CLINICAL TRIAL PROTOCOL

1. Proposed Project

FORMULATION AND EVALUATION OF LIPOSOMAL CARRIER SYSTEM OF CYCLOSPORINE-A FOR TOPICAL DELIVERY

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Title of the Project
Formulation and Evaluation of Liposomal Carrier System of Cyclosporine-A for Topical Delivery

1. Name, designation and address of the guide

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2. Objectives

The principle objective identified under the current project includes design and development of novel liposomal carrier system for topical delivery of the selected drug, i.e., Cyclosporine A (CsA), for the treatment of some very difficult to manage dermatological disorders like psoriasis, eczema and atopic dermatitis. These skin problems involve multiple, complex pathophysiological pathways, which complicate the understanding of their etiological mechanisms, and make it very difficult to choose a drug-candidate. However, in the entire armoury of therapeutic weapons, CsA has been identified as one of the most promising choices.1,2

However, despite the promising potential of this drug, it has not been able to be utilized for the direct on-site application (i.e., on the affected skin), which compels for the oral/systemic administration. The latter is undesirable for obvious reasons of wide distribution in the body, and a lot of side effects, while former i.e., topical route may serve the best. However, the topical delivery of CsA is not an easy task by virtue of the peptidal nature of the drug, unfavourable physico-chemical properties of the drug (its very high molecular weight and highly lipophillic nature), the structural vulnerability of the peptide, and the tough skin barrier.3,4
The conventional vehicles with traditional formulation approaches fail to address the above problems. Thus, it needs to work-upon some innovative and strategic formulation designs for modifying the penetration, partitioning and permeation of CsA across the skin barrier. For this, efforts are being made by adopting varied drug delivery approaches. Amongst them, phospholipids-based supramolecular self-assembled micro- or nano-range carrier systems i.e., liposomes\textsuperscript{5-7} have been conceived here in this project, to help deliver the CsA onto the target site, and thus to improve the dermato-kintetic profile to the drug molecules. Encapsulating the drug within the exquisitely built carriers help the drug molecules acquire a new set of physico-chemical microenvironment. The latter help navigate the active molecules to the site of action while preserving its originality en-route.

In order to achieve these objectives, following major goals have been framed:

- **Selection of formulation design**
  This entails the study of varied microstructures such as liposomes i.e., lamellar vesicles, which can produce, a carrier effect for the drug molecules as well as the desired changes within the skin compartments.

- **Selection of appropriate formulation components**
  To achieve the above formulation design, the tasks include the selection of appropriate constituents, techniques and experimental conditions.

- **Standardization of developed products**
  The prepared products are to be standardized for various characteristics, including the evaluation of product shelf-life.

- **Performance evaluation of developed products**
  Finally, the validated products are to be tested in-vitro and in-vivo, and at the clinical level for their on-site performance.

- **Industrial scale-up of the successful drug product**
  Finally, the attempts would be made to develop and scale-up the process for fabricating the product on industrial scale.
3. Introduction and review of literature

3a. Psoriasis

Psoriasis is a common, genetically determined, chronic relapsing inflammatory and proliferative disease of the skin, which affects 1-3% of the world population. In India it accounts for about 2.3% of the total dermatology outpatients. Men and women are almost equally affected; women have an earlier onset of disease. The skin lesions may appear as early as the age of 1 or as late as age 80 years. Regardless of the time of onset of the disease, the patient faces a lifelong struggle to eradicate the erythematous scaly plaques that are a source of anxiety and embarrassment. In the ancient times, people considered psoriasis a form of leprosy and many psoriatics suffered the same physical and mental abuses as lepers of that era.

Psoriasis is unique because it represents excessive but controlled cellular proliferation and inflammation, both occurring within 0.2 mm of the skin's surface. There is no perfect experimental model and the pathogenesis is not completely understood. The clinical lesion first emerges as a pinpoint erythematous spot. It enlarges, stabilizes, and becomes slightly papular and scaly or even clears. Microscopically, the lesion of psoriasis is characterized by parakeratosis, an absent granular layer, acanthosis, and an inflammatory infiltrate in the dermis and epidermis, as well as by changes in the capillaries of the papillary dermis. In considering the pathologic events in psoriasis, certain facets merit analysis. First, the role of immune mechanisms is documented by the great number of activated T cells within the altered epidermis and dermis, as well as by the linkage of psoriasis to certain class I and class II HLA antigens. Second, the predominant changes consist of highly increased, persistent keratinocyte proliferation in conjunction with a characteristic inflammatory pattern. Further, the genetic relationship represents a hallmark of psoriasis; the inheritance is polygenic, including probably a number of non-HLA-related genes.

Pathogenesis

The exact etio-pathogenesis of the disease is not understood. The evidence that psoriasis may be inherited is beyond doubt, and this rests on population surveys, twin and other family analyses and HLA studies. Evidence has been presented for single-gene autosomal dominant inheritance with reduced penetrance, for the presence of multifactorial genetic components, as well as for no genetic component. Basic research in psoriasis is the subject of a voluminous literature. In the recent past, investigations focussed on the roles of leukocyte-attractant mediators; keratinocyte growth factors; proto-oncogenes; polyamines; signal
transduction systems including cyclic nucleotides, the phosphatidyl inositol cycle and calmodulin; and epidermal proteases. Topics of particular interest at present include molecular genetics, T-cell-dependent mechanism and mechanisms of keratinocyte proliferation.

**Clinical features**

The first manifestation of psoriasis may occur at any age. Its duration may vary from a few weeks to a whole lifetime. The course is unpredictable and the variations numerous. Certain patterns are, however, more common than others. The most characteristic lesions consisting of chronic, sharply demarcated, dull-red, scaly plaques, particularly on the extensor prominences and in the scalp. Nail changes may herald the development of psoriasis elsewhere, or remain localized for several years. The appearance of a typical lesion is characteristic. Plaques of varying sizes are often found on the trunk and limbs. They vary in diameter from one to several centimetres and are oval or irregular in shape. There may be any number of lesions or only a single, and, when multiple, may be symmetrically distributed. Common clinical types are chronic plaque type psoriasis (psoriasis vulgaris), pustular psoriasis, psoriatic erythroderma and psoriatic arthritis.

Four distinct pathological alterations characterize this disorder:

- Hyperproliferation of the epidermis
- Altered maturation of the epidermis (resulting in scaling)
- Inflammation
- Vascular alterations (resulting in redness)

**3b. Problems with current therapies**

Despite a continued progress toward an elucidation of the genetic and pathophysiological pathways involved in psoriasis, definitive cure remain elusive. The patient with psoriasis represents both a challenge and an opportunity for drug treatment. Although we have a number of treatments that can induce remission in a high percentage of psoriatic patients, in the absence of a cure, we do not have both safe and effective therapy to maintain patients in remission.

For patients with mild to moderate psoriasis, topical therapeutic repertoire includes emollients and moisturizers, tars, anthralins, topical corticosteroids, and vitamin D analogue. However, for approximately 30% of patients seeking dermatologists' care, these treatment's are insufficient and systemic therapies are required. Systemic therapies generally tried are
methotrexate, acitretin, cyclosporine A, hydroxyurea, PUVA (psoralens+ UVA) with or without various topical therapies. These therapies are associated with potential severe toxicity, need extensive monitoring and are costly. PUVA is associated with increased long-term risk of increased skin cancers, extensive photodamage and other side effects related to this. Besides, PUVA is not suitable for treatment of psoriasis in pregnancy, children and patients with associated systemic diseases like hepatic, renal, ocular diseases etc.

3c. Cyclosporine A as potential candidate for psoriasis

**Drug profile**

CsA, a hydrophobic cyclic oligopeptide, is a clinically useful T-cell active immunosuppressant.\(^3,4\) CsA was isolated from the fungus *Tolypocladium inflatum*. It was first investigated as an anti-fungal antibiotic, but its spectrum was too narrow to be of any clinical use. Its immunosuppressive activity was discovered by J. F. Borel in the year 1976.\(^2,4\)

Recently, the dermal or cutaneous delivery of CsA has also been found to be effective for treating various autoimmune skin disorders such as psoriasis and atopic dermatitis.\(^1, 2, 16\) However, the topical delivery of CsA is restricted because of the inability of the molecules to cross over the horny layer (stratum corneum) of the skin. The drug being peptidal in nature, is devoid of the requisite physico-chemical characteristics such as solubility and partitioning properties (because of its high lipophilicity and large molecular weight), which restricts its entry through skin.\(^17-21\) However, in the recent times attempts have been made to deliver the
drug through topical route using different approaches and encouraging results have been obtained.\textsuperscript{22, 23}

**Mode of action**\textsuperscript{3, 4, 24}

CsA acts in the cell nucleus by binding to intracellular receptor termed cyclophilin. This CsA–cyclophilin complex inhibits the calcineurin (calcium and calmodulin dependent phosphatase), a key enzyme involved in calcium dependent signaling process. Inhibition of calcineurin prevents the transcription of Interleukin-2 (IL–2) and other lymphokines genes. Thus blockade of IL-2 and other lymphokine (T-cell growth factors) production by T-helper cells results in the impairment of IL-2 driven proliferation of activated T-cells in response to specific antigen in the allograft rejection.

**Toxicities and adverse effects**\textsuperscript{1, 2, 25}

The oral administration of CsA is associated with many limitations such as very low and variable bio-availability, considerable systemic toxicities and drug interactions. The drug exhibits dose- and time-dependent nephrotoxicity, and hypertension upon systemic administration. Another important adverse effect is the development of cutaneous and extra-cutaneous infections with the systemic treatment of inflammatory dermatoses. Because of the immunomodulatory effect, CsA also inhibits tumor defense mechanisms leading to higher incidence of skin cancers. Gastro intestinal disorders, hypertrichosis, vertigo, muscle cramps and tremors have also been reported to be associated with the oral drug administration.

**3d. Importance of liposomal carrier mediated topical drug delivery and potential benefits**

Traditional or conventional topical dosage forms invariably have been failing on pharmacokinetic or pharmaco-dynamic criterion. To alleviate the problems, the design and development of carrier-based topical systems promises multiple benefits. In this pursuit, the concept of selective or targeted drug delivery of topically administered agents by their incorporation in phospholipid-based carriers such as liposomes has shown tremendous promise.\textsuperscript{7}

**Liposomes** are multi-compartmental, water-filled lipoidal vesicular carriers composed of phospholipids. Their ability to entrap and transport therapeutic molecules selectively to enhance the efficacy and safety has been well documented.\textsuperscript{26} Due to their natural composition, these carriers are digested by enzymes in the skin, freeing their contents in a slow, gradual and programmed manner. Various topical liposomal products for corticosteroids, local anesthetics, anti-psoriatic drugs, antifungal agents, anticancer drugs,
retinoids, proteins, peptides, macrolides, monoclonal antibodies, enzymes along with liposomal topical vaccines, are in the different phases of clinical trials. Figure 2 depicts challenges and targets for targeting of cyclosporine molecules into and across stratum.

**Targeting of Cyclosporine: Mechanism of enhanced topical delivery using carriers**

**Cyclosporine targets and delivery difficulties**

1. Stratum Corneum
2. Intracellular molecular targets

**Fig 2a.** Cyclosporine A due to large molecular weight (1202.6 daltons and very high lipophilicity (log P = 2.92) is unable to penetrate the horny stratum corneum barrier.

**Fig 2b.** After penetrating the stratum corneum, the drug molecules need to be effectively internalized or taken up by the different immune cells of skin viz. T cells, langerhans cells, macrophages etc.

**How the micro-structured carrier(s) work to deliver?**

1. **Increased skin moisturization:** The aqua-lipoidal biphasic state of carriers improve the hydration state of the skin, which leads to loosening of the dense lipidic lamellae of stratum corneum, thus allowing enhanced penetration of the drug molecules.
2. **Penetration of drug-containing carriers:** Vesicular carriers, i.e., liposomes depending on their size, lamellarity and surface properties such as charge etc., loose some of its lamellae and pass intact through the stratum corneum, thus carrying the drug molecules along to the desired site of action.

3. **Transcutaneous hydration force effect:** There is enhanced penetration of the liposomes or reverse micelles (containing drug) under the “transcutaneous hydration force” caused by the water concentration gradient, between the skin surface and the skin interior.

4. **Adsorption and fusion of carriers to the stratum corneum:** The same promotes the penetration and accumulation of the drug in the skin strata.

5. **Molecular mixing and “swelling effect”:** Liposomal or micellar phospholipids mix with the intercellular lipids matrix of the stratum corneum and thus cause swelling of the intercellular spaces, accumulating the drug outside the cell membrane, which enhance the drug-target interactions, and also causes the formation of depots in the skin layers.

6. **Modification of stratum corneum barrier:** The phospholipid constituents of carriers, induce some physicochemical changes in the bilayer packing of stratum corneum and deeper skin strata, thereby causing a large increase in the “lecithin alkyl chain disorder”, thus leading to enhanced penetration of the drug molecules.

7. **Reduced clearance of the encapsulated drug from skin layers:** In general, highly lipophillic drugs are immediately cleared away by the epithelial blood capillaries, as soon as the drug molecules cross the stratum corneum. However, drug molecules entrapped in the vesicular or micellar carriers are less prone to clearance from the site of action, which results in enhanced and prolonged presence of the drug at the site of action.

Thus, the above achievements would add a remarkable value to the treatment of dermatological disorder such as psoriasis, as it is one of the most potent and most frequently used drug while skin afflictions are in question. The favourable results with topical Cyclosporine A, would be a big breakthrough to obtain a topical product, as the drug until now is being administered through oral route. And for topical problems like psoriasis, this kind of topical product will be a more relevant and meaningful approach.
4 Study-design for clinical evaluation: Clinical trials with novel topical formulations (Liposomal gel) of Cyclosporine A.

A. Preliminary open-label clinical trial

The assessment of clinical level performance would be carried out in accordance with standard operating procedures and after the approval by institutional ethical committee of PGIMER, Chandigarh. The developed liposomal formulation of cyclosporine A, would be evaluated in ‘stable chronic plaque psoriasis’.

Ten patients recruited for liposomal gel formulation, would be given an initial wash-off period of 2 weeks during which no topical antipsoriatic therapy would be given (to remove the effect of earlier treatment). Patients will be instructed to apply the formulation carefully over the selected psoriatic plaque(s) (of total area $\leq 100 \text{ cm}^2$), once daily. Treatment will be continued for eight weeks or till lesion clearance, whichever is earlier. Lesions will be examined clinically and disease severity assessed using using Dermatological Sum Score (DSS)$^{28}$ at base line and 2 weekly interval thereafter.

Finally, patients will be asked to grade the ease of use and overall acceptability of the each formulation at the end of the treatment period. The grading will be based on a 0-10 visual analog scale (VAS) by the patients.

B. Double-Blind, Randomized, Vehicle Controlled Prospective Clinical Trials

Based on the experience with open label study a randomized, double blind, vehicle controlled trial with intra-patient comparison of two formulations will be carried out, as explained below.

Subject selection

Male or female patients aged 12 years or older, fulfilling the clinical and morphological criteria of stable plaque psoriasis, with bilaterally symmetrical plaques measuring $\leq 100 \text{ cm}^2$ of the body surface area, registered at the Psoriasis Clinic of the Dermatology outpatient department, PGIMER, Chandigarh, will be enrolled for the study.

Twenty-four patients will be recruited, for liposomal formulation of CsA and placebo lipogel. The nature of the study will be explained to the patients and written consent would be obtained. Before treatment with CsA formulations, a detailed history will be taken, and patients will be subjected to an initial wash-off for a defined time period to remove any residual effect of any other therapeutic treatment. In particular, patients will not receive any topical antipsoritic or any systemic antipsoritic therapy for a minimum of 4 weeks.
**Patient Exclusion criteria**

Major exclusion criteria for the study includes impaired kidney function, uncontrolled hypertension, past or present malignancy, infection, pregnancy and lactation, concomitant immunosuppressive therapy, primary or secondary immunodeficiency, and known hypersensitivity to drug or its ingredients.

The minor exclusion criteria will be recent or excessive photochemotherapy, and concomitant hypertension with drugs that are nephrotoxic, or with drugs, which have known pharmacological interaction with CsA and hence affect pharmacokinetics of CsA such as macrolide antibiotics, aminoglycosides, NSAIDs, and oral contraceptives etc.

**Study design**

The study will be divided in two parts, A and B.

**Part A**

Part A of the study will comprise, evaluation of the liposomal formulation, in a vehicle controlled, blinded randomized trial. Twenty-four patients will be included in the study based on the selection criteria as described in open-label trial. A total of 24 psoriatic plaques will be treated with liposomal formulation of CsA while the rest 24 with plain liposomal gel (i.e., placebo or without drug), based on randomization (Arm I).

**Part B**

In Part B of the study, the developed formulation will be compared with cyclosporine incorporated in conventional cream base at same concentration and clobetasol propionate 0.05% cream (CP) as part of the active comparator study. Fourteen patients will be recruited for this study arm and randomized in 1:1 ratio to treatment with cyclosporine lipogel vs. cyclosporine o/w cream (as Arm II), and cyclosporine lipogel vs. CP cream (as Arm III). The study design is depicted in Figure 3.
Study design

Figure 3: Outline of the protocol for double-blind, vehicle controlled, and clinical trial for assessment of the clinical performance of prepared liposomal formulation of Cyclosporine A.
Treatment

In part A of the study

Each patient would be given 2 identical tubes (Tube A and B), 20 g each, one of which will contain Cyclosporine A liposomal formulation and the other tube will contain placebo i.e., plain vehicle without drug. Each patient would be randomly assigned to apply one of the tubes plaque on right side of the body, and the other to the left side, once a day. Treatment will be continued for 14 weeks or till the total signs of lesions vanish, whichever is earlier. Patients will be examined at screening, baseline and at 2weekly intervals there after or till the total lesional clearance, whichever was earlier.

In part B of the study

Each patient would be given 2 identical tubes (Tube A and B), 20 g each, one of which will contain prepared Cyclosporine A liposomal formulation, and the other will contain cyclosporine o/w cream (as Arm II) or CP cream. Each patient would be randomly assigned to apply one of the tubes on the plaque of right side, and the other to the left side, once a day. Treatment will be continued for 14 weeks or till the total signs of lesions vanish, whichever is earlier. Patients will be examined at screening, baseline and at 2 weekly intervals thereafter.

Clinical evaluation criteria

Lesions to be examined clinically and disease severity to be assessed using Dermatological Sum Score (DSS)\textsuperscript{28} to assess the erythema, scaling and plaque elevation, scored on a 4-point scale (0-absent, 1-minimal, 2-moderate and 3-severe) with measurement at base line and 2 weekly interval thereafter till the termination of study. Physician Global Assessment (PGA) will be used to assess treatment response on a 7-point scale on each bi-weekly visit (0-complete clearance, 1-almost clear (90%), 2-marked improvement (75%), 3-moderate (50%), 4-slight improvement (25%), 5-no change, 6-worse).Photographs of the lesions will also be taken at the baseline as well as at different periodic intervals thereafter. DSS and PGA served as the primary and secondary outcome measures respectively. The side effect profile (if any) (at baseline and every two weeks thereafter), and CsA blood trough levels would also be measured, in patients, enrolled for the study.
5. Statistical Analysis
Depending upon the data obtained for all patients at end of study (i.e., normal or not normal), parametric (e.g. ANOVA test or T-test) or non-parametric statistical tests (for example Wilcoxon Signed Ranks test) will be used to assess the therapeutic efficacy of different cyclosporine formulations. The effectiveness of cyclosporine dose will be assessed at different concentrations by comparing reduction in DSS score compared to baseline, in different treatment arms using Wilcoxon Signed Rank with 95% confidence interval test within the group. Correlations between patients’ baseline character and different treatments will be established by Spearman’s rank correlation coefficient. Mann-Whitney test will be used to analyze differences between the different treatment arms of a group.
6. Study proforma

Proforma for Clinical Trial
UIPS, Panjab University
&
PGIMER, Chandigarh

Investigator(s): Dr. O. P. Katare (UIPS) &
Dr. Sunil Dogra (PGI, Dermatology)

Product: Cyclosporine A gel

Trial Type: Prospective Randomized, Double blind Clinical Trail

Skin disorder: Stable Plaque Psoriasis

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Data Recording

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7. References:


