of mice inoculated with 10^6 Lewis lung cells and treated daily for 10 days. Control and delta-9-THC mice treated orally. Pyran injected i.p.

b. Whole body weight changes determined after 10 days of treatment.

c. Percent increased life span over control.

d. 0.01 ml/g of 7.5% bovine serum albumin.

* $p < .05$.

These studies have been expanded (Munson et al., 1975) to include a number of other tumor systems, such as the Friend leukemia virus-induced splenomegaly and the C3H mammary tumor where inhibitory activity is also seen. However, delta-9-THC is inactive in mice hosting the L-1210 murine leukemia. This tumor system grows rapidly and follows an exponential function.

A number of other natural cannabinoids have also been examined to ascertain their antitumor activity. Delta-8-THC is quite active (Figure 4), as is cannabinol, but cannabidiol produces a marked stimulation of tumor growth (Figure 5). To date, we have tested a wide variety of natural and synthetic cannabinoids as well as some heterocyclic analogs. None of these has proven to be either much more potent or efficacious than delta-9-THC.

To enhance our ability to examine large numbers of compounds and to attempt to determine mechanisms of action, we have turned to an in vitro

system (Harris et al., in press; Munson et al., 1975). In this system, normal bone marrow or tumor cells are incubated with either the drug or drug vehicle and their ability to incorporate tritiated thymidine into DNA is examined.

Experiments with bone marrow and isolated Lewis lung cells incubated in vitro with delta-9-THC showed a dose-dependent inhibition of the incorporation of tritiated thymidine into DNA. Of great interest was an observed differential between the tumor and the bone marrow cells (Figure 6). The importance of this finding is twofold. First, few anti-tumor agents have such a selective effect. Second, if we can find an explanation for the differential effect we may discover some important difference between the tumor and normal cells which will allow a new rational approach to chemotherapy.

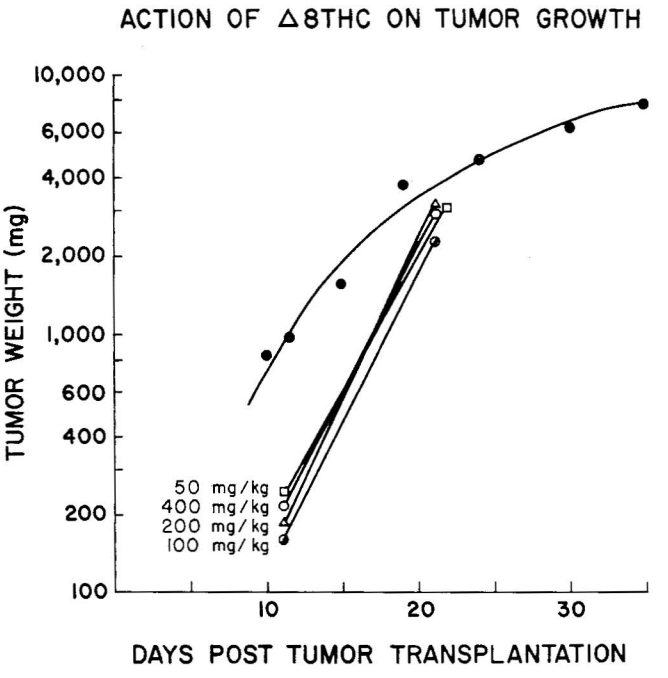


Figure 4

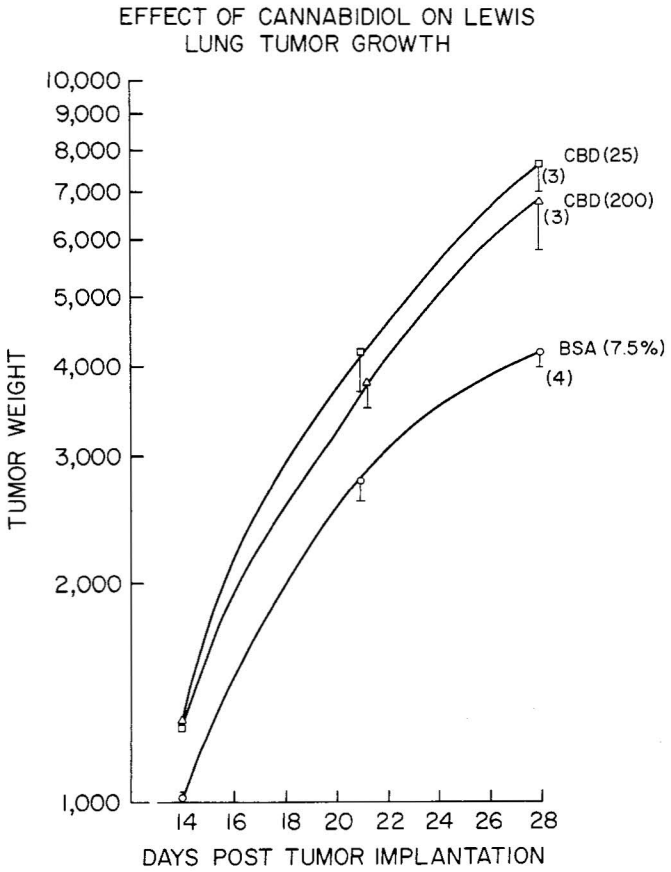

EFFECT OF CANNABIDIOL ON LEWIS
LUNG TUMOR GROWTH
TUMOR WEIGHT
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
14
16
18
20
22
24
26
28
DAYS POST TUMOR IMPLANTATION
CBD (25)
(3)
CBD (200)
(3)
BSA (7.5%)
(4)

Figure 5

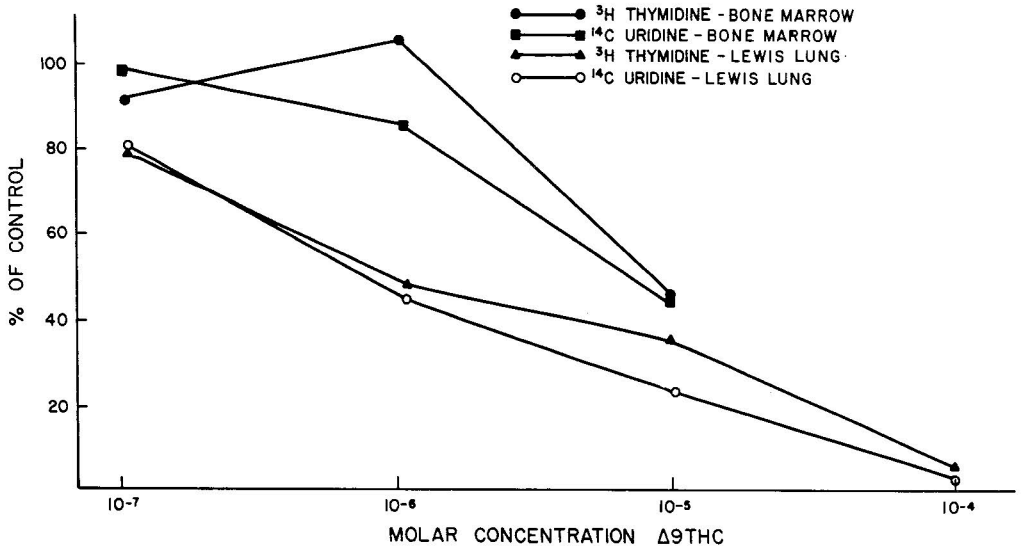


Figure 6

This in vitro system has allowed us to screen a wide variety of compounds as illustrated in Table 5. As can be seen, many compounds have inhibitory activity but none are much more potent than delta-9-THC. More importantly, none show the differential effect between tumor and normal cells that delta-9-THC does. It is also of interest to note that cannabidiol produces an enhancement of thymidine incorporation. This may reflect the mechanism for enhanced tumor growth observed in the in vivo system (Munson et al., 1975).

As to translation of these findings to tumor chemotherapy in man, there is a long way to go. We would like to have more efficacious compounds with greater potency than delta-9-THC and with little or no central nervous system activity. It may be that the cannabinoids will be useful only as adjuncts to other chemotherapy. We are currently investigating combination medication with a variety of antitumor agents and some of these appear promising. More important findings, however, will probably come from investigation of the basic mechanisms involved in the action of these drugs on tumor cells.

Table 5. The Effect of Cannabinoids and Cytosine Arabinoside on in vitro DNA Synthesis

Cannabidiol	3.37×10^{-5}M	*********	4.89×10^{-4}
ABN Cannabidiol	9.28×10^{-5}M	1.29×10^{-2}	5.51×10^{-6}
Cannabichromene	$>10^{-4}$M	$>10^{-4}$M	———
β,11/di/OH Δ^9-THC	$>10^{-4}$M	$>10^{-4}$M	———
Cannabicyclol	$>10^{-4}$M	———	———

DRUG	STRUCTURE	LEWIS LUNG	E.D. 50* L1210	BONE MARROW
Δ^9-THC		4.18×10^{-6}M	3.26×10^{-5}	2.06×10^{-5}
Δ^8-THC		2.99×10^{-6}M	8.70×10^{-6}	1.26×10^{-6}
ABN Δ^8-THC		1.48×10^{-6}M	5×10^{-6}M	3.56×10^{-6}
Cannabinol		2.3×10^{-6}M	2.2×10^{-6}	3.08×10^{-7}
ARA - C		1.36×10^{-7}	2.53×10^{-8}	1.57×10^{-7}M

Source: Carchman, R. A. et al. Cancer Res., 1976, 36, 95-100

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