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Inhibition of contact sensitivity in the mouse by topical application of corticosteroids

D. P. EVANS, MARGARET HOSSACK AND D. S. THOMSON

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

Summary

1. Topical application of oxazolone to the ears of mice previously sensitized to this hapten resulted in a marked increase in ear weight 24 h later.

2. Topical application of hydrocortisone, betamethasone 17-valerate, fluocinolone acetonide or triamcinolone acetonide inhibited this response in a dose dependent manner.

3. The free alcohols of the three fluorinated compounds were only weakly active.

4. Activity in this model was truly local. Oral administration of hydrocortisone or fluocinolone acetonide at doses comparable to those used topically failed to inhibit the ear swelling.

5. Some steroids with poor anti-inflammatory properties had no significant effect.

Introduction

Topical application of steroids is used for the treatment of various skin complaints such as eczema and contact dermatitis. The laboratory evaluation of the local anti-inflammatory activity of these compounds has involved models such as the granuloma pouch (Dipasquale, Rassaert & McDougall, 1970) or ear irritation induced by croton oil (Tonelli, Thibault & Ringler, 1965). There is a continuing search, however, for an additional model having a closer aetiological resemblance to human disease which might be even more predictive of a clinical response.

Contact sensitivity to simple haptens can readily be provoked in laboratory animals, but few workers have used this approach for assessing the activity of topically applied corticosteroids. Asherson & Ptak (1968) have described the use of oxazolone (4-ethoxymethylene-5-oxo-2-phenyl-4,5-dihydrooxazole) for inducing contact sensitivity in the mouse. The animals were sensitized by painting the shaved abdomen with the agent and challenged 7 days later by application of oxazolone to the ears. The severity of the reaction was assessed by measuring the thickness of the inflamed ear 24 h later. This procedure appeared to be a possible basis for the laboratory study of the topical effect of steroids and experiments designed to assess this possibility are reported below.

Methods

The experiments were carried out in adult mice (20–30 g) of either sex (S.P.F. random-bred colony at Alderley Park) which were allowed free access to water and food. In each experiment, the weights of the mice used were within a 2 g range.

Oxazolone was prepared by the method of Cornforth (1949) and was stored at +4° C in a desiccator. Due to its potent sensitizing properties, disposable plastic gloves were worn when the compound or the treated animals were handled. It was dissolved in olive oil B.P. to a concentration of 2% w/v with gentle warming (*c.* 50° C). The abdominal region of the mice was carefully shaved with electric clippers and 0·1 ml of the oxazolone solution applied with a syringe and gently rubbed into the shaved area. Seven days after sensitization, 10 μl of an oxazolone solution in acetone (2% w/v) was applied to one or both ears using a Micro-repette syringe assembly (Jencons). In experiments in which oxazolone was applied to only one ear, acetone was applied to the other.

The anti-inflammatory drugs used in this study were hydrocortisone (B.D.H. Ltd.), betamethasone alcohol and betamethasone 17-valerate (Glaxo Laboratories Ltd.), fluocinolone alcohol (Syntex Research), fluocinolone acetonide (I.C.I. Ltd., Pharmaceuticals Division), triamcinolone alcohol and triamcinolone acetonide (Lederle Laboratories Ltd.). In addition, aldosterone (Sigma), corticosterone and deoxycorticosterone (Steraloids), oestradiol (B.D.H. Ltd.), progesterone (Searle) and testosterone proprionate (Organon) were tested. The steroids were dissolved in acetone at double the concentration required and mixed with an equal volume of 4% oxazolone in acetone. Preliminary experiments established that, in the case of hydrocortisone, betamethasone 17-valerate and fluocinolone acetonide at least, chemical interaction between oxazolone and the steroids was not responsible for the anti-allergic activity of the steroids since the separate formulation and application of the sensitizing agent and the drug produced identical responses to those following a single application of oxazolone plus steroid in the same solution.

Twenty-four hours after challenge, or at other times as indicated in the text, the animals were killed with chloroform. Both ears were removed by cutting along the base and weighed on a torsion balance. This procedure was more reliable than measuring ear thickness with a micrometer.

Results

In preliminary experiments twelve groups of ten unsensitized mice of either sex were used. To one ear of each animal acetone was applied and to the other a 2% solution of oxazolone in acetone. The animals were killed 6, 24 or 48 h later and the ears removed and weighed. There was no significant difference in the various groups between the mean weights of the ears treated with oxazolone alone and the untreated ears ($P<0·5$, Student's t test). Only in one group of female mice killed 24 h after the treatment was the mean weight of the oxazolone treated ears 30% ($P<0·01$) higher than that of the control (acetone treated) ears. These results confirm the findings of Dietrich & Hess (1970) that oxazolone is only mildly irritant in the mouse.

In a second experiment, groups of ten mice of either sex were sensitized with oxazolone and challenged on one ear 7 days later. Acetone alone was applied to

the other ear. The different groups were killed 6, 24 or 48 h later, the ears removed and weighed. In all groups (Table 1) the mean weights of the acetone treated ears were significantly lower ($P<0.05$) than those of the oxazolone treated ears.

The maximal reaction to oxazolone occurred at 24 h in both sexes and this time was therefore chosen to study the action of topically applied corticosteroids in subsequent experiments. Both male and female mice gave adequate responses to oxazolone.

Effect of topically applied anti-inflammatory steroids

Groups of five sensitized mice of either sex were used. Each animal was challenged by the application to one ear of oxazolone solutions containing steroids in different concentrations and to the other of acetone alone. Control animals received oxazolone alone on one ear and acetone on the other. Six experiments

TABLE 1. *Ear weights of mice sensitized to and challenged with oxazolone*

Sex	Time (h after challenge)	Treatment	Mean ear weight (mg+S.E.M.)
Male	6	Acetone	57·2±6·1
		Acetone+oxazolone	108·0±9·5
	24	Acetone	61·0±2·3
		Acetone+oxazolone	109·9±5·7
	48	Acetone	56·3±3·5
		Acetone+oxazolone	77·7±3·8
Female	6	Acetone	56·6±1·8
		Acetone+oxazolone	106·1±2·8
	24	Acetone	66·8±1·7
		Acetone+oxazolone	119·6±4·8
	48	Acetone	64·1±3·5
		Acetone+oxazolone	98·6±5·4

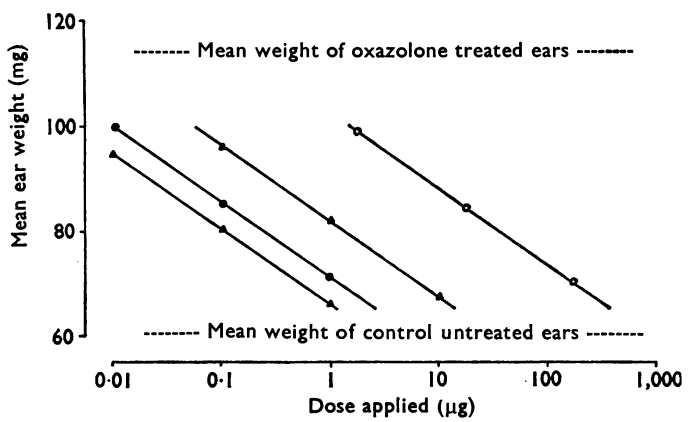

FIG. 1. Inhibitory effect of betamethasone 17-valerate (△——△), fluocinolone acetonide (▲——▲), hydrocortisone (○——○) and triamcinolone acetonide (●——●) on oxazolone-induced ear swelling. Regression lines calculated from the results of six experiments (thirty mice at each point). The relative potencies (hydrocortisone=1) were: betamethasone 17-valerate 38 (19–76); fluocinolone acetonide 481 (242–960); triamcinolone acetonide 213 (113–402). 95% confidence limits are in parentheses.

were performed and regression lines (Fig. 1) calculated by the method of least squares (Finney, 1964). Based on these data an estimate of potencies relative to hydrocortisone was made.

Comparison of local and systemic activity of topically applied steroids

Experiments were carried out in order to determine whether the ability of topically applied steroids to reduce oxazolone-induced ear swelling was due to a true local activity or whether it was necessary for the drug to be absorbed systemically to an appreciable extent before producing its effect. The drug could be absorbed into the general circulation after topical application, or it could be rubbed off the ear on to the paws during the normal washing habits of the animal and then ingested orally. If the steroids were absorbed from the ear to reach a sufficiently high plasma concentration then their application to the normal, uninflamed ear should inhibit oxazolone-induced swelling on the other ear. Thus in groups of five sensitized mice, oxazolone was applied to one ear and acetone containing varying amounts of fluocinolone acetonide (0·01, 0·1 and 10 μg) or hydrocortisone (2·5, 25 and 250 μg) to the other. The mean weights of the oxazolone treated ears from those groups that had received drug on the opposite ear did not differ significantly ($P=0·5$) from the mean ear weight of the oxazolone treated ears of a control group receiving only acetone on the other ear.

As it has been shown that percutaneous absorption of corticosteroids is often enhanced in the presence of inflammation (Fitzpatrick, Griswold & Hicks, 1955), in the second series, oxazolone was applied to both ears and fluocinolone acetonide or hydrocortisone, at the same doses as in the previous experiment, to only one ear. Application of either drug to one inflamed ear resulted in a dose dependent reduction in weight of that ear, but this was not accompanied by a concomitant reduction in the weight of the ear to which the drug was not applied.

To determine whether the steroids given orally were capable of reducing oxazolone-induced ear swelling, two experiments were performed using groups of five sensitized mice. Hydrocortisone (0·25 and 2·5 mg/mouse) or fluocinolone acetonide (0·001 and 0·01 mg/mouse), suspended in Dispersol*, were administered orally by a stomach tube at the same time as oxazolone was applied to both ears. A control group received oxazolone on both ears and either hydrocortisone (250 μg) or fluocinolone acetonide (1 μg) on one. The largest dose administered orally was chosen to be 10 times greater than that given topically. Only in those groups to which the drugs were applied topically was there any significant reduction in mean ear weight ($P<0·01$). Thus the ability of topically applied steroids to inhibit oxazolone sensitivity was due to local as opposed to systemic activity.

Comparison of the effect of C_{16}-C_{17} substituted steroid derivatives and the free alcohols

In man, betamethasone and triamcinolone as the free alcohols are much less active as anti-inflammatory agents than betamethasone 17-valerate and triamcinolone acetonide, the corresponding C_{16}-C_{17} substituted derivatives (McKenzie & Atkinson,

*'Dispersol': 'Lissapol' NX 1 ml, 'Lissapol' C 1 g and 'Dispersol' O.G. (30%) 3·3 ml (each from I.C.I.) per l., adjusted to pH 7.

1964; Schlagel, 1965). Therefore the ability of these compounds to inhibit oxazolone hypersensitivity in the mouse was compared with that of the free alcohols. Two experiments were performed. Each animal received acetone on one ear and oxazolone with the steroid alcohol or the substituted steroid on the other. Control animals received oxazolone alone. The combined results in Fig. 2 are expressed in terms of percentage inhibition.

In the case of fluocinolone and triamcinolone, the free alcohol showed little activity. Betamethasone as the free alcohol showed a dose-dependent reduction in the mean ear weight but was approximately 10 times less active than its 17-valerate.

Effect of some miscellaneous steroids on oxazolone-induced ear swelling

Groups of ten mice were treated with several steroids (aldosterone: 50 μg; corticosterone: 250 μg; deoxycorticosterone: 250 μg; progesterone: 100 μg; oestradiol: 250 μg; testosterone proprionate: 250 μg) having little if any anti-inflammatory activity. Of these compounds, only corticosterone showed a significant ($P<0.05$) anti-allergic action (37% inhibition) at 250 μg, but was much less active than hydrocortisone (cf. Fig. 1).

Discussion

Although the skin of man is different from that of the common laboratory animals and hence the ease with which drugs will penetrate to the deeper layers might be different, useful information can still be gleaned from animal experiments. There is a need for a technique suitable for the preliminary assessment of the effects of anti-inflammatory agents after topical application which is simple and reliable. The induction of contact sensitivity in the mouse using oxazolone is a straightforward procedure with a 100% 'take' and the increase in the size of the treated ear over the normal is of the order of 100%. The technique gave reproducible

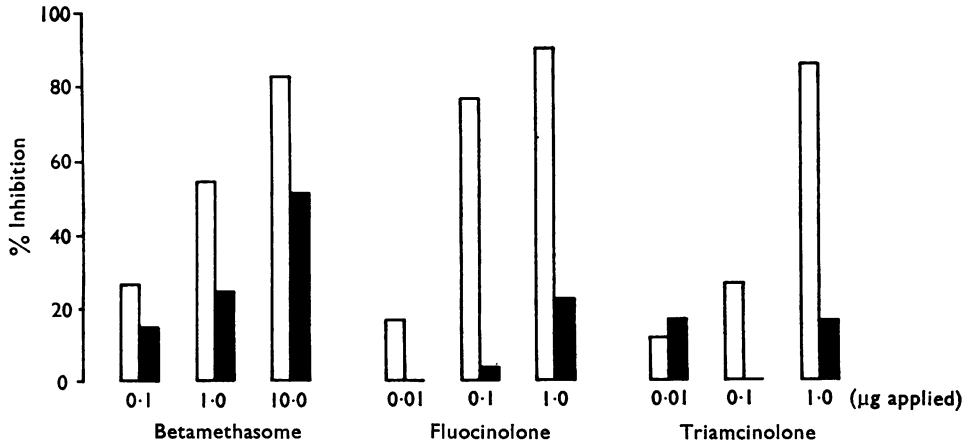

FIG. 2. Comparison of the activities on oxazolone sensitivity of betamethasone 17-valerate, fluocinolone acetonide and triamcinolone acetonide (open columns) with the corresponding free alcohol (solid columns). The results are the mean from two experiments (a total of ten mice per group). Percentage inhibition was calculated from: percentage inhibition $=100[a-b)/(a-c)]$, where a=mean oxazolone treated ear weight (control); b=mean oxazolone and drug treated ear weight; and c=mean weight of ears treated with acetone.

results and standard drugs showed a regular dose-dependent inhibition of the inflammatory response. Assessment of the anti-inflammatory potency of steroids in man is difficult. McKenzie (1962) and McKenzie & Atkinson (1964) have ranked them in the order: fluocinolone acetonide>triamcinolone acetonide>betamethasone 17-valerate>>hydrocortisone. A similar rank order of activity was found in inhibiting oxazolone hypersensitivity in the mouse although the potencies relative to hydrocortisone were much higher (see Fig. 1). The lower activity in mice of the free alcohols of betamethasone, fluocinolone and triamcinolone compared to their C_{16}-C_{17} substituted derivatives agrees with the observations in man. The local mode of action of hydrocortisone and fluocinolone acetonide in contrast to a systemic one was established by their failure to inhibit the ear swelling if applied to the opposite ear and by their ineffectiveness after oral administration at relatively high doses.

The use of chemical sensitizing agents does involve some hazard to the experimenter due to the risk of skin sensitization following, for example, accidental spillage. Precautions, including the wearing of gloves and safety glasses and the rinsing of contaminated glassware with dilute ammonia, which degrades the compound to non-sensitizing products (D. M. O'Mant, personal communication), must be adopted.

Dietrich & Hess (1970) have shown that during the development of oxazolone-induced ear swelling in the mouse, histological changes similar to those in allergic and toxic dermatitis occur. The cellular infiltration is primarily mononuclear and probably represents a typical type IV allergic response mediated by appropriately sensitized lymphocytes. These authors have studied the ability of steroids and immunosuppressive agents to inhibit oxazolone hypersensitivity after systemic administration. Their results complement our findings on the activity of steroids following topical application.

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