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Intraocular Pressure, Organ Weights and the Chronic Use of Cannabinoid Derivatives in Rabbits for One Year

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Three synthetic cannabinoid derivatives were applied topically to rabbit eyes twice daily for a year and intraocular pressure and body weight were measured every two weeks. Twelve rabbits initially received each drug. The intraocular pressure always showed a fall of about 15–25%, depending on the drug, which was sustained for about 3 hr before rising slightly; topical administration of a second drop 4 hr after the first usually caused the intraocular pressure to return to the level of the maximum fall. No tolerance was noted since the intraocular pressure fall was the same at the end of the year as it was the first week. Organ weights were measured at the end of the year and revealed that the liver weight (g/100 g body weight) of all treated groups was significantly decreased. One drug, the least effective in terms of intraocular pressure reduction, caused a weight decrease in four of the seven organs examined. The most effective intraocular pressure-reducing drug (a water soluble compound) caused only a change in liver weight. Histological examination of tissues revealed no pathological changes that were related to the experimental treatment.

Key words: rabbit; intraocular pressure; organ weights; body weight; cannabinoids; chronic effects; ganglionectomy.

1. Introduction

Several studies have been made of the acute effects of the primary active ingredient of marihuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabinoid derivatives on intraocular pressure (IOP) in animals (Blumenthal, Yankelev and Dikstein, 1976; Dren, 1976; Green, Bigger, Kim and Bowman, 1977; Green and Bowman, 1976; Green and Kim, 1976, 1977). All of these studies have concentrated on the immediate effects of single topical applications of drugs to animal eyes, although Green et al. (1977) reported data following 60-day applications of synthetic cannabinoid derivatives to rabbit eyes. This study was continued with twice-daily drug administration over the following 10 months. Some other studies have been made of the relative toxicity of several new cannabinoids (Thompson and Yang, 1976), where severe ocular changes were described in dogs over a three day treatment period.

In preclinical tests, Δ^9-THC has been given by smoking or intragastric routes to rats over periods up to 180 days (Rosenkrantz and Braude, 1976). The doses used were relevant to human usage (4 mg/kg by inhalation) and the results of a variety of tests indicated changes in behavioral, physiological, biochemical and morphological characteristics. Organ weight increases were also found, particularly in the testes, adrenals, brain, kidneys, heart, liver and lungs, with respect to control animal organ weights over an 87-day test period.

The present study was undertaken to examine the chronic (one year) effects of daily topical administration of three new synthetic cannabinoids on intraocular pressure and organ weights of rabbits. The cannabinoid derivatives have been previously described (Green, 1977; Pars et al., 1976; Pars and Razdan, 1976a,b; Razdan et al., 1976).

2. Methods and Materials

Albino rabbits, New Zealand strain, weighing approximately 2 kg were obtained from a commercial supplier. Under urethane anesthesia (25% w/v in 0·9% NaCl solution, administered intravenously) the animals were subjected to left superior cervical ganglion ectomy. At 10 days post-surgery the completeness of the ganglionectomy was tested by topical application of 0·1% norepinephrine solution to both eyes. Ganglionectomized animals were divided into three groups of 12 rabbits per group and housed individually.

The drugs tested were SP-1, an oil soluble nitrogen containing-compound with the form of white crystalline solid; SP-106, which is a water-soluble compound with an ester group on the phenolic hydroxy and SP-204, which has a methyl group on the phenolic group and is also a basic, water soluble, ester (Pars et al., 1976; Razdan et al., 1976). The drugs were synthesized and supplied by SISA, Inc. of Cambridge, Massachusetts. These three compounds have been shown to have quantitatively different effects on a variety of pharmacological tests including induction of sedation, depression of motor activity and analgesia (Razdan et al., 1976).

The drugs were solubilized, to a concentration of 0·1% (Green et al., 1977), in one of two vehicles. The oil soluble SP-1 was dissolved in paraffin oil (Fisher Scientific Company) and the other two drugs were solubilized in a commercial artificial tear solution (Ultra-tears, Alcon Laboratories, Fort Worth, Texas). The drugs were made fresh twice weekly, and administered as a 50 μl drop. The drop was applied in either the normal or the ganglion-ectomized eyes, but always in the same eye on each successive occasion twice daily, on every working day. Initially, half of each group received the drop in the normal eye and half in the ganglionectomized eye; due to the loss of some animals this division could not be maintained. Since some animals died, primarily due to respiratory problems, the remaining rabbits were treated with tetracycline in the drinking water for four or five days each month as an effective prophylactic measure.

The IOP and total body weight of the animals were measured at approximately 2-week intervals through the study period, although body weights were not recorded over the first 6–10 weeks. The IOP was measured using an Alcon Pneumotonograph following the application of a drop of 0·5% proparacaine hydrochloride to the eye for 5–10 sec followed by washing with 1 ml of 0·9% NaCl solution. At the beginning of each day during which the IOP was determined, two series of IOP measurements were made 30 min apart to provide a base-line IOP for that day. A 50 μl drop of the drug was applied and the IOP was measured 30 min after drop application, at 1 hr and at each succeeding hour over the next 6 hr (giving a total of 7 hr of IOP measurements after the initial drug administration). A 50 μl drop was administered 4 hr after the first drop.

At the termination of the experiment two-thirds of each group were taken for the determination of organ weights. Animals were killed with carbon dioxide and various organs were immediately removed, emptied (if applicable) washed and weighed on a single pan Sartorius balance to the nearest 10 mg. Some tissues were taken from each animal, particularly those which showed large weight changes and fixed in 10% neutral-buffered formalin for histological examination. Normal rabbits of approximately the same weight and about 8–9 months old were used as controls for organ weight determinations.

3. Results

The average maximum fall in IOP in the normal and ganglionectomized eyes of each group of rabbits is shown in Table I. In normal eyes, the order of efficacy of the drugs in reducing IOP on a chronic basis is SP-106 > SP-1 > SP-204. In ganglion-ectomized eyes it is apparent that the differences between the drugs are not as clear and that the fall in IOP is not as great as found in the normal eye (Table I) but primarily the following order applied. SP-106 > SP-204 > SP-1. With both normal

and ganglionectomized eyes there is a reasonable degree of variation in the value for maximum fall in IOP from week to week, although the fall is always greater than 15%, with maxima up to 33% (in the normal eye). Despite the inherent variation, however, drug administration caused an IOP fall in both eyes and no evidence of tolerance is found. The baseline IOP for normal and ganglionectomized eyes was essentially unchanged from week 1 through the last measurement in all series of animals, with little week to week variation. Body weights were not determined until 6–10 weeks had passed but showed an asymptotic rise to a maximum.

TABLE I

Average maximum percentage fall in intraocular pressure

	SP-1		SP-106		SP-204	
Time in weeks	N	Gangx	N	Gangx	N	Gangx
0–10	24·5	9·9	21·5	9·5	16·6	8·4
	±2·1	±1·4	±1·5	±1·0	±0·5	±1·3
10–20	18·9	7·3	18·4	8·5	14·4	6·5
	±1·0	±0·5	±1·3	±0·8	±0·1	±1·0
20–30	18·3	8·7	19·8	8·4	17·9	9·0
	±0·9	±1·6	±1·7	±0·8	±0·7	±0·8
30–40	19·8	10·0	23·1	8·0	19·9	12·7
	±1·5	±0·7	±2·0	±1·2	±1·7	±1·2
40–50	19·7	13·9	21·8	13·3	15·5	13·5
	±0·5	±1·2	±1·9	±0·7	±0·9	±0·5

All values are the mean ± S.E.M.

The mean organ weights of the treated groups are given in Table II as both raw data and as a fraction of the body weight, together with data obtained from normal, untreated animals. The liver weight, irrespective of the animals sex or treatment, expressed either as total organ weight or g/100 g body weight is significantly lower than that of normal animals. In some cases the liver, as well as being smaller, tended to be friable and easily fractured. Gross tissue examination showed that the hearts of treated animals tended to be soft and to have a lack of the normal rigidity and firmness. The only other organ affected is the heart of SP-106 and SP-204 females which also shows a weight loss. The importance of other differences is unclear, especially due to the body weight disparity between groups. Histological examination of tissues taken from various organs revealed normal cellular distribution and patterns with no immediately obvious pathological changes caused by the drug treatment; the appearance of all organs sampled fell within the variability observed within the normal group.

4. Discussion

Some reports have been made of chronic marihuana use by smoking or oral administration in man (Hepler, Frank and Petrus, 1976; Jones and Benowitz, 1976). In one study, of 94 day duration, volunteers smoked a minimum of two marihuana cigarettes per day and were examined for ocular changes (Hepler et al., 1976). Obviously, systemic pathological changes were impossible to observe, although some

TABLE II

Body and organ weight for cannabinoid-treated and normal rabbits

	Normal		SP-106		SP-1		SP-204
	Male	Female	Male	Female	Male	Female	Female
Body weight	3·667± 0·066	4·340± 0·225	3·930	4·035± 0·343	4·310±0·203	4·824±0·228	4·978±0·147
Organs							
Gonads	9·87 ± 1·00	0·78 ± 0·19	7·50	0·77 ±0·13	9·16 ±0·81	0·53 ± 0·12	0·51 ±0·08
	(0·270± 0·031)	(0·018± 0·004)	(0·191)	(0·020±0·006)	(0·213±0·014)	(0·011± 0·002)	(0·010± 0·002)*
Kidneys	20·65 ± 2·73	29·39 ± 3·06	20·94	23·99 ±2·94	20·26 ±1·91	28·07 ± 2·26	24·43 ±1·20
	(0·565± 0·083)	(0·679± 0·066)	(0·533)	(0·629±0·143)	(0·473±0·053)	(0·586± 0·051)	(0·494 ±0·033)‡
Liver	147·44 ±18·37	184·76 ±20·84	83·55*	88·70 ±9·51‡	90·82 ±1·94‡	111·60 ±11·33‡	96·39 ±1·87†
	(4·041± 0·564)	(4·301± 0·513)	(2·127)‡	(2·205±0·175)‡	(2·115±0·099)‡	(2·301± 0·151)‡	(1·949 ±0·085)‡
Heart	9·09 ± 1·28	15·35 ± 3·19	8·00	7·57 ±0·39‡	7·62 ±0·60	8·48 ± 0·54	8·10 ±0·58‡
	(0·249± 0·039)	(0·370± 0·100)	(0·203)	(0·193±0·025	(0·178±0·009)	(0·175± 0·004)*	(0·162 ±0·010)‡
Lungs	27·38 ± 8·26	18·13 ± 5·55	11·11	11·43 ±0·48	12·72 ±1·11	12·07 ± 0·51	11·12 ± 0·39
	(0·755± 0·235)	(0·414± 0·121)	(0·283)	(0·291±0·032)	(0·294±0·016)	(0·252 ±0·012)	(0·223 ±0·003)
Brain	9·78 ± 0·25	10·25 ± 0·27	9·41	9·49 ±0·32	9·59 ±0·21	9·65 ± 0·10	9·86 ± 0·14
	(0·267± 0·008)	(0·239± 0·015)	(0·239)	(0·239±0·015)	(0·223±0·006)‡	(0·202± 0·011)*	(0·199 ±0·005)‡
Adrenals	0·38 ± 0·06	0·45 ± 0·11	0·45	0·63 ±0·12	0·79 ±0·10	0·58 ± 0·06	0·42 ± 0·07
	(0·011± 0·001)	(0·010± 0·002)	(0·011)	(0·017±0·005)	(0·018±0·002)‡	(0·012± 0·001)	(0·008 ±0·001)
n	3	5	2	4	3	5	6

Body weight is expressed as kg, organ weights are expressed as grams and in parentheses as g/100 g body weight.
All values are the mean ± S.E.M. (except for SP-106 males, where only two were utilized). Significance values are: †, $P < 0.001$; ‡, $0.001 < P < 0.05$; *, $0.05 < P < 0.1$.

of these volunteers were noted to have decreased circulating plasma testosterone levels (Kolodny, Lessin, Toro, Masters and Cohen, 1976). In the other study, oral Δ^9-THC was given over a 30-day period and tolerance to the drug was noted (Jones and Benowitz, 1976).

The initial base-line IOP was remarkably constant when measured throughout the year, with as little as ± 2 mmHg between the bi-weekly determinations. This result illustrates two points: (1) that the IOP had returned to base-line following the previous day-drop application regimen and (2) there were no long-term drug effects on the base-line IOP. In terms of the IOP, therefore, chronic drug use had no apparent effect except for the daily fall in IOP following drug administration. The average maximum fall in IOP for all drugs was greater than 15%, and for SP-1 and SP-106 was usually at or about 20% at the concentration employed (0·1%).

No tolerance was noted to any of the drugs since the fall in IOP was repeatable and showed no evidence of decreasing during the year (Table I). The fall in IOP showed a similar daily pattern on each occasion it was tested, namely the reduction in IOP occurred rapidly after the administration of the first drop and remained low for about 1 or 2 hr before beginning to increase. The addition of the second drop, 4 hr after the first caused the IOP to show a slight fall before rising again at the 7 hr cessation of measurement. There was no statistically significant difference between normal eyes which received a drop and those which did not (the drop was placed in the contralateral ganglionectomized eye in the latter animals). The onset of the fall in IOP, however, tended to be slightly later in its initiation in the eye which did not receive the drop. The results were similar in the ganglionectomized eyes, although the IOP fall was less than that in the normal eye (Table I).

The total organ weight data show that the only organ affected is the liver which loses weight after all drug treatments (Table II). When the data are expressed as g/100 g body weight, another factor is introduced which markedly influences the data. Data already in the literature (Jelenko, Anderson, Scott and Wheeler, 1971; Latimer and Sawin, 1955, 1957; Levine, Mann, Hodge, Ariel and DuPont, 1941) indicate that there is a tendency for organ weights (expressed as g/100 g body weight) to decrease as body weight increases. It is immediately obvious that part of the organ weight decrease (g/100 g body weight) with the drugs is caused by the high body weights which relatively decrease the organ weight/body weight ratio, and it is obvious from the total organ weight data that significant differences with the drugs only exist for liver and heart (Table II).

The order of efficacy of the drugs with regard to the fall in IOP was SP-106 $>$ SP-1 $>$ SP-204. The significance of these observations relates to the low daily dose of drug which was administered, which was only 0·1% twice daily. A 50 μl drop contained only 50 μg and in 150 ml of plasma (50 ml/kg body weight) would provide a peak plasma concentration, in the unlikely event of complete systemic absorption, of 33×10^{-5} mg/ml (plasma volume would be relatively unchanged with body weight). The total daily quantity of drug presented to the animal was 100 μg or 25–40 μg/kg (depending on the weight); at least one-hundredth of the daily dose of Δ^9-THC used by Rosenkrantz and Braude (1976) in rats (4 mg/kg).

It appears, therefore, that despite the fact that the IOP fall is both substantial as well as occurring daily in response to drug application over periods up to one year, other factors must be considered regarding the potential clinical use of such drugs. All the drugs tested in this series caused a decrease in liver weight relative to normal both on an absolute and relative basis, and one drug caused significant organ weight

decreases in four of seven organs measured on a relative basis. Such changes are important in terms of potential clinical utilization for they indicate that long-term use may cause deleterious changes in organ systems, although no pathological changes were seen in the histological examinations.

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