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Anti-inflammatory activity of the steroid alkaloid glycoside, tomatine

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1. Tomatine, isolated from extracts of crown gall-infected tomato plants or obtained commercially, was tested for anti-inflammatory activity using three different methods.

2. Tomatine administered to intact rats intramuscularly in a dose range of 1–10 mg/kg or orally in doses of 15–30 mg/kg exerted a significant dose dependent inhibition of carrageenan induced paw oedema. The inhibitory effect of tomatine when given in a dose of 10 mg/kg intramuscularly to intact rats lasted more than 24 hr.

3. In adrenalectomized rats significant dose-related inhibition of paw oedema was obtained with tomatine and the inhibition at each dose level (0·5–10 mg/kg) was found to be greater than that found in intact animals.

4. Tomatine administered subcutaneously to intact rats daily for 7 days in doses of 5 or 10 mg/kg exerted a significant, dose dependent inhibition of granulation tissue formation induced by the subcutaneous implantation of carrageenan impregnated cotton pellets.

5. Tomatine administered to intact mice in a dose of 10 mg/kg subcutaneously 1 hr before the intraperitoneal injection of acidified saline and intravenous pontamine sky blue significantly decreased the leakage of the protein bound dye into the peritoneal cavity.

6. Tomatidine, the aglycone of tomatine, was not effective at dose levels of 10–20 mg/kg in any of the three tests.

The isolation of two antihistamine-like substances from crown gall-bearing tomato stems has been recently reported (Wakkary, Goodfriend & Kovacs, 1966) and one of the active principles has been identified as tomatine (Calam & Callow, 1964 ; Kovacs, Wakkary, Goodfriend & Rose, 1964 ; Wakkary *et al.*, 1969a). Tested on isolated organ preparations, tomatine was found to exert a non-specific effect in antagonizing, equally well, the contractions induced by histamine, bradykinin, SRS-A, 5-hydroxytryptamine and acetylcholine (Wakkary, Kovacs, Goodfriend & Rose, 1967). When injected into guinea-pigs, tomatine was found to be highly active in preventing the effects of intradermally injected histamine and bradykinin on capillary permeability and it exerted some protection against the effects of a lethal histamine aerosol (Wakkary *et al.*, 1967 ; Calam & Callow, 1964).

It is a widely accepted theory that, at least in the initial stage of inflammation,

the vasoactive amines (histamine, kinins, etc.) might play an important role as mediators (Wilhelm, 1962 ; Whitehouse, 1965). Since tomatine inhibited the effects of these substances it seemed of interest to investigate its possible action on inflammatory reactions.

The results presented in this paper deal with the effect of tomatine and its aglycone, tomatidine, in three different types of experimentally induced inflammation, and the data obtained are compared whenever possible with the effect of hydrocortisone or dexamethasone.

Methods

Male Wistar rats weighing 125–185 g, intact, or adrenalectomized 24 hr before use, and male albino CF No. 1 mice weighing 25–30 g were obtained from Canadian Breeding Laboratories (St. Constant, Quebec, Canada). All animals were fed on a standard Purina cube diet (except animals used for oral drug trials, which were fasted for 24 hours before testing) and water (or 1% saline if adrenalectomized) ad libitum both before and during experiments.

Tomatine produced in our laboratory and recrystallized at least five times or commercially prepared (Koch-Light Laboratories, Colnbrook, England) was dissolved in acidified distilled water (adjusted to pH 2·0 with 12 N hydrochloric acid) to give a concentration of 0·1–10 mg/ml. The final pH of the solution was 3·0 and it was stored at −20° C until required. Tomatidine (Koch-Light Laboratories, Colnbrook, England) was freshly suspended in a mixture of 1 part absolute ethanol and 2 parts distilled water (adjusted to pH 2·0 with 12 N hydrochloric acid) to give a concentration of 20 mg/ml. Hydrocortisone acetate suspension (Hydrocortone, Merck, Sharp & Dohme, Montreal, Canada) was diluted with sterile physiological saline from an initial concentration of 25 mg/ml. to 5 mg/ml. Dexamethasone (Decadron, Merck, Sharpe & Dohme, Montreal, Canada) was diluted with Decadron Vehicle (Merck, Sharp & Dohme, Montreal, Canada) from an initial concentration of 4 mg/ml. to 0·1 mg/ml.

Rat paw oedema formation

0·05 ml. of 1% (w/v) solution of carrageenan (Viscarin, Marine Colloids Inc., Springfield, New Jersey, U.S.A.), in sterile physiological saline was injected into the plantar surface of the left hind paws 1 or more hr after the administration of the drug or vehicle to groups of rats. Paw volumes were measured immediately and again 3 hr later, using the method of Harris & Spencer (1962) with slight modification. The animals were lightly anaesthetized with ether to ensure paw flaccidity. 1% aqueous Triton X-100 (A and C American Chemicals, Montreal, Canada) was used as the wetting agent. On the reservoir an additional line was drawn perpendicular to the horizontal line described by Harris & Spencer. The paw was so placed in the reservoir that the tip of the foremost toenail came to the intersection of these lines while the heel just rested against the reservoir wall. This procedure helped to ensure uniformly reproducible paw volume measurements.

Cotton pellet induced granuloma formation (Bush & Alexander, 1960)

Cylindrical cotton pellets (8 mm × 8 mm) cut from 14 cm absorbent cotton dental rolls were each soaked for 1 min in 1% (w/v) aqueous carrageenan (Viscarin,

Marine Colloids Inc., Springfield, New Jersey, U.S.A.), dried in air overnight on glass plates, paired ±2 mg and autoclaved for 1 hr at 30 lb/in². Pellets were implanted subcutaneously one into each axillary fold of groups of male rats under light ether anaesthesia. Each animal received 100,000 i.u. penicillin G potassium intramuscularly both on the day of implantation and 1 day later. The compound or the vehicle was then administered subcutaneously once daily for 7 days, the first dose being given immediately after implantation. Twenty-four hours after the final dose, the animals were killed and the pellets, together with the surrounding granulomatous tissue, were dissected out, dried at 60° C for 48 hours and weighed. If either granulomatous pellet was found to contain serum or blood, both were discarded. The extent of granulomatous tissue formation was calculated by subtracting the original pellet dry weight.

Peritoneal capillary permeability test (Northover, 1963)

Groups of mice received the compound or vehicle subcutaneously. Three hours later each mouse received 4 ml. of 0·05 N acetic acid in 0·9% saline intraperitoneally. followed as quickly as possible by 0·1 ml. of 4% pontamine sky blue intravenously. One hour after the administration of the dye the animals were killed and the peritoneal cavity was opened and drained. The exudate was centrifuged for 10 min at 2,000 rev/min and 0·5 ml. of the supernatant was diluted with 4·5 ml. of 0·9% saline. Dye concentrations were measured in a Perkin-Elmer spectrophotometer at 625 mμ, saline being used as a blank. The mean percentage of light absorption was then calculated for both the treated and control groups.

Student's *t* test was used to evaluate the results in all experiments.

Results

Rat paw oedema

Groups of twenty intact rats were given tomatine in doses of 0·1, 1, 5 or 10 mg/kg ; tomatidine in a dose of 20 mg/kg, hydrocortisone in a dose of 5 mg/kg, or dexamethasone in a dose of 0·1 mg/kg intramuscularly, 1 hr before the administration of carrageenan into the left hind paw. Identical numbers of animals received the corresponding vehicles. The results obtained in these experiments are summarized in Table 1. Tomatidine given in doses of 1 mg/kg or more induced a significant dose dependent inhibition of paw swelling. In this test, the anti-oedema effects of

TABLE 1. *Comparative inhibitory effects of intramuscularly administered tomatine, tomatidine, hydrocortisone and dexamethasone on carrageenan-induced paw oedema formation in intact rats*

Drug	Dose (mg/kg)	Number of animals		Mean paw volume Increase in ml. ± S.D.		% Inhibition of paw swelling	*P* value
		Test	Control	Test	Control		
Tomatine	10	20	20	0·29 ± 0·11	0·51 ± 0·14	44·6	< 0·001
Tomatine*	5	20	20	0·26 ± 0·12	0·39 ± 0·12	31·6	< 0·01
Tomatine†	5	20	20	0·31 ± 0·16	0·48 ± 0·15	35·4	< 0·01
Tomatine	1	20	20	0·43 ± 0·11	0·53 ± 0·18	19·3	< 0·05
Tomatine	0·1	20	20	0·40 ± 0·12	0·37 ± 0·13	−7·9	—
Hydrocortisone	5	20	20	0·23 ± 0·15	0·32 ± 0·12	28·6	< 0·05
Dexamethasone	0·1	20	20	0·18 ± 0·12	0·55 ± 0·16	67·6	< 0·001
Tomatidine	20	19	20	0·40 ± 0·13	0·45 ± 0·12	11·1	N.S.

* Tomatine isolated from crown gall-infected tomato stalks in our laboratory.
† Commercial tomatine (Koch-Light Laboratories).

5 mg/kg of tomatine and hydrocortisone were approximately equal. Tomatidine, on the other hand, in the dose administered, did not exert significant anti-oedema effects.

In order to see if the effect of tomatine was mediated through stimulation of the adrenals, a second set of experiments was performed. Table 2 summarizes the results obtained when groups of twenty adrenalectomized rats received tomatine in doses of 0·1, 0·5, 1, 5 or 10 mg/kg, hydrocortisone in a dose of 5 mg/kg, or the corresponding amount of vehicle (controls) intramuscularly 1 hr before the carrageenan administration. Again, tomatine administered in doses of 0·5 mg/kg or more brought about a significant dose dependent inhibition of oedema formation. Further, in these experiments tomatine appeared to be much more potent than hydrocortisone.

The next series of experiments, the results of which are shown in Table 3, was designed to determine the duration of the oedema inhibitory effect of tomatine. Carrageenan was injected 8, 24 and 48 hr after tomatine 10 mg/kg or the vehicle (controls) was given intramuscularly to one or more groups of twenty rats. The activity of tomatine was not significantly reduced after 8 or 24 hr and a slight inhibitory effect could perhaps be observed even after 48 hr.

To find out if tomatine would also be effective when administered orally, groups of twenty animals received 15 or 30 mg/kg of tomatine or the same volume of vehicle by stomach tube 1 hr before the carrageenan injection.

TABLE 2. *Comparative inhibitory effects of intramuscularly administered tomatine and hydrocortisone on carrageenan-induced paw oedema formation in adrenalectomized rats*

Drug	Dose (mg/kg)	Number of animals		Mean paw volume Increase in ml. ± S.D.		% Inhibition of paw swelling	*P* value
		Test	Control	Test	Control		
Tomatine	10	20	20	0·13±0·12	0·42±0·10	68·4	<0·001
Tomatine	5	20	20	0·26±0·10	0·47±0·11	44·4	<0·001
Tomatine	1	20	20	0·16±0·12	0·38±0·10	57·5	<0·001
Tomatine	0·5	20	20	0·38±0·13	0·48±0·11	20·5	<0·02
Tomatine	0·1	20	20	0·34±0·11	0·33±0·09	−0·9	—
Hydrocortisone	5	20	20	0·25±0·12	0·38±0·15	34·2	<0·01

TABLE 3. *Effect of pretreatment time on the inhibitory action of intramuscularly administered tomatine on carrageenan induced paw oedema in intact rats*

Drug	Pre-treatment time (hr)	Dose (mg/kg)	Number of animals		Mean paw volume Increase in ml. ± S.D.		% Inhibition of paw swelling	*P* value
			Test	Control	Test	Control		
Tomatine	8	10	20	20	0·26±0·13	0·46±0·16	43·5	<0·001
Tomatine	24	10	20	20	0·24±0·13	0·36±0·14	33·3	<0·01
Tomatine	48	10	30	28	0·37±0·20	0·39±0·20	5·1	N.S.

TABLE 4. *Inhibitory effect of orally administered tomatine on carrageenan-induced paw oedema in intact rats*

Drug	Dose (mg/kg)	Number of animals		Mean paw volume Increase in ml. ± S.D.		% Inhibition of paw swelling	*P* value
		Test	Control	Test	Control		
Tomatine	30	20	20	0·28±0·13	0·43±0·13	36·3	<0·001
Tomatine	15	20	20	0·33±0·13	0·41±0·13	19·5	=0·05

TABLE 5. *Effects of tomatine, tomatidine and dexamethasone on granulation tissue formation in intact rats*

Drug administered subcutaneously	Dose (mg/kg)	Number of animals		Mean % weight change of rats		Mean dry weight of granulation tissue in g. ± S.D.		% Inhibition of granulation tissue formation	*P* value
		Test	Control	Test	Control	Test	Control		
Tomatine	10	22	19	+9·2	+12·2	0·102±0·037	0·137±0·039	25·5	<0·001
Tomatine	5	15	20	+12·0	+15·2	0·123±0·036	0·141±0·032	12·8	<0·05
Tomatidine	10	11	14	+12·9	+4·9	0·126±0·025	0·121±0·034	−4·1	N.S.
Dexamethasone	0·1	18	10	−15·3	+13·8	0·053±0·027	0·153±0·037	65·4	<0·001

The results obtained are summarized in Table 4. As can be seen, orally administered tomatine in the dose range used exerted a significant dose dependent inhibitory activity upon oedema formation.

Cotton pellet induced granuloma formation

Groups of ten rats were treated daily for 7 days with tomatine in a dose of 5 or 10 mg/kg, tomatidine in a dose of 10 mg/kg, dexamethasone in a dose of 0·1 mg/kg, or the corresponding vehicle subcutaneously. Table 5 summarizes the results obtained. At both dose levels tomatidine induced a significant inhibition of granulation tissue formation. The effect was dose dependent, but as the data show, in this test tomatine was much less potent than dexamethasone. The daily injections were well tolerated by the animals and at the time of pellet removal no signs of local purulence were found at the site of the tomatine injection. Tomatidine in the dose administered did not inhibit granulation tissue formation. Animals treated with dexamethasone lost about 15% of their original body weights, whereas all of the other animals which were treated with tomatine, tomatidine or the various vehicles showed an average weight gain of about 12%.

Peritoneal capillary permeability test

Groups of 11 mice were injected subcutaneously with 1, 5 or 10 mg/kg of tomatine, 10 or 20 mg/kg of tomatidine or the corresponding vehicle in a total volume of 0·15 ml. Table 6 summarizes the absorption at 625 mμ for the diluted peritoneal fluids. Only at a dose of 10 mg/kg, did tomatidine significantly inhibit the leakage of dye into the peritoneal cavity while tomatidine in the dose range administered did not exert any significant inhibitory activity.

Discussion

The results obtained in these experiments indicate that tomatidine not only exhibits a fairly potent anti-inflammatory activity in the tests used but also manifests several rather unusual properties not previously described for any other anti-inflammatory compound in general use. The anti-inflammatory activity of tomatine was most extensively studied in the carrageenan-induced rat paw oedema test because it permits a valid quantitative assay and because most of the known anti-inflammatory drugs seem to possess anti-carrageenan activity (Winter, Risley & Nuss, 1962 ; 1963 ; Niemegeers, Verbruggen & Janssen, 1964 ; Winter, 1964).

In its oedema inhibiting activity, tomatine showed a good dose-response relation in the dose range used. In adrenalectomized animals at each dose level tested, the

TABLE 6. *Influence of tomatine and tomatidine on capillary permeability in the mouse peritoneum*

Drug administered subcutaneously	Dose (mg/kg)	Number of animals		Absorbance at 625 mμ ± S.D.		% Inhibition of dye leakage	*P* value
		Test	Control	Test	Control		
Tomatine	10	20	19	0·216 ± 0·095	0·319 ± 0·139	34·4	< 0·01
Tomatine	5	21	21	0·444 ± 0·157	0·470 ± 0·158	5·5	N.S.
Tomatine	1	19	19	0·406 ± 0·142	0·377 ± 0·182	−7·7	N.S.
Tomatidine	10	20	20	0·408 ± 0·178	0·457 ± 0·231	10·7	N.S.
Tomatidine	20	21	21	0·371 ± 0·144	0·409 ± 0·147	8·8	N.S.

relative percentage inhibition brought about by tomatine was higher than in intact animals. These results are very unusual and at present cannot be explained.

Another unusual result was the long duration of action of a single dose of tomatine in inhibiting carrageenan-induced rat paw oedema. This effect was, however, anticipated since Wakkary *et al.* (1967) have previously demonstrated a very long-lasting but reversible effect of tomatine in the inhibition of histamine or bradykinin induced smooth muscle contractions.

In order to evaluate the possible anti-inflammatory activity of tomatine and tomatidine on the later stages of inflammation, the widely used carrageenan impregnated cotton pellet granuloma test was selected. Granulation tissue formation has been shown to be depressed by nearly all anti-inflammatory drugs, but the steroids are most active while phenylbutazone and salicylates show poor activity and chloroquine is almost inactive (Whitehouse, 1965 ; Adams & Cobb, 1967). The effect of tomatine in suppressing granulation tissue formation seems to be similar to that of steroids, since the inhibition of the granuloma formation was directly proportional to the dose of tomatine used. Unlike the dexamethasone treated animals, which lost about 15% of their original body weights by the end of the experiment, the animals treated with tomatine and tomatidine showed no change in body weight as compared with controls. Tomatine therefore appears to be effective in suppressing granulation tissue growth without overt toxic effects.

Tomatine has a steroid nucleus, and yet the entire molecule may be functioning in a manner which differs from that of the steroid compounds. Consequently, as a further test, the mouse peritoneal capillary permeability test of Northover (1963) was chosen, since it has been shown (Northover, 1963, 1964) that steroids are ineffective in this situation while non-steroid substances are generally active.

Tomatine was found to exert a significant inhibition of dye leakage when given in a dose of 10 mg/kg subcutaneously. Therefore, according to the criteria of the method in this test, tomatine seems to act like a non-steroid drug.

Interestingly, tomatidine, the aglycone of tomatine, showed no significant inhibitory activity in any of the three tests. Previously, Calan and Callow (1964) also observed that tomatidine, unlike tomatine, did not protect animals against the lethal effects of a histamine aerosol.

The present studies did not include systemic toxicological investigations, but Wilson, Poley & De Eds (1961) showed that tomatine is a relatively non-toxic agent. It has also been shown by several groups of workers that tomatine exhibits antifungal and antibacterial activity (Irving, Fontaine & Doolittle, 1945 ; Schuster & Tarrade, 1969). Should tomatine prove to be effective as an anti-inflammatory agent in humans without exerting the serious side effects observed with both the steroid and non-steroid anti-inflammatory drugs, it may be of use in the treatment of chronic inflammatory diseases.

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