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Evaluation of Antiulcer Activity of Δ^9-Tetrahydrocannabinol in the Shay Rat Test

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Abstract. Δ^9-Tetrahydrocannabinol (THC) inhibited ulcer formation in the pylorus-ligated rat (Shay rat test). However, this antiulcer activity of THC was substantially less than for the anticholinergic substance tridihexethyl chloride both in terms of degree of activity and potency. In addition, the results of the present study suggested different mechanism(s) of action for the antiulcer effects of these substances.

The use of anticholinergic drugs such as tridihexethyl chloride as adjunctive therapy to diet and antacids in the treatment of ulcers is well documented (4). *Schwarz* (8) and *Lieberman and Lieberman* (6) reported clinical anticholinergic symptoms, including dry mouth, tachycardia and decreased salivation, after smoking or oral ingestion of marihuana or Δ^9-tetrahydrocannabinol (THC), its major psychoactive constituent (7). Moreover, *Bose et al.* (1) demonstrated *in vitro* anticholinergic activity of marihuana extract against acetylcholine-induced contractions of isolated rat intestine and uterine strips. Contrary to this, however, *Sofia et al.* (10) reported that THC was unable to block the muscarinic effects of tremorine in the mouse after doses as high as 80–120 times the effective dose of atropine.

Because of these observations suggesting an anticholinergic component of activity for marihuana or THC, the following studies utilizing the Shay rat antiulcer test were conducted. Tridihexethyl chloride was used as the positive anticholinergic standard test substance.

Materials and Methods

The experiments were conducted using male Charles River CD rats (Sprague-Dawley strain) weighing 200–300 g. Experiments were begun only after an acclimation period of at least 5 days to the laboratory environment which consisted of automatically controlled illumination with 12 h of light alternating with 12 h of dark, regulated temperature at 22–24 °C and a relative humidity range of 40–60%.

THC and tridihexethyl chloride were dissolved or suspended in undiluted propylene glycol and administered subcutaneously or orally in a constant volume of

1.0 ml/kg body weight. Control rats received a corresponding volume of the vehicle only.

Effects of each test substance on ulcer formation, mortality and gastric juice parameters including volume, pH and free and total acid content in rats with pylorus ligation described by *Shay et al.* (9) were studied. Groups of 10 rats each were utilized following a 48-hour fasting period during which time only water was available *ad libitum*. The surgical procedure was carried out under light ether anesthesia.

Drug effects were determined at two time intervals following ligation, i.e., 6 and 18 h. In the 6-hour test, drugs or vehicle were administered as single doses half an hour prior to pyloric ligation. In the 18-hour test similar doses were used, but half the total dose was given half an hour prior to ligation and the other half immediately following the surgical procedure. Animals dying during the course of the experiment were noted. 6 or 18 h following ligation of the pylorus, all surviving animals were sacrificed by an ether overdose and the intact stomach removed. The stomachs were opened along the greater curvature and examined under a dissecting microscope for the presence of ulcers in the rumen or non-glandular portion of the stomach. Stomachs exhibiting one or more ulcers were considered positive responders. These rumenal ulcers varied in size from tiny round necrotic lesions to large irregularly shaped necrotic areas or even perforations. For statistical comparison among groups, χ^2 analyses were performed.

Prior to the morphological evaluation described above, the gastric contents were collected and their volume (ml) and pH measured. Immediately thereafter, the contents were titrated with 0.1 *N* NaOH for free acid content using Toepfer's reagent and for total acid using 1.0% phenolphthalein as indicators. The

Table I. Comparative effects of THC and tridihexethyl chloride in the 6-hour pylorus-ligated rat

Test group and route of administration	Dose mg/kg	No. with ulcers / No. tested	Mortality	Gastric content parameters (mean ± SE)				
				volume ml	free acid		total acid	
					mEq/6 h sample time	mEq/liter	mEq/6 h sample time	mEq/liter
Subcutaneous								
Vehicle	–	9/10	0/10	12.0 ± 0.9	0.85 ± 0.06	72.6 ± 4.3	1.03 ± 0.10	87.5 ± 4.6
THC	100.0	1/10***	0/10	7.3 ± 1.3**	0.70 ± 0.09	104.8 ± 9.9**	0.83 ± 0.10	123.1 ± 10.2**
Tridihexethyl chloride	25.0	0/10***	0/10	2.1 ± 0.7***	0.00 ± 0.00***	0.0 ± 0.0***	0.06 ± 0.01***	23.0 ± 5.3***
Oral								
Vehicle	–	9/10	0/10	10.8 ± 1.2	0.60 ± 0.10	53.9 ± 6.2	0.77 ± 0.10	71.4 ± 5.6
THC	100.0	5/10*	0/10	9.3 ± 0.7	0.66 ± 0.08	71.2 ± 7.2	0.83 ± 0.08	90.7 ± 6.5*
Tridihexethyl chloride	25.0	0/10***	0/10	8.6 ± 1.1	0.18 ± 0.03***	20.6 ± 3.8***	0.31 ± 0.03***	38.9 ± 3.3***

When compared to respective vehicle control value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

results are expressed as mEq/sample time and mEq/liter. Mean (± SE) values were calculated and statistically evaluated using Student's t test.

Results

Table I reveals that 100.0 mg/kg of THC, whether administered subcutaneously or orally, produced marked reductions in gastric ulcer formation in the 6-hour pylorus-ligated rat. This protective effect of THC was more pronounced following subcutaneous administration. On the other hand, 25.0 mg/kg of tridihexethyl chloride given by either route of administration afforded complete protection against ulcer formation. Although 9 of 10 control rats in each experiment developed ulcers, no animals died. After subcutaneous drug administration both THC and tridihexethyl chloride significantly reduced gastric juice volume by 39.2 and 82.5%, respectively. However, this effect was less pronounced after oral administration. Table I shows that for tridihexethyl chloride a marked depression of both free and total acid concentration accompanied the decreased volume of gastric content. On the other hand, THC either increased or produced no change in the gastric free and total acid content, despite its depressant effect on volume.

Table II. Comparative effects of THC and tridihexethyl chloride in the 18-hour pylorus-ligated rat

Test group and route of administration	Dose mg/kg	No. with ulcers / No. tested	Mortality	Gastric content parameters (mean ± SE)				
				volume (ml)	free acid		total acid	
					mEq/6 h sample time	mEq/liter	mEq/6 h sample time	mEq/liter
Subcutaneous								
Vehicle	–	10/10	9/10	6.0 ± 1.1	0.12 ± 0.06	14.3 ± 7.2	0.31 ± 0.70	51.2 ± 6.4
THC	100.0	7/10	4/10*	5.3 ± 1.0	0.26 ± 0.10	47.6 ± 9.9**	0.38 ± 0.09	72.9 ± 9.3
Tridihexethyl chloride	25.0	0/10***	0/10***	6.1 ± 1.0	0.10 ± 0.05	19.7 ± 7.1	0.27 ± 0.07	49.0 ± 13.2
Oral								
Vehicle	–	10/10	9/10	2.8 ± 0.5	0.02 ± 0.02	11.7 ± 8.8	0.21 ± 0.03	80.3 ± 18.1
THC	100.0	10/10	8/10	10.8 ± 4.3	0.35 ± 0.32	20.7 ± 15.7	0.99 ± 0.55	81.0 ± 14.0
Tridihexethyl chloride	25.0	9/10	0/10***	11.8 ± 4.7	0.17 ± 0.07	13.3 ± 3.6	0.31 ± 0.10	45.9 ± 6.0

When compared to respective vehicle control value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the 18-hour pylorus-ligated rat experiments (table II) THC administered subcutaneously or orally (100.0 mg/kg) did not significantly reduce ulcer formation, but did reduce mortality by the former route of administration only. Unlike the 6-hour test (table I) THC had no significant effect on the volume of gastric content. However, a trend toward an increase in the concentration of free and total acid was observed in THC-treated rats. Using the same experimental design, we observed that tridihexethyl chloride completely inhibited ulcer formation and mortality after a subcutaneous dose of 25.0 mg/kg, whereas a similar oral dose only protected against mortality. Although all gastric content parameters remained statistically unaltered after oral administration of tridihexethyl chloride, a great deal of variability in the data was observed. This most likely resulted from increased lethal activity observed and higher incidence of perforated ulcers in the 18-hour pylorus-ligated rat.

Discussion

The results of this investigation suggest a potential antiulcer activity for THC in the Shay rat test. Activity was more pronounced after subcutaneous than oral administration and more effective in the 6-hour than the 18-hour pylorus-ligated rat. In all experiments THC was less potent and effective than the anticholinergic drug tridihexethyl chloride.

The mechanism for the antiulcer activity of THC, at least in the Shay rat, probably differs from that of the anticholinergic drug tridihexethyl chloride. One reason for this conclusion is the difference between these two compounds on free and total acid concentration of the gastric contents, despite the fact that both THC and tridihexethyl chloride significantly reduced gastric volume. Some published data supporting an anticholinergic component of activity for marihuana or THC are equivocal (1, 6, 8, 10); other data suggest a different effect. For example, *Layman and Milton* (5), using pure THC, demonstrated inhibition of the twitch response of the isolated guinea pig ileum to electrical stimulation, but no alteration in response to exogenously administered acetylcholine, suggesting blockade of acetylcholine release from nerve endings rather than an anticholinergic action. *In vivo* experiments by *Domino* (3) in cats showed that THC decreased neocortical release of acetylcholine. In addition, *Cavero et al.* (2) found parasympatholytic activity of THC in the dog attributable neither to alteration in ganglionic transmission nor to blockade of muscarinic receptors.

The results of the present study indicate that further experiments must be conducted to determine the mechanism for the antiulcer activity of THC.

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