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The effect of levamisole on gastric ulcers induced in the rat by anti-inflammatory and necrotizing agents

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Levamisole, in doses similar to those required to observe immunostimulation in animals, antagonized dose-dependently, in rats, gastric ulcers produced by necrotizing agents (HCl and ethanol) and NSAIDs (indomethacin and piroxicam) without affecting the anti-inflammatory properties of these latter drugs.

Levamisole, which possesses immunopotentiating, thymomimetic and antianergic properties (Symoens & Rosenthal 1977; Symoens et al 1979; Renoux 1980; Schnieden 1981) is currently used in association with non-steroidal anti-inflammatory drugs (NSAIDs) for treatment of rheumatoid arthritis in patients with persistently active or progressive disease that failed to respond to NSAIDs (Huskinsson & Adams 1980). Since levamisole stimulates healing of skin lesions in patients with intertrigo inguinalis as well as surgical wounds in guinea-pigs (Symoens et al 1979), it appeared worthwhile to determine its effects on NSAID-induced gastric lesions and anti-oedema properties as well as gastric lesions induced by necrotizing agents such as ethanol and HCl.

Materials and methods

Male albino rats, Sprague-Dawley Morini strain, 180–200 g, were housed in plastic cages with wire bottom to minimize coprophagy. Indomethacin and piroxicam were suspended in an aqueous vehicle containing NaCl 0·9%, Tween 80 0·4%, carboxymethylcellulose 0·5% and benzyl alcohol 0·9% while levamisole was dissolved in H_2O. All substances were administered by gavage in a volume of 5 ml kg^{-1}.

Effect of levamisole on necrotizing agents induced gastric lesions. After 24 h fasting the animals received levamisole (10–20–40–80 mg kg^{-1}) and 30 min later 1 ml of absolute ethanol or 0·6 M HCl was administered by gavage (Robert et al 1979). One h later the animals were autopsied and degree and incidence of gastric ulcers scored according to Del Soldato et al (1979).

Effect of levamisole on indomethacin or piroxicam induced gastric ulcers. After an overnight fasting the animals received oral indomethacin (15 mg kg^{-1}) or piroxicam (100 mg kg^{-1}) with or without levamisole (10–20–40 mg kg^{-1}). Animals were autopsied 5 h after oral dosing and degree and incidence of gastric ulceration scored according to Del Soldato et al (1979).

Effect of levamisole on indomethacin or piroxicam anti-oedema properties. Paw oedema was induced according to Winter et al (1962) by injecting into the plantar aponeurosis of right hind paw 0·1 ml of 0·5% suspension of carrageenan. Paw volume was measured immediately before carrageenan and again 3 h later by means of a mercury plethysmometer. Indomethacin (1 mg kg^{-1}) or piroxicam (2 mg kg^{-1}) with or without levamisole (40 mg kg^{-1}) were administered by gavage 1 h before carrageenan.

Statistical analysis. Percent incidence and degree of gastric lesions were analysed according to the Chi square method. Percent inhibition values relative to

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Table 1. Effect of levamisole on necrotizing agents induced gastric lesions.

Lesion model	Treatment (mg kg^{-1} oral)	n =	Gastric ulcers: Percentage incidence		Gastric ulcers: Degree (mean score)	
HCl 0·6 M	Control	30	86·6	ED50 and 95%	1·46	ED50 and 95%
	Levamisole 10	22	72·7	c.l. = 25·9 (12·2–	1·18*	c.l. = 18·6 (17·0–
	Levamisole 20	20	60	55·0)	0·65**	20·2)
	Levamisole 40	20	25***		0·25***	
Ethanol	Control	32	93·7		2·50	
	Levamisole 20	20	89·6		1·79*	
	Levamisole 40	29	80		1·45***	
	Levamisole 80	22	77·2		1·45***	

* $P < 0·05$ compared with control group. ** $P < 0·01$ compared with control group. *** $P < 0·001$ compared with control group.

Table 2. Effect of levamisole on indomethacin or piroxicam-induced gastric ulcers.

Treatment	Dose mg kg^{-1} oral	n =	Gastric ulcers % Incidence		Degree (mean score)	
Indomethacin	15	19	78·9	ED50 and 95%	1·26	ED50 and 95%
Indomethacin + levamisole	15 + 10	20	50	c.l. = 27·1 (15·0–	0·71	c.l. = 19·5 (10·4–
Indomethacin + levamisole	15 + 20	20	40*	49·3)	0·60*	36·7)
Indomethacin + levamisole	15 + 40	19	36·8*		0·57*	
Piroxicam	100	18	100	ED50 and 95%	2·77	ED50 and 95%
Piroxicam + levamisole	100 + 10	18	100	c.l. = 26·5 (17·6–	2·60	c.l. = 18·4 (8·0–
Piroxicam + levamisole	100 + 20	18	44***	39·7)	0·55***	42·7)
Piroxicam + levamisole	100 + 40	18	22***		0·33***	

* $P < 0·05$ compared with control group. *** $P < 0·001$ compared with control group.

incidence and degree of gastric ulcers (treated vs control animals) were plotted against the log dose and regression analysis calculated according to the least square method. ED50 and 95% confidence limits were determined according to Litchfield & Wilcoxon (1949). Data relative to carrageenan paw oedema were analysed by means of Student's *t*-test for unpaired data.

Results and discussion

Levamisole reduced both incidence and degree of HCl-, indomethacin- or piroxicam-induced gastric lesions (Tables 1, 2). The degree, but not incidence of ethanol-induced gastric lesions (Table 1), was reduced by the concomitant administration of levamisole (Table 1). Administered in doses that effectively antagonized gastric ulceration, levamisole neither affected paw oedema formation nor the anti-inflammatory properties of indomethacin or piroxicam (Table 3).

The observation that levamisole reduces experimentally-induced gastric lesions at doses that do not possess antisecretory properties (unpublished observation) suggests that it exerts a cytoprotective action on rat gastric mucosa similar to that described for sodium salicylate (Robert 1981) and prostaglandins (Robert et al 1979). Although the mechanism(s) responsible for cytoprotection is as yet unknown (Robert 1979), non-protein sulfhydryls appear to be involved in gastric cytoprotection (Szabo et al 1981). This hypothesis is consistent with the observation that sulfhydryl-containing substances protect rats from ethanol-induced gastric erosions (Szabo et al 1981). Levamisole has a sulphur-containing ring which is easily cleaved in-vitro and possibly in-vivo giving rise to thiol OMPI ((±)-2-oxo-3-(2-mercapto-ethyl)-5-phenyl-imidazoline) which is even more effective than levamisole in protecting glutathione-depleted cells from auto-oxidative necrosis (De Brabander et al 1979). In view of this, it is tempting to speculate that the formation of thiol OMPI is the mechanism responsible for the cytoprotective effect exhibited by levamisole.

The observation that levamisole also reduces NSAID-induced gastric ulcers might be interpreted as an indication that it counteracts prostaglandin imbalance produced by NSAIDs (Whittle 1981; Ezer et al 1976). Although the effective doses of levamisole used in this study are in excess of those used in man (Symoens et al 1979; Huskisson & Adams 1980), they are in line with those necessary to induce immunostimulation in animal models (Symoens et al 1979). In addition, levamisole antagonized indomethacin- or piroxicam-induced gastric ulcers in doses that did not affect their antioedema properties. These findings suggest that it could be interesting to determine whether patients being treated with levamisole in combination with NSAIDs have a lower incidence of gastric distress and/or ulceration than those treated with NSAIDs alone.

Table 3. Effect of levamisole on indomethacin or piroxicam antioedema properties.

Treatment	Dose (mg kg^{-1})	n =	Paw volume increase after carrageenan (mm-Hg) mean ± s.e.	Inhibition %
Control	—	44	12·11 ± 0·86	—
Levamisole	40	18	11·66 ± 0·98	3·7
Indomethacin	1	20	9·30 ± 0·53*	23·2
Indomethacin + levamisole	1 + 40	20	9·75 ± 0·89*	19·5
Piroxicam	2	24	8·29 ± 0·78**	31·5
Piroxicam + levamisole	2 + 40	24	7·35 ± 0·82**	39·3

* $P < 0·05$ compared with control group.
** $P < 0·01$ compared with control group.

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The effect of garlic extracts on contractions of rat gastric fundus and human platelet aggregation

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Garlic has been extracted and separated chromatographically into various fractions which show different degrees of activity as inhibitors of platelet aggregation and smooth muscle. The most potent smooth muscle inhibitor fraction had little activity on platelet aggregation, but μg ml^{-1} concentrations greatly reduced the contractions of rat gastric fundus to prostaglandin E_2 and acetylcholine. Material in this fraction may contribute to some of the claimed therapeutic effects of garlic involving smooth muscle. Its identity is not known, but is different from allyl sulphide, dimethyl sulphide and diallyl disulphide. These compounds eluted earlier on liquid chromatography than the most active fraction, and they showed only modest inhibitory activity against prostaglandin E_2 and acetylcholine on rat fundus.

Garlic (*Allium sativum* L) has been used world-wide as a folk medicine since the time of Hippocrates (Culpeper 1653). It is still used today both for prophylaxis and treatment of various diseases including infections and vascular disorders (Martindale's Extra Pharmacopoeia, 1982). The antibacterial and antifungal properties are due to allicin (diallyl disulphide) (Cavallito & Bailey 1954; Tansey & Appleton 1975). Garlic extract can also reduce serum cholesterol levels, increase plasma fibrinolytic activity and increase blood coagulation time (Bordia et al 1975, 1977). More recent work shows that garlic extracts reduce platelet aggregation by inhibiting thromboxane synthesis (Makheja et al 1979, 1980), and inhibit prostaglandin synthetase prepared from sheep seminal vesicles (Vanderhoek et al 1980). Of the garlic oil components that inhibit platelet aggregation, methyl allyl trisulphide was identified as the most potent (Ariga et al 1981). Lio & Agnoli (1927) reported that garlic briefly stimulates and then depresses smooth muscle. We have fractionated garlic extracts, and obtained material that preferentially blocks responses of rat gastric fundus to prostaglandin E_2 (PGE_2) and acetylcholine (ACh).

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Methods

Extraction of fresh garlic cloves. In two separate extractions fresh garlic cloves were dehusked, chopped finely and homogenized in 0·15 M NaCl (saline) at 4 °C (138 g Extract 1, and 158 g Extract 2 in 400 ml). Garlic oil was obtained by extraction with chloroform (400 ml × 2) followed by rotary evaporation under reduced pressure, the yield being about 2·8 mg oil g^{-1} garlic cloves.

Silicic acid column chromatography. Garlic oil (40 mg) was loaded on to a silicic acid column (40 g) which was eluted sequentially with hexane : diethyl ether, 9 : 1, 4 : 1, 2 : 1, 1 : 1, ether, and methanol (200 ml). The eluant was monitored at 225 nm in a Pye Unicam spectrophotometer (SP6-550) fitted with 100 μl flow cell. Twelve 100 ml fractions were collected, evaporated to dryness, dissolved in 1·5 ml diethyl ether and placed in preweighed tubes. The ether was blown off using oxygen-free nitrogen and the tubes reweighed. Each fraction was then made up as a suspension using a rotary whirler to give a fine dispersion in saline.

Commercial garlic oil prepared by steam distillation (Zimmerman Hobbs and Hofel Pure Foods), allyl sulphide, and dimethyl disulphide (Sigma), and diallyl disulphide (ICN Pharmaceuticals Inc) were chromatographed similarly.

Bioassay. Fractions, in saline, were assayed for antagonist activity against PGE_2 on rat fundus strips bathed in Krebs solution at 37 °C and bubbled with 5% CO_2 in O_2. Amine-blocking drugs (hyoscine, mepyramine, methysergide, phenoxybenzamine and pronethalol) and indomethacin (0·2, 0·2, 0·1, 0·1, 1 and 1 μg ml respectively) were included in these experiments to increase selectivity and sensitivity of the assay (Bennett et al 1973). Consistent submaximal control contractions to PGE_2 were obtained using a 10 min cycle time. One aliquot of a garlic fraction was added after a response to PGE_2 had returned to baseline following washout, and