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Effects of topical pimecrolimus 1% on high-dose ultraviolet B-irradiated epidermal Langerhans cells

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ABSTRACT

Some studies reported no changes in the number of epidermal Langerhans cells (LC) that were observed in mice treated with pimecrolimus, and low-dose stimulated solar radiation (once)-induced changers in LC are minimally affected by pimecrolimus. This study is to investigate the effects of topical pimecrolimus 1% on high-dose ultraviolet B (UVB)-irradiated epidermal LC. Forty human foreskin tissues were randomly divided into 4 groups of 10 tissues each: Group A, control; Group B, pimecrolimus 1% (once)-only; Group C, 180 mJ/cm^2 UVB (once)-only; Group D, UVB + pimecrolimus. Each tissue was cut into 4 pieces corresponding to 4 time points. All the tissues were cultured at 37 °C. After being treated, the tissues were collected respectively and processed for immunohistochemical staining and immunofluorescence staining. For UVB-only group, epidermal CD1a$^+$ LC number at 18 h decreased from 39.6 ± 8.30 to 22.3 ± 2.26/5 high magnification, compared to CD1a$^+$ LC number at 0 h ($P<0.01$). The CD1a$^+$ LC number of UVB-only group was significantly less than other groups at 18 h, 24 h and 48 h ($P<0.05$, respectively). Similar results were obtained with immunofluorescence staining for CD 1a and immunohistochemical staining for Langerin. The numbers of epidermal HLA-DR$^+$ LC had no significant differences among all groups at different time points. Our study found a single 180 mJ/cm^2 UVB irradiation significantly reduced epidermal LC numbers at 18 h, 24 h and 48 h, however, topical pimecrolimus could reverse these changes. UVB plus pimecrolimus treatment did not affect human LC maturation.

1. Introduction

Topical calcineurin inhibitors (TCIs), pimecrolimus cream and tacrolimus ointment, can block T-cell proliferation [1] and have been mainly used for the treatment of atopic dermatitis. Epidermal Langerhans cells (LC)-mediated immunosurveillance is one important pathway against tumorigenesis. Meingassner JG, et al. [2] reported no changes in number or morphology of LC were observed in epidermal sheets of mice treated for 5 days with pimecrolimus. Martires KJ, et al. [3] reported a single low-dose stimulated solar radiation-induced changers in LC are minimally affected by pimecrolimus, compared with triamcinolone. Herein, we designed a study to investigate the effects of topical pimecrolimus 1% on high-dose ultraviolet B (UVB)-irradiated human epidermal LC.

2. Material and methods

2.1. Skin tissues

Forty fresh human foreskin tissues were obtained from Department of Urology by circumcision, consented by the patients (18–30 years old). The tissues were randomly divided into 4 groups of 10 tissues each. The subcutaneous tissue was removed by scraping with forceps, and each tissue cut into 4 pieces of 0.5–1.0 cm. After three washings with 0.9% NaCl for 5 min, all the separate tissues ($n = 160$) were cultured dermal side down in 1 ml media (RPMI with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 55 mM β-mercaptoethanol) per well of 24 well tissue culture plates [4]. Only the bottom of tissue was immersed in the culture medium.

2.2. UVB irradiation and topical treatment

The source of UVB was BLE-1T158 (Spectronics Co., Westbury, NY, USA). A Kodacel filter (TA401/407, Kodak, Rochester, USA) was used

Abbreviations: LC, Langerhans cells; UVB, ultraviolet B; TCIs, topical calcineurin inhibitors; HM, high magnification.

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to block wavelengths of less than 290 nm (ultraviolet C).The UVB dosage was quantified using a Waldmann UV meter (model no. 585100: Waldmann Co., VS-Schwenningen) and 180 mJ/cm^2 UVB was delivered once to the epidermal side of the tissues. The lid was removed from the 24 well plates prior to UVB irradiation.

Topical pimecrolimus 1% (Elidel; Novartis Pharma GmbH, Germany) was applied on the epidermal side of the tissues after UVB irradiation (or without UVB irradiation). Pimecrolimus was delivered to the skin with a thin layer using sterile cotton swab, after that we kept daubing for 30 s with a certain pressure.

2.3. Study design

The obtained 40 fresh tissues were randomly divided into 4 groups of 10 tissues each as follows: Group A, control; Group B, tissues topically applied once with pimecrolimus 1% on epidermal side; Group C, tissues irradiated once with 180 mJ/cm^2 UVB on epidermal side; Group D, tissues topically applied pimecrolimus after UVB irradiation.

Ten minutes after irradiation or application, each well of tissue culture plate was added 1 ml culture medium to immerse the whole tissue in medium. All the tissues were cultured at 37 °C. After that, four time points were set as follows: (a) at 0 h; (b) at 18 h; (c) at 24 h; (d) at 48 h. Each tissue was cut into 4 pieces (above mentioned) corresponding to 4 time points, for each group.

After being treated, the tissues were collected at every time point respectively. Afterwards, each skin specimen was cut in two. One part was fixed with formalin until further processing for immunohistochemical staining. Another part was prepared for epidermal sheet that processed for immunofluorescence staining.

2.4. Immunohistochemistry

Slides were prepared using a Ventana autoimmunostainer (Loche, USA) and an available CD1a monoclonal antibody (Mouse anti-human; Maixin, Maixin.BiO, China, ready-to-use). Detection utilized Polymer-HRP, with 3,3′-diaminobenzidine chromogen. Similar procedures were followed for and Langerin (Mouse anti-human; Maixin, Maixin.BiO, China, ready-to-use) and HLA-DR (Mouse anti-human; Maixin, Maixin.BiO, China, diluted 1:80). Slides were visualized at 40× with a Nikon Eclipse microscope.

2.5. Immunofluorescence

Epidermal sheets obtained by incubation with EDTA-contained separation medium for 2 h at 37 °C were fixed with acetone for 5 min at −20 °C. After three successive washings with PBS for 5 min, the epidermal sheets were incubated with CD1a monoclonal antibody (Mouse anti-human; Maixin, Maixin.BiO, China, ready-to-use) for 1 h at 37 °C. The sheets were then washed with PBS for 15 min and incubated with fluorescein isothiocynate-conjugated secondary antibody (Goat anti-mouse; Bioworld Technology Co., USA, diluted 1:100) for 30 min at 37 °C. The specimens were viewed under a fluorescence microscope.

2.6. Statistical analysis

The numbers of epidermal CD1a$^+$, Langerin$^+$ and HLA-DR$^+$ LC were all counted for five successive fields in high magnification (HM, ×400) with light microscopy. The total number of LC was calculated and expressed as the numbers of LC/5 HM. The average LC number of each group at different time point was expressed as mean ± SD and analyzed using SPSS version 18.0. All tests were 2-sided, and $P<0.05$ was considered significant.

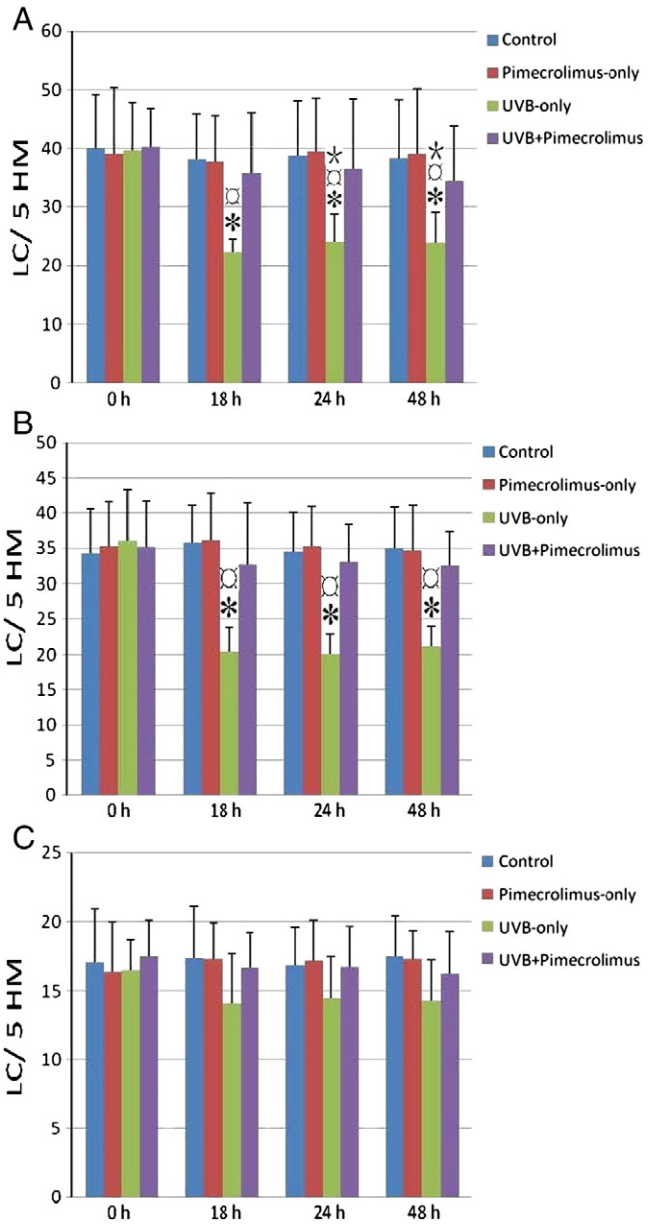

Fig. 1. The number of epidermal LC (immunohistochemistry), for each group. Panel A, CD1a$^+$ LC: *P<0.001, UVB-only (18 h, 24 h and 48 h, respectively) *versus* UVB-only (0 h); ¤P<0.001, UVB-only (18 h) *versus* other groups (18 h), UVB-only (24 h) *versus* control (24 h), UVB-only (24 h) *versus* pimecrolimus-only (24 h), UVB-only (48 h) *versus* control (48 h), UVB-only (48 h) *versus* pimecrolimus-only (48 h); *P<0.05, UVB-only (24 h) *versus* UVB + pimecrolimus (24 h), UVB-only (48 h) *versus* UVB + pimecrolimus (48 h). Panel B, Langerin$^+$ LC: *P<0.001, UVB-only (18 h, 24 h and 48 h, respectively) *versus* UVB-only (0 h); ¤P<0.001, UVB-only (18 h) *versus* other groups (18 h), UVB-only (24 h) *versus* other groups (24 h), UVB-only (48 h) *versus* other groups (48 h). Panel C, HLA-DR$^+$ LC: epidermal HLA-DR$^+$ LC numbers had no significant differences at different time points, for each group; there were no significant differences among all groups at the same time point.

3. Results

Figs. 1A and 2A–D show the epidermal CD1a$^+$ LC numbers (immunohistochemistry) had no significant differences at different time points, for control and pimecrolimus-only group. Epidermal CD1a$^+$ LC numbers of UVB + pimecrolimus group (18 h, 24 h and 48 h, respectively) had a slight but not significant decrease, compared to UVB + pimecrolimus group (0 h) ($P=0.109$, 0.414 and 0.112, respectively). For UVB-only group, the epidermal CD1a$^+$ LC number at 18 h substantially decreased from 39.6 ± 8.30 to 22.3 ± 2.26/5 HM,