

Topical tacrolimus 0.1% ointment (Protopic®) reverses nickel contact dermatitis elicited by allergen challenge to a similar degree to mometasone furoate 0.1% with greater suppression of late erythema

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The aim of this study was to evaluate the ability of topical tacrolimus 0.1% under occlusion for 48 h to suppress nickel-elicited allergic contact dermatitis in a randomized, petrolatum- and mometasone furoate 0.1% ointment-controlled double-blind, intra-individual study which included 28 women volunteers. 3 closed patch tests (Finn Chambers® on Scanpor, Epitest Ltd Oy, Tuusula, Finland) containing 0.1 ml of 5% nickel sulfate in petrolatum were applied on day 0. After removal on day 2, the study compounds were applied under occlusion for 48 h. The eczema reaction and the degree of erythema were evaluated clinically and by reflectance spectrophotometry at days 4 and 7, respectively. Mean visual scores corresponding to petrolatum-treated sites were significantly higher than those corresponding to both mometasone furoate and tacrolimus at days 4 ($P < 0.001$) and 7 ($P < 0.001$). In both tacrolimus- and mometasone furoate-treated sites, there was a significant decrease in visual scores with time ($P < 0.001$) from day 2 to day 7, and the corresponding mean decreases in scores were 0.73 and 1.04, respectively. The difference between both was 0.30 in favour of tacrolimus (95% confidence intervals, -0.04 and 0.65), although this did not reach statistical significance ($P = 0.084$). Mean erythema index values were similar at day 2. Significant differences among treatment sites were seen at days 4 ($P < 0.001$) and 7 ($P < 0.001$). The decrease was significantly more pronounced on day 7 in patches where tacrolimus had been supplied ($P < 0.5$). This method might provide useful means to compare different concentrations and/or presentations of tacrolimus or other calcineurin inhibitors and topical anti-inflammatory agents.

Key words: allergic contact dermatitis; mometasone furoate; nickel sulfate; tacrolimus. © Blackwell Munksgaard, 2003.

Accepted for publication 10 November 2003

Topical corticosteroids are nowadays the mainstay of therapy for allergic contact dermatitis, but the need for potent formulations carries an associated risk of atrophy, telangiectasia, other local adverse effects and systemic absorption. In addition, the development of tachyphylaxis or tolerance to their therapeutic effect and the potential for delay of epidermal regeneration, as suggested by late transepidermal water loss measurements (1), are significant drawbacks to the use of topical corticosteroids and justify the search for better therapeutic alternatives. Topical calcineurin inhibitors, which have recently been introduced to our therapeutic armamentarium, are promising in this respect.

The first topical immunomodulator approved for human use, tacrolimus ointment, has been shown to be effective and safe in the treatment

of both children and adults with atopic dermatitis, and consequently approved for that indication in many countries. Topical tacrolimus has also been reported to be of benefit in other immunologically mediated skin diseases, including hand dermatitis, contact dermatitis, eyelid dermatitis and steroid-induced rosacea (2), where topical corticosteroid treatment provides suboptimal results or is contraindicated.

Betamethasone-17-valerate and similar corticosteroids with well-known atrophogenic potential have been used in most comparative studies of topical calcineurin inhibitors. Mometasone furoate 0.1% belongs to a class of corticosteroids, characterized by their intrinsic potency, which are widely prescribed nowadays and purported to have an improved therapeutic ratio. A recent study has shown mometasone furoate 0.1% and tacrolimus

0.1% to be approximately equipotent in the treatment of dyshidrotic palmar eczema (3), a relatively corticosteroid-resistant dermatosis.

Thus, the aim of our study was to evaluate the ability of topical tacrolimus 0.1% under occlusion for 48 h to suppress nickel-elicited allergic contact dermatitis in a randomized, petrolatum- and mometasone furoate 0.1% ointment-controlled within-volunteer study.

Materials and Methods

This investigation was designed as a single-centre, double-blind, intra-individual, comparative study.

Volunteers

28 white, women volunteers attending our Contact Dermatitis Clinic at Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, took part in the study (mean age 28.3 years; range 20–51 years) after giving their informed consent. All had a history of nickel allergic contact dermatitis confirmed by the results of patch tests with TRUE Test™ (Mekos Laboratories AS, Hillerød, Denmark), which were applied on day 0.

3 closed patch tests (Finn Chambers® on Scanpor, Epitest Ltd Oy, Tuusula, Finland) were performed, which included a filter paper disc that were cut from the sheets provided by the manufacturer and applied on the back of the volunteers. The patch tests, containing nickel (0.1 ml of 5% nickel sulfate in petrolatum), were applied on day 0 and removed 48 h later (day 2). Only those volunteers who developed a clear-cut positive response with similar clinical scoring on the 3 patch tests were included in the study for further evaluation.

Topical agents

On day 2, the study compounds (0.1 ml of tacrolimus 0.1% (Protopic®) ointment, mometasone furoate 0.1% (Elocom®) ointment and petrolatum) were applied by a nurse using 3 closed patch tests as above. The test compounds were contained in coded opaque syringes and blinded for both investigators and volunteers. The order of application was determined by sequential allocation to a computer-generated random permutation series. The 3-patch-test cut-outs were applied on the same location to where nickel patch tests had originally been applied, secured with adhesive tape and removed after 48 h (day 4).

Evaluation

Clinical evaluation (visual assessment) recorded the presence of erythema, papulation, vesicula-

tion and blistering using a scale from 0 to 3, as used in patch-test result reading (0, absent reaction; 1, weak positive reaction, with well-delimited erythema, infiltration and discrete papules; 2, strong positive reaction, with well-delimited erythema, infiltration, papules and discrete vesicles; 3, severe positive reaction, with coalescing vesicles and bullae).

The degree of erythema was quantified in 16 volunteers by determination of the erythema index with reflectance spectrophotometry (Cortex DermaSpectrometer, Cortex Technology, Hasund, Denmark).

All measurements (visual assessment and colourimetry) were carried out on days 2 and 4, 30 min after removal of the patch tests, and on day 7.

The Friedman test was applied to investigate visual scores and erythema index values. The latter were also analysed using the general linear model analysis of variance. The level of significance was set at $P < 0.05$. All the tests were 2-sided. Statistical analysis was performed using the SPSS (V10.0) (SPSS Inc., Chicago, IL, USA) software.

Results

Mean visual scores corresponding to all treatment sites were practically identical at day 2 and thereon differed significantly according to time and treatment. The corresponding means are detailed in Table 1. In petrolatum-treated sites, visual scores increased at day 4 with respect to day 2 ($P = 0.007$) and decreased at day 7 with respect to day 2 ($P < 0.001$) and day 4 ($P < 0.001$). Visual scores corresponding to petrolatum-treated sites were significantly higher than those corresponding to both mometasone furoate and tacrolimus at days 4 ($P < 0.001$) and 7 ($P < 0.001$). In both tacrolimus and mometasone furoate-treated sites, there was a significant decrease in visual scores with time ($P < 0.001$), but the differences between both treatments were not significant at any point in time; from day 2 to day 7, the corresponding mean decreases in scores were 0.73 and 1.04, respectively. The difference between both was 0.30 in favour of tacrolimus (95% confidence intervals, -0.04 and 0.65), which did not reach statistical significance ($P = 0.084$).

Mean erythema index values are detailed in Table 2. They were similar at day 2. Significant differences among treatments were seen at days 4 ($P < 0.001$) and 7 ($P < 0.001$). There was a trend towards decreasing erythema index values with time in sites treated with mometasone furoate

Table 1. Visual assessment scores (mean $\pm$ standard deviation and significance values of statistical tests)

	Day 2	Day 4	Day 7	P
Petrolatum	2.21 $\pm$ 0.63	2.57 $\pm$ 0.57	1.82 $\pm$ 0.60	<0.001
Mometasone furoate 0.1%	2.14 $\pm$ 0.52	1.89 $\pm$ 0.92	1.41 $\pm$ 0.81	<0.001
Tacrolimus 0.1%	2.18 $\pm$ 0.67	1.75 $\pm$ 0.93	1.14 $\pm$ 0.84	<0.001
P	0.607	<0.001	<0.001	

or tacrolimus, which approached statistical significance in the case of tacrolimus ($P=0.076$). Only treatment ($P=0.004$) and treatment/time interaction ($P=0.025$) were significant variables on the general linear model analysis of variance. There was a trend for greater decrease in erythema index values in tacrolimus-treated sites when compared with mometasone furoate, which reached statistical significance only on day 7 ($P=0.02$).

Comment

Models of contact dermatitis have been used to test the efficacy of tacrolimus in decreasing symptoms. Lauerma et al. (4) performed an investigation where humans were sensitized to 1-chloro-2,4-dinitrobenzene and then pretreated with either tacrolimus ointment 1%, 0.1% or 0.01%, or a vehicle. The skin areas that were treated with the vehicle displayed dermatitis, whereas the areas treated with tacrolimus did not display any changes in histopathology.

Animal models of contact dermatitis have also been employed. Meingassner and Stutz (5) utilized pig skin, which resembles human skin in permeability properties, to determine the effect of topically applied tacrolimus ointment 0.4 and 0.04% in comparison with rapamycin, cyclosporin, dexamethasone and clobetasol propionate, following the application of dinitrofluorobenzene (DNFB). Treatment was performed twice, 30 min and 6 h after elicitation of challenge reaction. Topical application of 0.4–0.04% tacrolimus caused a pronounced inhibition (similar to the activity of 0.13% clobetasol) of inflammatory skin reactions of hypersensitivity to DNFB. Dexamethasone (1.2%) was less active than clobetasol, whereas rapamycin and cyclosporin were inactive at concentrations of 1.2 and 10%, respectively.

Duncan (6) used guinea pigs to determine if DNFB-induced hypersensitivity could be controlled using topical tacrolimus 0.02 and 2%, cyclosporin A and rapamycin. It was found that *in vivo*, only tacrolimus suppressed T-cell infiltration and erythema. However, compared with the other compounds, tacrolimus did not have effects on keratinocyte growth, which the other agents inhibited. According to Lauerma et al. (7), topical tacrolimus suppresses both irritant and allergic patch-test reactions in the guinea-pig model, but the most prominent suppressive effect was seen when skin sites were pretreated with tacrolimus.

Topical administration of tacrolimus hydrate dramatically suppressed ear swelling and epidermal hyperplasia, as well as the increase in interferon- γ expression, in a chronic allergic contact dermatitis induced in rat ear by repeated application of oxazolone (8). Topical tacrolimus demonstrated suppressive effects as potent as those of betamethasone valerate in this model.

The anti-inflammatory activity of topical tacrolimus appears to be rather specific for T-cell-mediated inflammatory responses, as shown by the results of studies on experimental human-skin inflammation models (9). When tacrolimus was applied 30 min after sodium lauryl sulfate irritation, an increased inflammation in comparison to controls was observed visually and quantified by non-invasive bioengineering methods, namely chromametry and measurement of trans-epidermal water loss, but only the latter data reached statistical significance. Once daily application of tacrolimus for 5 days, starting at the end of the 4-day irritation period, was without any effect. Similarly, no effect of tacrolimus was seen in the UVB model.

Topical pimecrolimus has also been shown to be effective after topical application in allergic contact dermatitis of domestic pigs (10). The

Table 2. Erythema index values (mean $\pm$ standard deviation and significance values of statistical tests)

	Day 2	Day 4	Day 7	P
Petrolatum	17.25 $\pm$ 5.00	20.06 $\pm$ 6.63	17.13 $\pm$ 5.67	0.144
Mometasone furoate 0.1%	16.88 $\pm$ 5.04	16.88 $\pm$ 5.68	15.88 $\pm$ 3.72	0.393
Tacrolimus 0.1%	16.69 $\pm$ 4.83	15.63 $\pm$ 4.41	13.56 $\pm$ 4.93	0.076
P	0.197	<0.001	<0.001	

first published experimental study with topical pimecrolimus on established nickel contact dermatitis elicited by allergen challenge in human beings was performed in 66 healthy volunteers with previous patch tests positive to nickel who were challenged with 5% nickel sulfate in water patches applied under occlusion for 48 h (11). The therapeutic effect of 0.6% pimecrolimus following twice daily application for up to 12 days has been reported to be similar to that of 0.1% beta-methasone-17-valerate cream.

Topical tacrolimus and other calcineurin inhibitors might provide an alternative treatment of allergic contact dermatitis, and the development of experimental models can provide suitable means to compare the potency and time course of effect of topical tacrolimus in different concentrations and in comparison to alternative treatments. In the experimental model we used, the time course of the inflammatory reaction was assessed by mean visual scores and erythema index values. Following occlusive application of petrolatum for 48 h, they peaked at day 4 and decreased at day 7 to baseline (visual score) or below baseline (erythema index) levels (i.e., allergic contact eczema following occlusive application of 5% nickel sulfate in petrolatum for 48 h). The application of topical tacrolimus 0.1% and mometasone furoate 0.1% caused a decrease in mean visual scores at days 4 and 7, although there was no statistically significant difference between the 2 in this period. The decrease in mean erythema index, a quantitative determination, was significantly more pronounced on day 7 in patches, where tacrolimus 0.1% was applied.

Because tacrolimus appears to be devoid of any known vasoconstrictive effect, the decrease in erythema might represent a direct reflection of its anti-inflammatory effect, with a different time course than that of corticosteroid-induced vasoconstriction. Irrespective of the mechanisms involved, and pending further studies that are required to quantify the blood flow in the microcirculation of the treated areas and to confirm this hypothesis, this assay might provide a useful

means to compare different concentrations and/or presentations of tacrolimus and other calcineurin inhibitors.

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