

Tacrolimus enhances irritation in a 5-day human irritancy *in vivo* model

M. FUCHS, S. SCHLIEMANN-WILLERS, C. HEINEMANN AND P. ELSNER

Department of Dermatology, Friedrich-Schiller-University, Jena, Germany

Tacrolimus (FK 506) is a macrolide discovered in 1984 as a metabolic product of *Streptomyces tsukabaensis*. It has been used successfully in treating atopic dermatitis, allergic contact dermatitis, lichen planus mucosae and pyoderma gangrenosum. In the present study, we evaluated the anti-inflammatory activity of FK 506 in 2 human skin inflammation models. FK 506 as Protopic[®] cream was tested (i) in a 4-day repetitive irritation test with 2 × daily application of sodium lauryl sulphate (SLS), and (ii) in a UVB erythema model. The effect was evaluated visually and quantified by non-invasive bioengineering methods, namely chromametry and tewametry (TEWL). When FK 506 was applied 30 min after SLS irritation, an increased inflammation in comparison to controls was observed with all 3 methods, with only the TEWL data reaching statistical significance. 1 × daily application of FK 506 for 5 days, starting at the end of the 4-day irritation period, was without any effect. Similarly, no effect of FK 506 was seen in the UVB model. In conclusion, FK 506 was shown to enhance experimentally induced irritant contact dermatitis and not to accelerate healing of irritant contact dermatitis and UVB erythema.

Key words: anti-inflammatory action; atopic dermatitis; bioengineering methods; chromametry; FK 506; human irritancy *in vivo* model; transepidermal water loss. © Blackwell Munksgaard, 2002.

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A large number of compounds are available for the treatment of inflammatory skin diseases such as atopic dermatitis (1), dermatitis solaris or psoriasis (2), the most effective external anti-inflammatory compounds being glucocorticoids. Their side-effects (3) have motivated continuing research for other therapeutic compounds, and fungal metabolites like tacrolimus (FK 506) have figured prominently in the literature (4).

The macrolide antibiotic FK 506 was discovered in 1984 as a product of *Streptomyces tsukabaensis* (5). Lawrence et al. (6) described its immuno-suppressive potency that is used therapeutically to prevent rejection of liver and kidney transplants. In short, the mechanism of action may be attributed to a direct inhibitory effect on the transcription of the essential autocrine IL-2 cytokine in T-lymphocytes (7). While, on systemic application, many effects and side-effects of FK 506 mimic those of cyclosporin A, topical skin application of FK 506 was found to be more effective due to its smaller molecular size and consequently better percutaneous penetration (2).

Several studies have documented a benefit of topical FK 506 treatment in inflammatory dermatoses, e.g. atopic dermatitis (1,7–9), psoriasis (4), allergic contact dermatitis (5), and erosive lichen planus mucosae (11), but there are no human *in vivo* studies on its effect on irritant contact dermatitis (ICD).

Nasr et al. (4) found that FK 506 serum concentration is 5–20 ng/mL during systemic therapy and rarely above 1 ng/mL under topical application. Accordingly, the side-effects of FK 506 observed after systemic application, including increase in serum creatinine, arterial hypertonus, headache, hirsutism, gastrointestinal irritation and abnormal glucose tolerance (4), are not seen during external therapy. During the latter, skin irritation with burning, itching and erythema is the most common complaint. However, collagen synthesis is not impaired, and skin atrophy does not occur (12).

The present study aimed at evaluating the anti-inflammatory efficacy of FK 506 in sodium lauryl sulphate (SLS)-induced ICD and UVB-induced erythema.

Materials and Methods

The investigation was designed as a controlled and single-blind study under standardized laboratory conditions. 20 volunteers (16 F, 4 M) with healthy skin participated, after written informed consent in accordance to the Helsinki declaration. The study was approved by the ethics committee of Friedrich Schiller University, Jena.

The mean age of the subjects was 26.3 (± 1.48) years (range 18–40). The study compound, FK 506 0.1% (Protopic®, Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan), was applied in a randomized fashion onto test areas on the back. Untreated areas served as controls. During the study period, volunteers were allowed to shower as usual but they were asked to avoid bathing, and application of surfactants, moisturizers or emollients on their backs. They were also asked to avoid sunbeds and solar radiation.

Inclusion criteria were

- voluntary participation;
- age between 18 and 40 years;
- either sex;
- absence of major internal diseases, and;
- absence of major dermatological diseases.

Exclusion criteria were

- severe general internal diseases,
- pregnancy, lactation, and uncertain contraception,
- dermatological diseases,
- immunosuppressive therapy (e.g. corticosteroids),
- topical dermatological treatment within the last 2 weeks,
- very fair complexion and light sensitive skin type I,
- lack of co-operation.

Test product: FK506 0.1% (Protopic®, Fujisawa Pharmaceutical Co. Ltd)

Each gram contains 1.02 mg of tacrolimus hydrate (1 mg as tacrolimus) in a base of white petrolatum, high-viscosity paraffin, bleached wax, hard paraffin and propylene carbonate.

Induction of SLS irritation

Repetitive irritation was performed from day 1 to day 4 at the same time (± 1 h). The application area was the skin of the paravertebral mid-back. 4 test areas were marked with a stencil. Cumulative irritation was induced by occlusive application of 50 μ L of sodium lauryl sulphate of highest purity (SLS, dissolved in water 0.5%, Serva Feinbiochemie, Heidelberg, Germany) by using large Finn

Chambers (12 mm) with filter discs (filling volume 50 μ L, Epitest Ltd, Hyrlä, Finland). The irritant was applied 2 $\times$ daily at a 3 h interval (11), and the chambers were removed after 30 min. After removal of the chambers, the skin was dried carefully with paper tissues.

Induction of UVB erythema

Before inducing the experimental erythema, the minimal erythema dose (MED) was determined with increasing UVB doses (Waldmann 800, Villingen-Schwenningen, Germany) exposure on 6 test areas on the gluteal skin (diameter 1 cm²). Visual reading was performed after 24 h (12). Thereafter, 2 more fields were irradiated with double the MED.

Application of FK506

10 min after removal of the Finn Chambers, 1 area on the back received 50 μ L of FK 506, which was gently distributed with a glove-covered finger.

In another test field, application of FK 506 started on day 4, 10 min after the last irritation (parallel treatment). The test substance was applied 1 $\times$ daily at the same time (± 1 h) a total of 5 $\times$ until day 8. Another field served as control with irritation only.

In 1 of the 2 gluteal fields with UVB-induced erythema, treatment was started 24 h after irradiation and was continued 1 $\times$ daily for 2 more days. The resolution of erythema was compared to the 2nd field, which was irradiated only (control).

To investigate the impact of FK 506 on healthy skin, 1 additional field in the back was exclusively treated 2 $\times$ daily (3½ h apart) with FK 506 for 4 days.

All volunteers participated in both the ICD and UVB studies.

Clinical evaluation and skin measurements

Prior to visual scoring and the measurements, volunteers rested for at least 15 min under standardized laboratory conditions (temperature 20–22°C, relative humidity 30–40%). Measurements were performed by the same investigator at the same time of day (± 1 h), 10 min before application of test substances. The final measurements were made on day 9, 24 h after the last application.

Clinical assessment was based on visual scoring according to Frosch & Kligman (15). Transepidermal water loss (TEWL), as an indicator of epidermal barrier function, was measured using the Tewameter TM 210® (Courage + Khazaka electronic GmbH, Cologne, Germany), and followed

the guidelines of Pinnagoda et al. (16). The intensity of erythema was quantified using the chromameter CR-300® (Minolta GmbH, Ahrensburg, Germany) according to the recommendations of Elsner (17). Only the a^* -score, which represents the red–green axis of the three-dimensional assay system, was considered.

Statistics

Statistical analysis was performed using the SPSS 10.0 for Windows software package. The mean values of the chromametry and TEWL data points were derived from 3 and 2 measurements, respectively. The comparisons of the test and control data were accomplished with the non-parametric Wilcoxon test, and the results were labelled in the figures as n.s. = not significant, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Results

Effects of FK506 on healthy skin

No effects of a total of 8 applications from day 1 to day 5 were observed. The visual score remained at zero, and neither the chromametric a^* values nor the TEWL delta values increased during the study period.

Effects of FK506 on SLS-induced ICD (parallel treatment)

The FK 506-treated field showed a pronounced increase in the visual score (Fig. 1) and the chromametric a^* value (Fig. 2) in comparison with the control field, though the difference was not statistically significant. Furthermore, the TEWL data (Fig. 3) revealed an additional irritant effect of FK 506, indicated by a pronounced increase in the TEWL on the FK 506-treated field ($p \leq 0.05$).

Effects of FK506 on SLS-induced ICD (treatment after irritation period)

When FK 506 treatment was started just after the end of the SLS irritation period, an accelerated resolution of the ICD could not be observed with any method (data not shown).

Effects of FK506 on UVB-induced erythema

UVB irradiation with double the MED induced a moderate erythema in all test fields. We did not observe an accelerated decrease in erythema in the fields being treated with FK 506 in comparison with the control field, either by visual scoring or

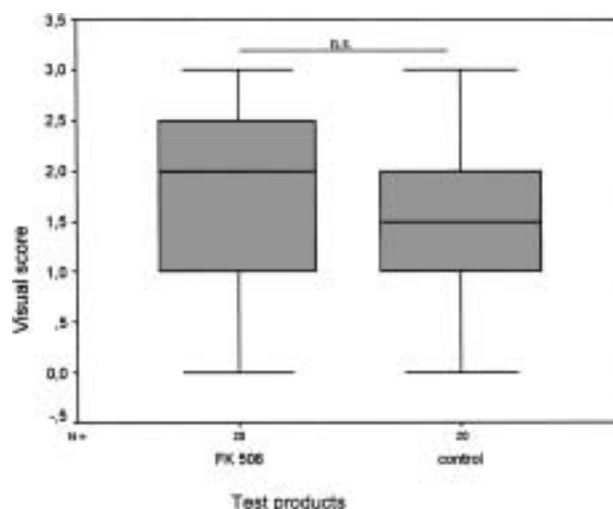


Fig. 1. Boxplots of the visual score at day 5 compared with the control field with irritant only. Cumulative SLS irritation model with FK 506 application immediately after irritation: Visual Score. Control = untreated control field. FK 506 = tacrolimus-treated field. n.s. = not significant. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$

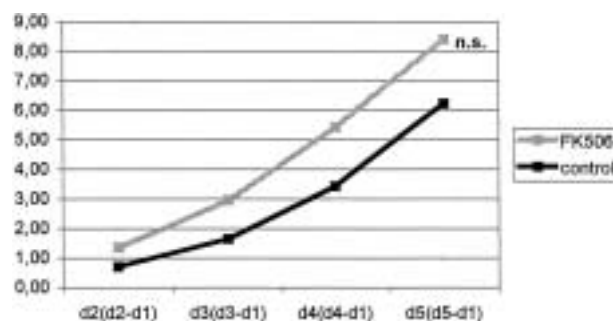


Fig. 2. Mean chromametric a^* values from day 1 to day 5 compared with the control field with irritant only. Cumulative SLS irritation model with FK 506 application immediately after irritation: Chromametry. Control = untreated control field. FK 506 = tacrolimus-treated field. n.s. = not significant. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$

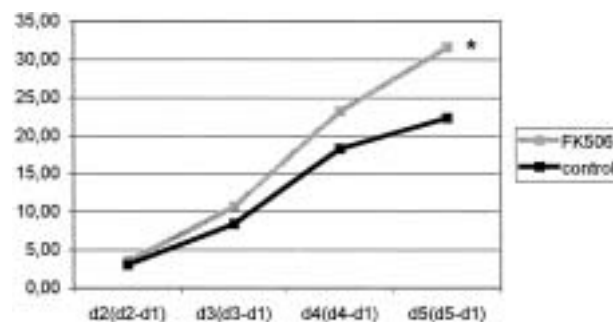


Fig. 3. Mean TEWL delta values from day 1 to day 5 compared with the control field with irritant only. Cumulative SLS irritation model with FK 506 application immediately after irritation: TEWL. Control = untreated control field. FK 506 = tacrolimus-treated field. n.s. = not significant. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$

by the use of chromametry. The epidermal barrier function was not significantly impaired after UVB irradiation with double the MED, which was indicated by a mean TEWL of $8.58 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ after irradiation, and there was no influence of FK 506 on TEWL.

Discussion

Our study confirmed an earlier report (18) that FK 506 does not cause irritant contact dermatitis on healthy skin. However, in the 2 models of our study, SLS-induced ICD and UVB-induced erythema, we could not confirm the widely reported anti-inflammatory effect of tacrolimus (19–26), which has been explained by a direct inhibitory influence on the transcription of IL-2 in T-lymphocytes. On experimentally induced ICD, a pronounced disturbance of the epidermal barrier function and a higher degree of erythema, respectively, was produced by FK 506. According to Reitamo et al. (9), skin irritation is the most prevalent (47%) side-effect of topical FK 506, which appears mostly in the early phase of treatment. It may be assumed that any irritant effects would have ceased on longer duration of our study. We conclude from these data that topical application of FK 506 should be regarded with reservation as far as ICD with ongoing exposure to the irritant is concerned.

Skin irradiation with UVB is known to induce gene expression of the proinflammatory cyclooxygenase 2 (COX2) and, as a consequence, the synthesis of prostaglandins and the liberation of mediators of inflammation (27, 28). Therefore, it appears worthwhile to study the effects of repeated applications of FK 506 started immediately after irradiation.

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Address:

Silke M. Fuchs, MD

Department of Dermatology and Allergology
Erfurter Str. 35

07743 Jena

Germany

Tel: +49 3641 937390

Fax: +49 3641 937430

e-mail: silke.fuchs@derma.uni-jena-de