Original Investigation

Efficacy of Novel Topical Liposomal Formulation of Cyclosporine in Mild to Moderate Stable Plaque Psoriasis: A Randomized Clinical Trial

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IMPORTANCE Attempts to use topical cyclosporine in treatment of psoriasis have failed because of unfavorable physicochemical properties and inappropriate formulation design of the conventional dosage forms.

OBJECTIVE To evaluate the efficacy of topical cyclosporine using liposomal nanocarriers (lipogel) in limited chronic plaque psoriasis.

DESIGN, SETTING, AND PARTICIPANTS A single-center randomized clinical trial was conducted using a 3-arm parallel group, double-blind, vehicle and active comparator design and included 38 patients with chronic plaque psoriasis measuring less than or equal to 100 cm² performed in a tertiary care hospital.

INTERVENTIONS In the first arm, a total of 24 patients were randomized to receive either cyclosporine lipogel, 2.0% weight by weight (w/w), or placebo lipogel. In the second arm, 7 patients were randomized to receive cyclosporine lipogel, 2.0%, or conventional cyclosporine cream, 2.0% w/w. The third arm comprised 7 patients randomized with cyclosporine lipogel, 2.0% or standard clobetasol propionate cream, 0.05% w/w. Patients were examined twice weekly for 14 weeks, or until total lesional clearance was observed, whichever was earlier.

MAIN OUTCOMES AND MEASURES The primary outcome measure was the dermatological sum score (DSS) assessing erythema, scaling, and plaque elevation on a 4-point scale (0, absent; 1, minimal; 2, moderate; 3, severe).

RESULTS In 38 patients (23 men and 15 women with a mean [SD] age range from 35 [8] to 40 [13] years), a 19% decrease in DSS score from a mean (SD) of 8.45 (0.67) to 6.82 (0.77) compared with baseline was observed after 2 weeks of treatment with cyclosporine lipogel, 2.0% w/w (P < .001; 95% CI, 13.77-24.51) in 59% of psoriasis lesional sites. At the end of the eighth week, a significant reduction (approximately 83%) in DSS was seen in all sites treated with cyclosporine lipogel. (P < .001; 95% CI, 77.48-88.22). At the end of the study period, complete clearance (ie, DSS = 0) was observed in 16 (41%) psoriasis lesional sites treated with cyclosporine lipogel, 85.7% of sites treated with clobetasol propionate cream, and none of the sites treated with conventional cyclosporine cream or placebo gel.

CONCLUSIONS AND RELEVANCE Topical liposomal formulation of cyclosporine, 2.0% w/w, is effective in treatment of limited chronic plaque psoriasis with a satisfactory safety profile. Future clinical trials should assess liposomal cyclosporine in larger study populations.

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The pathogenic role of T-cell–derived cytokines in psoriasis has led to considerable interest in exploring T-cell–specific calcineurin inhibitors in its treatment.1,2 Cyclosporine is approved by the US Food and Drug Administration (FDA) to treat recalcitrant chronic plaque psoriasis in nonimmunosuppressed patients at an oral dose of 2.5 to 5.0 mg/kg/d.3 Several attempts to use topical cyclosporine in varying dose regimens (0.1%-10% weight by weight [w/w]), in different topical vehicles, have failed to yield expected clinical response in treatment of various inflammatory dermatoses, including psoriasis.4–10 This has been attributed to the poor topical absorption resulting from the unfavorable physicochemical properties of the drug, such as poor aqueous solubility, large molecular weight, and high lipophilicity.9,11,12

Liposomal carriers have been extensively investigated as a means of enhancing the clinical efficacy of several topical drugs. Liposomes, first described by Bangham et al,13 are smectic mesophase vesicles composed of 1 or more bilayers of phospholipids (PLs) enclosing aqueous compartments. The liquid-crystalline architecture of liposomes provides an effective platform for incorporating different types of drugs.14 The similarity of lipid composition of liposomes to membranes of intercellular lamellae and keratinocytes enables the liposomes to penetrate the stratum corneum barrier, allowing for increased drug permeation into deeper skin layers and decreased drug clearance from the epidermis, thus promoting better sustained release of drugs in the epidermis and dermis.15 The current study assesses the clinical efficacy and safety of developed topical liposomal systems of cyclosporine in a single-center, 3-arm parallel group, double-blind, vehicle and active comparator controlled study of 14 weeks in patients with limited plaque-type psoriasis.

**Methods**

**Cyclosporine Lipogel Formulation**

Cyclosporine-loaded liposomes were prepared by optimal entrapment of drug, that is, cyclosporine in multilamellar liposomes, composed of high-purity phosphatidyl choline and other appropriate liposomal constituents, using standard thin-film technique.16 The formulation consisted of cyclosporine-loaded liposomes of submicron size (mean vesicle size, d95 950 nm) with optimum drug payload of 142 μg/mg and percentage of drug entrapment of 97.4%, dispersed in a hydrophilic carbopol 940 gel to provide optimal rheological properties. Liposomal gel without cyclosporine, prepared analogously, served as the placebo (ie, placebo lipogel) for this study. Cyclosporine dispersed in conventional oil in water (o/w) cream (cyclosporine o/w cream) base (prepared in accordance with European Medicines Agency guidelines)17 served as one of the active comparators, while clobetasol propionate 0.05%, w/w cream, available commercially, was used as the standard active comparator.

**Patient Selection**

The trial protocol is provided in the Supplement. Patients 12 years or older (23 males and 15 females) fulfilling the clinical and morphological criteria of stable plaque psoriasis with bilaterally symmetrical plaques measuring 100 cm2 or smaller, registered at the psoriasis clinic of our institute were enrolled from January 2008 to March 2010. The ethics committee of the Postgraduate Institute of Medical Education and Research approved the study. All patients gave written informed consent for participation, and they were not compensated for their participation. Bilateral symmetrical plaques were selected to facilitate side-by-side comparison of developed cyclosporine lipogel formulation on 1 side and the corresponding vehicle (negative control) or active comparator on the other side. Major exclusion criteria were impaired renal function, uncontrolled hypertension, past or present malignant abnormalities, infection, pregnancy and lactation, and concomitant immunsuppressive therapy.

**Clinical Assessment**

Lesions were examined clinically and disease severity was assessed using the dermatological sum score (DSS)18 to assess the erythema, scaling, and plaque elevation, scored on a 4-point scale (0, absent; 1, minimal; 2, moderate; and 3, severe) measured at baseline and twice-weekly intervals thereafter until the termination of study. Physician Global Assessment (PGA) was used to assess treatment response on a 7-point scale on each biweekly visit (0, complete clearance; 1, almost clear [90%]; 2, marked improvement [75%]; 3, moderate [50%]; 4, slight improvement [25%]; 5, no change; 6, worse).19 Photographs of the lesions were also taken at the baseline and at different periodic intervals thereafter. DSS and PGAs served as the primary and secondary outcome measures, respectively.

**Blinding and Treatment Allocation**

Efficacy of cyclosporine lipogel, 2.0%, formulation was evaluated against placebo lipogel as well as 2 active comparators: in a within-patient and parallel-group study. A total of 38 patients with symmetrical plaque-type psoriasis were enrolled and entered into a 4-week prestudy washout period, wherein all active antipsoriatic topical and systemic therapies were stopped (demographics are enlisted in the Table). In the first arm (arm 1), 24 patients (ie, total of 48 psoriatic plaques on left and right sides) were randomized in an equal ratio to treatment with cyclosporine, 2.0%, topical lipogel and placebo lipogel using a random number table. The remaining 14...
patients were randomized in a 1:1 ratio to treatment with cyclosporine lipogel vs cyclosporine o/w 0.2% cream (as arm 2), and cyclosporine lipogel vs clobetasol propionate cream (as arm 3), as part of the active comparator study. The study design is depicted in Figure 1.

Each patient was provided with 2 identical 20-g tubes, one of the tubes containing cyclosporine lipogel, 2.0% w/w, and the other containing placebo lipogel, cyclosporine o/w cream, or clobetasol propionate cream. One of the investigators (RK, SD, or RM) as well as the patients remained blinded about the composition and identification of treatment dispensed. Pharmacy-controlled randomization was used to conceal the random allocation of treatment using random number tables. This was performed by different team participants (BA, BS), who were neither directly involved in patient registration nor in assessing the outcomes. The formulation codes were broken only after complete results were obtained from all the participating patients on completion of treatment period.
Treatment and Follow-up
All the patients were asked to apply a standard amount of lipogel, that is, 1 fingertip unit (FTU) equivalent to approximately 0.5 ± 0.2 g, over a lesion area of no greater than 100 cm² once a day, preferably at bedtime. The method for measurement of lesion area (ie, area of 1 palm equivalent to 100 cm²) was taken as the standard for lesion area measurement. Patients were followed up twice weekly for 14 weeks, or until total lesional clearance, whichever was earlier. Patients were monitored for any relapse of the disease for a period of 8 weeks following discontinuation of treatment.

Safety Studies
As part of the safety evaluation, the extent of systemic absorption of cyclosporine following topical application of cyclosporine lipogel was determined after single and multiple application(s) at 2 different concentrations (ie, 2 g of lipogel = 40 mg of cyclosporine, and 5 g lipogel = 100 mg of...
cyclosporine). Quantification of cyclosporine in collected blood samples was carried out using fluorescence polarization immunoassay method.19

Statistical Analysis
Because the sample size was small and the data obtained did not exhibit normal distribution, nonparametric statistical tests were used to assess the therapeutic efficacy of different cyclosporine formulations. Primarily, the effectiveness of the cyclosporine dose was assessed at different concentrations by comparing reduction in DSS score with baseline in different treatment arms using Wilcoxon signed ranks with 95% CIs within the group. Correlations between patients’ baseline characteristics and different treatments were established by Spearman rank correlation coefficient. The Mann-Whitney test was used to analyze differences between the treatment arms of a group.

Results

Efficacy Outcomes
The clinical efficacy of cyclosporine lipogel, 2.0%, w/w was evaluated in 38 enrolled patients against placebo and active comparators under 3 different arms, as shown in the Table. Final outcomes (ie, DSS and PGA) on treatment have been graphically represented using median box whisker plots in terms of percentile frequencies from baseline and second week, respectively, for DSS and PGA in Figure 2.

In study arm 1, comparison of percentage of decrease in DSS from the baseline was done using the Wilcoxon signed rank test, and the comparison of percentage of decrease in DSS with cyclosporine lipogel vis-à-vis placebo lipogel was carried out using the Mann-Whitney test (P < .001). The results indicated that lesions treated with cyclosporine lipogel showed marked improvement compared with lesions treated with placebo lipogel formulation over the study period (P < .001). It was observed that 19 (50%) of the 38 lesion sites began to respond to the treatment at the second week, with an appreciable (approximately 19%) reduction in DSS from a mean (SD) of 8.45 (0.67) to 6.82 (0.77) observed after 2 weeks of treatment with cyclosporine lipogel. Almost complete lesion clearance (ie, DSS and PGA scores ≤1) was observed in 23 (60%) of the 38 cyclosporine lipogel–treated lesions after the eighth week, with an 83% reduction in DSS from baseline. At the end of 14th week, 34 (90%) of the sites treated with cyclosporine lipogel showed more than 90% lesion clearance (Figure 3). The placebo–treated sites, however, did not show significant lesion clearance (Figure 4A). Overall, reduction in scaling was most prominent, followed by erythema and plaque elevation.

Approximately 10% to 20% reduction in DSS in the lesions treated with placebo lipogel was observed at the second week onward until the end of the study period, attributable to the unique skin hydration and emollient effect of PLs, the major constituent of liposomes present in the placebo lipogel (Figure 3B).20

In study arm 2, all lesions treated with cyclosporine lipogel, 2.0%, showed significant fall in DSS after the second week (Wilcoxon signed rank test; \( P = .02 \)). Around 60% of the 7 cyclosporine lipogel–treated sites showed approximately an 80% decrease in DSS (Figure 4B) at the end of the eighth week. Only mild erythema (DSS = 1) without any induration or scaling remained in 6 of 7 cyclosporine lipogel–treated sites after 10 weeks. In cyclosporine o/w cream–treated sites, there was no significant difference in DSS and PGA scores at the end of the treatment period. Statistical analysis revealed that the clinical response using conventional o/w cream of cyclosporine at equivalent concentration was not significant compared with the clinical response observed using cyclosporine lipogel (Mann-Whitney test; \( P = .98 \)).

In arm 3 of the parallel-group study, almost total lesion clearance (ie, DSS approximately 0) was observed at the end of the sixth week in 5 of 7 lesions treated with clobetasol propionate cream compared with an approximate 90%
reduction in DSS score at the end of the eighth week in 5 of 7 cyclosporine lipogel–treated sites (Figure 4C). Treatment with cyclosporine lipogel reduced scaling the most, followed by induration and erythema, whereas maximum reduction was observed in erythema, followed by induration and scaling on the sites treated with clobetasol propionate cream. The latter can be attributed to the marked anti-inflammatory property of the corticosteroid.

Four of 7 patients receiving clobetasol propionate cream application, however, reported mild itching in the perilesional area.

Following completion of the treatment period of the double-blind study, 25 of 34 patients who completed the treatment were monitored for a period of at least 2 months to record the remission of disease over the cyclosporine lipogel–treated psoriatic sites. It was observed that the mean scores
for individual psoriasis lesions remained stable in most of the patients during this period.

Safety Studies of Cyclosporine Lipogel
The results of the safety study confirmed that cyclosporine did not accumulate in the systemic pool of any of the patients even after repeated applications. In all the patients, blood pressure and other vital laboratory parameters, like liver function, renal function, and electrolyte levels, remained well within the normal limits throughout the study period.

Adverse Effects
Mild adverse effects in the form of erythema, irritation, and dryness were observed in 3 of 34 sites treated with cyclosporine lipogel and 1 patient reported increased fissuring at the treated sites. However, none of the patients discontinued the therapy because of the adverse effects. No secondary infections (bacterial, viral, or fungal) were seen in any of the patients who completed the treatment period.

Discussion
Cyclosporine acts on psoriasis by its capacity to modulate (1) proliferation and differentiation of the keratinocytes, (2) inflammation, (3) cellular-mediated immune response, and (4) angiogenesis. However, the clinical effects of topical cyclosporine are limited by the inherent physicochemical characteristics of the cyclosporine, such as high lipophilicity (log rankFoctanol/water = 2.92), large molecular weight (approximately 1202 Da), very low aqueous solubility (approximately 2-6 μg/mL), and cyclic molecular structure. Moreover, the barrier properties of the skin and the inability of conventional vehicles to provide the desired vehicular effect (ie, partitioning of drug molecules across stratum corneum and its localization in dermis) are also impediments in the development of suitable topical formulation.

Despite these failures, Brown et al4 reported that intralesional injection of cyclosporine on 6 alternate days produced clinically significant resolution of psoriasis plaques within 2 weeks. Electrospun nanofibers of cyclosporine have also been evaluated for attaining local immunosuppression. These findings suggest that the ineffectiveness of topical formulations results from inadequate penetration of cyclosporine into skin. Therefore a vehicle that facilitates adequate transdermal permeation of cyclosporine could be as effective as systemic cyclosporine. Several attempts have been put forth in this regard, such as the use of penetration enhancers, chemical modifications in the drug molecule, iontophoresis, lipid micelles. All such approaches, however, have been unsuccessful owing to improper formulation design and approach.

For the current study, liquid-state liposomes composed of high-purity unsaturated PLs of submicron range were formulated and optimized for critical quality attributes such as liposomes size, surface charge, drug payload, and rheological properties. The results obtained in the current studies are in close agreement with the findings reported by various researchers, showing higher drug penetration across the stratum corneum for liposomes containing unsaturated PLs. Apart from PLs, the optimized liposomal formulation contained antioxidant α-tocopherol (2.0% w/w of total lipids) and stearic acid as surface charge imparting agent (0.2% w/w of total lipids). The ζ potential determination of optimized liposomal formulation yielded a value of 12.4 mV. The value of ζ potential showed that prepared liposomes have sufficient surface charge and mobility to inhibit aggregation of vesicles. Also, positively charged cyclosporine liposomes are considered to be superior to neutral or negatively charged liposomes for dermal applications because of the presence of negative charge on skin.

It was observed that the patients who received cyclosporine lipogel formulation had a positive response at the end of treatment, with 36 (95%) of the patients receiving a physician rating of at least “good improvement.” The results show that clinically significant lesion clearance (75%-100%) was seen in more than 23 (60%) of psoriasis sites after about 4 weeks of treatment.

Conclusion
Based on the results obtained in the study, it was concluded that the clinical performance of cyclosporine liposomal gel was superior to that of cyclosporine incorporated in conventional cream formulation at equal drug concentrations. This could be attributed to the ability of liposomal carriers to provide enhanced interaction of cyclosporine at the cellular level, besides forming the cutaneous drug depots at the early stage of applications. The latter might have served as slow drug releasing microreservoirs in the psoriatic sites leading to higher drug accumulation locally.

The results of pharmacokinetic studies and other safety evaluation protocols followed in the current investigation clearly indicate tolerability of topical cyclosporine lipogel. Moreover, the studies also revealed that application of topical cyclosporine lipogel resulted in uniform reduction of plaque elevation and was not associated with the development of hot spots. Although the results reported herein are from a small therapeutic exploratory study, the findings suggest that future research may lead to clinical applications with positive outcomes.
Conflict of Interest Disclosures: A patent (2514/DEL/2005) for “A Multicompartimental Liposomal System For Topical Drug Delivery” has been filed with Indian patent office by M/l. Suresh Innovations Private Limited.

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