CANNABINOIDS IN GLAUCOMA III: THE EFFECTS OF DIFFERENT CANNABINOIDS ON INTRAOCULAR PRESSURE IN THE MONKEY*

C. W. Waller, D. A. Benigni
E. C. Harland, J. A. Bedford
J. C. Murphy, M. A. ElSohly

The Research Institute of Pharmaceutical Sciences
School of Pharmacy
University of Mississippi
University, Mississippi

I. INTRODUCTION

The use of the constituents of Cannabis sativa as therapeutic agents in glaucoma was initiated by Hepler et al., who observed the lowering of intraocular pressure in normal and glaucomatous subjects when marijuana was smoked and when delta-9-tetrahydrocannabinol (THC) was given (1,2). The ocular effects of the cannabinoids were reviewed in 1979 by Green (3) and more recently in the Institute of Medicine's report "Marijuana and Health" (4). The extensive literature on marijuana was annotated by Waller et al. (5,6).

In previous communications we reported on a screening procedure using the rabbit for the lowering of intraocular pressure (IOP) by certain constituents of Cannabis sativa (7,8). Since there is no ideal methodology for the screening of compounds for IOP lowering effects in animals that simulate the pathological condition of glaucoma, a second animal model was deemed necessary. The monkey has been used in glaucoma research. Green and Kim (9) used five juvenile (3 kg) rhesus monkeys which were immobilized with ketamine and averaged the results from the group. Our experience with ketamine-immobi

*Supported in part by National Eye Institute grant EY-03353 and the Research Institute of Pharmaceutical Sciences.

lized rhesus monkeys (unpublished obervations) showed that it was difficult to control the degree of anesthesia during the time of IOP measurements. Excessive intersubject variability in baseline readings were observed.

Thus it was necessary to develop the use of conscious monkeys (M. arctoides) to validate the rabbit data for the testing of cannabinoids for IOP lowering effects.

II. METHODS

A. Animals

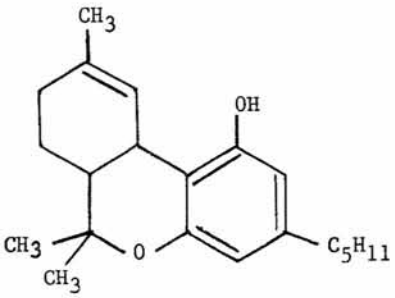
Groups of six stumptailed macaque (M. arctoides) monkeys (3 males and 3 females) were used. The animals wre maintained in individual cages with free access to water. They were given monkey chow (Purina) twice daily and each animal was given a multiple vitamin tablet daily. On the day preceding testing, each animal was anesthetized with ketamine, weighed and placed in a restraint chair (Plas. Labs). During the test day, intraocular pressure readings were taken in the conscious animals using a Digilab R 30 instrument preceded by one drop of 0.5% proparacaine HCl solution in each eye.

A period of 4 to 6 weeks was spent in adapting the animals to IOP measuring procedures. A positive reward of banana pellets or fruit was given to encourage cooperation. The goal of the procedure was to use as little restraint as possible in obtaining IOP readings.

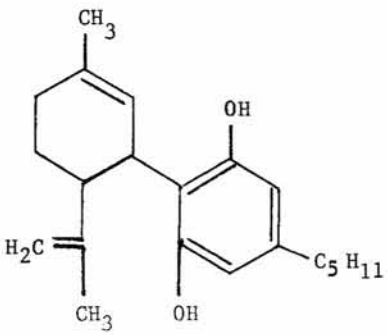
Compounds were tested by obtaining IOP readings as follows: At least two baseline readings separated by more than 30 minutes were taken. Following a uniform baseline reading, a dose of the drug was given orally by intubation. Readings were taken hourly for up to 6 hours.

B. Compounds

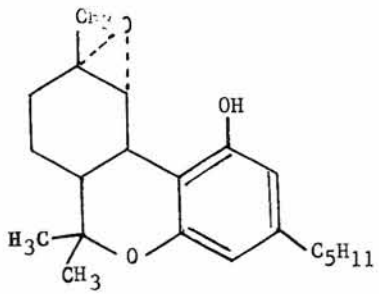
The major cannabinoids (THC, delta-8-THC and CBD) were obtained through the National Institute on Drug Abuse. The epoxides of delta-8-THC and the epoxide of delta-9-THC were prepared by literature procedures (10,11). Their structures are shown on the opposite page. Dosage forms were prepared as previously described (7).



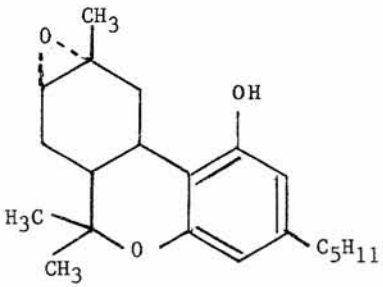
Δ^9-Tetrahydrocannabinol
Δ^9-THC



Cannabidiol
CBD



9α,10α - epoxyhexahydrocannabinol
9α,10α - EHHC



8α,9α - Epoxyhexahydrocannabinol
8α,9α - EHHC

C. Analysis of the Data

Data were converted to mean percent of control by the following formula:

$$\text{Mean \% of control} = \text{Sigma(n)} \quad \frac{(IOP_{(t)D}/IOP_C \times 100)}{n}$$

where $IOP_{(t)D}$ is the $IOP_R + IOP_L/2$ at time t, IOP_C is the $IOP_R + IOP_L/2$ at time 0, IOP_R and IOP_L are the IOP readings for the right and left eye, respectively, and n is the number of animals used (6 in this case).

Statistical comparisons were accomplished via the Wilcoxon Matched-Pairs Signed Ranks (12).

III. RESULTS

When THC was given orally, IOP was reduced for 1 to 4 hours post dosage. A dose of 3 mg/kg of THC lowered IOP from 20.3 to 15.9 mm of mercury or 22% in 2 hours (Fig. 1). Figure 2 shows the dose response curves for THC dosage range of 0.25 - 3.0 mg/kg.

The epoxide of THC (9alpha,10alpha-EHHC) was found to be active orally in the conscious monkeys. Figure 3 shows the effect of 3 mg/kg dose in absolute IOP values in mm of mercury while Figure 4 shows the dose response curves at doses of 0.25 - 3.0 mg/kg.

The epoxide 8alpha,9alpha-EHHC was tested in this model at 1 mg/kg and found to have slight activity at this dose (Figure 5). Cannabidiol was found to be inactive at a dose of 10 mg/kg orally.

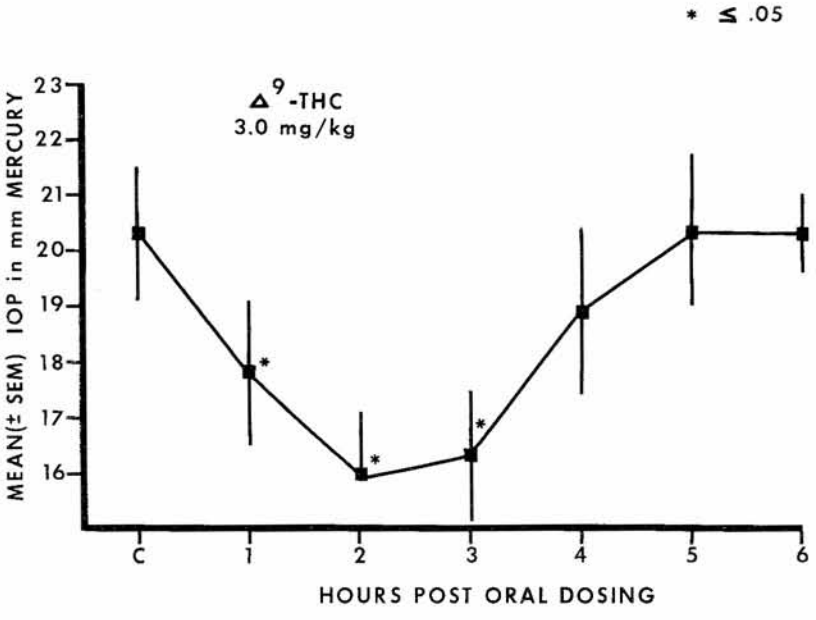


FIGURE 1. Effects on IOP in mm of mercury of 3 mg/kg of THC given orally to 6 conscious Macaque monkeys. (*p < 0.05)

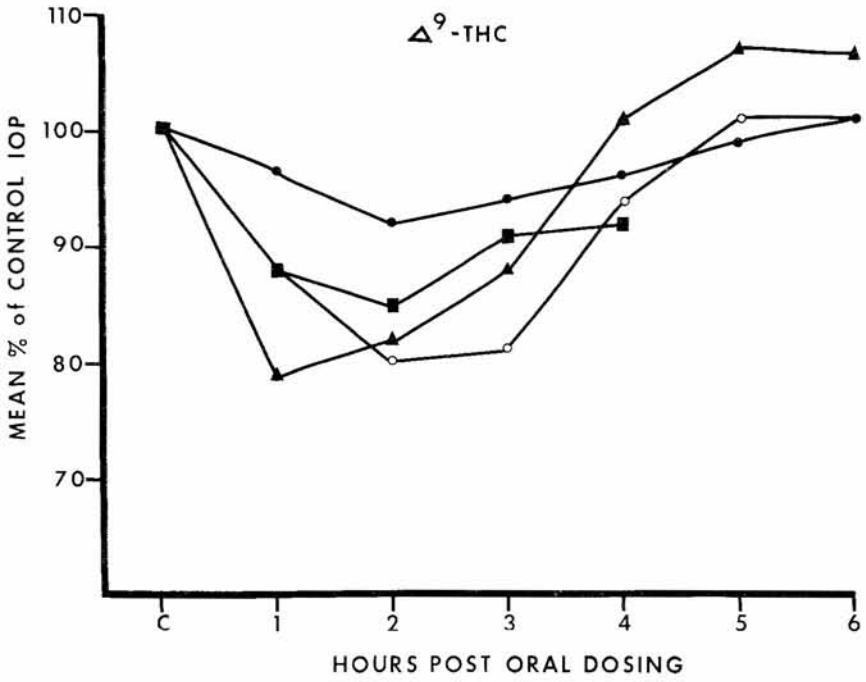


FIGURE 2. The dose response effects of THC on IOP when given orrally to conscious Macaque monkeys: (●) 0.25 mg/kg; (■) 1.0 mg/kg; (▲) 2.0 mg/kg; (O) 3.0 mg/kg.

IV. DISCUSSION

Cannabis and its constituents have evolved as a lead for the development of new and hopefully useful chemotherapeutic agents for the treatment of wide angle glaucoma. In one respect, it is an ideal lead since its major constituent, THC, has been shown to lower IOP in glaucomatous patients. Yet, it is a difficult problem to execute since there is no good laboratory animal or biological system to follow the chemical progress through a stepwise determination of the structure to activity relationships. The use of the rabbit as a test animal for screening compounds for their effects on IOP has been reported by several investigators. Green publised several reports on the effects of cannabinoids on IOP. However, Green's methodology using the rabbit as an animal model was

deficient in that cannabidiol (CBD), which he reported as active, was found to be inactive in both rabbits (7) and in humans (13). Perez-Reyes et al., compared several cannabinoids (including CBD) in normal humans as to their psychoactivity, effects on heart rate and IOP lowering when given by intravenous infusion (13). They suggested that CBD was inactive in all of these parameters and it could be used as a placebo drug.

We have reported on a refined methodology using the rabbit for primary screening of cannabinoids for their ability to lower IOP (7) with the following conclusions (8):

1. THC is active by the IV route.
2. The IOP effects appear to parallel the psychoactivity.
3. Most compounds related to THC that have psychoactivity show lowering effects on IOP.
4. The activity appears to be biphasic.
5. Topical activity is erratic and both eyes are affected even when the drug is applied to only one eye.
6. The activity needs to be confirmed in other animal species.

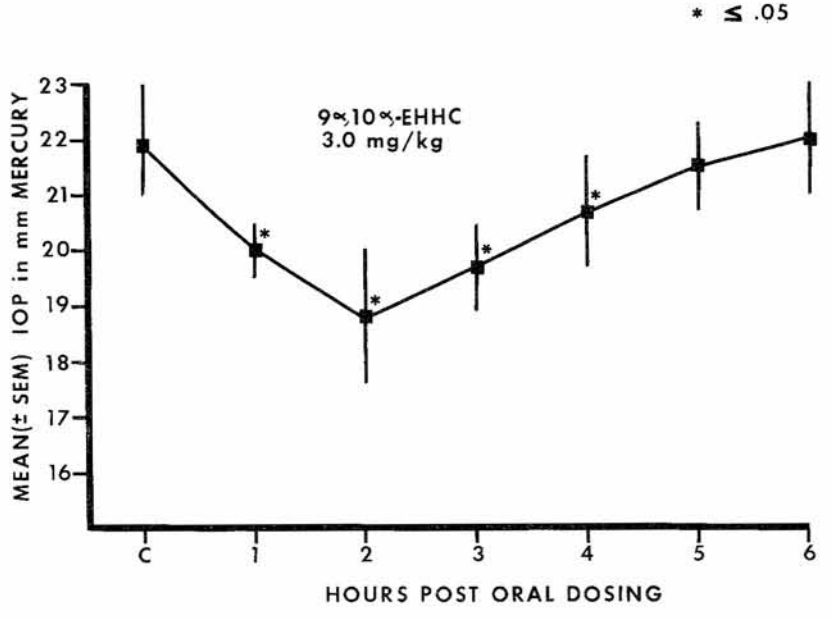


FIGURE 3. Effects on IOP in 6 conscious monkeys when 3 mg/kg of 9alpha,10alpha-EHHC was given as a single dose orally. (*$p < 0.05$)

The use of the monkey as a second animal model was investigated. The anesthetized rhesus monkey was unacceptable because of the inter-subject variability in the IOP baseline readings. It was decided to attempt to train monkeys to accept IOP measurements while conscious.

The stumptailed macaque was chose over the rhesus macaque because this species is generally more docile. The oral route of administration was selected over i.v. since the i.v. route is impractical.

The compounds used in this investigation were previously tested in rabbits and in rhesus monkeys by the i.v. route. All compounds that showed activity in these models were found to be active in the present model. Cannabidiol was found to be inactive in this investigation as it was in other studies.

Examination of Figure 2 shows the biphasic nature of the activity of THC which is in agreement with our previous observations (7,8). The 9alpha,10alpha-epoxyhexahydrocannabinol appeared to have a longer duration of action than did THC.

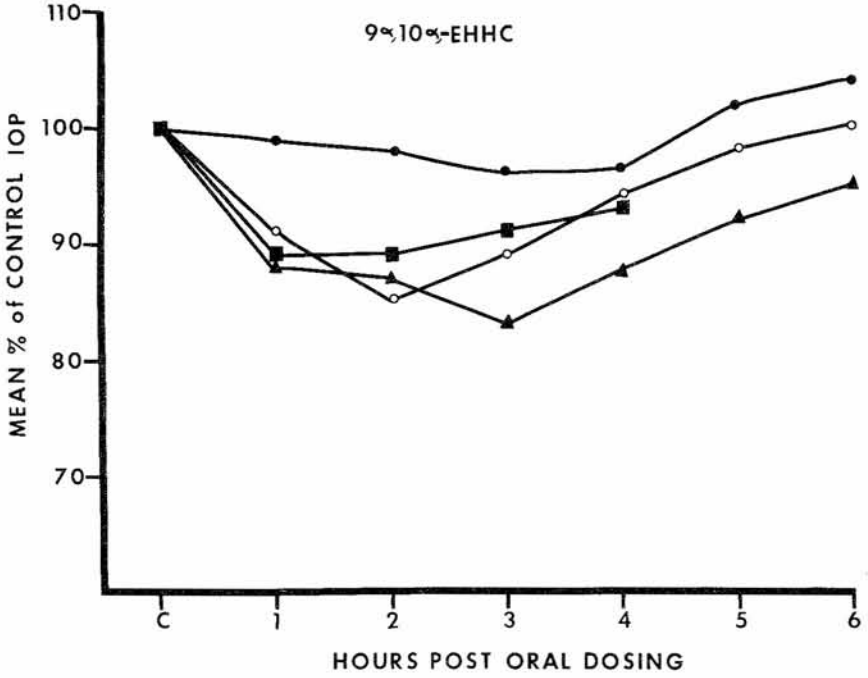


FIGURE 4. Average percentage change of IOP in 6 conscious Macaque monkeys/group when given 9alpha,10alpha-EHHC orally at (●) 0.25 mg/kg; (■) 1.0 mg/kg; (▲) 2.0 mg/kg; (O) 3.0 mg/kg.

IV. CONCLUSION

The data in both rabbits and monkeys confirm that THC is an active agent for the lowering of IOP in these normal laboratory animal models as has been reported for humans.

The 9alpha,10alpha-epoxyhexahydrocannabinol is a minor metabolite of THC and it has pharmacological properties very similar to THC (14) and delta-8-THC (15). The data reported herein and by ElSohly et al. (8) show that 9alpha,10alpha-EHHC is an active agent in lowering IOP in both the rabbit and monkey models.

The rabbit and the monkey models will be used in our laboratories to continue the search for useful agents to treat glaucoma in humans. Within this program is a search for methodology with suitable dosage forms to affect IOP when the drug is given topically.

ACKNOWLEDGMENT

The authors thank the National Institute on Drug Abuse for all the cannabinoids used in this work. Special thanks go to

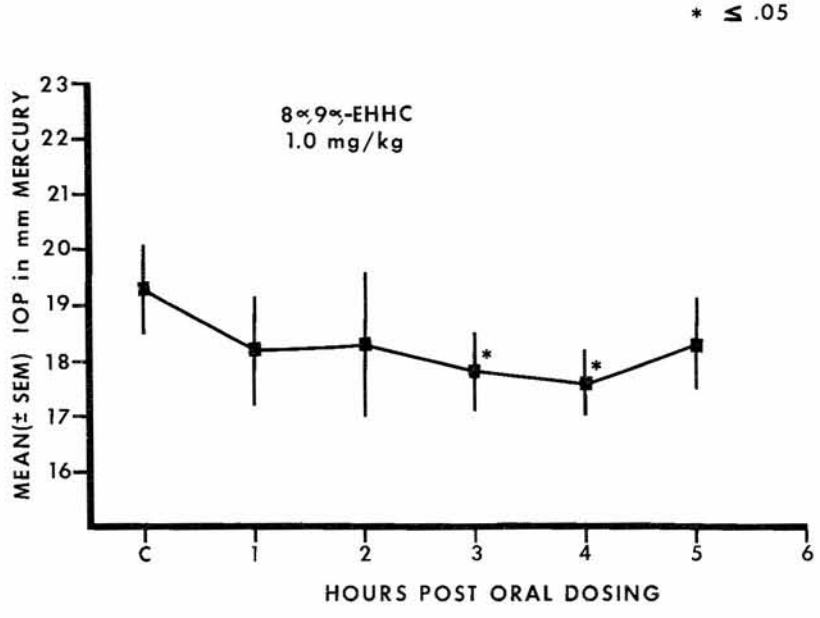


FIGURE 5. Effects on IOP of 8alpha,9alpha-EHHC at 1 mg/kg orally in 6 conscious Macaque monkeys. (*$p < 0.05$)

Dr. Carlton E. Turner for continued assistance and to Dr. Theodore Krupin, Department of Opthalmology, Washington University School of Medicine, for consulting with us on the screening procedures.

REFERENCES

1. Hepler, R. S., Frank, I. M., Marijuana smoking and intraocular pressure, J. Amer. Med. Assoc. 217:1392 (1971).
2. Hepler, R. S., and Petrus, R. J., Experiences with administraton of marijuana to glaucoma patients, in "The Therapeutic Potential of Marijuana" (S. Cohen and R. Stillman, eds.), pp. 63-75. Plenum Medial Book Co., New York, 1976.
3. Green, K., Current status of basic and clinical marijuana research in Opthalmology, in "Offprints from Symposium on Ocular Therapy", Vol. II (I. H. Leopold and R. P. Burns, eds.), pp. 37-49. Wiley, New York, 1979.
4. Marijuana and Health", (A. S. Relman, Chairman), pp. 140-142. Institute of Medicine, National Academy Press, Washington, D. C., 1982.
5. Waller, C. W.., Johnson, J. J., Buelke, J., and Turner, C. E., "Marijuana - An Annotated Bibliography", Vol. I. Macmillan, Riverside, N. J., 1976.
6. Waller, C. W., Nair, R. S., McAllister, A. F., Urbanek, B., and Turner, C. E, "Marijuana - An Annotated Bibliography", Vol. II. Macmillan, Riverside, N. J., 1982.
7. ElSohly, M. A., Harland, E., Murphy, J. C., Wirth, P., and Waller, C. W., Cannabinoids in glaucoma: A primary screening procedure, J. Clin. Pharm. 21:472S-478S (1981).
8. ElSohly, M. A., Harland, E., Murphy, J. C., Wirth, P., and Waller C. W., Cannabinoids in glaucoma II: The effects of different cannabinoids on intraocular pressure, A. Ph. A., Academy of Pharmaceutical Sciences 128th Annual Meeting, March 31, 1982.
9. Green, K., and Kim, K., Acute response of intraocular pressure to topical and oral cannabinoids, Proc. Soc. Exp. Biol. Med. 154:228-231 (1977).
10. Yamamoto, I., Narimatsu, S., Watanabe, K., and Yoshimura, H., Synthesis of 8alpha,9alpha- and 8beta,9beta-epoxyhexahydrocannabinols, Chem. Pharm. Bull. 29:3378-3381 (1981).
11. Mechoulam, R., Ben Zvi, Z., Varconi, H., and Samuelov Y., Cannabinoids rearrangements. Synthesis of delta-5-tetrahydrocannabinol, Tetrahedron 29:1615-19a (1973).
12. Siegal, S., "Nonparametric Statistics for the Behavioral Sciences". McGraw-Hill, New York, 1956.

13. Perez-Reyes, M., Wagner, D., Wall, M. E., and Davis K. H., Intravenous administration of cannabinoids and intraocular pressure, in "The Pharmacology of Marijuana" (M. C. Braude and S. Szara, eds.) pp. 829-832. Raven Press, New York, 1976.
14. Mechoulam, R., and Edery, H., Structure - activity relationship in the cannabinoids series, in "Marijuana, Chemistry, Pharmacology, Metabolism and Clinical Effects" (R. Mechoulam, ed.), pp. 101-136. Academic Press, New York, 1973.
15. Ohlsson, A., Widman, M., Carlsson, S., Ryman, T., and Strid, C., Plasma and brain levels of delta-6-THC and seven monooxygenated metabolites correlated to the cataleptic effect in the mouse, Acta Pharmacol. et Toxicol. 47:308-17 (1980).