



# *Chapter 1*

## *Introduction*

Steroid-Responsive Dermatoses, or SRD, refers to skin conditions that respond to treatment with topical corticosteroids. These conditions are a diverse group —plaque psoriasis, eczema, atopic and contact dermatitis, pityriasis rosea etc. Atopic dermatitis is one of the common steroid responsive dermatoses affecting over 10% of children, and it is the most common cause of occupational disability in adults (Shiohara et al, 2004).

### **Atopic Dermatitis**

Atopic dermatitis (AD) is a chronic allergic inflammatory disease which manifests itself as eczematous skin lesions. The clinical phenotype that characterizes atopic dermatitis is the product of interactions between susceptibility genes, the environment, defective skin barrier function, and immunologic responses (Leung, 2004). Although the precise mechanism underlying atopic dermatitis has remained unclear, it appears that immediate Immunoglobulin E IgE -mediated mast cell type , late IgE-mediated Th2 type and delayed IgE- independent Th1 type allergic reactions are involved in atopic dermatitis. Immunological analyses of the pathogenesis of atopic dermatitis have revealed that activated mast cells and eosinophils and an excess of differentiated T-helper (Th) 2 cells might play important roles in the development of dermatitis. Both Th cell subsets may contribute to the pathology of atopic dermatitis (Novak et al, 2003a and b, Tamura et al, 2005). Atopic dermatitis is a chronic and relapsing inflammatory skin disease characterized by episodes of intense pruritus, multiple lesions with erythema, excoriation, erosions, lichenification, papules, dry skin, and susceptibility to cutaneous infection. A major factor exacerbating AD is xerosis (Leung, 2003).

### **Topical Corticosteroids**

Topical corticosteroids remain one of the most important treatments available for AD. Patients' irrational fear about using topical corticosteroids has become

a major barrier to effective long term management of severe AD. Topical corticosteroids are available in a variety of vehicles— creams, ointments, lotions, gels, and more recently, foam. The vehicle used can substantially affect the individual agent's clinical action, potency, and acceptability to the patient. Moreover, some vehicles are better suited for specific body areas.

The corticosteroids have a multiplicity of actions; anti-inflammatory, immunomodulatory, vasoconstrictor, gluconeogenic, anti-mitotic to name a few. It is believed that several of these actions contribute to the therapeutic efficacy of these drugs in the treatment of skin disease. Indeed it is often this multi-pronged attack that endows the Corticosteroids with the considerably greater therapeutic potency above other modes of treatment. The major anti-inflammatory effects are mediated through suppression of transcription of various genes encoding proinflammatory proteins. Besides, they also activate production of anti-inflammatory Lipocortin-1. Corticosteroids also exert their antiproliferative effects on T cells, fibroblasts, eosinophils etc (Barnes, 1998, Norris, 2005).

Topical corticosteroids are effective, easy to administer, acceptable to patients, safe when used correctly, and provide superior results considered despite their demonstrated effectiveness as treatment for psoriasis or AD, topical corticosteroids are associated with various side effects that may limit their use. These include localized skin reactions occurring at the site of application and generalized adverse effects from systemic absorption of corticosteroid. Local cutaneous reactions are more common than systemic side effects and are largely due to the antiproliferative effects of these agents. They include atrophy or thinning of the skin, striae, telangiectases, acneiform eruption, rosacea, and contact dermatitis. Systemic side effects, although uncommon, may occur when locally applied corticosteroids become absorbed through the

skin and enter the general circulatory system and includes the suppression of Hypothalamus pituitary adrenal axis (Hengge et al, 2006).

Corticosteroid potency is defined in terms of drug's maximum dermal vasoconstriction effect. It encompasses ultra-high potency (Class I) drugs, including clobetasol-and halobetasol-propionate, and ends with low potency (Class VII) drugs, such as dexamethasone, prednisolone, and especially hydrocortisone (Ahluwalia, 1998).

Halobetasol propionate is an ultra potent corticosteroid available as ointment and cream for clinical use, out of which ointment being occlusive is more potent. Being an ultrapotent steroid, a potential for serious local and systemic side effects is associated with its use.

### **Topical Calcineurin Inhibitors**

Apart from the topical corticosteroid, calcineurin inhibitor, retinoid, vitamin D3 analogues are also used for the treatment of dermatoses.

Topical calcineurin inhibitors (tacrolimus and pimecrolimus) are recommended as second-line treatment for moderate-to-severe atopic eczema not controlled by topical corticosteroids, or when there is a high risk of adverse effects such as skin atrophy. The main side-effects are skin irritation, burning, erythema, infections and alcohol intolerance (Del Rosso et al, 2005).

The mechanism of action is different from that of topical corticosteroids. There is a series of complex immunodysregulatory responses in some patients who have AD. Tacrolimus acts directly on the T-lymphocytes, especially CD4+ cells, by binding to the FK-binding protein (FKBP). This tacrolimus-FKBP complex then binds to and competitively inhibits calcineurin which in

turn inhibits transcription factor, NFAT (nuclear factor of activated T-cells). It is this transcription factor that activates the promoter region of the gene for various inflammatory cytokines that participate in the early immune response and are postulated to play a role in AD pathogenesis. Tacrolimus inhibits the release of mast cell and basophil preformed mediators, downregulates IL-8 receptor expression, and decreases chemokines. This broad range of the inflammatory inhibition mechanism may reduce antigen recognition and downregulate the entire inflammatory cascade leading to clinical disease (Sengoku, 1999, Norris, 2005).

### **Enhanced Dermal Delivery with Nanocarriers**

Human skin is an important target site for the application of drugs. Especially in the treatment of local diseases, a topical drug delivery is an appropriate strategy to restrict the therapeutic effect on the affected area and to reduce systemic incrimination. In order to reach therapeutic drug concentrations in certain skin layers, the uppermost barrier, the stratum corneum (SC), has to be overcome.

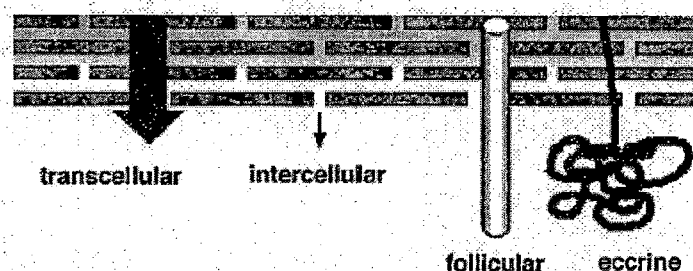


Fig 1.1: Possible pathways for dermal delivery of drug

This process is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for application, condition of the epidermal barrier etc. Thus, an ideal topical drug for treatment of inflammatory skin diseases should be able to pass the stratum corneum and

to reach therapeutically relevant concentrations in the epidermis/dermis without leading to high serum levels and systemic exposure (Peltola et al, 2003).

Methods for improving cutaneous delivery rely on the use of chemical penetration enhancers, novel vehicle systems (e.g., microemulsions, liposomal-based delivery systems and supersaturated formulations), or more complex physical enhancement strategies (e.g., iontophoresis , sonophoresis and electroporation).

Most corticosteroid dermatics contain skin permeation enhancers to promote drug absorption. Agent transfer across the skin is further modified by changing the thermodynamic activity of the drug on the skin, e.g. by choosing a less potent (e.g. cream/lotion) or a more potent (e.g. ointment) formulation, and occasionally by drug supersaturation.

Lipid vesicles (liposomes) were proposed by some but not confirmed by other researchers to increase corticosteroid concentration in the skin (Yu et al, 1996). Therapeutic benefit of liposome-based corticosteroid dermatics in humans was also found to be inconsistent. Specially designed vesicles overcome the skin permeability barrier in the stratum corneum. The prerequisites are carrier stability and self-deformation under stress and virtual pathways opening through the organ. Transdermal drug permeability is influenced mainly by three factors: the mobility of the drug in the vehicle, the release of the drug from the vehicle, and drug permeation through skin. The drug solute must first diffuse out of the vehicle to the skin surface and then it must penetrate through the external cornifying layer (stratum corneum) of the skin; it should then diffuse to its target (local tissue or systemic circulation). Both steps are

dependent upon the physicochemical properties of the drug, vehicle/ carrier, and skin barrier (Biruss et al, 2007, Cevc et al, 2004, Barichello et al, 2007).

Modern drug carrier systems microemulsions (MEs) are thermodynamically stable, low viscous, transparent and optical isotropic formulations with a dynamic microstructure that form spontaneously by combining appropriate amounts of a lipophilic and a hydrophilic ingredient, as well as a surfactant and a co-surfactant. A number of investigations have been carried out which demonstrated that drugs incorporated into microemulsions penetrate efficiently into the skin and through the SC-barrier. Compared to conventional vehicles such as emulsions and hydrogels, microemulsions have shown larger drug solubility due to coexistence of hydrophilic and lipophilic solubilization sites, and larger oil/water interfacial area. There are several permeation enhancement mechanisms of microemulsions such as an increased concentration gradient and thermodynamic activity toward skin and the permeation enhancement activity of the components of microemulsions (Teichmann, 2007 and Schmalfub, 1997).

Important features of ME are their high drug solubilization capacity, which leads to high concentration gradients towards the skin and a microstructure that allows free and fast drug diffusion. Having low or no interfacial tension, MEs are thought to rapidly penetrate into the stratum corneum where they will blend into skins mantle. The oil, surfactant and the cosurfactant can act as permeation enhancers. They are relatively stable and can solubilize a considerable amount of hydrophobic drugs in their lipophilic domain. The mechanism of enhancement via drug supersaturation is based simply on the increased thermodynamic activity of the drug in the vehicle, that is, an increased driving force for transiting out of the formulation and going into and through stratum corneum. In microemulsions, the co-surfactant lowers

the interfacial tension of the surfactant film, resulting in a more flexible and dynamic layer as suggested by previous reports. The drug in this energy-rich system can diffuse across the flexible interfacial surfactant film between the phases, a thermodynamic process that increase partitioning and diffusion into the stratum corneum (Kreilgaard, 2002, Kogan et al, 2006).

### **Combination Therapy**

More specific approaches to minimize side effects associated with topical corticosteroids include combination. The rationale assumes that agents are selected on the basis of their individual mechanisms of action, which may offer the possibility of one or more of the following: (1) additive or synergistic efficacy, (2) reduction in the dosage of either or both products, and (3) reduction in the occurrence of side effects. The possibility of additive toxicity and incompatibility between the combined agents must be considered. More recently, investigators have started to explore the possibility of combining topical corticosteroids with topical calcineurin inhibitors (TCIs) for the treatment of psoriasis or AD (Abramovits, 2005). Few studies have evaluated this combination. In vitro studies using fresh human dermatomed skin showed that co-application of Betamethasone valerate foam and tacrolimus ointment or pimecrolimus cream on the same site may positively affect penetration of the calcineurin inhibitors. The optimal combination therapy would allow reduced use of both drugs (eg, weekday use of a TCI with weekend use of a topical corticosteroid) to minimize the risks of adverse effects. The addition of a topical corticosteroid to monotherapy with a TCI offers the advantage of immediate relief of symptoms and reduction in inflammation as the effects of the TCI commence (Tanojo et al, 2003).

Studies of combination therapy in AD are almost nonexistent; however, two small trials examining topical corticosteroid/TCI combinations demonstrated



greater efficacy compared with monotherapy with either agent. In one study (N = 17), intermittent topical betamethasone butyrate propionate/tacrolimus sequential therapy improved lichenification and chronic papules more effectively than intermittent topical betamethasone butyrate propionate/emollient sequential therapy (Nakahara et al, 2004). In the other study (N = 59), concomitant treatment with clocortolone pivalate cream and tacrolimus was superior to monotherapy with either agent in improving overall dermatologic sum score, excoriation, and erythema and in reducing transient pruritus and burning or stinging. The corticosteroid/TCI combination for AD is favored by many practitioners because the two drug classes have different and possibly complementary mechanisms of action (Torok et al, 2003). It is also supported by the recent guidelines from the International Consensus Conference on Atopic Dermatitis, which recommended corticosteroids for acute control of disease progression and as intermittent treatment in maintenance therapy with TCIs. Rigorous clinical trials are clearly warranted to explore the efficacy of combination therapy, particularly to establish optimal dosages (which may differ markedly from those used in monotherapy).

### **Research Envisaged**

The present studies focuses on development and characterization of microemulsion based topical creams of a corticosteroid and calcineurin inhibitor. It is also aimed to develop a microemulsion based cream for the combination of two drugs.

### **Hypothesis**

The microemulsion based combination cream will enhance dermal penetration of the individual agents and thus it will be possible to achieve equivalent therapeutic effect with lower dose of the drugs. Ointments of

corticosteroids are known to give higher dermal penetration than aqueous creams but are less patient acceptable. The present work will develop an aqueous cream giving higher penetration and will be having higher patient acceptability. A combination of Topical corticosteroid and calcineurin inhibitor is not available commercially, although the two agents have different mechanism of action, are each others alternative and have not been reported to be incompatible chemically. Since the two drugs are have non-overlapping mechanism of action and corticosteroid can control the side effects produced by calcineurin inhibitor, it is expected to achieve a synergistic effect and with less side effects:

#### **Proposed Plan of Work**

- Review of literature with reference to Atopic Dermatitis, Dermal Delivery and strategies for its enhancement, topical corticosteroids, topical calcineurin inhibitors, microemulsions, animal models of atopic dermatitis, analytical profile and physico-chemical properties of the selected therapeutic agents.
- Preparation of solutions containing selected drugs, preparation and optimization of microemulsions with the help of pseudo ternary diagrams and titration method.
- Characterization of drug loaded microemulsions for their globule size, zeta potential, % transmittance, drug content, pH, viscosity, transmission electron microscopy and evaluation of stability of the formulations under normal and accelerated conditions.
- Incorporation of optimized drug loaded microemulsion in cream base and its characterization.
- Comparative *in-vitro* diffusion studies of formulations across artificial membrane and *ex-vivo* diffusion across skin.

- Toxicity and irritation studies of the optimized formulation by sub acute dermal toxicity studies, MTT assay on excised tissue and human acceptability test.
- Pharmacodynamic studies of the drugs on suitable animal models (Hapten induced mice model of dermatitis) in clinical and sub-clinical dose. The evaluations include disease characterization, biochemical investigation, histopathology and cytokine gene expression studies.
- Clinical studies include skin blanching bioassay for corticosteroid in human volunteers and pilot clinical study in dermatitis patients.
- Development and characterization for combination of corticosteroid and calcineurin inhibitor.

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