Pharmacological Effects of a Specific Leukotriene B₄ Receptor Antagonist (VML 295) on Blood Leukocytes, Cutaneous Inflammation and Epidermal Proliferation

Bianca A.M.P.A. Seegers^a Monique P.M. Andriessen^a
Candida A.E.M. van Hooijdonk^a Els S.M. de Bakker^a
Ivonne M.J.J. van Vlijmen-Willems^a Gerry L. Parker^b
Piet E.J. van Erp^a Peter C.M. van de Kerkhof^a

^aDepartment of Dermatology, University Hospital, Nijmegen, The Netherlands, and

^bVanguard Medica Limited, Surrey Research Park, Guildford, UK

Key Words

CD11b · Inflammation · Proliferation, epidermis · Leukotriene B₄ · Leukotriene B₄ receptor · LY 293111 · Tape-stripping · VML 295

Abstract

VML 295 (LY 293111) is a potent and specific leukotriene₄ receptor antagonist. It has previously been shown in human volunteers that VML 295 at a dosage of 48 mg twice daily inhibits the ex vivo leukotriene B₄ (LTB₄)-induced upregulation of CD11b on peripheral blood neutrophils. A clear dose-response relationship was shown. In addition, VML 295 inhibits various inflammatory aspects resulting from LTB₄ challenge of the skin, again showing a dose-response relationship. In

view of the large variation in the elimination half-life of VML 295 (25–88.5 h) in individual human subjects, the present pharmacological study was designed to provide information on the pharmacodynamics of the drug by the assessment of VML 295 plasma concentrations, ex vivo LTB₄-induced CD11b upregulation of neutrophils, neutrophil accumulation in the skin following epicutaneous application of LTB₄ and epidermal regeneration following standardized surface trauma. A group of 36 healthy volunteers were treated in a double-blind study with VML 295 at 200 mg twice daily, VML 295 at 200 mg once daily or placebo for 7 days. Before treatment, at the end of treatment and following discontinuation of treatment, VML 295 plasma concentrations and CD11b upregulation of blood neutrophils were assessed. In 18

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2000 S. Karger AG, Basel
1422–2868/00/0132–0075\$17.50/0

Accessible online at:
www.karger.com/journals/sph

P.C.M. van de Kerkhof
Department of Dermatology, University Hospital Nijmegen
PO Box 1901
NL–6500 HB Nijmegen (The Netherlands)
Tel. +31 24 3613247, Fax +31 24 3541184

subjects, the effects of the three treatments on LTB₄-induced inflammatory were assessed before and at the end of treatment, and in the remaining 18 subjects the effects of these treatments on epidermal regeneration were assessed similarly. VML 295 at 200 mg either twice or once daily has a profound inhibitory effect on ex vivo LTB₄-induced CD11b upregulation of blood neutrophils, LTB₄-induced neutrophil accumulation in the skin, trauma-induced hyperproliferation of the epidermis and regenerative keratinization. The twice daily dose schedule was significantly more effective than the once daily regimen in reducing ex vivo CD11b stimulation of neutrophils, in blood samples collected 24 h after discontinuation of VML 295 treatment. The twice daily schedule tended to be more efficient in skin biopsies, although this difference was not statistically significant in the number of subjects investigated. A plasma concentration of 100 ng/ml proved to be the threshold for these effects. The profound biological effects, both systemically and cutaneously, as well as the safety profile, make VML 295 a promising drug for skin disorders characterized by epidermal proliferation and neutrophil accumulation.

Copyright © 2000 S. Karger AG, Basel

Introduction

VML 295 (LY 293111) is a novel diaryl ether carboxylic acid derivative which has been characterized as a potent and specific antagonist at the leukotriene B₄ (LTB₄) receptor. In vitro, VML 295 demonstrates a range of pharmacological actions against LTB₄-induced human neutrophil stimulation including inhibition of chemotactic activity, calcium generation and induction of CD11b/CD18 adhesion receptor expression [1]. VML

295 also antagonized LTB₄- and fMLP-induced human neutrophil aggregation.

VML 295 has been administered to a total of 211 human subjects and has proven to be safe and well tolerated with no serious drug-related adverse events reported.

Single and multiple oral doses of VML 295 have been administered to healthy human subjects and patients up to a maximum dose of 200 mg twice daily for 8 weeks. At the highest dose, almost complete suppression of ex vivo human neutrophil LTB₄-induced CD11b upregulation was demonstrated. In an antigen challenge study in mild asthmatics, 1-week treatment with VML 295 at 112 mg 3 times daily resulted in a significant reduction in the number of neutrophils in bronchoalveolar lavage fluid [2]. Additionally, in a study involving epicutaneous administration of LTB₄ in healthy volunteers, VML 295 demonstrated (relative to placebo) a significant dose-related suppression of cutaneous hyperproliferation and a reduction of inflammatory cell infiltrate with effects (which were most marked at 200 mg twice daily, although a dosage of 48 mg twice daily already inhibited the ex vivo LTB₄-induced upregulation of CD11b on peripheral blood neutrophils) on dermal and epidermal polymorph migration, perivascular T lymphocyte accumulation and Langerhans cell migration [3].

Previous pharmacology data have indicated the individual subjects' terminal elimination half-life to range between 25 and 88.5 h [Vanguard Medica, data on file]. Therefore, a pharmacological study in healthy volunteers was designed to provide information on the pharmacodynamics of VML 295 itself and, in particular, to investigate the dynamics of the cellbiological effects of this compound in peripheral blood leukocytes ex vivo, LTB₄-induced cutaneous inflammation in vivo, and regenerative epidermis in vivo.

The following study aims were defined: to evaluate the effects of once daily (o.d.) and twice daily (b.i.d.) treatments of VML 295 at 200 mg on LTB₄-induced upregulation of CD11b expression in peripheral blood neutrophils before, during and 2–36 h after dosing, in healthy volunteers *ex vivo*; to assess the effects of o.d. and b.i.d. treatments of VML 295 at 200 mg on elastase-positive cells, predominantly neutrophils, following epicutaneous application of LTB₄; and to measure the effects of o.d. and b.i.d. treatments of VML 295 at 200 mg on epidermal cell characteristics during regenerative recovery of the epidermis following adhesive tape-stripping.

Patients and Methods

Subjects, Blood Samples and Biopsies

The design of the present study was a double-blind, randomized, placebo-controlled, parallel-group phase-I study. Thirty-six healthy volunteers without a clinically significant medical history were included in this study, after they had given informed consent. All volunteers were treated with either VML 295 at 200 mg o.d. (4 active 50-mg capsules before noon and 4 placebo capsules after noon), 200 mg b.i.d. (4 active capsules taken twice daily), or placebo (4 placebo capsules taken twice daily). Randomization was carried out before using a computerized program for randomization. All subjects were treated for 8 days (from day 1 up to and including day 8). All medications were prohibited 7 days prior to and during the entire study, unless required for emergency or supportive care. Paracetamol was allowed for minor headaches at the discretion of the investigator. Women of childbearing age had to take (oral) contraceptives.

Blood specimens (n = 36) for assessment of *ex vivo* neutrophil CD11b surface expression were obtained on day 1 (immediately prior to the study medication), day 8 (prior to the last evening medication), and day 9 (2, 12 and 24 h after the last dose of the study medication). Unfortunately, in subjects in the VML 295 200 mg o.d. group, blood sampling 2 h after the last dose on day 9 was missed due to an error in the sampling schedule. Peripheral blood (5 ml) was collected through venipuncture, kept in EDTA at 4°C and processed within 2 h after collection to prevent nonspe-

cific upregulation of CD11b expression as a result of neutrophil activation.

In one group of 18 volunteers, epicutaneous LTB₄ application was carried out, 24 h before day 1 and 24 h before day 8. The other group of 18 subjects attended the clinic 48 h prior to both day 1 and day 8 for induction of the standardized surface trauma by tape-stripping. In all volunteers, two punch biopsies (3 mm) were taken from affected areas under local anesthesia on days 1 and 8.

Ex vivo CD11b Integrin Upregulation Assay

Blood samples were processed in triplicate using 90-μl aliquots which were incubated with LTB₄ (10 μl, 1×10^{-7} M) in Hanks' balanced salt solution (HBSS; Sigma Chemical Corp., St. Louis, Mo., USA) containing 0.1% bovine serum albumin (HBSS-BSA) for 30 min at 37°C or with HBSS-BSA alone. Samples were then cooled and incubated in the dark for 30 min at 4°C with 10 μl (0.045 g/l) anti-human CD11b fluorescein conjugate (Mo-1-FITC; Coulter Corp., Hialeah, Fla., USA). Erythrocytes were lysed (with 10% FACS[®] Lysing Solution in demineralized water; Becton Dickinson, San Jose, Calif., USA), and the remaining cells were washed with HBSS-BSA, fixed in 1% paraformaldehyde solution and stored at 4°C until analysis. The analysis was always performed within one week of preparation of the leukocyte suspensions in order to prevent the possibility of artefacts caused by prolonged storage.

All specimens were analyzed with an Epics[®] Elite flow cytometer (Coulter, Luton, UK). Cells were excited with an air-cooled 488-nm Argon laser set at 15 mW. Green fluorescence (FITC) was measured through a 525-nm band pass filter (band width 30 nm). Calibration and sensitivity were checked in every experiment by using FITC-labelled beads (Standard-Brite; Coulter Source, Hialeah, Fla., USA). Forward and side scatter were used for gating only granulocytes. For each sample 5,000 gated cells were analyzed.

LTB₄ Application and Tape-Stripping

LTB₄ (Peasel, Frankfurt, Germany) was administered at a dose of 100 ng diluted in 10 μl of ethanol, and applied to the test sites on the upper arm of the subject using a plastic cylinder (5.5 mm diameter) and the ethanol evaporated under a stream of nitrogen (n = 18). The test sites were covered with impermeable dressings (silver patch; van der Bend, Brielle, The Netherlands) and held in place with leukosilk tape (Beiersdorf, Hamburg, Germany) after application of LTB₄.

Tape-stripping was carried out over a 2×2 cm area of the upper arm of the subject ($n = 18$) by repeated application of adhesive tapes (Sellotape). The procedure was stopped when the skin reached a glistening appearance. At this moment, the stratum corneum was virtually totally removed.

Immunohistochemical Staining Procedures

From the biopsies ($n = 18$) taken 24 h after LTB₄ application, cryostat sections of $7 \mu\text{m}$ were cut and fixed for 10 min in acetone for staining with anti-elastase (IgG₁ kappa, clone NP57 mouse; Dakopatts, Copenhagen, Denmark). Anti-elastase has a specificity for neutrophil elastase which is present in PMNs and sporadic in monocytes. Slides were incubated with the monoclonal antibody for 30 min, followed by 2 washes with PBS and incubation for 30 min with rabbit-anti-mouse antibody conjugated with peroxidase (RAM-PO, Dakopatts). After 2 more washes with PBS and preincubation with sodium acetate buffer (pH 4.9), slides were stained with sodium acetate buffer containing 200 mg/l 3-amino-9-ethyl-carbazole (AEC solution) and 0.01% H₂O₂ for 10 min at 37°C in the dark. All slides were washed in demineralized water and lightly counterstained with Mayer's hematoxylin (Sigma). Slides were finally mounted on glycerol gelatin (Sigma) and studied by light microscopy.

All stainings were assessed before the study was unblinded. The density of infiltrate cells was assessed semi-quantitatively in epidermis and dermis using a 5-point scale and a 7-point scale, respectively: epidermis: 0 = no positive cells observed, 1 = sporadic staining, 2 = minimal presence, 3 = moderate presence, 4 = pronounced presence; dermis: 0 = no positive cells, 1 = sporadic, 2 = 1–25% of infiltrate cells stained, 3 = 26–50%, 4 = 51–75%, 5 = 76–99%, 6 = 100%.

Cell Suspensions and Flow Cytometry

Epidermal cell suspensions were derived from the biopsies taken 48 h after tape-stripping of the skin ($n = 18$). Dermoepidermal separation was performed by overnight incubation with thermolysin, and an epidermal cell suspension was prepared by trypsinization according to standardized procedures [4]. To assess epidermal proliferation and inflammation, the cell suspensions were stained with the following agents: RKSE 60 (a gift from Prof. F.C. Ramaekers, Department of Molecular Biology, University of Maastricht, The Netherlands) which recognizes cells of the suprabasal compartment, expressing keratin 10 [5]; TOPRO-3-IODIDE (TB3; Molecular Probes, Eugene, Oreg., USA) which allows quantification of the percentages of cells in S and G₂M phases [6], and anti-vimentin

(VIM3B4; Novocastra Laboratories, Newcastle-upon-Tyne, UK) which is a monoclonal antibody binding to vimentin [7].

The triple labelling of these two antibodies and DNA stain have been described before [4]. In brief, 3-color fluorescence was obtained with the fluorochromes fluorescein-isothiocyanate (FITC) and phycoerythrin (PE) which are conjugated to monoclonal goat antibodies against mouse IgG_{2a} and mouse IgG₁, respectively, in combination with TP3.

From each sample 5,000 gated cells were measured and analyzed using an EPICS[®] Elite flow cytometer (Coulter) equipped with a dual laser system. PE and FITC were excited with an air-cooled Argon ion laser (15 mW, 488 nm) and TP3 was excited with a HeNe laser (10 mW, 633 nm). After setting appropriate gates with the EPICS[®] Elite software, the percentages of vimentin- and keratin-10-positive cells were calculated. Using Multicycle[™] software, the percentages of basal keratinocytes in the S and G₂M phase of the cell cycle were calculated from the DNA histograms.

Clinical Laboratory Safety Measurements and VML 295 Plasma Levels

Prior to starting medication, a general physical examination and laboratory measurement consisting of hematology, serum chemistry, serum electrolytes, random blood glucose and urinalysis were performed. During the study period, the hematology, serum chemistry and urinalysis examinations were repeated on days 1, 8, and at the follow-up visit (between day 9 and 23). On days 4, 8 and 9 (12, 18 and 24 h after dosing), additional blood samples (10 ml) were taken for assay of VML 295 concentrations in plasma.

Statistical Analysis

Statistical comparisons were carried out with two-tailed tests at the 5% level of significance. Comparisons between the VML 295 at 200 mg b.i.d. group and the placebo group were conducted for the CD11b ratios 2 and 12 h after the last dose using Wilcoxon rank sum tests with normal approximation. For the data 24 h after the dose, all three treatment groups were compared by the Kruskal-Wallis test with the χ^2 approximation. For all time points, the median differences between treatment groups were estimated from the analysis and 95% confidence intervals were constructed around these estimated differences. The data obtained from the flow-cytometrical analysis of the cell suspensions and the data obtained from the immunohistochemical assessments were statistically analyzed by the paired Student *t* test.

Fig. 1. Mean CD11b stimulation ratio (mean $\pm$ 2 SD) in volunteers treated with VML 295 once daily (n = 12), volunteers treated with VML 295 twice daily (n = 11), and volunteers treated with placebo (n = 13). **a** Before and during treatment. **b** Following discontinuation of treatment.

Results

Demographic Characteristics

A total of 36 subjects (20 females and 16 males) were recruited to the study, 12 (33.3%) were assigned to the VML 295 at 200 mg o.d. group, 11 (30.6%) to the VML 200 mg b.i.d. group and 13 (36.1%) to the placebo group. A total of 35 volunteers completed the study. One patient dropped out for reason of a gastrointestinal (viral) infection. Overall, the av-

erage age of the subjects was 27.1 years, the youngest subject being 18.1 and the oldest 56.8 years.

Ex vivo CD11b Upregulation of Blood Neutrophils

Figures 1a and b summarize the mean CD11b stimulation ratio before, during and after discontinuation of treatment with VML 295 at 200 mg o.d., 200 mg b.i.d. and placebo. It can be seen that those subjects who were

Fig. 2. a VML 295 plasma concentrations during and after discontinuation of VML 295. **b** CD11b stimulation ratio versus VML 295 plasma concentration.

treated with VML 295 had a markedly reduced stimulation ratio during treatment. As long as 36 h after discontinuation, the stimulation ratio remained reduced. CD11b stimulation ratios were, on average, 0.21-fold greater in the o.d. group compared to the b.i.d. group. At both 2 and 12 h after the final dose of study medication, the differences between the VML 295 b.i.d. group and the placebo group were highly significant ($p < 0.001$). However, from 24 h after discontinuation of VML 295 onward, the o.d. schedule had permitted a slight recovery in LTB₄-induced ex vivo CD11b stimulation, whereas the b.i.d. schedule maintained complete inhibition of the CD11b stimulation. At 24 h after the last

dose, a statistically highly significant difference was determined between the three treatment groups ($p < 0.001$). Pairwise comparisons revealed that results from the VML 295 o.d. treatment group and the VML 295 b.i.d. treatment group were both significantly lower than those obtained from the placebo group ($p < 0.001$). Comparison between the o.d. and b.i.d. groups indicated that there was a difference which was statistically significant ($p < 0.012$).

VML 295 Plasma Concentrations

VML 295 plasma concentrations were measured during and following discontinuation of treatment. It can be seen that during

Fig. 3. Elastase-positive cells 24 h after epicutaneous application of LTB₄. **a** Epidermal accumulation of elastase-positive cells (mean ± SEM). **b** Dermal accumulation of elastase-positive cells (mean ± SEM).

treatment (day 8, 2 h after dosing) no overall correlation could be observed between CD11b stimulation and plasma VML 295 levels, although the inhibition of the CD11b upregulation proved to be most apparent at VML 295 VML plasma concentrations >100 ng/ml (fig. 2b). Interestingly, 24 h after discontinuation, a correlation coefficient of

-0.578 ($p = 0.008$) could be shown between both parameters.

Elastase-Positive Cells 24 h after Epicutaneous Application of LTB₄

In total, 18 subjects were included for LTB₄ application. In 5 subjects, placebo treatment was given, 6 subjects received VML 295

o.d. and 7 subjects were treated with VML 295 b.i.d. Both dose regimens of VML 295 completely inhibited infiltration of elastase-positive cells ($p = 0.0005$ o.d. and $p = 0.002$ b.i.d.) into the epidermis following application of LTB₄ (fig. 3a). The placebo treatment had no statistically significant effect ($p = 0.09$). Both dose regimens of VML 295 also markedly reduced the infiltration of elastase-positive cells (fig. 3b) into the dermis ($p = 0.006$ o.d., $p = 0.0007$ b.i.d.).

Epidermal Cell Characteristics 48 h after Tape-Stripping

In total, subjects were included for tape-stripping. Eight subjects were treated with placebo, 6 subjects with VML 295 o.d. and 4 with VML 295 b.i.d. Both o.d. and b.i.d. VML 295 dose regimens were effective in reducing the percentage of basal cells (keratin 10-negative cells) in S and G2M phases ($p = 0.006$ and $p = 0.04$, respectively), whereas placebo had no effect ($p = 0.36$) on this parameter. These results are shown in figure 4a. VML 295 b.i.d. (fig. 4b) showed a slight trend towards a reduction in vimentin-positive cells ($p = 0.06$), whereas placebo or VML 295 o.d. showed no consistent effect ($p = 0.29$ and $p = 0.22$, respectively). Figure 4c shows the percentages of suprabasal cells (keratin 10-positive cells) following tape-stripping. Remarkably, placebo and VML 295 o.d. did not have any effect on the relative decrease of suprabasal cells 48 h after tape-stripping ($p = 0.47$ and $p = 0.18$, respectively). After VML 295 b.i.d., the percentage of keratin 10-positive cells is increased 48 h after tape-stripping ($p = 0.05$), as compared to their expression after tape-stripping before VML 295 medication.

Safety and Tolerability

The three treatment groups were comparable with respect to vital signs at screening. There were no clinically significant changes in

vital signs, i.e. none were reported as adverse events. There were no major changes in laboratory values. A total of 23 adverse events were reported by 17 (47.2%) subjects. Of these, 10 events were reported by 7 subjects (58.3%) in the VML 295 at 200 mg o.d. group, 6 by 4 subjects (36.4%) in the VML 295 b.i.d. group and 7 by 6 subjects (46.2%) in the placebo group. The most frequently reported event was headache, which was reported by 6 subjects, 3 (25%) in the VML 295 o.d. group and 3 (23.1%) in the placebo group. Diarrhea was reported by a total of 4 subjects, 2 (16.7%) in the VML 295 o.d. group and 2 (18.2%) in the VML 295 b.i.d. group. No serious adverse events were reported.

Discussion

The present study reconfirms the marked efficacy of VML 295 in blocking the ex vivo LTB₄-induced upregulation of CD11b on peripheral blood neutrophils. In a previous study, this inhibition was shown after 5 days of treatment with a dose of 200 mg b.i.d. in healthy volunteers [3].

The present study provides further information on the pharmacodynamics of VML 295. The reduction of CD11b upregulation remained manifest as late as 36 h following discontinuation of treatment. Interestingly, the o.d. schedule showed a small diminishment of this profound inhibition of the LTB₄-induced CD11b upregulation 24 h after discontinuation of treatment. Therefore, the b.i.d. schedule was selected for further study as a more effective dosing regimen for reduction of neutrophil functioning.

Integration of the observations of VML 295 plasma concentrations and CD11b upregulation revealed that a concentration of VML 295 of 100 ng/ml is sufficient to cause a substantial inhibition of neutrophil functioning.

Fig. 4. Epidermal cell characteristics 48 h after tape-stripping. **a** Percentage of basal cells in SG₂M phase (mean $\pm$ SEM). **b** Percentage of vimentin-positive cells (mean $\pm$ SEM). **c** Percentage of keratin 10-positive cells (mean $\pm$ SEM).

It is possible that the lack of correlation between VML 295 and CD11b upregulation is caused by a full occupancy of receptors at this concentration of VML 295.

In order to find to what extent VML 295 might have a pharmacological effect on the skin and to what extent the advantage of VML 295 b.i.d. is also reflected in an enhanced efficacy on the skin, two quantitative well-reproducible *in vivo* models for cutaneous inflammation were evaluated. The inflammatory response to epicutaneous application of LTB₄ has been evaluated extensively [8–10]. LTB₄ in ethanol at concentrations between 10 and 100 ng/10 µl induces accumulation of neutrophils, with a sharp maximum between 16 and 24 h. In this dose range a clear dose-response relationship has been shown. The response to tape-stripping has been evaluated before as a well-reproducible *in vivo* model to study a clearly defined wave of recruitment of cycling epidermal cells and as such is adequate for studies on the induction of the epidermal proliferation *in vivo* [4, 11]. Again, the present study reconfirms these *in vivo* models as adequate to study the interference of an experimental drug with neutrophil accumulation and epidermal proliferation, respectively.

The pronounced accumulation of neutrophils following the relatively high dose of 100 ng/10 µl of LTB₄ was totally blocked in the epidermis by the o.d. and b.i.d. dose schedule of VML 295, whereas a partial inhibition was observed in the dermis. The b.i.d. schedule tended to inhibit the dermal accumulation of neutrophils more than the o.d. schedule, although both schedules were not statistically significantly different. In a previous communication [3], VML 295 b.i.d. was reported to produce a pronounced reduction of various LTB₄-induced inflammatory events.

The effect of VML 295 on epidermal growth and differentiation characteristics fol-

lowing tape-stripping further substantiates the profound effect of VML 295 on epidermal cell behavior. In a previous study, epidermal proliferation *in vivo* proved to be markedly stimulated following epicutaneous application of LTB₄. *In vitro*, LTB₄ was shown to stimulate epidermal proliferation [3]. The present investigation again suggests that LTB₄ has a direct effect on epidermal proliferation. Although the percentage of vimentin-positive cells tended to be reduced by VML 295 b.i.d., a consistent and profound inhibition of the percentage of basal cells in the S and G₂M phase and an increase of keratin 10-positive cells was evident for both the o.d. and b.i.d. dose schedule of VML 295. The b.i.d. dose schedule tended to be more effective as compared to the o.d. schedule, although a statistically significant difference between both schedules was not shown.

The profound effects of VML 295 on neutrophil accumulation and epidermal proliferation makes this compound an attractive candidate for the treatment of skin disorders characterized by epidermal proliferation and neutrophil accumulation. In view of the safety data, so far, further clinical studies are worthwhile.

References

- 1 Marder P, Scott Sawyer J, Froelich LL, Mann LL, Spaethe SM: Blockade of human neutrophil activation by LY293111, a novel leukotriene B₄ receptor antagonist. *Biochem Pharmacol* 1995;49:1683–1690.
- 2 Evans DJ, Barnes PJ, Spaethe SM, van Alstyne EL, Mitchell MI, O'Connor BJ: Effect of a leukotriene B₄ receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* 1996;51:1178–1181.
- 3 van Pelt JP, de Jong EMG, van Erp PEJ, Mitchell AL, van de Kerkhof PCM: The regulation of CD11b integrin levels on human blood leukocytes and leukotriene B₄ stimulated skin by a specific leukotriene B₄ receptor antagonist (LY293111). *Biochem Pharmacol* 1997;53:1005–1012.
- 4 Glade CP, Seegers BAMPA, Meulen EFJ, van Hooijdonk CAEM, van Erp PEJ, van de Kerkhof PCM: Characterization of epidermal cell suspensions prepared from normal and hyperproliferative human skin using an optimized thermolysin-trypsin protocol. *Arch Dermatol Res* 1996;288:203–210.
- 5 Ramaekers FCS, Puts JJG, Moester O, Kant A, Huysmans A, Haag D, Jap PH, Herman CJ, Vooijs GP: Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors: An overview. *Histochem J* 1983;15:691–713.
- 6 van Hooijdonk CAEM, Glade CP, van Erp PEJ: TO-PRO-3 iodide, a novel HeNe laser-exitable DNA stain as an alternative for propidium iodide in multiparameter flow cytometry. *Cytometry* 1994;17:185–189.
- 7 de Waal RMW, Semeijn JT, Cornelissen IMH, Ramaekers FCS: Epidermal Langerhans cells contain intermediate-sized filaments of the vimentin type: An immunocytologic study. *J Invest Dermatol* 1984;82:602–604.
- 8 Camp R, Russell Jones R, Brain S, Woollard P, Greaves M: Production of intraepidermal microabscesses by topical application of leukotriene B₄. *J Invest Dermatol* 1984;82:202–204.
- 9 Lammers AM, van de Kerkhof PCM: Response of polymorphonuclear leukocytes to topical leukotriene B₄ in healthy and psoriatic skin. *Br J Dermatol* 1987;116:521–524.
- 10 van de Kerkhof PCM, Copius Peereboom-Stegeman JHJ, Boeyen J: An ultrastructural study of the response of normal skin to epicutaneous application of leukotriene B₄. *J Dermatol* 1991;18:271–278.
- 11 Boezeman JBM, Bauer FW, de Grood RM: Flow cytometric analysis of the recruitment of G₀ cells in human epidermis in vivo following tape stripping. *Cell Tissue Kinet* 1987;20:99–107.