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Percutaneous absorption and anti-inflammatory activity of indomethacin in ointment

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Indomethacin is widely used in therapy for various inflammatory diseases. In an attempt to avoid the systemic side effects, we have prepared a topical preparation of the drug and tested its effects on inflamed tissue in experimental models.

Materials and methods

Materials. The preparation was a gel made by dissolving indomethacin (0·3–3%) in a gel base containing water, ethanol, propylene glycol and carboxyvinyl polymer (Nagai et al 1979). We also prepared reference ointments of ibuprofen and phenylbutazone in petroleum jelly and purchased other ointments.

Carrageenan-induced paw oedema. Experimental carrageenan oedema (Winter et al 1962) was induced in groups of seven male Wistar strain rats. The gel, 100 mg, was applied to the surface of the right hind paw. The gel without the drug was similarly applied to a control group. The treated area was immediately covered by thin vinyl sheet and gauze. Two hours later, the covers were removed and 1% carrageenan solution was injected subcutaneously into the treated area. Three hours later the rats were killed and the paws were excised at the joint and weighed. Oedema weight was calculated from the difference between the injected and non-injected paw.

To study the influence of the gel on the left foot, oedema of both hind paws was induced by carrageenan and its volume was measured hourly using a plethysmometer (Ugo Basile, Italy) while the gel was applied only to the right foot.

To study percutaneous absorption and distribution of the drug in inflamed tissue, right hind paws of five male Wistar strain rats were treated with 100 mg ointment containing 1% of [^{14}C]indomethacin as above. The remaining ointment was removed and 1% carrageenan solution was injected subcutaneously in both hind paws 2 h after application. Three hours later the rats were decapitated and systemic blood, local exudate and muscle from inflamed tissues was collected for assay of their radioactivity.

Adjuvant arthritis. Adjuvant arthritis was induced by injection of killed and dried *M. butyricum* (Difco Lab. USA) suspended in paraffin oil into female Sprague-Dawley rats (Graeme et al 1966). Rats with established adjuvant arthritis were taken 14 days after the injection of adjuvant (7 rats in each group) and gel was applied to the right hind paw for 2 h daily for 5 days, as described above. The placebo gel was applied to control animals. The right hind paw volume of these treated rats was measured before ointment application, as well as on the 4th and 6th days following the initiation of therapy, as carrageenan oedema method.

Ultraviolet-induced erythema. Nine Hartley strain guinea-pigs in each group were depilated and 2 h later, 50 mg of gel was applied and again 1 h later, while the control group was treated with placebo gel. The animals were put in a rubber glove with four holes (diameter, 9 mm) at point corresponding to the middle of an animal's back 1 h after a second application of gel. The animals were then exposed to ultraviolet light 18 cm away for 5 min using a Sun lamp (Fuji Roentgen, Tokyo). Two hours after irradiation, the erythema intensity was graded by three observers unaware of the treatment, according to the scale: 0—no redness; 1—minimum erythema; 2—more pronounced erythema; 3—intense erythema.

Results

Inhibitory effects on carrageenan oedema and percutaneous absorption of indomethacin gel. The gel with 0·3–3% drug showed dose-dependent inhibition (Table 1). At 1%, the gel produced significant inhibitory effects and the activity

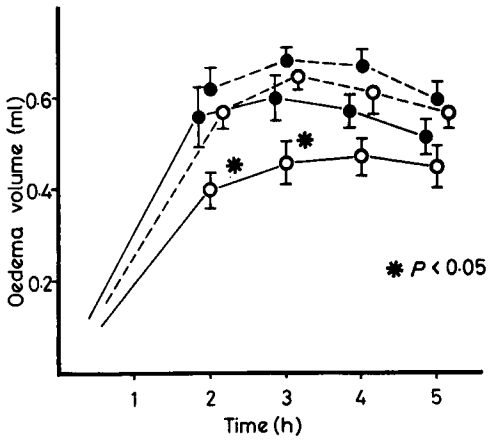

FIG. 1. Effect on carrageenan-induced paw oedema of both feet of indomethacin ointment applied topically to one foot and its influence on the contralateral non-treated foot. Control animals were treated with placebo ointment. ●——● and ● - - - ● placebo ointment treated foot and contralateral non-treated foot, respectively; ○——○ and ○ - - - ○ IND gel treated foot and contralateral non-treated foot, respectively. Means ± s.e. of 12 rats.

* Correspondence.

Table 1. Dose-response of indomethacin ointment applied topically on carrageenan-induced oedema in rats.

Sample		Oedema weight (g)	Inhibition (%)
Control		0·64 ± 0·04	
Indomethacin gel	0·3%	0·61 ± 0·03	4·7
	1%	0·52 ± 0·03*	18·8
	3%	0·39 ± 0·02***	39·1

Control animals were applied with placebo ointment. Each value indicates the mean ± s.e. of 12 rats.
* $P < 0·05$, * $P < 0·001$ compared with the control.

Table 2. Inhibitory effect of indomethacin and reference ointments applied topically on carrageenan-induced oedema in rats.

Sample	Concentration (%)	Inhibition (%)
Indomethacin	1	22·2*
Ibuprofen	3	21·5*
Phenylbutazone	5	14·4
Mobilat		17·9*
Prednisolone valerate acetate	0·3	22·6*
Betamethasone valerate	0·12	19·7*
Hydrocortisone butyrate	0·1	9·7
Beclomethasone propionate	0·025	23·4*

* $P < 0·05$ as compared with the control.

Table 3. Concentration of indomethacin in carrageenan-induced paw oedema by topical application of [^{14}C]indomethacin ointment in rats.

	Concentration (μg equivalents of indomethacin g^{-1})		
	Exudate	Muscle	Blood
Applied foot	60·4 ± 15·5	6·09 ± 1·47	0·84 ± 0·13
Non-applied foot	1·68 ± 0·23	0·61 ± 0·08	

Each value indicates the mean ± s.e. of 5 rats.

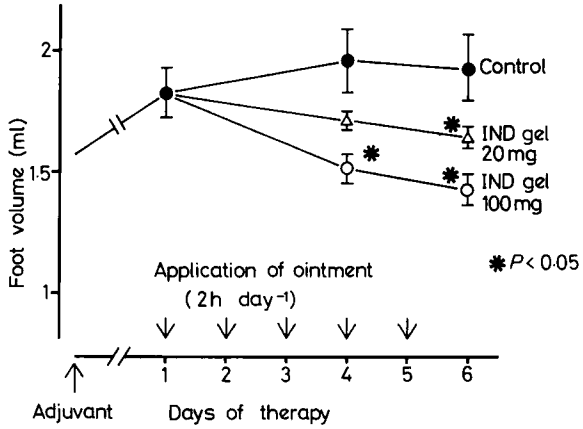

FIG. 2. Therapeutic effect of indomethacin ointment applied topically to foot on established adjuvant arthritis in rats. Control animals were treated with placebo ointment. Each mark represents the mean ± s.e. of 7 rats.

was approximately equivalent to or more effective than that of the reference ointments (Table 2). In this experiment, the placebo gel and the petroleum jelly base had no effect on carrageenan oedema. The gel significantly inhibited inflammation only in the treated paw but had no influence on oedema of non-applied paw (Fig. 1). The placebo gel also did not modify the response of the paw to carrageenan-induced oedema. When the gel containing 1% of [^{14}C]indomethacin was used, the concentration of radioactivity was very high in the exudate and muscle of the treated paw but was scarcely detectable in the tissue of the other paw or in the blood stream (Table 3).

Effects on adjuvant arthritis and ultraviolet-induced erythema. When the gel was applied to paws with established adjuvant arthritis for 2 h daily for 5 days, therapeutic effects were observed at doses of 20 mg. After application of 100 mg gel daily, the therapeutic effects were observed by the 4th days (Fig. 2).

The gel also reduced the ultraviolet-induced erythema in guinea-pigs (Fig. 3).

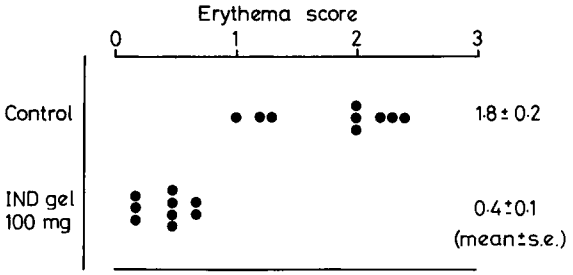

FIG. 3. Effect of indomethacin ointment applied topically on the ultraviolet-induced erythema in guinea-pigs. Each circle represents the erythema score of treated animals. Control animals were treated with placebo ointment.

Discussion

Ishihama et al (1979) reported that indomethacin penetrated percutaneously into the skin and muscles after topical application of the gel containing 1% drug to guinea-pigs. This study has also shown that indomethacin penetrated percutaneously into the inflammatory site, and that its preparation produced local effects in rats. But the effects did not extend to the contralateral paw tissues and the blood level of drug was markedly less than that following oral administration, as reported by Hucker et al (1966). The gel also had a definite effect in the therapy of adjuvant arthritis and in suppressing ultraviolet-induced erythema in guinea-pigs.

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