Drug-induced suppression of phosphorylase kinase activity correlates with resolution of psoriasis as assessed by clinical, histological and immunohistochemical parameters

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Summary  
Background Phosphorylase kinase (PhK), also known as adenosine triphosphate (ATP)-phosphorylase b phosphotransferase, integrates multiple calcium/calmodulin-dependent signalling pathways, including those involved in cell migration and cell proliferation, while coupling these pathways to glycogenolysis and ATP-dependent phosphorylation, thus ensuring continuing energy supply for these activities.

Objectives Our laboratory recently reported correlation of elevated PhK activity with psoriatic activity. This study further evaluates the significance of drug-induced suppression of PhK activity on psoriatic activity.

Patients and methods PhK activity was assayed in four groups, each with 10 patients: (i) active untreated psoriasis; (ii) resolving psoriasis treated by calcipotriol (Dovonex®. Bristol Myers Squibb, Princeton, NJ, U.S.A.), a vitamin D₃ analogue and an indirect inhibitor of PhK; (iii) curcumin (diferuloylmethane), a selective PhK inhibitor; and (iv) 10 normal non-psoriatic subjects.

Results PhK activity in units mg⁻¹ protein was highest in active untreated psoriasis (1204 ± 804·3; mean ± SD), lower in the calcipotriol-treated group (550·7 ± 192·9), lower in curcumin-treated group (207·2 ± 97·6), and lowest in normal skin (105·4 ± 44·6). One-way analysis of variance performed on log-transformed PhK activity measure showed significant differences among the four groups, F₅,₅₀ = 48·79, P < 0·0001. Decreased PhK activity in curcumin-and calcipotriol-treated psoriasis was associated with corresponding decreases in keratinocyte transferrin receptor (TRR) expression, severity of parakeratosis and density of epidermal CD8+ T cells.

Conclusions Our results demonstrate that drug-induced suppression of PhK activity is associated with resolution of psoriatic activity as assessed by clinical, histological and immunohistochemical criteria, and support the hypothesis that effective antipsoriatic activity may be achieved through modulation of PhK activity.

Key words: calcipotriol, cell migration, cell proliferation, curcumin, phosphorylase kinase, psoriasis

Psoriasis is a hyperproliferative skin disease¹–³ with an inflammatory cell component.⁴–⁸ The increased keratinocyte cycling in psoriasis is associated with increased migration of immature keratinocytes towards the stratum corneum, resulting in marked parakeratosis.⁹ The presence of inflammatory cells within the epidermis correlates with psoriatic disease activity and T cells, activated by external stimuli such as trauma¹⁰ or bacterial superantigens,¹¹–¹³ are believed to trigger the psoriatic process in genetically predisposed individuals.¹⁴–¹⁷ Compartmentalized epidermal T cells in psoriatic skin are thought to release cytokines that induce the psoriatic keratinocyte phenotype,¹⁸ which in turn is associated with epidermal growth factor (EGF) -dependent keratinocyte proliferation.¹⁹–²¹ EGF-dependent proliferation is stimulated by phosphorylation of its receptor, tyrosine kinase, which in turn is dependent on the activity of phosphorylase kinase.
PhK, also known as adenosine triphosphate (ATP) -phosphorylase b phosphotransferase, integrates multiple calcium/calmodulin-dependent signalling pathways while coupling these pathways to glycogenolysis and ATP-dependent phosphorylation, thus ensuring a continuing energy supply for activities such as cell migration and cell proliferation.

PhK is a multimeric enzyme, with four subunits (α, β, γ and δ). Initially, this enzyme was thought to function mainly in coupling muscle contraction with energy production. The expression of the catalytic activity is achieved through activation of its γ subunit, regulated by the phosphorylation of the α and β subunits. The PhK molecule, however, is initially activated by the binding of Ca2+ to its calmodulin-containing δ subunit. Conformational change of the molecule is achieved by phosphorylation of the β subunit, catalysed by type I cyclic adenosine monophosphate (cAMP)-dependent protein kinase. Phosphorylation of the α subunit, catalysed by type II cAMP-dependent protein kinase results in further conformational change, which contributes to its deactivation. PhK activity has been reported to be elevated in psoriasis, with elevated levels of PhK correlating with increased glycolysis, phosphorylation reactions and psoriatic activity.

PhK achieves its capacity to influence and therefore integrate multiple pathways simultaneously by means of its wide substrate specificity. Besides phosphorylating serine residues on glycogen phosphorylase and phosphorylase b in the presence of Mg2+, PhK has been shown to phosphorylate growth factor receptor tyrosine kinase in the presence of Mn2+. In addition, PhK phosphorylates threonine residues on troponin. Furthermore, PhK is implicated in the phosphorylation of inositol in the activation of phosphatidylinositol kinase, implicating its involvement in signalling pathways triggered by external stimuli. The α subunit of PhK has been noted to be homologous with regions in α-tropomyosin and human EGF receptors, with biological implications in the regulation of cell locomotion and cell proliferation by this enzyme. PhK has established involvement through glycogen phosphorylase activity in muscle contraction, presumably through the phosphorylation of actomyosin in muscle cells; therefore, increased activity of PhK in psoriatic epidermis is also suspected to be involved through similar actomyosin interactions in migratory non-muscle cells. Consequently, perhaps explaining the rapid egress of inflammatory cells into uninvolved psoriatic skin following tape-stripping and the increased migratory activity of keratinocytes following experimental trauma. Unlike other signalling molecules that are expected to decrease following a time interval of hours to days, PhK levels in psoriasis not only fail to decrease after tape-stripping, but rise to extraordinarily high levels with the maturation of the psoriatic plaque (Heng, unpublished observations). In view of this observation, together with our previous report that PhK activity correlates with psoriatic activity, we hypothesize that the persistence of high levels of PhK may contribute to the chronicity of the psoriatic plaques.

To test the hypothesis that drugs that specifically suppress PhK activity may result in the suppression of psoriatic activity, we assayed PhK levels in skin biopsies from 10 patients each with: (i) untreated active psoriasis; (ii) resolving psoriasis treated with curcumin, a selective PhK inhibitor; and (iii) resolving psoriasis treated with a known antipsoriatic drug, calcipotriol (vitamin D3 analogue). While curcumin is a selective PhK inhibitor, calcipotriol is proposed to inhibit PhK indirectly through stimulation of the PhK deactivator, type II cAMP-dependent protein kinase.

Materials and methods

The study was conducted at the Sepulveda Campus of the Veterans Affairs Greater Los Angeles Health Care System, and approved by its Internal Review Board.

Participants and clinical groups

With consent, we collected punch biopsies (6 mm) from: (i) active plaques of 10 patients with active untreated psoriasis; (ii) resolving plaques from 10 patients each treated with either curcumin or calcipotriol; and (iii) normal skin from 10 non-psoriatic patients to monitor the results. The patients were all men, and their mean ages were not significantly different across groups: 57.2 ± 13.3 years (SD: untreated group); 58.2 ± 14.5 years (curcumin-treated); and 62.5 ± 14.7 years (calcipotriol-treated group). The affected untreated patients had chronic disease resistant to steroids and calcipotriol: eight of 10 of these had chronic plaques of moderate severity over the limbs and/or trunk, and two of 10 had severe generalized erythrodermic psoriasis. The curcumin-treated group had chronic disease resistant to steroids, methotrexate and calcipotriol: nine of 10 patients had chronic plaques (moderate = 5; severe = 4) involving the trunk and limbs; the remaining patient had severe,
generalized erythrodermic psoriasis. The calcipotriol-treated group had chronic plaques resistant to steroids and tars, but which responded to calcipotriol; nine of 10 patients had chronic plaques of moderate severity, with the remaining patient suffering from severe generalized erythrodermic psoriasis.

Based on our hypothesis that persistently high levels of PhK contribute to the chronicity of the psoriatic plaque, we studied chronic psoriatic plaques resistant to multiple therapies. Active psoriatic plaques were plaques of moderate to severe thickness with bright erythema and no evidence of clearing or resolution. Withdrawal of therapy for 4 weeks constituted the clinical status of untreated in the active group. In the treated group, patients had a hiatus of 4 weeks with no treatment before starting either calcipotriol or curcumin. Resolving psoriatic plaques were plaques with >50–95% clearance of the plaque observed following treatment with either calcipotriol or curcumin. Biopsies were taken from areas of relative clearance in the drug-treated groups.

As curcumin is only soluble in alcohol, we used an alcoholic gel preparation containing 1% curcumin. The commercially available calcipotriol ointment (Dovonex®, 0.005% calcipotriol) was used. We also evaluated the effect of curcumin in alcoholic gel compared with the vehicle alone in six additional male patients, ages 62–81 ± 7.8 years (mean ± SD). In each of these, two untreated plaques of similar activity were selected—one treated with curcumin gel, and the other with the alcoholic gel base. These were biopsied at 4 weeks for PhK activity assays; and the results reported separately.

Cytosolic preparation of epidermal cells

From each site, skin biopsy samples (6 mm punch) were stored at −70°C until ready to be used, and then processed by techniques previously described.25 Briefly, each frozen sample was placed, with the epidermal surface upwards, in a glass tube to which 3 mL Tris–HCl buffer [10 mmol L−1 Tris–HCl, pH 7.8, 1 mmol L−1 dithiothreitol, 3 mmol L−1 MgSO4 and 1 mmol L−1 ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid] was added, and homogenized vigorously with a Teflon plunger in a Tris-R model K44a homogenizer for 1 s. Homogenization was repeated if necessary. The lysate of epidermal cells, separated from the dermis, was in the cytosolic solution. The fibroblasts that were inadvertently detached, together with the relatively intact piece of dermis, were removed by decanting the cytosolic solution into a 5-mL capacity polypropylene test-tube. The lysates were centrifuged at 3000 g for 15 min. Membranes and other cytosolic organelles that formed a pellet at the bottom of the tube were removed. The supernatant, which contained the cytosolic component of epidermal cells, was then subjected to biochemical analysis.

Assay of phosphorylase kinase activity

The serine kinase activity of PhK was assayed by measuring the incorporation of 32P into phosphorylase b according to a modification of the method of Cohen as previously described.25

Immunohistochemistry

Biopsy specimens were processed for immunocytchemistry using standard DAKO LSAB2 kit and endogenous biotin block technique using the DAKO Biotin Block system (Dako, Carpinteria, CA, U.S.A.). The biotinylated monoclonal antibodies used are listed in Table 1. The slides were incubated with streptavidin-conjugated peroxidase, with diaminobenzidine or 3-amino-9-ethylcarbazole as the chromogen. Negative controls were carried out using non-immune (mouse) serum in place of the primary monoclonal antibody.

<table>
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BD, Becton Dickinson; LC, Langerhans cells; MHC, major histocompatibility complex; NK, natural killer cells.

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Assessment of transferrin receptor expression by epidermal keratinocytes

The expression of transferrin receptor (TRR) on basal and suprabasal keratinocytes was quantified as the percentage of TRR+ keratinocytes per rete ridge. The mid-points between adjacent rete ridges at the epidermal–dermal junction (usually corresponding to the apices of the papillary dermis) were defined. Through these points on either sides of the rete ridge, lines were extended at right angles to the surface stratum corneum. These lines defined the lateral intrapidermal margins of the rete ridge for TRR enumeration. The keratinocytes of 10 consecutive rete ridges were assessed for each biopsy and the results averaged. Assessment of reliability of measurements showed a test–retest intra-rater reliability of Pearson’s $r = 0.91$, and an inter-rater reliability of 0.83.

Assessment of Ki-67 expression by epidermal keratinocytes

The expression of Ki-67, a cell proliferation marker expressed by the nucleolus on basal and suprabasal keratinocytes, was quantified as the percentage of Ki-67+ keratinocytes per rete ridge (number of Ki-67+ keratinocytes divided by total keratinocytes with nuclei per rete ridge × 100). The keratinocytes of 10 consecutive rete ridges were assessed for each biopsy and the results averaged. Assessment of reliability of measurements showed a test–retest intra-rater reliability of Pearson’s $r = 0.97$, and an inter-rater reliability of 0.95.

Assessment of parakeratosis

Parakeratosis, assessed histologically by the loss of the granular layer and presence of nuclei of immature keratinocytes within the stratum corneum, reflects in part the migratory capacity of basal keratinocytes towards the stratum corneum. The severity of parakeratosis was assessed as the percentage involvement (presence of parakeratosis) of a 4-mm linear strip of stratum corneum. An average of three sections per biopsy specimen was used for data analysis. Assessment of reliability showed a test–retest intra-rater reliability of Pearson’s $r = 0.96$, and an inter-rater reliability of 0.93.

Assessment of epidermal T-cell density

The T-cell (CD8+ subset) population within the epidermal compartment represents the activated cytokine-secreting CD8+ T-cell population that has migrated from the vascular compartment in the dermis, across the basement membrane, into the epidermis. The density of the epidermal CD8+ T cells was enumerated and quantified as the number of epidermal CD8+ T cells per high power field (h.p.f.). This value was the average of 10 h.p.f. measurements per specimen. Assessment of reliability showed a test–retest intra-rater reliability of Pearson’s $r = 0.98$, and an inter-rater reliability of 0.95.

Assessment of HLA-DR+ cells

T-cell activation results in the expression of HLA-DR, major histocompatibility complex (MHC) class II molecules by inflammatory cells (T cells, macrophages, Langerhans cells). HLA-DR is also expressed by cytokine-activated target cells. The activated HLA-DR+ inflammatory cell population was assessed as abundant (200–500 or more cells per h.p.f.), moderate 50–200 cells per h.p.f., and sparse 1–50 cells per h.p.f. This value was the average of 10 h.p.f. measurements per specimen. Cytokine-activated non-inflammatory cells that express HLA-DR molecules in untreated psoriasis include epidermal keratinocytes and capillary endothelium. The expression of HLA-DR molecules on endothelial cells and/or keratinocytes was quantified as: (i) strongly positive (clumps of endothelial cells and/or keratinocytes showing HLA-DR positivity); (ii) weakly positive (occasional endothelial cells and/or keratinocytes showing HLA-DR positivity); and (iii) negative (no HLA-DR+ observed) based on assessments of 10 h.p.f. per specimen.

Statistical analysis

SPSS version 7.5 (Statistical Package for the Social Sciences, Chicago, IL: SPSS Inc.) was used. A one-way ANOVA with Tukey’s post hoc tests were used to compare differences among the three patient groups. Where appropriate, comparisons with normal epidermis were also made. The results were considered statistically significant when $P < 0.05$ for the two-tailed test. All results in this report are expressed as mean ± SD.

Results

Clinical data

In the curcumin-treated group, five of 10 patients had a 90% resolution of psoriasis after 2–6 weeks of
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treatment; with the remainder showing 50–85% improvement after 3–8 weeks of curcumin. In the calcipotriol-treated group, three patients had 70–80% resolution after 4–6 months of treatment, and seven of 10 had 50–65% improvement after 6–18 months of calcipotriol. In the six patients with untreated plaque psoriasis treated with curcumin gel compared with vehicle (alcoholic gel) for 3–4 weeks, six of six curcumin-treated plaques improved by 25–70%, compared with no improvement (two of six) or worsening (four of six) in vehicle-treated plaques.

Biochemical and immunohistochemical data

Elevated PHK activity was previously reported to correlate with increased psoriatic activity. We assessed PHK activity in terms of PHK-dependent functions, i.e. cell cycling and cell migration, using the following: (i) TRR+ keratinocytes as a measure of keratinocyte cycling cell population; (ii) parakeratosis as a marker of surface migratory capacity of immature keratinocyte population; and (iii) epidermal CD8+ T-cell population as a measure of migrated T-cell population. Expression of HLA-DR/MHC class II on inflammatory and non-inflammatory cells was also assessed as a marker of cytokine-dependent activity, including the expression of migration-dependent adhesion molecules in psoriatic skin.

Phosphorylase kinase activity in active and treated psoriasis. The results of the curcumin/calcipotriol study are summarized in Figure 1(a). PHK activity was: highest (1204 ± 804·3 units mg⁻¹ protein, mean ± SD, n = 10) in active untreated psoriasis; lower in the calcipotriol-treated group (550·7 ± 192·9, n = 10); lower in the curcumin-treated group (207·2 ± 97·6, n = 10); and lowest in untreated skin (105·4 ± 44·6; n = 10). Levene’s test of homogeneity of variance was significant (P < 0·001), indicating that the assumption of homogeneity of variance across these groups was rejected. This was primarily due to the large variability in the untreated psoriasis relative to the small variability in normal skin. Following natural log (ln) transformation of the data, Levene’s test was no longer significant (P = 0·752). An ANOVA was then performed on the log-transformed PHK activity measure, and showed significant differences among the four groups. F₃,₁₆ = 48·79, P < 0·0001. Post-hoc Tukey honestly significant difference (HSD) tests showed that each group was significantly different from the others, P < 0·05.

Correlation between PHK activity and clinical disease was also determined. The clinical plaque severity was categorized numerically as 1 (minimal), 2 (mild), 3 (moderate) and 4 (severe). PHK activity showed a positive correlation with the severity of the psoriatic plaque (Pearson’s r = 0·80).

Transferrin receptor positive keratinocytes in active and treated psoriasis

Transferrin and iron are required for the function of ribonucleotide reductase in the S phase of DNA synthesis. That TRR expression serves as a marker for DNA synthetic cells, i.e. cycling cells, is supported by studies showing that iron is required by ribonucleotide reductase for the S phase of DNA synthesis. In activated T cells, TRR expression is a prerequisite for the stimulation of DNA synthesis by the T-cell growth factor, interleukin-2.

We evaluated TRR+ keratinocytes as a cell-surface marker of epidermal cycling cells. As illustrated in Figure 2, the percentage of TRR+ keratinocytes per rete ridge (mean ± SD; n = 10 per group) was highest in untreated psoriasis (60·1% ± 9·3, Fig. 2a), lower in calcipotriol-treated (17·0% ± 6·1, Fig. 2b) and lowest in curcumin-treated psoriasis (4·3% ± 2·2, Fig. 2c) and normal skin (4·3% ± 1·2). One-way ANOVA on log-transformed data (Levene’s test P = 0·06) showed significant differences among the groups. F₃,₁₆ = 147·22, P < 0·0001. Post-hoc Tukey’s HSD comparisons showed significant differences between untreated psoriasis and the other three groups (P < 0·0001); the values in curcumin-treated psoriasis were significantly lower than the calcipotriol-treated psoriasis (P < 0·0001), but were not different from normal skin (P = 0·960).

Keratinocyte TRR+ expression also correlated with clinical disease in that TRR+ keratinocytes were more numerous in biopsies from severe (thick) active clinical psoriasis (Fig. 2a) than biopsies from more moderate untreated disease.

Ki-67+ keratinocytes in active and treated psoriasis. We also evaluated the proliferative activity of keratinocytes using Ki-67 antigen, a nuclear cell proliferation marker. One-way ANOVA was performed following the square-root transformation of the mean percentage of Ki-67+ keratinocytes per rete ridge (Levene’s statistic = 2·54; P = 0·098) and showed significant differences among the groups: F₂,₂₇ = 11·27, P < 0·0001. Post-hoc Tukey’s comparisons showed that each group was significantly different from the other two, P < 0·0001.
Figure 1. Summarizes the following parameters evaluated in skin biopsy specimens taken from active untreated psoriasis (n = 10), calcipotriol-treated resolving psoriasis (n = 10), curcumin-treated resolving psoriasis (n = 10) and normal non-psoriatic subjects (n = 10). (a) Phosphorylase kinase activity (units mg⁻¹ protein) was highest (1204 ± 804: SD) in active untreated psoriasis, lower in calcipotriol-treated group (550:7 ± 192:9), lower in curcumin-treated group (207:2 ± 97:6) and lowest in normal skin (105:4 ± 44:6), P < 0.0001 (ANOVA). (b) The percentage of transferrin receptor positive keratinocytes per rete ridge was highest in active untreated psoriasis (60:1 ± 5:3), lower in the calcipotriol-treated group (17:0 ± 6:1) and lowest in curcumin-treated psoriasis (4:3 ± 2:2) and normal skin (4:3 ± 1:2), P < 0.0001 (ANOVA). (c) Percentage linear involvement of stratum corneum by parakeratosis was highest in active untreated psoriasis (94:6 ± 4:9), lower in calcipotriol-treated psoriasis (8:4 ± 6:8), still lower in curcumin-treated psoriasis (1:4 ± 2:4), P < 0.0001 (ANOVA), parakeratosis was not observed in normal skin. (d) The number of epidermal CD8+ T cells (migratory population) per high power field was highest in active/untreated psoriasis (38:2 ± 6:1), lower in calcipotriol-treated psoriasis (8:7 ± 3:7) and still lower in curcumin-treated psoriasis (0:6 ± 0:8), P < 0.0001 (ANOVA). CD8+ T cells were not observed in normal skin. The results are expressed as mean ± SD.

for each comparison. The percentage of Ki-67+ keratinocytes per rete ridge (mean ± SD) was highest in active untreated psoriasis (45:5 ± 8:4; Fig. 3a), lower in calcipotriol-treated psoriasis (15:7 ± 7:6; Fig. 3b), and lowest in curcumin-treated psoriasis (3:7 ± 2:1; Fig. 3c). In curcumin-treated psoriasis, decreased Ki-67 antigen expression was associated with loss of parakeratosis, and was observed to precede shortening of the rete ridges (Fig. 3c).

Severity of parakeratosis in active and treated psoriasis. Percentage parakeratotic involvement of stratum corneum was 94:6 ± 4:9% (mean ± SD) in untreated psoriasis, lower in the calcipotriol-treated (8:4 ± 6:8%), still lower in curcumin-treated psoriasis (1:4 ± 2:4%) and completely absent in normal skin. One-way ANOVA comparing the three psoriatic groups showed significant differences among them: F₂, 2₇ = 1065, P < 0:0001. Tukey’s HSD comparisons showed significant improvements in parakeratosis in calcipotriol-treated (P < 0:0001) and curcumin-treated specimens (P < 0:0001) relative to active untreated psoriasis, with less parakeratosis in curcumin-treated compared with calcipotriol-treated specimens (P < 0:012). The results are summarized in Fig. 1(c).
Figure 2. Immunocytochemical labelling of transferrin receptors (TRR) on surface membranes of cycling keratinocytes (single arrows) and lymphocytes (double arrows) in the following skin biopsy specimens: (a) severe active untreated psoriasis; (b) calcipotriol-treated resolving psoriasis; (c) curcumin-treated psoriasis early in the course of resolution; observe marked decreased in TRR expression in curcumin-treated psoriasis prior to flattening of the elongated rete ridges. Original magnification ×250.

Compartmentalized (epidermal) T-cell population in active and treated psoriasis

In evaluating cell locomotion (PheK-dependent activity) in psoriatic biopsies, the compartmentalized (epidermal) CD8+ T-cell population was used as an indicator of T cells migrated from the dermal vasculature into the epidermis. As it has been reported that activated compartmentalized (epidermal) T cells in psoriatic skin release lymphokines that induce the psoriatic keratinocyte phenotype, the epidermal CD8+ T-cell population may be used to assess the cytokine-secreting T-cell population important in inducing the psoriatic

Figure 3. Immunocytochemical labelling of Ki-67 proliferating cell nuclear antigen in (a) active untreated psoriasis, (b) calcipotriol-treated psoriasis and (c) curcumin-treated psoriasis, showing fewer Ki-67+ keratinocytes in calcipotriol-treated and curcumin-treated resolving psoriasis. Observe marked decreased Ki-67 expression in curcumin-treated psoriasis prior to flattening of the elongated rete ridges. Original magnification ×250.

Figure 4. Immunohistochemical labelling of epidermal CD8+ T cells (single arrows) in (a) active/untreated psoriasis, (b) calcipotriol-treated psoriasis and (c) curcumin-treated psoriasis showing a decrease in the density of epidermal CD8+ T cells in the treated specimens; (d) and (e) are adjacent sections from active untreated psoriasis, showing (d) CD3+ T cells and (e) CD8+ T cells. Observe that the density of epidermal CD3+ T cells (d) is similar to the density of epidermal CD8+ T cells (e), suggesting that most of the migratory CD3+ T cells within the epidermis belong to the CD8+ subset. Original magnification × 250.

phenotype. The density of the migratory T-cell population was highest in active untreated psoriasis (Fig. 4a), with $38.2 \pm 6.1$ CD8+ T cells per h.p.f. (mean ± SD) within the epidermal compartment. Density of the CD8+ lymphocytes was lower in both treatment groups with $8.7 \pm 3.7$ CD8+ T cells per h.p.f. in the calcipotriol group (Fig. 4b) and $0.6 \pm 0.8$ CD8+ T cells per h.p.f. in the curcumin-treated group (Fig. 4c). CD8+ cells were not observed in samples of normal epidermis. A square root transformation of epidermal CD8+ T-cell density was performed to achieve homogeneity of variance (Levene statistic = 0.976, $P = 0.396$). One-way ANOVA showed significant differences among the three psoriatic groups, $F_{2,27} = 217.5$, $P < 0.0001$. Post hoc Tukey's HSD comparisons showed significant improvements in both
the calcipotriol- and curcumin-treated groups ($P < 0.0001$) relative to untreated psoriasis. Labelling with the CD3+ epitope showed that most of the epidermal (presumed migratory) CD3+ T cells belonged to the activated CD8+ subset (Fig. 4d,e). The results are summarized in Figure 1(d).

**HLA-DR expression in active and treated psoriasis**

The expression of HLA-DR molecules is a reflection of the presence of cytokines, in particular interferon-γ, a cytokine secreted by activated T cells. In active untreated psoriatic skin, HLA-DR expression was strongly positive both on endothelial cells (Fig. 5a) and keratinocytes (Fig. 5a). HLA-DR expression on endothelial cells and keratinocytes was weakly positive in calcipotriol-treated psoriasis (Fig. 5b), and not observed in curcumin-treated (Fig. 5c) and normal skin. Overall, HLA-DR expression was as follows: (i) abundant in 10 of 10 skin specimens (Fig. 5a) from untreated psoriasis; (ii) moderately abundant (Fig. 5b) in eight of 10 specimens, abundant in one of 10 specimens and sparse in one of 10 specimens from calcipotriol-treated psoriasis; and (iii) sparse (Fig. 5c) in 10 of 10 specimens from curcumin-treated psoriasis. HLA-DR+ cells were not observed in normal non-psoriatic skin.

**Phosphorylase kinase activity in curcumin gel compared with vehicle**

To determine the anti-PhK activity of curcumin vs. vehicle, a separate study using six patients was performed, comparing the effect of 3–4 weeks of twice daily treatment of plaques of similar clinical severity with either 1% curcumin in alcoholic gel vs. vehicle alone. The PhK values were compared in the curcumin-treated and vehicle-treated groups using a two-tailed $t$-test for independent groups. The results are presented in Table 2. Two outlier values (range 490–1773) in the vehicle-treated group resulted in a SD that was significantly greater than that seen in the PhK-treated group (range 149–670); Levene’s test = 5.57, $P = 0.04$. Transformation of the PhK values, $\ln_{10}(\text{PhK})$.

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produced a more normal distribution without outliers, and met the assumptions of the analysis: Levene's test = 0.04. P = 0.8 (not significant) and analysis of the ln(PK) values showed a significant difference between the groups, t = 2.681, P = 0.023. Following log transformation, the PK values in the curcumin-treated group were significantly lower than those in the vehicle-treated group (P = 0.023).

Discussion

In this study, we report the antipsoriatic activity of a novel molecule, curcumin. Also known as diterpyrrol-methane, curcumin is a component in spices such as turmeric and ginger. This molecule has been reported to inhibit PK specifically.

The inhibition of EGFR-induced tyrosine phosphorylation of EGF receptors observed with curcumin may be achieved through its anti-PK effect.

We also report that treatment directed at lowering PK activity, either with curcumin, a specific PK inhibitor, or with calcipotriol (which is thought indirectly to lower PK activity by stimulating its deactivator, type II cAMP-dependent protein kinase), results in the resolution of psoriasis as determined by clinical, histological and immunohistochemical criteria. These results support our premise that elevated PK levels may be important in maintaining plaque chronicity in psoriasis. We cannot be sure that the lowering of PK activity is directly responsible for plaque improvement, nor that this is a specific effect of curcumin rather than simply a bystander effect related to the improvement in psoriasis; therefore, observations that decreased Ki-67 expression precedes shortening of the rete ridges in curcumin-treated psoriasis would appear to suggest that inhibition of PK activity by curcumin is associated with decreased proliferative activity. Moreover, the clinical effectiveness of curcumin in resolving recalcitrant psoriatic plaques appears also to support the hypothesis that PK inhibitors may be effective antipsoriatic agents.

The reasons for the abnormally high levels of PK in psoriasis are not clear, nor is it clear why these levels fall to decrease with expected triggering of homeostatic mechanisms. We speculate that regulatory mechanisms involved in (i) overactivation of PK (e.g. via Ca2+ activation of the calmodulin-containing γ subunit, or activation of the β subunit by type I cAMP-dependent protein kinase), or (ii) defective deactivation of PK (e.g. via deficiencies in type II cAMP-dependent protein kinase), may constitute possible fundamental anomalies in psoriasis contributing to persistently high levels of PK in chronic psoriatic plaques. The findings of low levels of type II cAMP-dependent protein kinase in psoriasis provides support for the hypothesis that psoriatic activity may result from overactivity of PK due, at least in some cases, to a defective deactivation or switch-off mechanism of PK from low levels of type II cAMP-dependent protein kinase. Current genetic studies also provide support for these premises. Genetic studies in psoriatic individuals show linkage of susceptibility loci encoding genes mapped to chromosome 17q. It is of interest that the regulatory subunit (RRA) of type II cAMP-dependent protein kinase has recently been shown to be encoded by genes also residing on chromosome 17q. The linkage to susceptibility loci on chromosome 16q have also been reported in psoriatic individuals. As genes encoding PK β subunits have been mapped to chromosome 16q, the linkage of susceptibility loci on chromosome 16q in psoriasis, although unconfirmed yet, suggests the possibility that individuals with overexpression of PK β subunits may also be predisposed to psoriasis.

These findings may lend credence to the unspoken belief by many clinicians that psoriasis may be a disease of multiple genetic types.

The mechanisms of action of current antipsoriatic drugs also support the crucial part of PK in psoriatic activity. The observed protection of calcium channel blockers against the development of psoriasis induced by the Koebner phenomenon supports the involvement of a calcium-containing molecule in psoriatic activity, as do the antipsoriatic properties of calmodulin inhibitors, such as cyclosporin A and anthralin, and previous observations of elevated calmodulin levels in psoriatic skin. Evidence also suggests that retinoids act also by stimulating type II cAMP-dependent protein kinase.

The possible site(s) of action of curcumin on PK molecule is still unclear. Curcumin has been shown to be a selective PK inhibitor. In addition, it has also been shown to inhibit cAMP-dependent protein kinase, albeit at higher concentrations. The known protein kinases possess a common structure that defines a catalytically competent domain termed the kinase catalytic core. The kinase catalytic core of PK has 33% identity with the kinase catalytic core of cAMP-dependent protein kinase. It is reasoned that any molecule that binds to the kinase catalytic core of PK, would also bind to the kinase catalytic core domain of cAMP-dependent protein kinase because of the 33% identity of these kinase catalytic core elements.
Because curcumin has been shown to inhibit both PhK,33 and at a higher concentration cAMP-dependent protein kinase,33,54 we deduced that the curcumin molecule may serve as a pseudosubstrate by directly binding, in whole or in part, to the kinase catalytic core domains of both PhK and cAMP-dependent protein kinase. The data in this study support current data33 that curcumin, rather than calcipotriol, may be a preferred substrate of PhK.

Two antigenic markers that have been associated with cell proliferation are, namely, transferrin receptors (TRR/CD71, a cell-surface protein) and Ki-67, a nuclear protein.57 Although widely used as a marker for cell-cycle-dependent cell proliferation, Ki-67 is of unknown function.58 The localization of Ki-67 to the nucleolus has been shown to be phosphorylation-dependent, as dephosphorylation causes its movement to cytoplasmic sites.58 TRR expression is also cell-cycle-dependent, increasing from 106–177% on S phase cells to 118–233% on G2/M cells.59 PhK may affect the expression of TRR in several ways. First, by enhancing inositol phosphorylation PhK affects the activity of phosphatidylinositol 3’ kinases; antibodies blocking the activity of these kinases have been shown to block cycling of TRR and growth factor receptors and to inhibit both the actin cytoskeletal functions and growth factor-dependent DNA synthesis.60 Second, by promoting tyrosine phosphorylation, PhK increases epidermal growth factor receptor tyrosine kinase activity with triggering of signalling pathways leading to tyrosine-kinase-dependent activation of the TRR gene promoter.61 Because the expression of TRR has been shown to be dependent on the phosphorylation of both inositol62 and tyrosine,61 i.e. PhK-dependent phosphorylation sites, we have used TRR as a PhK-dependent proliferation marker in this study. Moreover, the use of TRR as a cell proliferation marker in psoriasis is particularly appropriate since both psoriatic activity and TRR expression are tumour necrosis factor (TNF)-α-dependent,62,63 and curcumin has been shown to inhibit TNF-α-induced molecules.64 It is significant that while activation of serine/threonine protein kinase C is known to downregulate protein tyrosine kinase,55 activation of PhK results in the activation of EGF receptor tyrosine kinase.40

In conclusion, this study presents data in support of the antipsoriatic properties of a novel drug, curcumin (diferuloylmethane). This study provides preliminary data supporting the hypothesis that effective antipsoriatic effect may be achieved through the modulation of PhK activity.

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