

Acetone has anti-inflammatory effects on experimental contact reactions

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The effects of a topically applied corticosteroid and its acetone vehicle on experimental allergic, toxic and irritant reactions are presented. The corticosteroid budesonide in acetone or acetone alone was applied to reactions immediately after and at different time intervals within the 1st h after provocation. Classical naked eye observation was performed and the dermal cellular infiltrate was differentiated and counted using a previously well-characterized method. "Treatment", whether with the steroid in acetone or acetone alone, had anti-inflammatory effects. For all reaction types, erythema and oedema diminished and a significant decrease in mononuclear cells was seen, when application occurred within the first 5 min after provocation. The effects were most marked for the toxic reaction to croton oil, the steroid and the vehicle being anti-inflammatory to the same extent. Application up to 60 min after provocation had anti-inflammatory effects for this reaction type. The mechanisms of acetone's anti-inflammatory effects are at present unclear. One possible explanation is that intercellular lipid organisation and, by extension, cellular membrane lipid organisation, are altered, influencing membrane receptor function. Possible anti-inflammatory effects of acetone should be considered in experimental and perhaps even clinical situations. Further investigation of the therapeutic possibilities of the finding seems warranted.

Key words: cellular infiltrate; organic solvents; corticosteroids; allergic; toxic; irritant; mononuclear cells; acetone; anti-inflammatory effect. © Munksgaard, 1999.

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The present grading of topical corticosteroid is based on the vasoconstriction response in normal skin (1), a physiological phenomenon not necessarily central to the most usual use for topical corticosteroids, the treatment of inflammatory dermatoses including contact eczema. The guinea pig is a traditional experimental animal for the study of contact reactions, since the naked eye appearance and the histology of the skin reaction is in principle the same as in man (2-4). Animals can be sensitized and local allergic inflammatory skin reactions provoked in test areas. By applying irritant and toxic chemical substances, other types of inflammatory reactions can be induced. By studying groups of animals, the provoked reaction can, more easily than in human experimentation, be studied microscopically at many different time points. We have previously developed a guinea pig model in which microscopic grading, i.e., differential counting of the infiltrating cells in the upper dermis, can be compared to the macroscopic assessment of redness and oedema (5-8). Using this experimental model, attempts were made (9) to grade the efficacy of topical corticosteroids in cream and ointment bases. This proved to be difficult, one problem being

that the vehicles in themselves irritated guinea pig skin causing erythema and mononuclear cell infiltration in the upper dermis, which interfered with the macroscopic and microscopic assessment of the corticosteroid effect. Since alcohols and acetone had been shown not to result in any visible or light microscopic effect on the dermis in normal skin (8), it was proposed that application of a corticosteroid in a solvent vehicle might overcome these problems.

The aim of this paper was thus to study the effects of a topically applied corticosteroid in an acetone solution and the vehicle itself on the dermal inflammatory cell infiltrate in allergic, toxic and irritant reactions. The influence of the interval between provocations and treatment within the 1st h after provocation was also studied.

Materials and Methods

Animals

Female albino Dunkin Hartley guinea pigs weighing around 300g at the time of sensitization were kept in separate cages with free access to standard pellets and water. The flanks of the guinea pigs were shaved with an electric razor.

Sensitization and testing

For the allergic (oxazolone) reactions, sensitization and testing were done as previously reported (6). In short, the animals were sensitized by painting 20% oxazolone (4-ethoxymethylene-2-phenyl oxazolone BDH Chemicals LTD Poole, England) in acetone (pro analysis Sigma Chemical Co. ST. Louis MO USA) on the neck for 2 consecutive days. Testing was performed 2 weeks later, by pipetting 10 μ l of 0.2% oxazolone in acetone on the shaven flanks once only. Elicitation of the toxic (croton oil Sigma Chemical Co.) and irritant (sodium lauryl sulfate, SLS Sigma Chemical Co.) reactions were done as previously described (7–9). Briefly, 10 μ l of 0.1% croton oil in acetone was applied to the shaved flanks 1 $\times$ only and 5 μ l of 2% sodium lauryl sulfate (SLS) in water was applied 3 $\times$ daily for three days (1 $\times$ 3 $\times$ 3). Normal skin was taken from every animal.

Treatment

As a model corticosteroid, budesonide (obtained from Draco AB, Göteborg Sweden) dissolved in acetone was used. Pilot studies on the toxic reaction to croton oil showed concentrations of 0.025–0.1% to be equally effective. Budesonide 0.05 or 0.1% was used for treatment. 10 μ l of budesonide solutions and pure acetone were pipetted onto the reaction sites immediately after the provoking dose was seen to have dried. Each animal served as its own control, i.e., an untreated test site was included for the different reaction types.

Macroscopic assessment

Macroscopic assessment according to the following scheme was performed by 2 experienced technicians prior to sacrifice. Parentheses were used to give further discrimination of the grade of the reaction. Scores were given numerical values to facilitate handling of group results.

Morphological score	Numerical score
0=no change or uncertain reaction	0
(+)	0.5
+ =redness, often slightly irregular in distribution	1
+(+)	1.5
++ =redness with slight palpable induration	2
++(+)	2.5
+++ =redness with obvious swelling	3

Group means were obtained by dividing the total numerical score for all animals by the number of animals in the group. This was termed the “macroscopic score” for the group.

Histological processing

After sacrifice, punch biopsies of the test sites and normal skin were fixed in 10% neutral phosphate buffered formalin, embedded in glycol methacrylate and polyethylene glycol (JB-4 Plastic Embedding Kit, Polysciences USA), cut in 3 μ sections and stained with 100% May-Grünwald (Diagnostica MERCK, Germany) and 3% Giemsa solution (Diagnostica MERCK, Germany).

Microscopic assessment

Mononuclear cells (a group comprising chiefly lymphocytes but also monocytes), mast cells (also a mononuclear cell but counted separately), basophils, eosinophils and neutrophils in the upper dermis were counted based on their morphology. The number of cells per high power field (1000 $\times$ oil immersion) for 20 fields was averaged. Details of the method can be found in (5).

The cell response

The mean number of cells in a test area minus the mean number of cells in normal skin in the same animal gives a “cell response”. This procedure compensates for normally infiltrating cells and other resident cells in the dermis, gives a qualitative and quantitative assessment of the cell traffic into (or out of) the reaction area (5) and is more easily presented in table or figure form. The cell response can be expressed as a “total cell response” or as a response for the individual cell types.

Analysis of the results

The statistical significance of changes in cell counts was assessed by comparing the count in the test area with the count in the control area by paired t-test analysis. The level of statistical significance is indicated as $*=p<0.05$, $**=p<0.01$ and $***=p<0.001$. In tables, the significance level is indicated besides the relevant cell response figure.

Changes in the “macroscopic score” have been analysed by comparison of group means with control reaction means. All changes seen in this paper were decreases in macroscopic score (i.e., less erythema and oedema). In order to differentiate the degree of change, the designations “slight”, “moderate” and “marked” have been used. A macro-

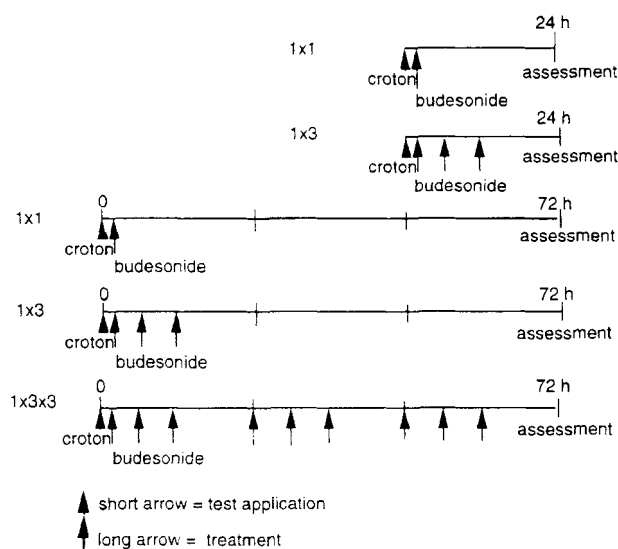


Fig. 1. Pilot series: determination of the effects of varying numbers of applications of 0.1% budesonide on toxic reactions to croton oil.

scopic change of less than 0.5 was deemed unchanged. —. Decreases were indicated by the direction of the arrows; 0.5–0.9 = “slight” ↓; 1.0–1.9 = moderate. ↓↓; 2.0+ = marked, ↓↓↓. No statistical analysis has been attempted.

Experimental design

3 experimental series were performed, a pilot series, the main series and a series to study the effect of the time interval between test and treatment.

Pilot studies: the effect of 0.1% budesonide in acetone and acetone alone applied 3× daily for 3 days was investigated on normal skin (not shown diagrammatically). The effects of different number of applications of budesonide (single, 1×3 or 1×3×3) on the 24 and 72 h toxic contact reaction elicited by croton oil were investigated (Fig. 1). The effect of varying budesonide concentrations (0.1% to 0.025%) applied to croton reactions 3× during the 1st day was assessed at 72 h.

Main series: in all 3 contact model reactions (toxic, allergic and irritant), budesonide and the vehicle acetone were applied immediately after application of the provoking agent (croton oil, oxazolone or SLS, respectively) (Fig. 2). The toxic reaction was studied 24 h after the standard single application and treatment. The allergic reaction was examined 24, 48 and 72 h after single application and treatment. The (cumulative) irritant reaction induced by the application of SLS 3× a day for 3 days (1×3×3) was treated with budesonide or acetone immediately after each application of SLS and assessed at 72 h.

Time interval between provocation and treatment: in all 3 contact model reactions, the provoking agent was applied to 5 areas (1 for each time interval) (Fig. 3). The toxic contact reactions were treated with 0.05% budesonide in acetone and acetone alone immediately (0 = as soon as the provoking agent had dried), 5, 15, 30 and 60 min after application. 24 h later the reactions were scored macroscopically and the animals sacrificed for histology. The allergic contact reactions were treated with 0.05% budesonide immediately (0), 5, 15, 30

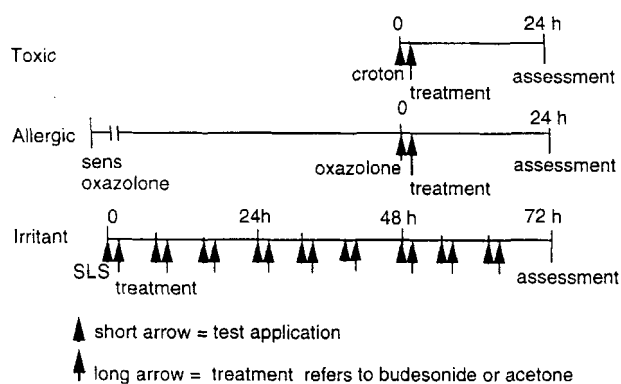


Fig. 2. A schematic diagram of the experimental design for the main study series.

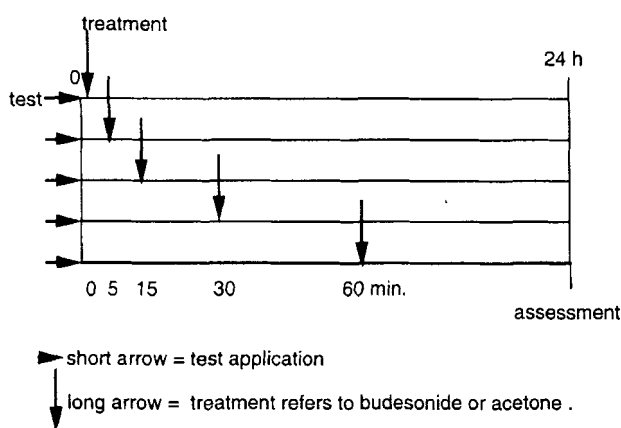


Fig. 3A. A schematic diagram of the experimental design for the time interval series. Test refers to croton or oxazolone.

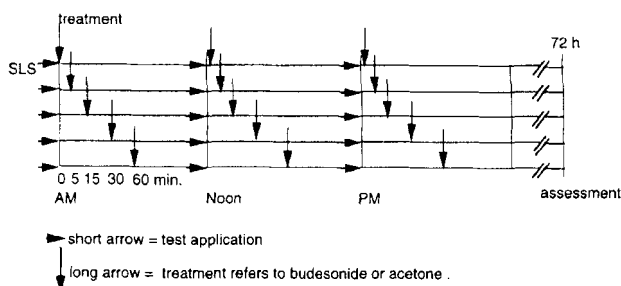


Fig. 3B. Application of SLS and treatment 3× (AM, Noon and PM) during the 1st day. The same procedure was repeated at 24 and 48 h.

Table 1. Normal skin; the effect on the naked eye appearance (macro-score) and the dermal inflammatory cell infiltrate (cell response) of 0.1% budesonide in acetone and acetone alone on shaved normal flank skin of 10 guinea pigs

Treatment (n=10)	Macro score	Cell response/high power view field					
		total	mono	mast	baso	eos	neutro
0.1% budesonide	0	3.4*	3*	0.3	0.02	0.07	0
acetone	0	1.8	1.5	0.07	0.03	0.2	0

The agents were applied 3× daily for 3 days (1×3×3); statistical significance, (see Methods) is indicated * $p<0.05$

and 60 min after provocation. The irritant reaction (SLS applied 3× a day for 3 days (1×3×3)) was treated with 0.1% budesonide 3× daily for 3 days (1×3×3) at the same time intervals as for the toxic and allergic reactions.

All studies were performed during 1983 and 1984. No further studies were undertaken to elucidate unanswered questions.

Results

Pilot studies

Neither 0.1% budesonide in acetone nor acetone alone had any effect on the macroscopic appearance of normal skin (Table 1). There was, however, an increase of the total cell response mostly comprising mononuclear cells. For the area treated with budesonide the total and mononuclear cell increase was statistically significant ($p<0.05$).

In the 24 and 72 h toxic reaction to croton oil, the macroscopic score and the total cell response for all applications of budesonide (1×1, 1×3, 1×3×3) decreased compared to the control reaction (Table 2). The differential cell response (raw data not shown) showed a significant decrease in the mononuclear cells at 24 h ($p<0.01$) and 72 h ($p<0.05$).

A decrease in the macroscopic score and the total cell response in the toxic reaction to croton oil was seen for all concentrations of budesonide

(0.1–0.025%) applied 3× the 1st day (raw data not shown). The decreases in the total cell response were statistically significant ($p<0.05$ –0.001).

Main series

Table 3 shows the effects of treatment with a single application of budesonide in acetone or acetone alone on toxic, allergic and irritant reactions. The 24 h toxic reaction to croton oil showed a decrease in the macroscopic score after both budesonide and acetone. The cell response after both budesonide and acetone was significantly decreased (total and mononuclear cells, $p<0.001$).

The 24 h allergic reaction to oxazolone was affected macroscopically only after application of acetone. Microscopically, both budesonide and acetone significantly decreased total and mononuclear cell counts ($p<0.05$). In the allergic reaction treated immediately with budesonide or acetone and assessed at 48 and 72 h after provocation (raw data not shown), a decrease in the macroscopic score and in the cellular infiltrate was seen for both time points and treatments. The decrease in the cell response (total and mononuclear) for both treatments was significant only at 48 h ($p<0.05$).

The irritant reaction to SLS was macroscopically decreased only after application of budesonide. Microscopically, however, both budesonide and acetone significantly decreased the total and mononuclear cell infiltrate ($p<0.001$ and $p<0.05$, respectively).

Table 2. The effect on the macroscopic score and the total cell response of 0.1% budesonide in acetone on the 24 and 72 h croton oil toxic reaction in 9 animals

(n=9)	Untreated	Budesonide		
		1×1	1×3	1×3×3
24-h				
macroscopic score	2.3	0.1↓↓↓	0.2↓↓↓	nd
total cell response	15.5	4.1 ***	3.8 ***	nd
72-h				
macroscopic score	1.0	0↓↓	0↓↓	0↓↓
total cell response	6.3	1.2 ***	2.1 **	3.6 *

nd: not done

Treatment, beginning immediately after provocation, was once only (1×1) or 3× during 1 day (1×3) or 3× daily for 3 days (1×3×3). Degree of change in the macroscopic score and the statistical significance of the cell response (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) is shown.

Time interval between provocation and treatment

For the toxic reaction to croton oil, the effect on increasing the interval between provocations and treatment from "0" to 60 min was studied for both budesonide and acetone. Marked effects both macroscopically and microscopically were seen at all time points (Table 4). The decrease in the macroscopic score was more marked for budesonide. The total cell and the mononuclear cell response showed a statistically significant ($p<0.01$ –0.001) reduction at all time points for both budesonide and acetone. The basophil gran-

ulocytes showed a decrease ($p < 0.05$) in numbers with budesonide treatment while eosinophils and neutrophils remained unchanged. With acetone treatment, basophils tended to decrease in number, compared to controls, while eosinophils increased. The increase in eosinophils at 60 min was statistically significant ($p < 0.05$).

Budesonide treatment of the allergic reaction to oxazolone (Table 5) resulted in a decrease in the macroscopic score and in the total cell and mononuclear cell response, which reached significance only when treatment was 0 or 5 min after provocation. Changes in the basophil, eosinophil and neutrophil counts were not significant.

The macroscopic score for the irritant reaction (Table 6) decreased slightly for budesonide application at 0 and 5 min. Microscopically the total

cell response decreased significantly at 0 and 5 min. For the mononuclear cell response, significant decreases were seen at all time points. Eosinophils and neutrophils tended to increase in numbers, particularly at later time points, though statistically significantly so only for neutrophil granulocytes at 60 min.

Discussion

Acetone as a vehicle produced, as expected (8), none of the irritation previously seen when corticosteroids were applied in cream or ointment bases (10).

The corticosteroid budesonide in acetone was shown to have anti-inflammatory effects which varied according to the reaction type. The toxic

Table 3. The effect on the macroscopic score and the cell response of a single application of budesonide or acetone on toxic, allergic and irritant reactions

Skin reaction	Macro-score		Cell response/high power viewfield					
	mean	change	total	mono	mast	baso	eos	neutro
croton oil 24 h								
untreated	1.6		11.3	9.9	0.3	0.8	0.2	0
budesonide	0.9	↓	1.3***	0.8***	0.3	0*	0.3	0
acetone	1.0	↓	3.0***	2.2***	0.5	0.1	0.3	0
oxazolone 24 h								
untreated	1.4		18.8	14.2	0.3	4	0.3	0
budesonide	1.2	—	8.5*	6.0*	0.3	2.2	0.1	0
acetone	0.9	↓	9.4*	7.0*	0.1	2.0	0.2	0
SLS 1×3×3 72 h								
untreated	1.9		16.4	14.1	0.3	0.7	0.3	1.1
budesonide	1.2	↓	8.4***	6.8***	0.2	0.6	0*	0.9
acetone	2.1	—	8.2*	7.3*	-0.8	0.2	0.1	0.6

The toxic reaction to 0.1% croton oil in acetone and the allergic reaction to 0.2% oxazolone in acetone was treated with 0.05% budesonide. The irritant reaction to 2% aqueous solution of SLS was treated with 0.1% budesonide in acetone. Acetone alone was applied to all reaction types. All applications occurred immediately after the provoking agent had dried. 10 animals were used in each reaction type.

Table 4. Effect of interval between provocation and treatment in the toxic reaction to croton oil

Croton oil (n=10)	Macro-score		Cell response/high-power viewfield					
	mean	change	total	mono	mast	baso	eos	neutro
untreated	1.6		11.3	9.9	0.3	0.8	0.2	0
budesonide								
0 min	0.9	↓	1.3***	0.8***	0.3	0*	0.3	0
5	0.5	↓↓	2.8***	2.2***	0.1	0.1	0.4	0
15	0.6	↓↓	4.6***	3.9***	0.4	0.1	0.2	0
30	0.3	↓↓	2.7***	2.1***	0.3	0*	0.3	0
60	0.4	↓↓	2.3***	2.2***	0	0*	0	0
acetone								
0 min	1.0	↓	3.0***	2.2***	0.5	0.1	0.3	0
5	0.8	↓	3.0***	2.0***	0.3	0.2	0.4	0
15	1.0	↓	4.7**	3.3***	0.2	0.6	0.6	0
30	1.1	↓	2.3***	1.5***	0.2	0.1	0.6	0
60	1.3	—	4.8**	3.4**	0.2	0.4	0.8*	0

The reaction was treated once only with 0.05% budesonide or acetone immediately (=0 min) after provocation and after the specified time intervals. 10 animals were tested. The reactions were assessed at 24 h.

Table 5. Effect of interval between provocation and treatment in the allergic reaction to oxazolone

Oxazolone (n=10)	Macro-score		Cell response/high-power viewfield					
	mean	change	total	mono	mast	baso	eos	neutro
untreated	1.5		18.8	14.2	0.3	4	0.3	0
budesonide								
0 min	1.2	—	8.5*	6.0*	0.3	2.2	0.1	0
5	0.8	↓	10.4*	7.2*	0.2	2.9	0.1	0
15	1.2	—	13.5	9.5	0.1	3.8	0.1	0
30	1.2	—	17.9	14.2	0.3	3.3	0.2	0
60	1.0	↓	20.0	15.4	0.3	4.1	0.2	0

The reaction was treated with 0.05% budesonide immediately (0=min immediately) after provocation and after the specified time intervals. 10 animals were tested. The reactions were assessed at 24 h.

Table 6. Effect of interval between provocation and treatment in the irritant reaction to SLS

SLS (n=10)	Macro-score		Cell response/high-power viewfield					
	mean	change	total	mono	mast	baso	eos	neutro
untreated	1.9		16.4	14.1	0.3	0.7	0.3	1.1
budesonide								
0 min	1.2	↓	8.4***	6.8***	0.2	0.6	0*	0.9
5	1.2	↓	10.0**	7.9**	0.1	0.8	0.2	1.1
15	1.8	—	14.2	10.6*	0.5	0.7	0.4	1.9
30	1.8	—	13.9	10.0*	0.3	1.1	0.5	2.1
60	1.9	—	15.1	9.5*	0.2	1.6	0.5	3.3*

The reaction was treated with 0.1% budesonide 3× daily for 3 days (1×3×3) at the specified time intervals. The reaction was assessed at 72 h. The experiment comprised 10 animals.

reaction to croton oil was the most reduced. Furthermore, a single application of budesonide on the toxic reaction was as effective as multiple applications, at least when the applications occurred immediately after provocation. Less pronounced effects were seen on the allergic and irritant reactions. It is tempting to speculate that this may be due to the differing pathogenetic mechanisms operative in the various contact reaction types.

That the acetone vehicle itself had an anti-inflammatory effect was an unexpected finding. Acetone, although a non-irritant in the present model, is reported to cause acute barrier disruption (11, 12) with increases in mRNA levels for cytokines in the epidermis (13). Acetone is a substance capable of removing intercellular lipids (14). In addition, the lipid mosaic model proposed by Forslind (15) explains the possible effects of exogenic agents on the lipid bilayer barrier structures in the stratum corneum. Since reorganization of lipid structures may affect not only the intercellular layers of the stratum corneum but also the cell membranes of the keratinocytes, it is conceivable that cell membranes may also be affected by acetone (16). In reactions such as the toxic reaction, in which the keratinocyte is a prime target cell (17), steric changes in the cell membrane could result in interrupted cell

membrane receptor and signal processing necessary for the development of an inflammatory response. The creation by acetone of a solvent front which might lower concentrations of the provoking agent needs also to be considered. Skin physiological parameters, such as the local cutaneous metabolism (18) or skin blood flow, may also be affected.

It should perhaps not be surprising that an organic solvent can be demonstrated to have anti-inflammatory effect. In veterinary practice, use of such solvents as anti-inflammatory agents, e.g., for inflamed fetlocks in horses, is traditional although perhaps hardly routine. Use of DMSO in musculoskeletal and orthopedic disorders to minimize inflammatory effects is also reported (19, 20). The relevance of the observation in the human is as yet uncertain, but in a manuscript under preparation, we will report anti-inflammatory properties of acetone on the UVB reaction in normal human skin. It is tempting to speculate that there may exist some therapeutic possibilities in the phenomenon.

In guinea pig contact reactions of various types, clear "late" differences – at 24, 48 and 72 h – have been observed in the dermal inflammatory infiltrate (5). In humans, results for studies of both epidermal and dermal aspects, have been less categorical (21, 22), and it is accepted that his-

tology at the individual test level has little clinical value in differentiating between allergic and non-allergic reactions. In both animal and human contact reactions it seems that specific pathogenic events occur within the 1st h after provocation of a reaction. In allergic reactions, early events might center on lymphocyte-epidermal cell interaction (23, 24). In non-allergic reactions, such as the toxic and irritant reactions here, keratinocytes may be key cells in the initiation of inflammation (17). Our results show a tendency for corticosteroid "effect" to be more marked when application occurred very early or immediately after provocation. This may explain why anti-inflammatory affects were not seen in our original paper (9) in which treatment was performed 24 h after provocation. It must be pointed out that the particular corticosteroid used (budesonide) was not included in the corticosteroid creams and ointments tested in the original paper (9). The lack of irritancy to acetone, compared to ointment or cream bases (8), is clearly also of importance.

The influx of inflammatory cells in response to the application of corticosteroids on normal skin of non-sensitized animals is an interesting finding without an obvious explanation. An association which can be made is the known response to application of a substance in an epicutaneous test (25), whether the subjects were allergic to the substance itself or not. The same influx was not seen for application of acetone alone.

In conclusion, the model used here, though an experimental one, has allowed the correlation of macroscopic and microscopic (cellular infiltrate) findings at precise time points after provocation. The information gained may be of use for the design of human studies of the effects produced by different therapeutic agents on contact reactions. The possible inherent anti-inflammatory properties of acetone should be considered whenever acetone is used as a vehicle – for instance, in patch testing. The possible use of organic solvents like acetone as anti-inflammatory agents, particularly early after exposure to an allergen or irritant agent, seems to be worth further investigation, given the present, sometimes total, reliance on corticosteroids for topical anti-inflammatory therapy.

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