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Research Paper

Vitamin D_3–Based Conjugates for Topical Treatment of Psoriasis: Synthesis, Antiproliferative Activity, and Cutaneous Penetration Studies

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Purpose. The goals of the experiments reported in this paper were to explore skin bioavailability and cell growth inhibitory activity of new vitamin D_3–based conjugates studied as a potential drug complex for psoriasis.
Methods. Conjugation was made between polyunsaturated fatty acids (PUFAs), such as linolenic acid or γ-linolenic acid, and calcipotriol—a vitamin D_3 analogue clinically used for topical treatment of psoriasis. These complexes were prepared by coupling the corresponding fatty acid with calcipotriol in the presence of dicyclohexyl-carbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) to obtain an ester bond.
Results. The conjugates were capable of enhancing the penetration of the vitamin into the skin as well as inhibiting proliferation of keratinocytes in cultures. The antiproliferative activity even increased after simulating the full hydrolysis of the conjugates. *In vitro* skin penetration studies revealed that the conjugates penetrated into the skin at higher levels relative to calcipotriol alone. It was also demonstrated that the conjugate containing n-3 fatty acid penetrated into the skin at higher levels as compared to the conjugate containing n-6 PUFA. High-performance liquid chromatography analysis has shown that after penetration, a major portion of calcipotriol-PUFA conjugate was first converted mainly into another isomer form, presumably by transesterification, and only then it was hydrolyzed to form apparently high local concentrations of both calcipotriol and PUFA.
Conclusions. The unique biotransformation that occurred after penetration into the skin indicates that these conjugates are mutual prodrugs that are able to be bioprocessed in the skin and fully converted to the parent therapeutic agents.

KEY WORDS: calcipotriol; polyunsaturated fatty acids; psoriasis; skin penetration; vitamin D_3.

INTRODUCTION

Psoriasis, a chronic inflammatory dermatosis characterized by scaling, infiltration, and erythema, affects approximately 2% of the worldwide population (1). The cause of psoriasis is unknown, but it is considered that disordered arachidonic acid metabolism may play a role in the pathogenesis of the disease through the chemotactic effect of the metabolites in human skin (2,3). It is well-known that vitamin D_3 analogues, 1α,25-dihydroxyvitamin-D_3 [1,25$(OH)_2D_3$] and calcipotriol, are useful for the treatment of hyperproliferative skin diseases, such as psoriasis vulgaris (4–7). Vitamin D_3 analogues exert their effects through interaction with vitamin D_3 receptor (VDR) located in epidermal keratinocytes (8). The efficacy of these compounds in inhibition of proliferation in a variety of cell types and their therapeutic potential in the topical treatment of psoriasis has been established in a large number of clinical trials (9,10). However, apart from their established beneficial activity, vitamin D_3 analogues have additional side effects. It is recognized that 1,25$(OH)_2D_3$ induces several biological effects influencing a number of signal transduction pathways, such as intracellular calcium increase and protein kinase C activation (11), and calcipotriol may lead to cutaneous irritant reactions in approximately 20% of the patients (12).

Polyunsaturated fatty acids (n-3 PUFAs) have been used for many years as a dietary supplement (mainly in fish oil) and have also been presumed to have beneficial effects in psoriasis. Although not completely established yet, there are still several indications that such PUFAs may possess a therapeutic potential for psoriasis. It was shown by Kew *et al.* (13) that n-3 PUFA can reduce markers of immune cell function. Some other studies reported on moderate beneficial effects of orally-administered fish oil (in capsules) on psoriasis (14,15). However, the intrinsic effectiveness of the n-3 PUFAs in clinical trials could not have been reflected by such protocols. Clinical trials using n-3 PUFAs given to patients by intravenous administration have shown to have beneficial and significant effect in psoriasis (16). In view of these results, it has been postulated that localized delivery of n-3 PUFAs into the skin by topical preparations may have advantages. The development of an efficient means of topical delivery can in-

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crease local soft-tissue and joint-drug concentration while reducing the systemic distribution of a drug (17,18).

Calcipotriol [and $1,25(OH)_2D_3$] has poor penetration through the stratum corneum into the epidermis (internal report) which may explain its limited efficacy and frequent treatment failures (19–21). In order to enhance drug-skin permeation of vitamin D_3 analogues, there is a need to modify the lipophilicity and to optimize partitioning into the skin and to maximize skin permeation. On a basis of a "mutual prodrug" in which each part (i.e., PUFA and calcipotriol) may act as a co-drug or as the promoiety bound to the drug, we synthesized and evaluated new molecules that combine calcipotriol and several PUFAs through an ester bond. The ester bond is partially and gradually hydrolyzed by skin esterases, releasing the free active co-drug at high levels in the deep skin layers, leading to sustained drug delivery followed by prolonged activity.

MATERIALS AND METHODS

Materials

Calcipotriol (calcipotriene) was a gift from Teva Pharmaceutical Industries Ltd. (Kfar Saba, Israel). Linolenic acid (18:3n-3; all-*cis*-9,12,15-octadecatrienoic acid), γ-linolenic acid (18:3n-6; all-*cis*-6,9,12-octadecatrienoic acid), dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)-pyridine (DMAP) were obtained from Sigma (Rehovot, Israel). High-performance liquid chromatography (HPLC) grade solvents were obtained from Merck (Darmstadt, Germany).

Synthesis of Conjugates

Dicyclohexylcarbodiimide (DCC) (33.3 mg; 0.16 mmol) and 4-(dimethylamino)-pyridine (DMAP) (1.975 mg; 0.016 mmol) were added into a solution of calcipotriol (100 mg; 0.24 mmol) in dry CH_2Cl_2 (20 ml). The mixture was stirred at 0°C and a solution of linolenic acid (45 mg; 0.1615 mmol) or γ-linolenic acid (45 mg; 0.1615 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise under nitrogen atmosphere. The mixture was stirred at room temperature for 3 h. The reaction mixture was washed with 0.5 N HCl, saturated $NaHCO_3$ solution, water, and then dried (with $MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel (eluting with 1% chloroform in methanol) to give the mono- and diester derivatives of the conjugates.

^{1}H NMR spectra were recorded on a Bruker DMX-500 operating at 500.1 MHz, and chemical shifts are reported in parts per million (δ) using TMS as the internal standard. Calcipotriol-PUFA mono conjugate: ^{1}H-NMR ($CDCl_3$): δ = 5.33–5.40 (m, 6H), 5.00 (s, 1H), 4.65–4.75 (m, 1H), 4.45 (bs, 1H), 4.25 (bs, 1H), 3.42–3.55 (m, 4), 2.70–2.90 (m, 4H). ^{13}C-NMR ($CDCl_3$): 66 (C-3); 71 (C-1); 86 (C-24). TLC (5% MeOH in $CHCl_3$): Rf = 0.33. LC-ESI-MS (see below): m/z = 672.2. Calcipotriol- di- PUFA conjugate: ^{1}H-NMR ($CDCl_3$): δ = 5.32–5.42 (m, 12H), 4.98 (s, 1H), 4.63–4.73 (m, 1H), 4.42 (bs, 1H), 4.22 (bs, 1H), 3.40–3.52 (m, 4), 2.68–2.88 (m, 8H). TLC (5% MeOH in $CHCl_3$): Rf = 0.53. LC-ESI-MS: m/z = 933.4.

LC-MS Instrument and Conditions

Liquid chromatography-mass spectrometry (LC-MS) Agilent 1100LC series (Waldbronn, Germany) and Bruker Esquire 3000plus MS (Bremen, Germany) instrument consisting of a C_{18} column (Betasil C_{18}, 5 μm, 250 × 4.6 mm; Thermo-Hypersil, UK) and methanol as the mobile phase. The UV detector was set at 265 nm, the flow rate at 1 ml/min, and injection volume at 10 μl. The MS conditions were optimized as follows: API electron spray interface, positive mode polarity, a drying gas flow of 10 L/min, a nebulizer gas pressure of 60 psi, a drying gas temperature of 335°C, a fragmentor voltage of 0.4 V, a capillary voltage of 4451 V, and a scan range of m/z 25–1000, at 1.15 s/scan.

In Vitro Skin Penetration Study

The permeability of calcipotriol-PUFA conjugates through pig skin was measured *in vitro* with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ, USA). The diffusion area was 1.767 cm^2 (15-mm diameter orifice), and the receptor compartment volumes varied from 11 to 12 ml. The solutions in the receiver side were stirred by externally driven, Teflon-coated magnetic bars. The testing was basically performed as previously described (22,23). Each set of experiments was performed with at least 4 diffusion cells ($n \geq 4$). Full-thickness porcine skin was excised from fresh ears of slaughtered white pigs (breeding of Landres and Large White, locally grown in Kibbutz Lahav, Israel). Skin sections (about 2 × 2 cm) were cut and subcutaneous fat was removed from the skin sections with a scalpel. Transepidermal water loss measurements (TEWL, Dermalab Cortex Technology, Hadsund, Denmark) were performed and only those pieces that the TEWL levels were within specification (<10 g/m^2 h) were mounted in the diffusion cells, ready for testing. The skin pieces were used for penetration studies within 2 weeks of preservation at −20°C, from the time of slaughtering. Each skin section was placed with the stratum corneum facing up on the receiver chambers, and then the donor chambers were clamped in place. The receiver chamber, defined as the side facing the dermis, was filled with phosphate buffer (4 mM, pH 7.4)-ethyl alcohol (analytical grade) (7:3). After 15 min of washing/conditioning the dermis side at 37°C, the buffer was removed from the cells and the receiver chambers were refilled with phosphate buffer (4 mM, pH = 7.4)-ethyl alcohol (analytical grade) (7:3). This receptor medium was chosen to provide *in vivo* equivalent "sink" conditions in the receiver compartment, or in other words to increase the circulation in the dermis and to prevent possible sedimentation of permeants in the dermis stagnant layer of the full-thickness skin. The presence of this percentage of ethanol in the receiver has not been expected to enhance the permeability of the porcine skin preparation (24), especially due to the finding that calcipotriol and its conjugates preferred to quantitatively accumulate in the skin and were undetected in the receptor medium as described in the results section. Calcipotriol (0.005%, 121 μM) and calcipotriol-PUFA conjugates at an equimolar concentration in isopropanol-propylene glycol (1:1 v/v) solutions, were applied on the skin, then the donor compartment was carefully occluded by Parafilm. A volume of 50 μl per cell (or 28 μl/cm^2 containing 3.4 nmols drug/cm^2) were applied to cover the entire diffusion area leaving a thin layer of solution on the skin. When inspected after 12 h, the skin surface area still remained wet. After 12 h, samples (1 ml) were taken from the receiver chambers into 2-ml vials, and the exposed skin pieces were extracted with ethanol containing 0.005% BHT (as described below). The receiver and the skin extract solu-

tions were transferred quantitatively into vials and dried under vacuum using DNA-mini concentrator apparatus (Heto Lab Equipment, Denmark). The dry samples were kept at –70°C until analyzed by HPLC. The analyses were usually performed within 2 days but never more than 7 days from the sampling time.

Skin Extraction

At the end of the diffusion process, the exposed skin was wiped off carefully, followed by 10 consecutive measures of tape stripping. The skin pieces were cut to small pieces and inserted in 2-ml vials. The pieces in each vial were extracted by 1-ml ethyl alcohol containing 0.005% BHT. Each extraction was performed by incubation in a 40°C shaking water bath (150 rpm) for 1 h. Aliquots of 0.8 ml of the skin extracts were evaporated to dryness (as described above), and just before analysis the residue was reconstituted with 200 μl of methanol followed by injection into the HPLC system. The total recovery of known quantities of calcipotriol impregnated in skin pieces and processed as above was 98.9 ± 1.9% (n = 4).

HPLC Analysis of Samples from Receiver Solutions and Skin Extracts

Aliquots of 20 μl from each sample were injected into HPLC system (Shimadzu VP series including SPD-M10Avp photodiode array detector), equipped with a prepacked C_{18} column (Betasil C_{18}, 5 μm, 250 × 4.6 mm, ThermoHypersil, UK). The quantitation of calcipotriol and its PUFA conjugates was performed by integration of peaks detected at 265 nm. The samples were chromatographed using an isocratic mobile phase consisting of water-isopropyl alcohol-methanol (10:9:81) for calcipotriol and methanol only for its PUFA conjugates. A flow rate of 1 ml/min was used. A calibration curve (peak area vs. drug concentration) was constructed by running standard calcipotriol solutions in methanol containing 0.005% BHT for every series of chromatographed samples. Identification of unknown calcipotriol and calcipotriol- PUFA conjugates was performed by analyzing their UV spectra. Calibration curves were linear over the range 0.1–5 μg/ml (0.1, 0.2, 0.5, 1, 2, 3, 5 μg/ml). Data were expressed as the permeating drug quantity per unit of skin surface area, Q_t/S ($S = 1.767\ cm^2$).

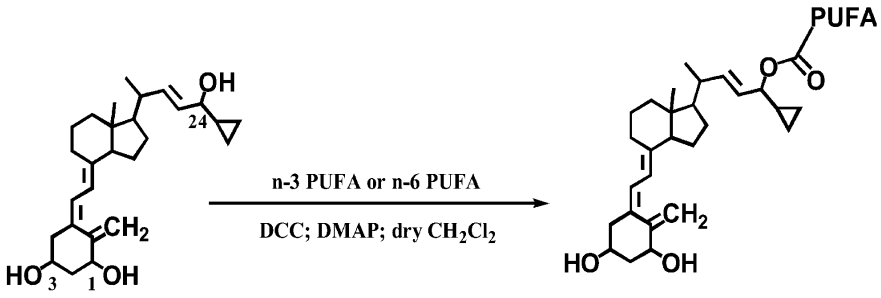
Determination of Growth Arrest and Differentiation of Keratinocytes

The direct effect of the new substances as compared to free PUFA and free calcipotriol on the proliferation level of keratinocytes was assessed *in vitro* using human keratinocyte cell lines. Cultures of normal human keratinocytes ((HaCaT)) were used as previously described (25). The cells (1.5×10^4) were inoculated in 0.2 ml Dulbecco modified Eagle medium (DMEM). After 48 h at 37°C, the medium was replaced with a fresh DMEM containing 1% ethanol and 30 μM test substance. DMEM containing 1% ethanol without test compounds was used for the control cells. Cell growth was assayed by vital dye assay (alamara blue and neutral red tests) after additional 24 h. It was evaluated by measuring the absorbance of the dye in the experimental cells at 540 nm as compared to the absorbance of the dye in the control cells.

RESULTS AND DISCUSSION

New complexes on the basis of "mutual prodrugs" built of calcipotriol and polyunsaturated fatty acids (PUFAs) were prepared. The corresponding fatty acid, either linolenic acid (18:3n-3 PUFA) or γ-linolenic acid (18:3n-6 PUFA), was condensed with calcipotriol in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) in dry dichloromethane (Scheme 1). Although calcipotriol structure includes three hydroxyl groups (1α, 3β, 24S), the preferable isomer during synthesis was found to be the esterification of the hydroxyl on carbon 24 (Scheme 1). The structures of the monoester derivatives were determined by ^1H and ^{13}C NMR and mass spectrometric analysis.

The synthetic conjugate, its bioforms (e.g., skin-formed isomers) and the free calcipotriol moiety (after skin hydrolysis) were identified and quantified by HPLC-UV from skin extracts 12 h after the conjugate solution (121 μM) was topically applied. The conjugate, its bioforms and the formed calcipotriol were found in the skin compartment only. Although ethanol was mixed with the receptor buffer solution to increase the solubility in this compartment, these compounds were not found in the receiver side at detectable concentrations. The free calcipotriol formed in the porcine skin was detected by HPLC after application of its monoesters bearing two different fatty acids, 18:3n-3 and 18:3n-6 PUFAs. The free calcipotriol levels found in the skin after the hydrolysis of each monoester were compared to calcipotriol permeated directly into the skin after application of an equimolar dosage of free calcipotriol as in the conjugates' solutions. Figure 1 presents the extent of formation of the free calcipotriol after hydrolysis of the conjugates by porcine skin and the cutaneous penetration of calcipotriol after its application in solution. As shown in Fig. 1, the accumulation of the free calcipotriol released from 18:3n-3 PUFA and 18:3n-6 PUFA mono con-



Calcipotriol (calcipotriene) **acyl ester of calcipotriol**

Scheme 1. Molecular structures of calcipotriol and its synthetic monoester with PUFA.

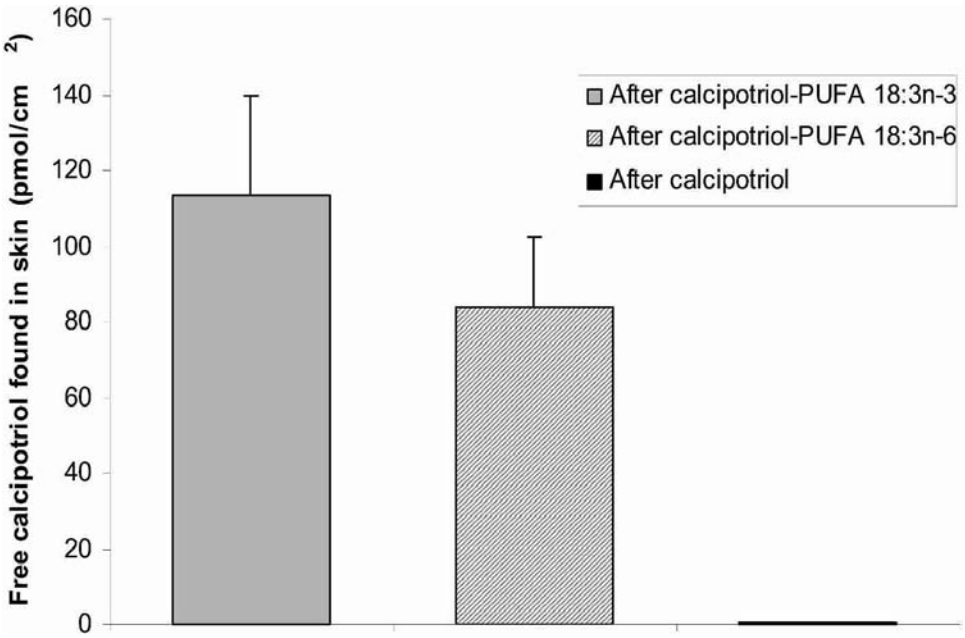


Fig. 1. Free calcipotriol generated in porcine skin 12 h after application of 12μM solutions of its monoesters with linolenic acid (18:3n-3) or γ-linolenic acid (18:3n-6). Note that no detected calcipotriol was found in the skin after calcipotriol 12 μM solution was applied for 12 h.

jugates was 113 and 84 pmol/cm^2 (or 47 and 35 ng/cm^2), respectively, while no penetration of calcipotriol was detected after free calcipotriol solution had been applied to skin. Interestingly, the percentages of calcipotriol formation from the different conjugates penetrated into the skin were similar, 14.6% and 16% of the 18:3n-3 PUFA and 18:3n-6 PUFA mono conjugates, respectively. It can obviously be deduced that (a) the depot formation of the free calcipotriol is due to the enhanced penetration process exerted by the lipophilic nature of the mono PUFA-calcipotriol derivatives and (b) the difference in this depot formation between the conjugates depends on their different skin permeability rather than any difference in the rate of hydrolysis. The significant difference ($p < 0.05$) in the extent of hydrolysis between the conjugates containing the 18:3n-3 and 18:3n-6 PUFA indicates that the n-3 fatty acid conjugate is superior over the n-6 fatty acid conjugate in generating drug depot in the skin (Fig. 1). This finding is apparently correlated to the enhancement in the penetration of the n-3 conjugate into the skin as shown in Fig. 2.

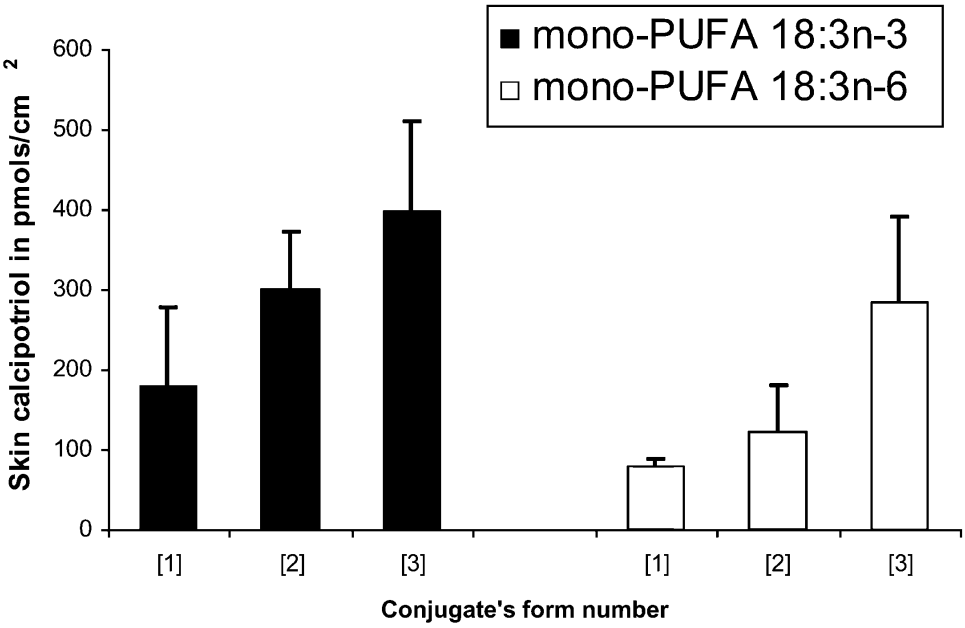


Fig. 2. Skin biotransformation into bioforms 1 and 3 of mono-PUFA 18:3n-3 and mono-PUFA 18:3n-6 after 12-h *in vitro* treatment with mono-PUFA 18:3n-3 (form 2) and mono-PUFA 18:3n-6 (form 2), respectively.

An interesting biotransformation activity occurred during and after penetration of n-3 and n-6 PUFA conjugates into the skin. It was found that each one of the penetrating conjugates was converted into two other isomer forms. The three different calcipotriol-PUFA ester derivatives were numbered according to their appearance in the reverse-phase chromatography (Fig. 3). The penetrating synthetic conjugate (the one that was applied on the porcine skin) was called form 2, a more hydrophilic compound was called bioform 1, and a less hydrophilic compound found in relatively large quantities was named as bioform 3. These compounds were identified and confirmed by UV spectra and by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Bioforms 1 and 3, in which the acyl group is bound to carbon positions 3 and 1, were formed presumably by transesterification of the synthetic form 2 (in which the acyl group is bound to carbon position 24 of the calcipotriol molecule) with nonspecific acyltransferases (26–28). The three forms, which accumulated and were detected in the skin (Fig. 2), then underwent enzymatic hydrolysis to release both free calcipotriol and PUFA (Scheme 2). The mass balance calculation of total conjugates plus the free calcipotriol that were found in the skin revealed that 29.2 ± 9.2 mol % of 18:3n-3 PUFA-CPT dose and 16.6 ± 5.9 ml % of 18:3n-6 PUFA-CPT dose actually penetrated into the skin.

The concept of specific skin transformation of the synthetic compounds to a preferred mono derivative was supported by a penetration study performed with *di*-PUFA ester of calcipotriol. As already demonstrated by the previous 12-h experiments, the synthetic mono-PUFA derivative of form 2 was found after 8 h of this experiment to be converted preferably into the mono derivative of bioform 3. However, the treatment with the corresponding *di*-PUFA derivative (in which two acyl groups were attached to two carbon positions - 1 and 24) led to almost exclusive hydrolysis into the mono derivative of bioform 3 (Fig. 4). This indicates that the formation of PUFA ester on the hydroxyl group of carbon position 1 of calcipotriol is the dominant route of skin biotransformation of these conjugates.

An enhanced antiproliferative activity in cultured keratinocytes of calcipotriol in combination of 18:3n-3 PUFA (linolenic acid) was also demonstreted. Figure 5 presents the antiproliferative activity of the mono calcipotriol-PUFA complexes (1:1 mol equivalent) in cultures of grown keratinocytes (cell number is expressed as absorbance of Alamara blue vital stain). A complete hydrolysis of the complexes was simulated by preparing a mixture of free calcipotriol and free PUFA at equimolar combinations. As shown, a significant antiproliferative activity was obtained after the complete hydrolysis of the monoester conjugate possessing 18:3n-3 PUFA compared to calcipotriol alone ($p < 0.05$), whereas no antiproliferative contribution was made by 18:3n-6 PUFA. It has been concluded therefore that apart of their enhanced skin permeability, conjugates consisting of n-3 PUFA produce a stronger inhibitory effect on cell proliferation than that obtained by conjugates of n-6 PUFA. The reason for this phenomenon still needs to be thoroughly explored, however, the fact that n-3 PUFA penetrates more effectively the lipid bilayer of the skin implies that this fatty acid has increased ability to intrude and interact with cellular memmbranes compared to n-6 PUFA.

Fatty acids, in particular oleic acid (18:1n-9 monounsaturated fatty acid), have been shown to be potent skin penetration enhancers of various drugs (29). It has been proposed that the predominant effect of the fatty acid is the formation of phase-separated domains, thereby forming permeability defects at liquid-solid interfaces (30). Unsatuarated C_{18} fatty acids have been found to be preferential for skin permeability over the saturated form. In addition, the branched isomer of C_{18} fatty acid (C_{16}-branched isostearic acid) was more effec-

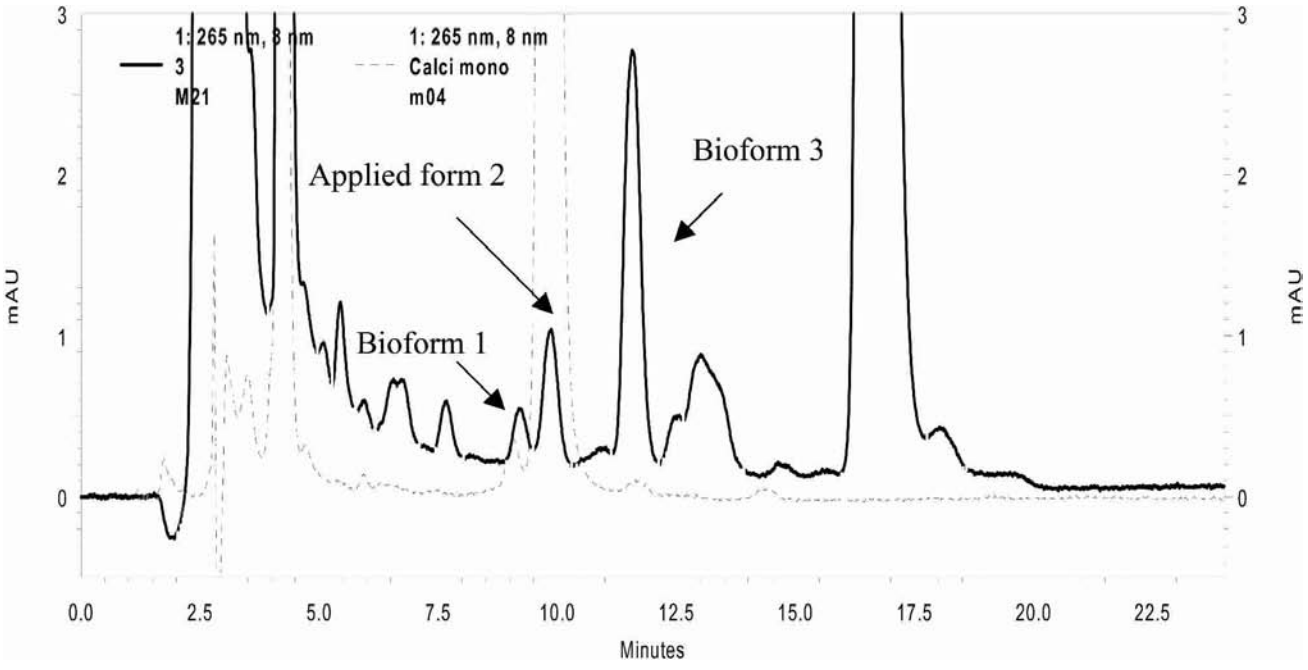
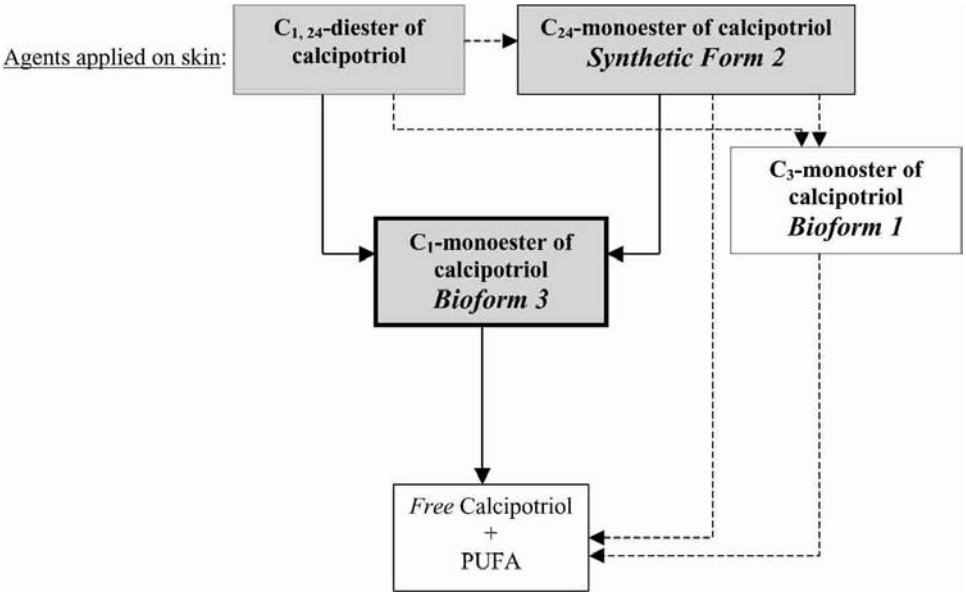


Fig. 3. Typical HPLC-UV chromatogram of skin extract showing peaks of bioform 1, the penetrating form 2 (synthetic ester of linolenic acid on C24 of calcipotriol), and bioform 3. The dotted chromatogram represents the synthetic monoester of linolenic acid and calcipotriol on C24 position (form 2). Note that the substance contains also very small amounts of bioforms 1 and 3 as impurities.



Scheme 2. Biotransformation of calcipotriol-PUFA in the skin.

tive in enhancing skin penetration than a differently branched (C_2-branched isostearic acid) or unbranched C_{18} isomer (stearic acid) (31). In a more recent study, polyunsaturated fatty acids such as linoleic, α-linolenic and arachidonic acids were found to enhance permeation of PABA more than monounsaturated fatty acids such as cis-9 and 13-octadecanoic acids (32). Esterified fatty acids have not been previously published as penetration enhancers while they are generally not as effective as their acids in enhancement of skin penetration. It is probably because the properties that dictate their ability to be disruptive of the skin lipid packing do not retain, although the properties enabling their own permeability (e.g., the three-dimentional structure, molecular volume, flexibility) may be preserved. Figure 6 illustrates the difference in three-dimensional structures between calcipotriol-18:3n-3 PUFA and calcipotriol-18:3n-6 PUFA conjugates. To evaluate the dimensions of these molecules, length measurements were performed. The length between two distant points in the PUFA moiety of the conjugates can be described by measuring the distance between the esteric oxygen (located on carbon 24 of calcipotriol) to the spatially most distant carbon atom on the chain of the fatty acid residue. Thus, in 18:3n-3 PUFA conjugate the distance between C12 and the esteric bond is 12.22Å, while in the case of 18:3n-6 PUFA conjugate the distance between the distant C9 and the esteric bond is 8.68 Å.

CONCLUSIONS

The current study shows that the permeation of calcipotriol through the skin can markedly be improved by the addi-

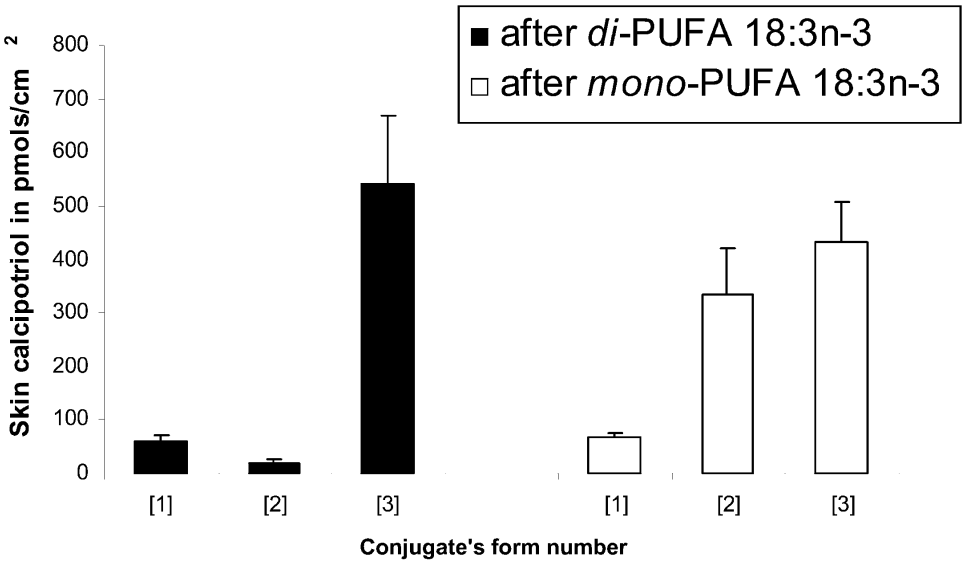


Fig. 4. Mechanism of action: a preferred mono derivative of bioform 3 was formed after hydrolysis of di-PUFA ester of calcipotriol in porcine skin after its 8-h application; after 8 h of mono-PUFA application (form 2), bioform 3 was dominantly generated from the penetrating ester of form 2.

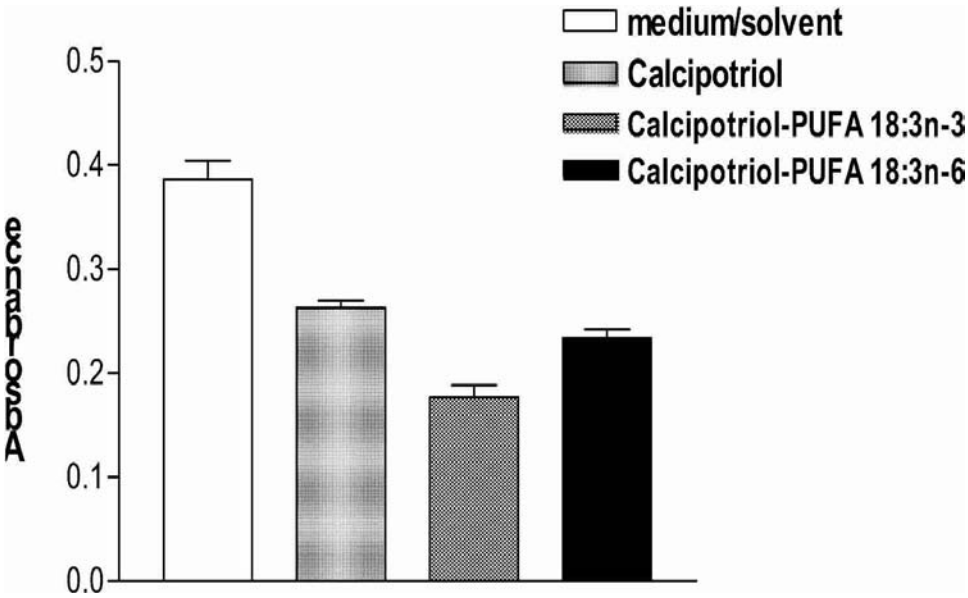


Fig. 5. Antiproliferative activity of calcipotriol and calcipotriol/ PUFA (1:1 mole equivalent) mixtures, simulating full hydrolysis, in cultures of grown keratinocytes.

tion of fatty acids. The desirable combination of the conjugate for topical treatment of psoriasis in terms of its enhanced lipophilicity and skin permeability can be achieved by proper selection of the acyl group. In this research, we have shown that derivation with n-3 PUFAs resulted in a better penetration with a concommitant formation of more dermally active drug than the derivation with n-6 PUFAs. One possible explanation can be derived from the three-dimentional structures of these molecules, showing more linear, more flexible and less bulky configuration of the calcipotriol ester of n-3 fatty acid (on carbon 24). The release of free calcipotriol and n-3 PUFA in the skin have been shown to occur by mainly two steps of enzymatic processes: one process has been presumed to occur by transesterification of the penetrating esters to new bioforms/isomers and the second process occurs by ester hydrolysis. The *in situ* released calcipotriol and n-3 PUFA have been found to be specifically and quantitatively localized in its target organ through topical treatment. It was also shown that the free n-3 PUFA by itself (rather than n- 6 PUFA) surprisingly possessed a significant antiproliferative

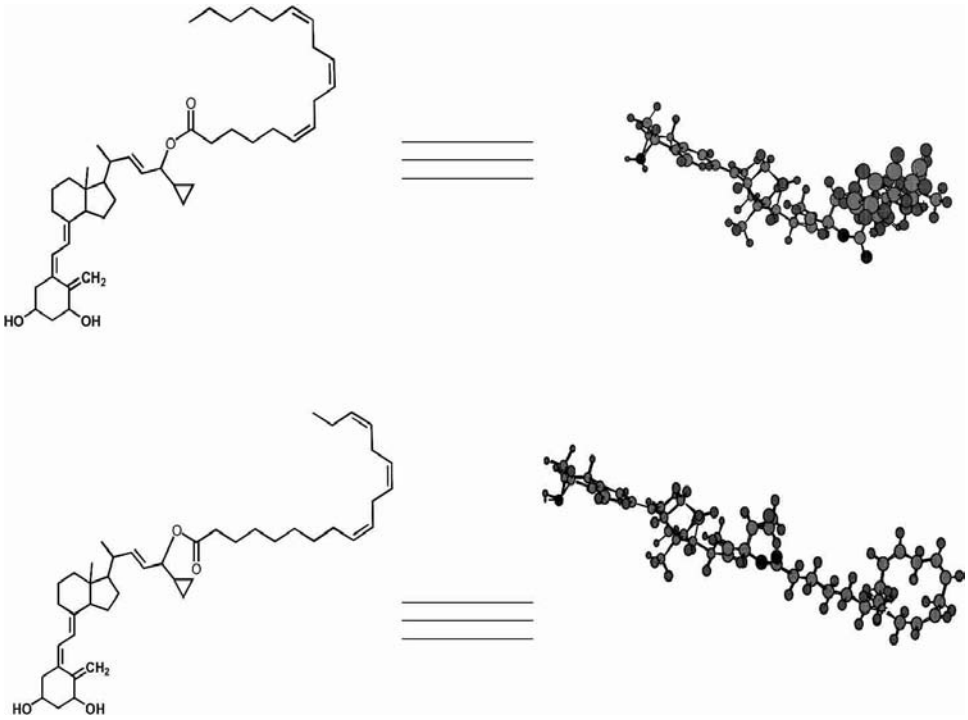


Fig. 6. Three-dimentional structures of the 18:3n-6 PUFA ester (upper) and the 18:3n-3 PUFA ester of calcipotriol (lower).

activity in cultured keratinocytes. This aspect should be further investigated in addition to the potential anti-inflammatory activity of topical PUFAs in the treatment of psoriasis. In view of the ongoing use of vitamin D_3 derivatives in psoriasis, this research may lead to a new approach in drug development for treatment of psoriasis and perhaps of other skin disorders as well.

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