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Anti-inflammatory activity of a polysaccharidic fraction of *Echinacea angustifolia*

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The anti-inflammatory activity of a polysaccharidic fraction (EPF) obtained from *Echinacea angustifolia* roots has been examined using the carrageenan paw oedema and the croton oil ear test. EPF (0·5 mg kg^{-1} i.v.) almost inhibited the carrageenan-induced oedema over 8 h and furthermore, EPF, topically applied, inhibited mouse ear oedema induced by croton oil. EPF also reduced the leukocytic infiltration of the croton oil dermatitis, evaluated both as peroxidase activity and histologically. After topical application EPF appears to be slightly inferior in potency to indomethacin. The results suggest that the anti-inflammatory activity of *E. angustifolia* resides in its polysaccharidic content.

Root preparations of *Echinacea purpurea* and *Echinacea angustifolia* have been used for the treatment of wounds, burns and other cutaneous affections, and to treat disorders such as viral infections, cutaneous illnesses and acute and chronic disease due to a deficiency of immunological responses. Also the cosmetic use of *Echinacea* preparations is widespread.

Polysaccharides present in the preparations were thought responsible for some responses. Bonadeo et al (1971) proposed that the roots' skin-repairing action may be due to the formation of a complex between hyaluronic acid and a polysaccharidic principle of the plant content.

Wagner et al (1984) demonstrated that a polysaccharidic fraction of *Echinacea purpurea* possessed immunostimulating properties. Purified polysaccharides obtained from *E. purpurea* were shown also to activate macrophages strongly (Stimpel et al 1984).

We found (Tragni et al 1985) that a partially purified aqueous extract of *E. angustifolia* (EAE), topically applied, inhibited the croton oil-induced oedema in mouse ear and that the intravenous administration of the extract inhibited carrageenan-induced oedema in the hind paw of the rat.

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The aim of the present work has been to verify if the activity can be ascribed to the polysaccharidic content of the plant.

Materials and methods

Chemicals. λ-Carrageenan, croton oil, indomethacin and guaiacol (*o*-methoxyphenol) were purchased from Sigma Chemical Co., St. Louis, MO, USA, hexadecyltrimethylammonium bromide (HTAB) was from Eastman Kodak Co., NY, USA, and ketamine hydrochloride from Parke Davis, Milan, Italy.

Preparation of the polysaccharidic fraction (EPF). The polysaccharidic fraction was obtained as described by Wagner et al (1984): 400 g of powdered roots of *E. angustifolia* yielded 1·2 g of EPF.

Animals. Male Sprague-Dawley rats (200 g) and male CD1 albino Swiss mice (28–32 g) (Charles River, Calco, Italy) were maintained on a standard laboratory diet with free access to tap-water. The animals were kept for at least two weeks at constant temperature (22 ± 1 °C) and humidity (50–60%) in an artificially illuminated room in the dark from 1900–0700h.

Carrageenan paw oedema. Paw oedema was induced by injection of 50 μL of 1% λ-carrageenan in 0·9% sodium chloride in the plantar region of the right hind paw of rats. Oedema was determined immediately after injection and at 1 h intervals thereafter, using a mercury plethismograph, as described by Winter (1965). EPF was injected intravenously 1 h before carrageenan injection at two doses: 0·5 or 0·1 mg kg^{-1}. Control animals received saline i.v.

Croton oil dermatitis. The inflammation was induced in anaesthetized mice (ketamine HCl 150 mg kg^{-1} i.p.) by application of 35 μg of croton oil dissolved in 15 μL of acetone to the inner surface of the right ear. EPF, and indomethacin as reference drug, were dissolved in the

irritant solution. Mice were killed 6 or 18 h later and a sample of 6 mm diameter was taken from both treated and untreated ears. The inflammatory response was monitored by measuring the differences in weight between the two samples as an index of the oedematous response.

The degree of granulocyte infiltration was assessed by measuring the peroxidase activity of the same samples homogenized in saline, containing 0·1% of HTAB. After centrifugation at 15 000*g* for 5 min, the supernatants (which contained more than 95% of the peroxidase activity) were assessed according to Dri et al (1982) and activity expressed as nmoles of tetraguaiacol min^{-1} at 25 °C.

Histological examination. The ear samples were fixed with 10% (v/v) formalin, embedded in paraffin and stained with haematoxylin and eosin.

Statistics. Data were analysed by means of the Dunnett test or the analysis of variance. $P < 0.05$ was accepted as significant.

Results

EPF after intravenous administration had a potent inhibitory effect on the carrageenan paw oedema. The two doses significantly reduced the oedema over the 8 h observation period. The higher dose (0·5 mg kg^{-1}) almost completely inhibited the response. The effect was quantified by calculating the areas under the curves (AUC) showed in Fig. 1. The ratio between AUC for treated animals and AUC for controls may therefore represent an index of the inhibition of the oedematous phenomenon. This evaluation gives 89·7 and 64·6% inhibition for the 0·5 and 0·1 mg kg^{-1} doses, respectively.

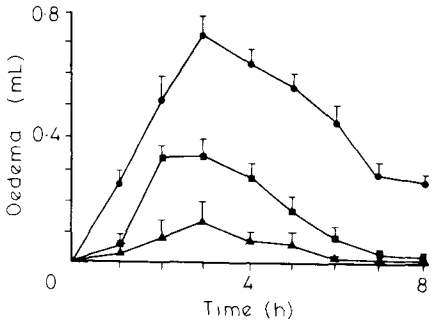

FIG. 1. Inhibitory effect of EPF on carrageenan oedema in rats. EPF was injected intravenously 1 h before carrageenan in rats. ● control (saline alone); ▲ EPF 0·5 mg kg^{-1}; ■ EPF 0·1 mg kg^{-1}. Each point represents the mean of 8 animals.

EPF also had a topical effect on the mouse ear. After 6 h contact (when the oedema of croton oil-treated ears reaches its maximum), both EPF and indomethacin induced a dose-dependent reduction of the oedematous response (Table 1). To compare the potency of EPF with that of indomethacin, the dose inhibiting 50% of the oedema (ID50) was determined for both substances by linear regression of the dose-response data. The values (μg/ear) are: EPF 100·8, indomethacin 41·6. Indomethacin appears to be about twice as active as EPF.

Table 1. Effect on oedematous response of EPF and indomethacin after 6 h induction of croton oil dermatitis.

Substances	Doses μg/ear	n	Oedema mg ± s.e.	Reduc. (%)	$P <$ *
EPF	0	39	8·0 ± 0·2	–	–
	15	14	7·4 ± 0·4	7·5	n.s.
	45	20	5·8 ± 0·5	27·5	0·001
	150	14	3·7 ± 0·6	53·8	0·001
	300	14	0·8 ± 0·2	90·0	0·001
	450	14	0·3 ± 0·1	96·3	0·001
Indomethacin	0	25	7·9 ± 0·2	–	–
	15	14	5·8 ± 0·5	26·6	0·001
	30	14	5·0 ± 0·4	36·7	0·001
	60	14	3·1 ± 0·4	60·8	0·001
	120	13	2·1 ± 0·2	73·4	0·001
	240	14	0·1 ± 0·1	98·7	0·001

* *P* at analysis of variance.

As expected for non-steroidal anti-inflammatory drugs (Tubaro et al 1985), neither EPF nor indomethacin affected the oedematous response after 18 h (data not shown).

To obtain further information about the topical anti-inflammatory activity of EPF, its influence on leukocytic infiltration was also evaluated. The analysis was carried out 18 h after the induction of inflammation, since previous studies have shown that, at this time, the leukocytic response to croton oil reaches its maximum (Tubaro et al 1985). The number of neutrophils in the inflamed ears was evaluated as peroxidase activity found in the tissues, as proposed by Bradely et al (1982) (Table 2). Both EPF and indomethacin induced a dose-dependent reduction of the granulocyte infiltration, with ID50 values higher than those related to the oedema inhibition (μg/ear EPF 184·8, indomethacin 121·3).

To substantiate the enzymatic data on leukocyte infiltration 18 h after treatment, histological examination showed a marked infiltration of neutrophils into the

Table 2. Effect on peroxidase activity of EPF and indomethacin after 18 h induction of croton oil dermatitis.

Substances	Doses μg/ear	n	Units* m ± s.e.	Reduc. (%)	$P <$ **
EPF	0	27	68·4 ± 7·1	–	–
	45	14	65·8 ± 8·2	3·8	n.s.
	150	14	38·0 ± 5·3	44·4	0·001
	450	13	15·0 ± 1·9	78·1	0·001
Indomethacin	0	26	54·9 ± 5·7	–	–
	45	14	37·7 ± 3·4	31·3	0·01
	150	13	26·9 ± 5·2	51·0	0·001
	450	13	11·2 ± 2·8	79·6	0·001

* U: nmoles of tetraguaiacol min^{-1} at 25 °C.
** *P* at analysis of variance.

dermal interstitium after the irritant alone, which was dramatically reduced when EPF (450 μg/ear) was applied with the croton oil. This demonstrated that the reduction in peroxidase activity observed was due to inhibition of leukocyte migration rather than to suppression of their enzymatic activity.

Discussion

The anti-inflammatory activity of EPF was tested with the same irritative tests used to evaluate the activity of EAE. The comparison of the inhibition of carrageenan paw oedema showed that 10 mg kg^{-1} of EAE induced 50% global inhibition (Tragni et al 1985), whereas 0·1 mg kg^{-1} of EPF caused 65% inhibition. It is evident that EPF is about 100 times more potent than EAE in reducing the response to the carrageenan paw oedema.

Comparison of the data previously obtained on the inhibition of croton oil-induced oedema by EAE (Tragni et al 1985) shows that after 6 h contact it had lower activity than the polysaccharidic fraction (*ID50* > 450 and ID50 = 100 μg/ear, respectively). However, the difference is not so marked as with carrageenan oedema, possibly because of the different route employed (i.v.).

The vasodilation and the vascular permeability changes associated with the oedematous response are only signs of an inflammatory reaction, the central feature being the infiltration of the cells into the injured tissue (Larsen & Henson 1983). Since the croton oil-induced dermatitis allows the effects of drugs on both the vascular and cellular phenomena to be examined (Tubaro et al 1985), it represents a suitable model for the evaluation of anti-inflammatory activity. EPF reduces both reactions with ID50 values slightly higher than those of indomethacin.

From our results it may be hypothesized that the EPF mechanism of anti-inflammatory activity could be similar to that of indomethacin on oxidation products of the arachidonic acid metabolism. This does not exclude the fact that EPF may interfere with other mediators involved in the inflammatory reaction.

As the data supplied indicate, the polysaccharidic fraction from *Echinacea angustifolia* has been shown to exert a noticeable anti-inflammatory activity both after systemic administration and topically. Polysaccharides may be considered as the main active principles involved in the anti-inflammatory activity of *E. angustifolia*.

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