Chapter 2
Literature Review

2.1 Skin Barrier

Mammalian skin is a complex transport barrier with special anatomical organisation and chemical structure. The skin is the largest organ of the body with an area of approximately 2 m^2 and is the interface between the organism and its environment. It prevents the loss of water and the ingress of foreign materials. In essence, the skin consists of three functional layers:

A. Epidermis

B. Dermis

C. Hypodermis.

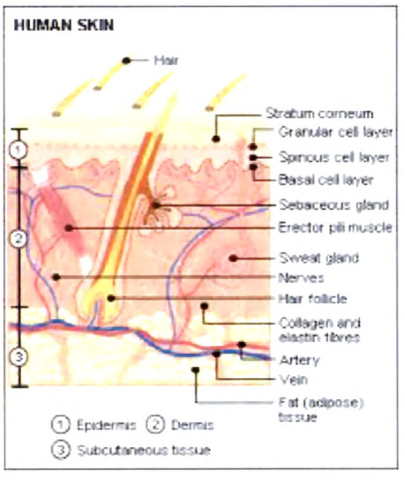


Fig 2.1: Anatomy of Skin

The hypodermis is a subcutaneous tissue consisting of fat and muscle and acts as a heat isolator, a shock absorber, and an energy storage region. The dermis is 2 mm thick and contains collagen, elastic fibres, blood vessels, nerves as well as hair follicles and sebaceous and sweat glands. The main cells in the dermis are fibroblasts, which are involved in the immune and inflammatory response. The dermis is the source of nutrients for the epidermis. Because the epidermis is avascular, essential substances are transported only by passive diffusion.

The epidermis has a multilayered structure reflecting different stages of differentiation of the skin cells (keratinocytes). From the proliferative, basal layer, cells change in an ordered fashion from metabolically active and dividing to dense, functionally dead, and fully keratinized, the so-called corneocytes. Most important in the respect is the outermost skin region, the SC, which comprises columns of tightly packed corneocytes organised into clusters of up to a dozen cells per corneocyte layer. Mammalian SC consists of ~15 corneocyte layers, with a total thickness of 6–8 μm [Anderson et al, 1973, Cevc, 2004].

In this lipid matrix, however, different enzymes (proteases) were found which are involved in the desquamation process. The terminally differentiated corneocytes (bricks) consist primarily of a highly organized keratin microfibrillar matrix, which provides mechanical resistance. In addition, protein junctions, the corneodesmosomes, link adjacent corneocytes and ensure the cohesiveness of this layer [Menon, 2002]. During the formation and maturation of the SC, desmosomes are modified and their number decreases towards the skin surface. Each corneocyte is surrounded by a 15–20 nm thick protein shell – the cornified cell envelope (CE), a 15 nm layer of defined structural proteins and a 5 nm thick layer of specialized lipids. The lipid monolayer provides a hydrophobic interface between the CE itself and SC lipid lamellae and helps maintain water barrier function [Marekov, 1998].

While typical biological membranes are mainly composed of phospholipids, the intercellular SC lipids (mortar) comprise primarily ceramides (~40% w/w), free fatty acids (~10% w/w) and cholesterol (~25% w/w), together with a small fraction of cholesterol sulphate and triglycerides [Elias, 1991]. The lipids originate from lamellar bodies that are synthesized in the upper viable layers

of the epidermis. These lipids, which are organized in multilamellar bilayers, regulate the passive flux of water through the SC and are considered to be very important for skin barrier function [Wertz, 1989]. [Grubauer, 1989]. This 'brick and mortar' arrangement of the SC creates a tortuous route for compounds to permeate the barrier.

The terminal differentiation of keratinocytes from granular cells to corneocytes is a critical step for the maintenance of skin barrier function. The formation of the cornified epithelium involves the sequential expression of several major structural proteins. During corneocyte differentiation, profilaggrin is dephosphorylated and proteolytically cleaved by serine proteases, into multiple filaggrin polypeptides. After cleavage, liberated filaggrin binds to keratin in a structure aligned parallel to the outer surface of the epidermis. Skin barrier function is determined by structural proteins, lipids, proteases, and protease inhibitors. A balance between proteolytic and antiproteolytic activities is essential for normal homeostasis in the skin

2.2 Topical Drug Delivery

Topical delivery has been used as a route of medicinal delivery for many thousands of years. The skin is accessible and it is relatively easy to interrogate it *in vivo*. Transdermal drug delivery has many advantages over the oral route of administration: it avoids hepatic metabolism, the administration is easier and more convenient for the patient, and there is the possibility of immediate withdrawal of the treatment if necessary. Despite the great potential of transdermal delivery of drugs, only a few drug formulations are available commercially. The main reason is the barrier function of human skin that is considered to be the most impermeable epithelium to exogenous

substances. The SC serves as the barrier to both the ingress of xenobiotics and the egress of water.

2.2.1 Routes of penetration

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. Therefore, the researchers are challenged to come up with formulations that increase the permeability of the drug without irreversibly changing the skin barrier function (Peltola et al, 2003).

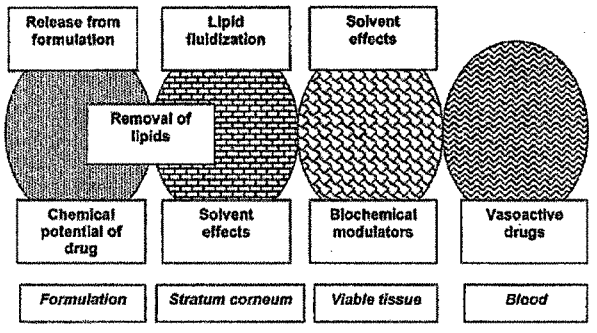


Fig. 2.2: A schematic representation of the skin and the way various enhancement strategies

Various potential mechanisms to enhance drug penetration through the skin include directly affecting the skin and modifying the formulation so the partition, diffusion, or solubility is altered (Barry, 2004).

Drug molecules in contact with the skin surface can penetrate by three potential pathways: through the sweat ducts, *via* the hair follicles and sebaceous glands (collectively called the shunt or appendageal route), or directly across the SC it is generally accepted that as the appendages comprise a fractional area for permeation of approximately 0.1%, their contribution to

steady state flux of most drugs is minimal. This assumption has resulted in the majority of skin penetration enhancement techniques being focused on increasing transport across the SC rather than via the appendages. Exceptions are iontophoretic drug delivery which uses an electrical charge to drive molecules via the shunt routes (Higuchi, 1962).

The SC consists of 10-15 layers of corneocytes and varies in thickness from approximately 10-15 μm in the dry state to 40 μm when hydrated. It comprises a multi-layered "brick and mortar" like structure of keratin-rich corneocytes (bricks) in an intercellular matrix (mortar) composed primarily of long chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulfate and sterol/wax esters. The intercellular lipid matrix is generated by keratinocytes and this extruded material rearranges to form broad intercellular lipid lamellae, which then associate into lipid bilayers. The hydrocarbon chains are arranged into regions of crystalline, lamellar gel and lamellar liquid crystal phases thereby creating various domains within the lipid bilayers. The presence of intrinsic and extrinsic proteins, such as enzymes, may also affect the lamellar structure. Water is an essential component of the SC, which acts as a plasticizer and is also involved in the generation of natural moisturizing factor (Wertz and Downing, 1989, Bouwstra, 1991, Anderson and Cassidy, 1973).

In order to understand how the physicochemical properties of the diffusing drug and vehicle influence permeation across the SC and thereby optimize delivery, it is essential to determine the predominant route of drug permeation within the SC. Traditionally it was thought that hydrophilic chemicals diffuse within the aqueous regions near the outer surface of intracellular keratin filaments (intracellular or transcellular route) whilst

lipophilic chemicals diffuse through the lipid matrix between the filaments (intercellular route). However, a molecule traversing via the transcellular route must partition into and diffuse through the keratinocyte and the molecule must partition into and diffuse through the estimated 4-20 lipid lamellae between each keratinocyte. This series of partitioning into and diffusing across multiple hydrophilic and hydrophobic domains is unfavorable for most drugs. Consequently, based on more recent data the intercellular route is now considered to be the major pathway for permeation of most drugs across the SC. As a result, the majority of techniques to optimize permeation of drugs across the skin are directed towards manipulation of solubility in the lipid domain or alteration of the ordered structure of this region (Scheuplein and Blank, 1971, Potts and Francoeur, 1991)

Drug permeation across the SC obeys Fick's first law where steady-state flux (J) is related to the diffusion coefficient (D) of the drug in the SC over a diffusional path length or membrane thickness (h), the partition coefficient (P) between the SC and the vehicle, and the applied drug concentration (C_0) which is assumed to be constant:

$$\frac{dm}{dt} = J = \frac{DC_0P}{h}$$

This equation aids in identifying the ideal parameters for drug diffusion across the skin. Molecules showing intermediate partition coefficients (log P octanol/water of 1-3) have adequate solubility within the lipid domains of the SC to permit diffusion through this domain whilst still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis. Optimal permeability has been shown to be related to low molecular size (ideally less than 500 Da) as this affects diffusion coefficient,

and low melting point which is related to solubility. When a drug possesses these ideal characteristics (as in the case of nicotine and nitroglycerin), transdermal delivery is feasible. However, where a drug does not possess ideal physicochemical properties, manipulation of the drug or vehicle to enhance diffusion, becomes necessary (Potts and Guy, 1992; Bos and Meinardi, 2000).

The approaches that have been investigated are summarized and discussed below. These potential mechanisms are interconnected with each other.

1. Direct effect on the skin

a. Denaturation of intracellular keratin or modification of its conformation causes swelling and increased hydration

b. Affection of desmosomes that maintain cohesion between corneocytes

c. Modification of lipid bilayers reduces resistance to penetration

d. Altering the solvent properties of the SC to modify drug partitioning

e. Use of solvent that can extract the lipids in the SC and decrease its resistance to penetration

2. Modification of the formulation

a. Supersaturation state produced by volatile solvent that leaves the active substance in a more thermodynamically active state

b. Choosing the enhancer molecules in the vehicle that are good solvents for the active ingredient and which enhance permeation through the skin

c. The diffusion of the active ingredient through the skin may be facilitated by using enhancers that create liquid pools within the bilayers like oleic acid, or disturb the bilayers uniformly as do the Azone® molecules (Azone® (1-dodecylazacycloheptan-2-one or lauro capram)

Sometimes the synergy between several enhancement effects causes a greater enhancement in permeability of the desired substance (Kreilgaard, 2002, Hadgraft, 2001 and Barry, 2004)

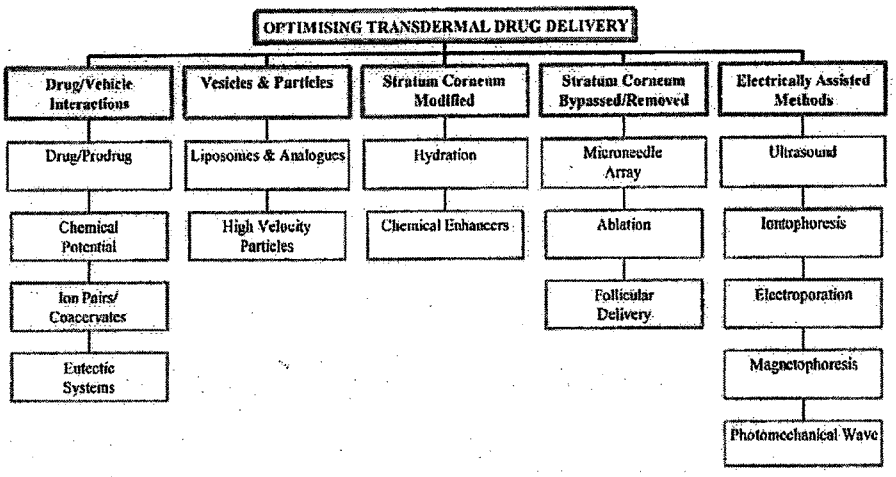


Fig. 2.3: Various techniques of optimizing transdermal drug delivery

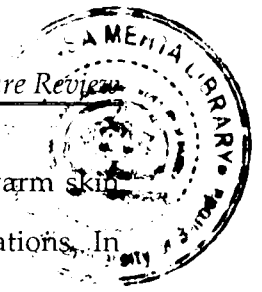
2.2.2 Penetration Enhancement through Optimization of Drug and Vehicle Properties

Chemical Potential of Drug in Vehicle – Saturated and Supersaturated Solutions

The maximum skin penetration rate is obtained when a drug is at its highest thermodynamic activity as is the case in a supersaturated solution. This can be demonstrated based on equation rewritten in terms of thermodynamic activities

$$\frac{dm}{dt} = \frac{aD}{\gamma h}$$

Where a is the thermodynamic activity of the permeant in its vehicle and γ is the effective activity coefficient in the membrane. Supersaturated solutions can occur due to evaporation of solvent or by mixing of cosolvents. Clinically,



the most common mechanism is evaporation of solvent from the warm skin surface which probably occurs in many topically applied formulations. In addition, if water is imbibed from the skin into the vehicle and acts as an antisolvent, the thermodynamic activity of the permeant would increase. Increases in drug flux of five- to ten-fold have been reported from supersaturated solutions of a number of drugs (Kemken et al, 1992 and Dias et al, 2003).

2.2.3 Penetration Enhancement by Stratum Corneum Modification

Hydration

Water is the most widely used and safest method to increase skin penetration of both hydrophilic and lipophilic permeants. The water content of the SC is around 15 to 20% of the dry weight but can vary according to humidity of the external environment. Additional water within the SC could alter permeant solubility and thereby modify partitioning from the vehicle into the membrane. In addition, increased skin hydration may swell and open the structure of the SC leading to an increase in penetration, Hydration can be increased by occlusion with plastic films; paraffins, oils, waxes as components of ointments and water-in-oil emulsions that prevent transepidermal water loss; and oil-in-water emulsions that donate water. Of these, occlusive films of plastic or oily vehicle have the most profound effect on hydration and penetration rate (Roberts and Walker, 1993, Scheuplein and Blank, 1973)

Lipid Disruption/Fluidisation by Chemical Penetration Enhancers

Many enhancers, such as Azone, DMSO, alcohols, fatty acids and terpenes, have been shown to increase permeability by disordering or 'fluidising' the lipid structure of the SC. In some cases, the enhancers penetrate into and mix

homogeneously with the lipids. However, others such as oleic acid and terpenes, particularly at high concentration, pool within the lipid domains to create permeable 'pores' that provide less resistance for polar molecules.

Interaction with Keratin

In addition to their effect on SC lipids, chemicals such as DMSO, decylmethylsulphoxide, urea and surfactants also interact with keratin in the corneocytes. It may result in a disruption of order within the corneocyte. This causes an increase in diffusion coefficient, and hence increases permeability (Walters, 1988)

Increased Partitioning and Solubility in Stratum Corneum

A number of solvents (such as ethanol, propylene glycol, Transcutol p and N-methyl pyrrolidone) increase permeant partitioning into and solubility within the SC, hence increasing P in Fick's equation. (Walters, 1988 and Liron and Cohen, 1984).

2.2.4 Determination of Percutaneous Absorption

Topical application to the skin usually aims at a local treatment. Therefore, the main interest lies in determining the drug level within the skin, in order to evaluate the dermal bioavailability of compounds or assess the bioequivalence between different formulations.

***In Vivo* Studies Using Pharmacodynamic Response**

Only a limited number of chemicals evoke a local quantifiable pharmacodynamic response, for example, the concentration-dependent vasoconstriction effect of corticosteroids. In these experiments, resulting skin blanching is scored visually by qualified investigators, using an ordinal data

scale. The lack of instrumentation has been criticized, because of possible subjective errors. However, approaches using the chromameter device, although recommended by the FDA, have failed to return the desired precision. (Adams and Singh, 1995, Smith et al, 1998).

***In Vivo* Dermatopharmacokinetic Approach**

Independent of any visible response, the extent of dermal absorption may be judged from the amount of substance missing within the recovered formulation after finishing incubation. Comprehensive mass balance including both drug and metabolite levels in the remaining formulation, washings, *SC*, blood, urine, and feces can provide a more detailed insight.

Skin Segmentation Studies – Tape Stripping Method

Sampling from the *SC* is usually performed by tape-stripping single corneocyte layers. Provided the test substance yields a unique infrared signal, attenuated total reflectance Fourier-transformation infrared (ATR-FTIR) spectroscopy is a fast, technically sophisticated, equipment-extensive method of quantification. It should be noted that with both tape-stripping and ATR-FTIR, the researcher's field of vision is limited to the nonviable part of the skin (Klimisch and Chandra, 1986 and Reddy et al, 2002).

***In Vivo* Dermal Microdialysis**

The technique consists of a microdialysis probe, a thin hollow tube made of a semi-permeable membrane usually around 200–500 μm in diameter, which is implanted into the skin and perfused with a receiver solution that recovers the unbound permeant from the local area. One of the most important parameters is the flow rate of the perfusate, which is normally in the range of 0.1–5 μl/min, inversely related to the amount of drug recovered in the

dialysate. Other factors that strongly influence are the lipophilicity and the extent of protein binding of the substance, and the molecular size of the compound. Nevertheless, the small sample size is a disadvantage since it requires very sensitive analytical methods (McCleverty et al, 2006, Davies, 1999 and Kreilgaard, 2002).

Perfused Skin Models

perfused skin models offer the benefits of living tissue with fully active microcirculation and metabolism. Ears of different animal origin, such as hairless mice, rabbits, or pigs, have been used. Because of their unusually high permeability, perfused ear models have been proposed to be predictive models of premature neonate skin (Riviere, 2006).

***In Vitro* Skin Permeation Studies**

In vitro percutaneous absorption methods have become widely used for measuring the absorption of compounds that come in contact with skin. For greatest acceptability of in vitro data, results from an in vitro method should come from a procedure that simulates in vivo conditions as closely as reasonably possible. Both static and flow-through diffusion cells are approved by the authorities. Basically, a donor and an acceptor compartment are separated by a membrane of either native skin or bioengineered materials. These materials can be of human, animal, or artificial origin. Sampling from the acceptor compartment is performed either continuously or at predetermined time intervals. Dosing is possible in infinite (typically >10 μl/cm2 or 10 mg/cm2) or finite manner (<10 μl/cm2 or 10 mg/cm2). The donor chamber may either be left open or be occluded. For the determination of membrane integrity, transepidermal water loss (TEWL) measurements are recommended in many guidelines. Temperature may be controlled by using a

water jacket around each permeation cell, an external water bath, or warm air in a drying oven. Usually, experiments are carried out at 32°C, that is, the temperature of the skin surface, Acceptor solutions preferably comprise buffer solutions of pH 7.4, spiked with preservatives, such as 0.05% sodium azide, if long-term incubation is desired. Viability of skin, composition of receptor fluid etc. are crucial parameters (Wagner et al, 2000 and Netzlaff et al, 2006).

Membranes

Various membrane types suitable for permeation experiments are listed below (Haigh and Smith, 1994).

Membranes of Human Skin Origin: For all in vitro skin experiments, human skin is considered to be the "gold-standard." Supply is usually provided from plastic surgery, amputations, or cadavers. Nonetheless, large intra and interindividual variations of up to 45% in vivo are documented.

Reconstructed Human Epidermis Equivalents

Because of the limited availability of human skin, reconstructed human epidermis equivalents are under investigation to serve as membranes in permeation experiments.

Membranes of Animal Skin Origin:

Species currently in use are mouse, hairless rat, hamster (cheek pouch), snake (shed skin), pig (ear, flank, abdomen, or back), and cow (udder). However, differences in *SC* thickness, number of corneocyte layers, hair density, water content, lipid profile, and morphology cause animal skin to be more

permeable than human skin. Human skin finds its closest match in porcine tissue (Bronaugh et al, 1982).

Membranes of Nonskin Origin:

Use of dialysis tubing and polymeric membranes, made up of regenerated cellulose, polycarbonate, polyethylene, or polydimethylsiloxane, to amphiphilic multilayer laminates and polymer networks has been reported (Hatanaka et al, 1992).

2.3 Microemulsions

Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multi component fluids composed of oil, water and surfactant (usually in conjunction with a cosurfactant). The droplets in a microemulsion are in the range of 1 nm-100 nm in diameter. Microemulsions appear transparent to the eye. The microemulsion concept was introduced as early in the 1943 by Hoar & Schulman who generated clear single phase solution by titrating a milky emulsion with hexanol. In recent years microemulsions have attracted a great deal of attention because of their biocompatibility, biodegradability, ease of preparation and handling and most importantly solubilization capacity for both water and oil soluble drugs. The differences between emulsions and microemulsions are enlisted in the following table 2.1:

Table 2.1: Differences between emulsions and microemulsions

CHARACTERISTICS	EMULSION	MICROEMULSION
Droplet size	100-100,000 nm	10-100 nm
Phase	Two	One
Appearance	Opaque	Transparent
Proportion of dispersed phase	30-60%	23-40% without corresponding to increase in viscosity
Energy requirement	Requires large energy input at the time of preparation	Forms spontaneously, so no energy requirement
Stability	Theoretically stable but thermodynamically unstable	Kinetically unstable but thermodynamically stable
Surfactant concentration	2-3% by weight	>6% by weight

Microemulsions can be oil in water, water in oil, bi continuous mixtures (Fig 2.9), ordered droplets or lamellar mixtures with a wide range of phase equilibria among them and with excess oil and/or water phases. This great variety is governed by variations in the composition of the whole system and in the structure of the interfacial layers.

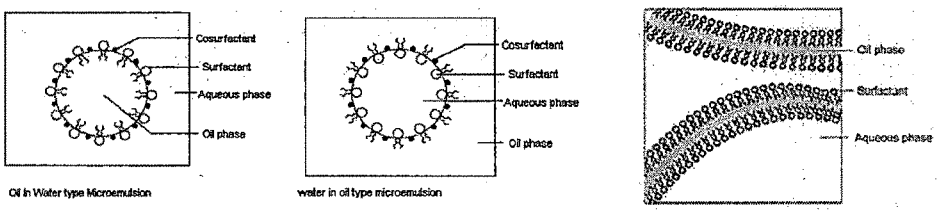


Fig. 2.4: O/W type, W/O type and bicontinuous microemulsion

The rationale for developing and using medicated microemulsions is listed in Table 2.2.

Table 2.2: Rationale for developing and using medicated microemulsions

Reason	**Drug Examples**
Solubilization of poorly water soluble drugs	Diazepam,VitaminA,VitaminE,Dexamethasone palmitate
Solubilization of hydrolytically susceptible compounds	Lomustine, Physostigmine salicyalate
Reduction of irritation, pain or toxicity of intravenously administered drugs	Diazepam
Potential for sustained release dosage forms	Barbiturates
Site specific drug delivery to various organs	Cytotoxic drugs

2.3.1 Theories of Microemulsion Formulation

Three different approaches have been proposed to explain microemulsion formation and the stability aspects. However, no single theory explains all aspects of microemulsion formation but each has its own significance in understanding of microemulsion formation. The important features of the microemulsion are thermodynamic stability, optical transparency, large overall interfacial area (about 100 m^2 /mL), variety of structures like ordered droplets on lamellar mixtures with wide range of phase equilibria with excess oil/water phases, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.

Thermodynamics of microemulsion

The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, it lowers the interfacial tension till CMC (Critical Micelle Concentration), after which micelles are formed. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system. In many cases, the interfacial tension is not yet ultra low when the CMC is reached. It has been studied and observed by Schulman and workers that the addition of a co surfactant (medium sized alcohol or amine) to the system reduces the interfacial tension virtually to zero and further addition of a surfactant (where γ is zero) leads to negative interfacial tension.

Mixed Film theories

The relatively large entropy of mixing of droplets and continuous medium explains the spontaneous formation of microemulsion. Schulman (Hoar and Schulman, 1942; Schulman & Strokenius, 1959) considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and cosurfactant. This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative) which is represented by following equation.

$$\gamma_i = \gamma_{o/w} - \pi_i$$

Where, $\gamma_{o/w}$= Oil-water interfacial tension without the film present

π_i= Spreading pressure

γ_i=interfacial tension

The interfacial film should be curved to form small droplets and to explain both the stability of the system and bending of the interface. Both sides of the interface expand spontaneously with penetration of oil and co surfactant until the pressures become equal. The side with higher tension would be concave and would envelop the liquid on that side, making it an internal phase. It is generally easier to expand the oil side of an interface than the waterside i.e. by penetration of the oil or co surfactant into the hydrocarbon chain area hence W/O microemulsion can be formed easily than O/W microemulsion (Tadros, 1983)

Solubilization theories

The group of Shinoda (Shinoda and Kunieda, 1973; Shinoda and Lindman, 1987; Friberg and Venable, 1983; Friberg and Lapcynska, 1976; Friberg and Buraczewska, 1977) considered microemulsion to be thermodynamically stable mono phasic solution of water-swollen (W/O) or oil swollen (O/W) spherical micelles.

Thermodynamic theories

This theory explains the formation of microemulsion even in the absence of cosurfactant. For microemulsion to form spontaneously, the free energy is

$$\Delta G = \gamma \Delta A$$

Where, ΔG= Free energy

γ = Interfacial tension

ΔA=Inverse in surface area

Thermodynamic theory takes into account entropy of droplets mixing and thermal fluctuations at the interface giving interfacial bending instability

(Ruckenstein and Chi, 1975; Ruckenstein and Krishnan, 1979; Ruckenstein and Krishnan, 1980). The dispersion of droplets in the continuous phase increases the entropy of the system and produces a negative free energy change, which is significantly important for very small droplets as in microemulsions. Ruckenstein and Chi, 1981 gave the equation for free energy (ΔG_m) in microemulsion formation.

$\Delta G_m = \Delta G_1 + \Delta G_2 + \Delta G_3$

Where, ΔG_m = Free energy

ΔG_1 = Interfacial energy

ΔG_2 = Free energy of inter droplet interactions

ΔG_3 = Entropy for dispersion of droplets in continuous medium

Later it was shown that accumulation of the surfactant and cosurfactant at the interface results in a decrease in chemical potential generating an additional negative free energy change called as dilution effect (Ruckenstein, 1981). This theory explained the role of cosurfactant and salt in a microemulsion formed with ionic surfactants.

2.3.2 Factors Affecting the Type of Microemulsion and Phase Behavior of the Microemulsion

Type of microemulsion

The formation of oil or water swollen microemulsion depends on the packing ratio, property of surfactant, oil phase, temperature, chain length, type and nature of cosurfactant.

Packing Ratio

The HLB (Hydrophilic Lipophilic Balance) of surfactant determines the type of microemulsion through its influence on molecular packing and film curvature. The analysis of film curvature for surfactant associations has been explained by Israclachvili, Mitchell and Ninham (1976) and Mitchell and Ninham (1981) in terms critical packing parameter.

Critical Packing (c.p.p.) =V/ (a*l)

Where, V-Volume of surfactant molecule

a = Head-group surface area

l =length

If c.p.p. has value between 0 and 1 interface curves towards water (positive curvature) and O/W systems are favoured, but when c.p.p. is greater than 1, interface curves spontaneously towards oil (negative curvature) so W/O microemulsions are favoured. At zero curvature, when the HLB is balanced (p is equivalent to 1), then either bi continuous or lamellar structures may form.

Property of surfactant, oil phase and temperature

Microemulsion is formed by the combination of dispersion and stabilization processes. The dispersion process involves a transient reduction of interfacial tension to nearly zero at which the interface expands to form droplets. Subsequently, they absorb more surfactant until the bulk phase is depleted enough to bring the value of interfacial tension positive. Stability of O/W emulsion system can be controlled by the interfacial charge. Type of surfactant also determines type of microemulsion formed. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these groups, which are a measure of the differential tendency of water to swell head group

and of oil to swell the tail area are important when estimating the surfactant HLB in a particular system.

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils, such as alkanes, penetrate the lipophilic group region largely than long chain alkanes and swelling of this region to a great extent results in an increased negative curvature. Temperature is important in determining the effective head group size of non ionic surfactants. Winsor studied the effect of temperature on the type of microemulsion formed. For the given amount of components in ternary system with nonionic surfactant, oil, and water, at relatively low temperatures, type I system (an oil in water with excess oil) is formed. At intermediate temperature type III system (microemulsion with excess of both oil and water) is present. At relatively higher temperature type II (water in oil microemulsion with excess water) system exist (Winsor PA, 1954, 1968)

The chain length, type and nature of cosurfactant

Alcohols are widely used as a cosurfactant in microemulsions. Addition of shorter chain cosurfactant (eg. ethyl alcohol) gives positive curvature effect, as alcohol swells the head region more than tail region and o/w type is favored, while longer chain cosurfactant (eg. cetyl alcohol) favors w/o type by alcohol swelling more in tail region than head region.

Phase behavior of microemulsion

Salinity: At low salinity, the droplet size of O/W microemulsion increases. As salinity further increases, the system becomes bi continuous over an intermediate salinity range. The microemulsion remains oil continuous with

the drop size decreasing with increasing salinity which causes complete phase transition.

Alcohol concentration: Increasing the concentration of low molecular weight alcohol leads to the phase transition from W/O to bi continuous and ultimately to O/W type microemulsion. The vice versa transition is visible in case of high molecular weight alcohol.

Surfactant hydrophobic chain length: The increase in length of hydrophobic chain length of surfactant shows change of O/W to W/O via bi continuous phase.

pH: Change in pH influences the microemulsions which contain pH sensitive surfactants and change the phase behaviour from W/O to O/W by increasing the pH.

Nature of oil: Increase in the aromaticity of oil leads to phase transition from O/W to W/O and is opposite to that of increase in the oil alkane carbon number.

Ionic strength: As the ionic strength increases the system passes from O/W microemulsion in equilibrium with excess oil to the middle phase and finally to W/O microemulsion in equilibrium with excess water.

2.3.3 Formation of Microemulsion and Phase Behavior

Preparation of a stable, isotropic homogeneous, transparent, non toxic microemulsion requires consideration of a number of variables. Phase diagrams help to find the microemulsion region in ternary or quaternary system and also help to determine the minimum amount of surfactant for microemulsion formation.

Phase Diagrams

Ternary systems

The phase behaviour of surfactant-oil-water (S/O/W) is best reported by using ternary diagram. Here, two independent composition variables are sufficient, since third one is complement to 100% (Fig 2.10). The phase diagram allows one to determine ratio of oil: water, surfactant-cosurfactant at the boundary of microemulsion region. To plot the composition of four component systems, a regular tetrahedron composed by fixing and varying the other three or by using a constant ratio of two components (surfactant and cosurfactant or co solvent). Fig. 2.5 shows the pseudo ternary diagram at constant surfactant to cosurfactant ratio. It also shows that single phase or multiphase regions of microemulsion domain are near the centre of diagram in areas containing large amounts of surfactant that is toxic. The phase behavior of surfactants, which form microemulsions in absence of cosurfactant, can be completely represented by ternary diagram.

***Winsor's regions*:** Winsor (1954) reported the relationship between the phase behaviour of amphiphiles-oil-water and nature of the different components of ternary system. Different regions of a phase diagram are shown in Fig. 2.5,6

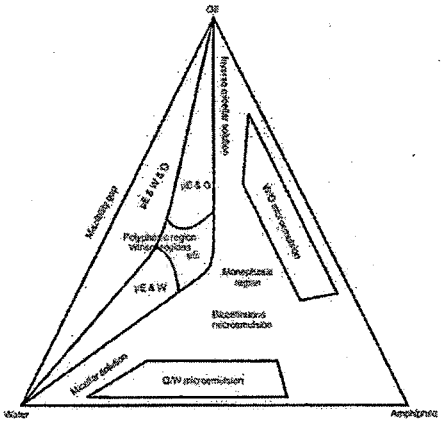


Fig. 2.5: Different regions of phase diagram

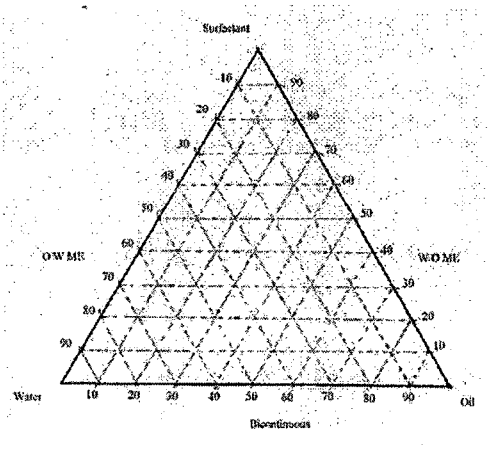


Fig. 2.6: Ternary system

Winsor I: The microemulsion composition corresponding to Winsor I is characterized by two phase, the lower oil/water(O/W) microemulsion phase in equilibrium with excess oil

Winsor II: The microemulsion composition corresponding to Winsor II is characterized maximal solubilization of oil and water for a given quantity of surfactant. Microemulsion coexists with both excess phases and no one can distinguish dispersed phase from continuous phase.

Winsor III: This phase comprises of three phases, middle microemulsion phase (O/W plus W/O, called bicontinuous) in equilibrium with upper excess oil and lower water.

Winsor IV: Microemulsions can be distinguished from the micelles by its inner core swollen with oil. The microemulsion structure depends on the chemical composition, temperature and concentration of the constituents.

Methods for constructing Phase diagram:

The microemulsion region is identified by its isotropic nature and low viscosityin quaternary phase diagrams. Other regions can be identified by their characteristic optical structure (Shinoda and Friberg, 1986). These diagrams are complicated and time consuming to prepare and provide a major drawback in the evaluation of a wide range of surfactant, co surfactant and other components.

In another method, microemulsion region can be located by titration method. At a constant ratio of S/CoS, various combinations of oil and S/CoS are produced. The water is added drop wise. After the addition of each drop, the mixture is stirred and examined through a polarized filter. The appearance (transparency, opalescence and isotropy) is recorded along with the number of phases. Thus, an appropriate delineation of the boundaries can be obtained

in which it is possible to refine through the production of compositions point-by point beginning with the four basic components.

The original method for construction of phase diagram developed by Bowcott and Schulman (1955) can be used for preparation of microemulsion. In this method, adding the oil surfactant mixture to some of the aqueous phase in a temperature controlled container with agitation makes a coarse macro emulsion as a first step. Then the system is titrated with cosurfactant until clarity is obtained and diluted with water to give a microemulsion of the desired concentration.

Formulation of microemulsions:

Generally, the microemulsion formulation requires following components:

a) *Oil Phase*: Toluene, Cyclohexane, mineral oil or vegetable oils, silicone oils or esters of fatty acids etc. have been widely investigated as oil components.

b) *Aqueous phase*: Aqueous phase may contain hydrophilic active ingredients and preservatives. Some workers have utilized buffer solutions as aqueous phase.

c) *Primary surfactant*: The surfactants are generally ionic, non ionic or amphoteric. The surfactants chosen are generally for the non ionic group because of their good cutaneous tolerance. Only for specific cases, amphoteric surfactants are being investigated.

d) *Secondary surfactant* (cosurfactant): co surfactants originally used were short chain fatty alcohols (pentanol, hexanol, benzyl alcohol). These are most often polyols, esters of polyols, derivatives of glycerol and organic acids. Their main purpose is to make inter facial film fluid by wedging themselves between the surfactant molecules.

2.3.4 Characterization of Microemulsions

The determination of microemulsion structure is difficult, although it is important for the successful commercial exploitation of microemulsions as a drug delivery system.

Phase Behavior Studies:

Visual observations, phase contrast microscopy and Freeze Fracture transmission electron microscopy can differentiate microemulsions from liquid crystals and coarse emulsions. Clear isotropic single phase systems are identified as microemulsions whereas opaque systems showing bifringence when viewed by cross polarized microscopy may be taken as liquid crystalline system. Phase behaviour studies provide information about the boundaries of different phases as a function of composition variables and temperature. They also allow comparison of the efficiency of different surfactant for given application.

Scattering techniques for microemulsions characterization:

Small angle X-ray scattering (SAXS), small angle neutron scattering (SANS) as well as static and dynamic light scattering are widely applied techniques in the study of microemulsions. In the static scattering techniques, the intensity of scattered radiation (q) is measured as a function of the scattering vector (q)

$$Q = (4\pi/\lambda) \sin\theta/2$$

Where, θ is the scattering and λ the wavelength of the radiation. The lower limit of size that can be measured with these techniques is about 2 nm. The upper limit is about 100 nm for SANS and SAXS and up to a few micrometers for light scattering. These methods are very valuable for obtaining quantitative information on the size, shape and dynamics of the components. The major drawback of these techniques is that samples need to be diluted in

order to reduce interparticulate interaction. This dilution can modify the structure and the composition of the pseudo phases. Nevertheless, successful determinations are carried out using a dilution technique that maintains the identity of droplets.

Static light scattering techniques have also been widely used to determine microemulsion droplet size and shape. Here, the intensity of scattered light is generally measured at various angles and for different concentrations of microemulsion droplets.

Dynamic light scattering also referred to as photon correlation spectroscopy (PCS) can analyze the fluctuations in the intensity of scattering by the droplets due to Brownian motion. This technique allows the determination of z-average diffusion coefficients, D. In the absence of inter particle interactions, the hydrodynamic radius of the particles R_H, can be determined from the diffusion coefficient using the Stokes-Einstein equation

$$D = kT/6\pi\acute{\eta}R_H$$

Where, k is Boltzmann constant, T is the absolute temperature and $\acute{\eta}$ is the viscosity of the medium and R_H is hydrodynamic radius.

Nuclear Magnetic Resonance Studies:

The structure and dynamics of microemulsions can be studied by using nuclear magnetic techniques. Self-diffusion measurements using different tracer techniques, generally radio labeling, supply information of the mobility of the components. The Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique uses the magnetic gradient on the samples and it allows simultaneous and rapid determination of the self diffusion coefficients (in the range of 10^4 to 10^{12} m^2/s) of many components.

Electron Microscopic Studies:

The microemulsion can be characterized by electron microscopic techniques even though high liability of the samples to the possibility of artifacts. The systems are observed under microscope either followed by chemical or thermal fixation methods. But the thermal fixation method, especially freeze fracture electron microscopy has also been used to study microemulsion structure; in which extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts. It has been reported that other than CRYO-TEM, the direct observation of the microemulsion over the grid followed by normal air drying is also an useful tool in the study of microstructure and size analysis (Sheikh Shafiq et al 2007).

Interfacial tension and electrical conductivity measurements:

Ultra low values of interfacial tension are correlated with phase behaviour, particularly the existence of surfactant phase or middle phase microemulsion in equilibrium with aqueous and oil phases. Spinning drop apparatus can measure the ultra low interfacial tension. A sharp increase in conductivity in certain W/O microemulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a 'percolative behaviour' or exchange of ions between droplets before the formation of bi continuous structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of microemulsion systems.

Rheological properties and viscosity measurements:

In general microemulsions have low viscosity and exhibit Newtonian flow behaviour. At very high shear rates shear thinning is observed. Viscosity data are helpful in determining the shape of the corresponding aggregates or extract information regarding the interaction potential between the droplets.

Even though microemulsions of bi continuous structure possess highly interconnected structure, they show newtonian flow with low viscosity because of their very short structural relaxation time (less than 1 millisecond). When there is transition from a droplet structure to a bi continuous structure, viscosity of the system increases.

Stability studies

The stability of the micro emulsion has been assessed by conducting long term stability study and accelerated stability studies. In long term stability study, the system is kept at room temperature and refrigeration temperature. Over the time period micro emulsion systems are evaluated for their size, zeta potential, assay, pH, viscosity and conductivity. On long term study, the activation energy for the system and shelf life of the system may be calculated as like other conventional delivery system (Nornoo et al 2007).

Accelerated stability studies are the essential tools to study the thermodynamic stability of micro emulsions. It can be done by centrifugation, heating/cooling cycle and freeze/ thaw cycles.

1. In the centrifugation, the system is subjected to centrifugation at 822 gm for 30 minutes and followed by the observation for phase separation
2. The Heating / Cooling cycle of keeping the system at 4ºC and 45ºC for not less than 48 hours at each stage.
3. Freeze/ Thaw cycles of micro emulsion can be done between - 21ºC and 25ºC or between 5ºC and 10ºC (Sheikh Shafiq et al 2007).

2.3.5 Microemulsions for Dermal Delivery

Drug delivery to or via skin presents both unique opportunities and obstacles due to skin structure, physiology and barrier properties. For dermal and

transdermal delivery, it is necessary to overcome this barrier without disrupting normal skin functions. Microemulsions have been widely investigated for dermal and transdermal drug delivery. They offer several advantages such as ease of preparation, excellent thermodynamic stability, long-term stability, high surface area, high solubilization capacity for hydrophilic and lipophilic drugs, and improved drug delivery [Kogan and Garti, 2006]. Some of the potential mechanisms by which microemulsions would improve the dermal or transdermal transport are (1) denaturation of intracellular keratin or modification of its conformation [Gloor et al, 2003], (2) perturbation/fluidisation of lipid bilayers [Changez et al, 2006], (3) creation of liquid pools and extraction of SC lipids, (4) increased partitioning and solubility in SC, (5) increased thermodynamic activity of the drug [Kemken et al, 1992], (6) higher concentration gradient [Peltola et al, 2003] and (7) appendageal transport[Biju et al, 2005]. The o/w formulation was supposed to accumulate drug in SC and epidermis whereas the w/o system delivered it primarily into deeper skin regions. Site-specific treatment of dermatological diseases is a classical field of topical administration. They enhance dermal and transdermal drug delivery, a lot of compositions have been created and tested in vitro and in vivo (Sintov, and Shapiro, 2004). A crucial point for the clinical use is the compatibility for which the surfactants, namely their type and amount, play the most important role.

The explored systems utilize a huge array of surfactants, co-surfactants and different pharmacological and chemical categories of drugs. A frequently used combination of surfactants is the mixture of Labrasol (caprylocaproyl polyoxylglycerides) and a Plurol-derivative (polyglyceryl fatty acid ester) for drugs like local anesthetics, analgesics, cardiovascular agnetns, vinpocetine etc (Kweon et al, 2004 and Hua et al, 2004). PEGylated fatty alcohols (e.g.,

Brij), another important class of non-ionic surfactants, offer the useful possibility of forming co-surfactant free microemulsions. Drugs investigated include antimicrobials, steroids like testosterone and several other classes (Kantarci et al, 2005). Colloidal drug carrier systems like microemulsions can be prepared with polysorbates (Tween) and Span, respectively, having either no co-surfactant or a short or medium chain alcohol. These are very frequently explored but often give toxicity related issues. Drugs like triptolide, lidocaine, methoxasalen and several others have been investigated and found to increase drug permeation (Chen et al, 2004 and Schmalfuss et al, 1997). Natural lecithin is one of the most promising and useful pharmaceutical agents in topical formulations. It is non-toxic even in high concentrations and does not lead to skin irritation. Furthermore, it is able to increase skin permeation. Due to their amphiphilic lipid structure, phospholipids are able to form liposomes, but also microemulsions—either on their own in narrow composition ranges or with co-surfactants like short chain alcohols. During the past several years, phospholipids have been used in different approaches as amphiphile for dermal drug delivery by microemulsions (Changez et al, 2006). In the 1990s a promising class of non-ionic surfactants came up in the development of colloidal carriers. Based on the renewable raw materials starch and natural oils, alkyl polyglycosides (APGs) show excellent environmental and skin compatibility that are known as sugar based surfactants (Lehmann et al, 2001). Microemulsions using ionic surfactants like AOT (sodium bis(2-ethyl hexyl)sulfosuccinate) is a frequently used anionic surfactant that forms stable w/o microemulsions without the addition of cosurfactants (Changez and Varshney, 2000).

2.4 Atopic Dermatitis

The prevalence of atopic dermatitis is on the rise all over the world, but particularly in Western and industrialized societies (Leung and Bieber, 2003; Novak et al., 2003). 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 and often associated with a family and/or personal history of allergy. Histopathology of AD skin lesions reveals an intense mononuclear cell infiltrate in the dermis with T cells, monocytes, macrophages, dendritic cells (DCs), mast cells, and eosiniphils or their products. In addition, there is fibrosis and collagen deposition in chronic skin lesions. The hallmarks of AD are skin barrier dysfunction, which results in dry itchy skin, which leads to scratching that inflicts mechanical injury and allergic sensitization to environmental antigens and allergic skin inflammation.

Atopic dermatitis develops as a result of a complex interrelationship between environmental exposure, genetic background, and immunologic factors. Immunologic factors that appear to play a role include the pattern of local cytokine release, nature of the antigen, differentiation of helper T cells, immunoglobulin (Ig)E antibodies, infectious agents, and superantigens (Grewe et al, 1998).

Serum IgE levels are elevated in about 80% of adult AD patients, who also show sensitization against airborne and food allergens and/or concomitant allergic respiratory allergy. This subtype is termed as extrinsic AD. The intrinsic subtype of AD with normal serum IgE levels has a late onset (older than 20 years) and a lack of these sensitizations.

Two hypotheses have been proposed for the pathogenesis of AD. One hypothesis holds that the primary defect is intrinsic to skin epithelial cells and results in a defective skin barrier function with a secondary immune response to antigens that enter via damaged skin barrier. The other hypothesis holds that the primary abnormality is in the immune system and results in a Th2/IgE-dominated immune response that causes a secondary defect in barrier skin function. In the majority of AD patients, a primary defect in barrier function in the context of a genetically inherited immune predisposition to mount a Th2/IgE-dominated immune response which in turn aggravates the skin barrier defect initiating a vicious cycle that triggers and perpetuates AD skin lesions (Oyoshi, 2003).

The skin of AD patients is dry and demonstrates increased TEWL. Scratching of dry itchy skin causes mechanical skin injury that activates keratinocytes to release proinflammatory cytokines and chemokines that induce the expression of adhesion molecules on vascular endothelium and facilitate the extravasation of inflammatory cells into the skin. In addition, damage by scratching to an already abnormally permeable skin enhances the entry of allergens and infectious agents. Several mediators are associated with itching in AD, including histamine, IL-31, neuropeptides, opioids, and serine proteases.

Acute AD skin lesions clinically show intensely pruritic, erythematous papules associated with excoriation and serous exudation. Pathological examination reveals spongiosis, that is intercellular epidermal edema, hyper-/parakeratosis, that is superficial epidermal hypertrophy and acantholysis, epidermal vesiculation/separation, with a marked infiltration of CD4+ activated memory T cells, antigenpresenting cells (APCs), including

Langerhans cells, inflammatory dendritic epidermal cells (IDECs), macrophages, and degranulated mast cell in acute lesional skin (Leung, 2000).

Chronic AD skin lesions clinically show thickened plaques with increased lichenification Pathological examination reveals marked epidermal hyperplasia, acanthosis with macrophage-dominated mononuclear cell infiltrate in the dermis, and perivascular accumulation of lymphocytes in smaller numbers than seen in acute AD (Leung and Bieber, 2003). Clinically unaffected skin in AD patients is not normal. It is frequently dry and has a greater irritant skin response to chemicals or physical agents.

2.4.1 Pathophysiology

Interactions between susceptibility genes, the host's environment, pharmacological abnormalities, and immunological factors contribute to the pathogenesis of atopic dermatitis.

The systemic immune response

Most patients with atopic dermatitis have peripheral blood eosinophilia and increased serum IgE concentrations. Peripheral blood mononuclear cells from patients with atopic dermatitis have a decreased capacity to produce interferon γ, which is inversely correlated with serum IgE concentrations. This association could be due to a deficiency of interleukin 18, an inducer of interferon γ production. An increased frequency of allergen-specific T cells producing IL- 4, 5, and 13, but little interferon γ, in the peripheral blood of patients with atopic dermatitis has also been recorded (Beck, 2000).

These cytokines also induce expression of vascular adhesion molecules such as VCAM-1, which are involved in eosinophil infiltration and downregulate

Th1-type cytokine activity. IL 5 plays a key part in development, activation, and survival of eosinophils. The cytokine micromilieu in which T-cell development takes place, pharmacological factors, the costimulatory signals used during T-cell activation, and the antigen-presenting cell determine the outcome of the T-cell response (Spergel, 1998).

Cytokine expression: Biphasic pattern of cytokine expression in atopic skin lesions

The pattern of local cytokine expression plays an important role in modulating tissue inflammation, and in AD this pattern depends on the acuity or duration of the skin lesion. The expression of IL-4, IL-5, IL-13, and IFN-γ messenger (m)RNA in skin biopsy specimens from clinically normal (uninvolved), acute (erythematous AD lesions of <3 days' duration), and chronic (>2 weeks' duration) skin lesions of patients with AD has been investigated. Uninvolved skin of patients with AD had significantly more cells expressing IL-4 and IL-13 mRNA than normal skin, but no increased expression of IL-5 or IFN-γ mRNA. Acute skin lesions had significantly more cells positive for IL-4, IL-5, and IL-13 mRNA than normal or uninvolved skin, but no significant expression of IFN-γ mRNA. Acute skin lesions also have an increased expression of IL-16.

In chronic AD skin lesions, expression of IL-4, IL-5, IL-13, and IFN-γ mRNA was significantly increased compared with normal and uninvolved skin. However, compared with acute skin lesions, chronic skin lesions had significantly fewer IL-4 and IL-13 mRNA-expressing cells and increased numbers of IL-5 and IFNγ mRNA-expressing cells. Other studies have demonstrated overexpression of IL-12 and GM-CSF in chronic AD lesions. These data indicate a biphasic pattern of cytokine expression in AD. IL-12

plays a key role in TH1-cell development, and its expression in eosinophils and/or macrophages is thought to initiate the switch to TH1-cell development in chronic skin lesions. In addition, IL-16 may promote the infiltration of CD4+ T cells into acute lesions and GM-CSF is likely to enhance cell survival of eosinophils and macrophages in chronic skin lesions. The biphasic pattern of T-cell activation has also been well demonstrated in studies on skin biopsy specimens of atopic patch-test reaction sites (Langveld et al, 2000; Reich, et al 2002).

Activated T cells also induce keratinocyte apoptosis, contributing to the spongiotic process seen in acute atopic dermatitis. This process is mediated by interferon γ derived from T cells. Keratinocytes play a role in innate immunity by expressing toll-like receptors and by producing antimicrobial peptides in response to invading microbes. AD keratinocytes secrete unique profile of chemokines & cytokines (lipozen and wolff, 2007)

Factors Affecting the Differentiation of Helper T Cells

Th0 cells can develop into either the Th1 or Th2 phenotype, and the pathway followed depends upon several factors, including the cytokine environment in which T-cell development takes place, the genetic background of the host, pharmacologic factors, and the costimulatory signals involved in T-cell activation.

Atopic dermatitis skin contains an increased number of IgE-bearing Langerhans cells and inflammatory dendritic epidermal cells expressing the high-affinity receptor for IgE. These antigen-presenting cells seem to have an important role in allergen presentation to Th2 and Th1 cells, respectively (von Bubnoff et al, 2001).

Interleukin 16, a chemoattractant for CD4 T cells, is more highly expressed in acute than chronic atopic dermatitis skin lesions. The C-C chemokines, T cells expressed and secreted (RANTES), monocyte chemotactic protein 4 (MCP4), and eotaxin are also increased in atopic dermatitis skin lesions and probably contribute to the chemotaxis of eosinophils expressing CCR3 and Th2 lymphocytes into the skin (Taha et al, 2000; Yawalkar et al, 1999, Galli et al , 2000, Bratton et al, 1995).

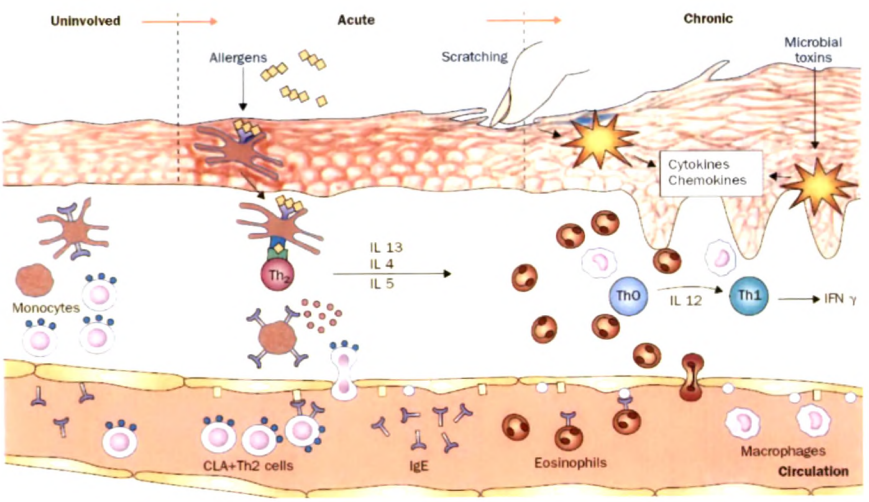


Fig. 2.7: Immunological pathways in atopic dermatitis

Genetics

Atopic dermatitis is a genetically complex, familially transmitted disease with a strong maternal influence. Parental atopic dermatitis confers a higher risk to offspring, suggesting the existence of genes specific to atopic dermatitis. Several chromosomal regions contain pathophysiologically relevant candidate genes, especially on chromosome 5q31–33 since it contains a clustered family of Th2 cytokine genes—ie, IL- 3, 4, 5, and 13, and GM-CSF. Similarly, variants of the interleukin 13 coding region and a functional mutation in the promoter region of RANTES (17q11) have a role in expression of atopic dermatitis.

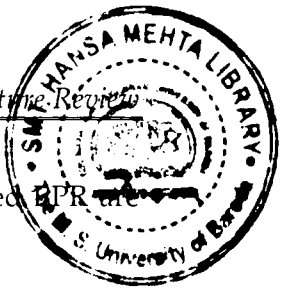
Cookson and coworkers (2001) reported that both atopic dermatitis and psoriasis were linked to loci on chromosome 1q21 and 17q25, suggesting that common candidate genes are involved in control of skin inflammation (Forrest et al, 1999).

2.4.2 Multifunctional Role for IgE in Atopic Skin Inflammation

IgE contributes to the inflammatory cell infiltrate in AD by several mechanisms, including a biphasic immediate/late phase reaction, allergen presentation by IgE-bearing Langerhans cells, allergen-induced activation of IgE-bearing macrophages, and IgE autoreactivity to human proteins.

Biphasic immediate/late phase reaction

Clinically significant allergen-induced reactions are associated with an IgE-dependent biphasic response. Mast cells bearing IgE directed against the relevant allergen release various mediators, cytokines, and leukocyte chemotactic factors into local tissue within 15 to 60 minutes of allergen exposure. This immediate reaction likely contributes to the acute pruritus and erythema observed after allergen exposure in patients with AD. Three to 4 hours after the immediate reaction starts to subside, there is the onset of an IgE-dependent late phase reaction (LPR). This reaction is characterized initially by expression of leukocyte adhesion molecules on postcapillary venular endothelium, followed by the infiltration of eosinophils, neutrophils, and mononuclear cells. The maximum cell accumulation of granulocytes is reached at 6 to 8 hours, and the cellular infiltrate consists predominantly of mononuclear cells by 24 to 48 hours after onset of the LPR. The cellular infiltrate in allergen-induced LPRs has been shown to express increased mRNA for IL-3, IL-4, IL-5, and GM-CSF, but no mRNA for IFN-γ. These



results suggest that the T cells infiltrating into the allergen-induced EPR are TH2-like cells (Leung, 2001).

2.4.3 Role of the Epidermis in Barrier Function

Skin barrier disfunction in AD is characterized by dry skin and increased transepidermal water loss even in nonlesional skin, and fewer ceramides in the cornified envelope of lesional and nonlesional skin are found AD patients. Changes in the SC pH in AD skin may impair lipid metabolism in the skin. Such alterations allow penetration and susceptibility of irritants and allergens, triggering the inflammatory response, cutaneous hyperreactivity, inflammation, and skin damage characteristic of AD. Filaggrin deficiency leads to mild or severe ichthyosis vulgaris. Impaired keratinocyte differentiation and barrier formation allow increased transepidermal water loss, and entry of allergens, antigens, and chemicals from the environment in AD. There is a reduction in ceramides in the epidermis of patients with AD, with an abnormal expression of sphingomyelin deacylase, which hydrolyzes sphingomyelin to yield sphingosulphosphorylcholine rather than ceramide. This leads to a reduction in ceramide and deleterious effects on the function of the SC. The reduction of ceramides and their ratios affects the function of the SC as a permeability barrier, with water loss and consequent dry skin. The dry skin may provide a portal of entry for allergens, irritants, and skin pathogens (Sator et al, 2003, Irvine and McLean, 2006, DiNardo, 1998, Murata et al, 1996)

2.4.4 Immunological Triggers

Foods

Food allergens induce skin rashes in nearly 40% of children with moderate to severe atopic dermatitis. Food allergies in patients with atopic dermatitis

might induce dermatitis and contribute to severity of skin disease in some patients, whereas in others urticarial reactions, or non-cutaneous symptoms are elicited (Sampson, 1999).

Aeroallergens

Pruritus and skin lesions can develop after intranasal or bronchial inhalation challenge with aeroallergens in sensitised (specific IgE) patients with atopic dermatitis. The degree of IgE sensitisation to aeroallergens is directly associated with the severity of atopic dermatitis (Tupker et al, 1996).

Autoallergens

Release of autoallergens from damaged tissues could trigger responses mediated by IgE or T cells.

Staphylococcus aureus

Increased numbers of *S aureus* are found in over 90% of atopic dermatitis skin lesions. One strategy by which *S aureus* exacerbates or maintains skin inflammation in atopic dermatitis is by secreting superantigens, which stimulate marked activation of T cells and macrophages (Leung et al, 1993).

AD frequently starts in early infancy and 45% of all cases of AD occur within the first 6 months of life. In about 85% of cases, disease becomes quiescent by age 5.

2.4.5 Animal models of Atopic Dermatitis

Over the last decade, animal models of AD have received increasing attention. The earlier models included NC/Nga mice and a hapten-induced mouse model. More recently, other mouse models of AD have been developed using knockouts or transgenes. They can be categorized into three groups: (1) models induced by epicutaneous application of sensitizers; (2) transgenic mice that either overexpress or lack selective molecules; (3) mice that

spontaneously develop AD-like skin lesions. They all have significant disadvantages, such as limited reproducibility, a requirement for repeated treatments, a long prodromal phase preceding overt disease and limited availability of these genetically-engineered mice (Shiohara et al, 2004, Jin et al, 2009).

Table 2.3: Comparison for mouse models of AD

Variables	NC/Nga mouse model	Hapten-induced model	IL-18 Tg mouse model
Incidence of AD-like lesions	~50%	100%	(~0)[a] 100%
Need for additional treatment to develop AD-like lesions	Hapten application	No	Hapten application[a]
Age of onset months	8–17 weeks	Anytime 30 days after starting hapten application	6
Reproducibility	Poor–fair	Excellent	Fair
Need for particular conditions	Conventional conditions	No	No
Variations	Impossible	Possible by changing haptens or mouse strains employed	Impossible
Availability	Limited	Excellent	Limited

NC/Nga mice

The NC/Nga mouse is the most extensively studied animal model of AD. It originated from Japanese fancy mice and was established as an inbred strain by Kondo et al. in 1957. NC/Nga mice have been reported to develop AD-like eczematous skin lesions when kept in an air-uncontrolled conventional room but not when maintained under specific pathogen-free (SPF) conditions. Clinical symptoms begin with itching, erythema, hemorrhage, scaling, dryness, and alopecia at the age of 8 weeks. Symptoms and serum IgE increases with age and reaches a maximum at around 17 weeks of age. Thickening of the epidermis with marked hyperkeratosis and parakeratosis, and a marked increase in mast cell and eosinophils with marked degranulation is noted. Infiltration of numerous CD4+ T cells and macrophages with a few CD8+ T cells is seen. The major drawback is the relatively low incidence of AD-like lesions even in NC/ Nga mice kept under conventional conditions. Additional treatments such as hapten application is

often needed to be 100% concordant for the development of AD-like lesions (Matsuda et al, 1997)

Hapten-induced model

During the process of developing a mouse model, it was noted that antigen specific IgE antibody is preferentially produced in mice repeatedly painted with a hapten. repeated application of 2,4,6-trinitrochlorobenzen (TNCB) at 2-day intervals for 24 days to the same skin site results in a site-restricted shift in the time course of antigen-specific hypersensitivity responses from a typical delayed-type to an ITH followed by a late reaction, a finding often seen in skin lesions of AD patients. This shift is associated with epidermal hyperplasia, accumulation of large numbers of mast cells and CD4þ T cells beneath the epidermis, and elevated serum levels of antigen-specific IgE. Acute lesion is driven by the production of Th1 cytokines (IFN-g and IL-12) while chronic is driven by the production of Th2 cytokines IL-4 and IL-10. This model allows study of events characterizing the progression from acute to chronic inflammation. By changing hapten and mouse strain, various types of chronic inflammation, probably reflecting heterogeneity in clinical presentation of AD, can be induced. It has significant disadvantages inherent to inducible models, such as the requirement for previous sensitization to a hapten and a concern about potential interactions between the hapten and therapeutic agents (Kitagaki et al, 1995, 1997, Thomas et al, 1978).

AD model induced by skin injury and EC sensitization with allergen.

A mouse model of AD induced by repeated EC sensitization of tape stripped skin with ovalbumin (OVA) has been developed (Spergel et al., 1998). This model operates in all five strains of mice including BALB/c and C57BL/6 mouse strains (Spergel et al., 1999). The back skin of mice is shaved and tape

stripped and OVA is painted and secured to the skin with a transparent bioocclusive dressing. EC-sensitized mice develop increased scratching behavior, and their skin develops lesions characterized by epidermal and dermal thickening, infiltration of CD4+ T cell and eosinophils and upregulated Th2 cytokines IL-4, IL-5, and IL-13, with no change in IFN-g.

AD model induced by EC application of house dust mite allergen.

BALB/c mice subjected to EC application of the recombinant mite allergen Der p8 exhibited features of dermatitis with epidermal hyperplasia and spongiosis, skin infiltration with CD4+ and CD8+ cells, and a skewed Th2 response locally and systemically (Huang et al., 2003).

Transgenic and knockout mice

A series of transgenic mice, characterized by a susceptibility to AD-like inflammatory skin lesions, has been created. Among them, the disease described in IL-18-transgenic mice is one of the closest available animal models of human AD. IL-18 is a potent inducer of IFN-g, it can also potentially induce Th2 cytokines and IgE and IgG1 production. Thus, IL-18 can act as a strong cofactor for both Th1 and Th2 cell development. Transgenic mice are overexpressing mature IL-18 in their skin. These KIL-18 Tg mice develop AD-like skin lesions at about 6 months after birth. The histology showed acanthotic epidermis, accumulation of mast cells, and prominent infiltration of lymphocytes and neutrophils. A marked elevation of serum IgE levels is observed in Tg mice (Hoshino et al, 2001, Tanaka et al, 2001, Xu et al, 2000, Kawase et al, 2003)

Another gene-targeted mouse with AD-like lesions is that lacking the transcription factor RelB, a member of the NF-kB/Rel family (Barton et al, 2000).

Transgenic mice overexpressing IL-4 in the skin develop spontaneous pruritus and chronic dermatitis at the age of 4 months (Chan et al., 2001). The onset and early progression of skin inflammation was found to correlate with the elevation of IgE and IgG1. The early skin lesions are characterized by prominent infiltration of T cells in the epidermis and dermis, whereas chronic lesions showed T-cell accumulation in the dermis. The chronic lesions also showed features of human AD, including acanthosis of epidermis with mild spongiosis, hyperkeratosis, and dermal eosinophils.

The expression level of IL-31 is associated with the magnitude of skin pruritus. Transgenic mice overexpressing IL-31, driven by the lymphocyte-specific promoter Lck or by the ubiquitous elongation factor-1a promoter, exhibited signs of dermatitis at the age of 2 months, including pruritus, mild-to-moderate hair loss, and considerable thickening of ear skin. Histological examination of skin lesions revealed hyperkeratosis, acanthosis, inflammatory cell infiltration, and an increase in mast cells, which resemble the skin lesions of human AD (Dillon et al, 2004).

Transgenic mice overexpressing the human CASP1 precursor gene in epidermal keratinocytes under the control of the human keratin 14 promoter (CASP1 transgenic mice) showed elevated serum levels of IgE and IgG1 at the age of 8 weeks, (Yamanaka et al., 2000). Histological examination showed prominent acanthosis, papillomatosis, parakeratosis, and intracellular edema with dense infiltration of lymphocytes, neutrophils, and mast cells, but not eosinophils in the skin lesion.

2.4.6 Management of Atopic Dermatitis

Successful management of atopic dermatitis requires a multipronged approach involving skin care, identification and elimination of flare factors, and anti-inflammatory treatment. Because the origin of atopic dermatitis is multifactorial and trigger factors differ among patients, treatment plans must be specific to the individual patient.

Skin care

In atopic dermatitis, the disturbed function of the skin barrier is probably the result of reduced ceramide concentrations and results in dry skin (xerosis) and enhanced transepidermal water loss. Xerosis contributes to development of epithelial microfissures and cracks, which allows entry of skin pathogens, irritants, and allergens. Wet dressings can be used on severely affected or chronic lesions refractory to skin care. Dressings can be an effective barrier against persistent scratching, allowing more rapid healing of excoriated lesions. Irritants such as soaps or detergents, contact with chemicals, smoke, alcohol and astringents found in toiletries, and abrasive clothing can worsen the xerosis. Soaps with minimum defatting activity and a neutral pH are preferred (Imokawa, 2001).

Identification and elimination of triggering factors

Immunologic triggers of AD vary for different patients and can include various foods, aeroallergens, irritants and contactants, hormones, stress, climate, and microorganisms such as Staphylococcus aureus. Potential allergens can be identified by taking a careful history and doing selective allergy tests. Negative skin prick tests or serum tests for allergen-specific IgE have a high predictive value for ruling out suspected allergens. Positive skin or in-vitro allergy tests, especially to foods, often do not correlate with clinical

symptoms and should be confirmed with controlled food challenges, elimination diets, or atopy patch tests. Avoidance of foods implicated in controlled challenges results in clinical improvement. As a rule, extensive elimination diets, which in some cases can be nutritionally deficient, are useless. Most children who are allergic to food outgrow their food hypersensitivity in the first few years of life, making it less relevant as trigger factor when older. Extended avoidance of house dust mites in sensitized patients with atopic dermatitis results in improvement of their skin disease (Jones, 2002)

Use of moisturizers/emollients

The regular use of emollients, together with skin hydration; represents the mainstay of the general management of AD. Emollients should be applied continuously, even if no actual inflammatory skin lesions are apparent. Emollients containing polidocanol are effective in reducing pruritic symptoms. Urea is used for intensive hydration of the skin, while salicylic acid can be added to an emollient for the treatment of chronic hyperkeratotic lesions. Hydration is achieved by daily soaking in a bath for 20–30 min. Addition of a small amount of disinfectant, such as Chlorox, is recommended to decrease bacterial skin colonization and helps to prevent superinfection. Hydration must be followed by application of a sealer such as petrolatum-based cream, or mineral oil, to prevent water loss (Loden, 2003).

Several topical barrier creams have become available for use in the management of AD. These physiologic moisturizers can be divided into three categories; the ceramide-dominant agents, formulations which contains the fatty acid palmitamide; and cream containing the antiinflammatory molecule glycyrrhetinic acid, telmesteine, shea butter, caprylol glycine, and hyaluronic

acid in a hydrolipidic base. Daily moisturizer therapy can also increase high-frequency conductance, a parameter for the hydration state of the skin surface. This allows for ranking the efficacy of moisturizers according to the duration of effects or the magnitude of increase in the hydration level of the SC (Chamlin et al, 2002).

2.4.7 Pharmacologic Treatments

Topical therapies

Topical corticosteroids form the cornerstone of pharmacologic treatment of AD and are the standard of care against which all other agents are compared. The topical calcineurin inhibitors (TCIs), tacrolimus and pimecrolimus have also demonstrated effectiveness in AD. Coal tar preparations also have been used to treat AD, but these agents are generally unacceptable to patients because of their propensity to stain clothing and other materials, and their offensive odor.

2.4.7.1 Topical Corticosteroids

For topical corticosteroids, the target cells are the keratinocytes and fibroblasts within the viable epidermis and dermis, where the glucocorticoid receptors are located [Marks et al, 1982]. Having attained the target, the cellular uptake and residence time of the steroid as well as its affinity for the glucocorticoid receptor will determine the clinical effect. Cellular uptake of glucocorticoids is non-mediated, passive diffusion process that involves two intrinsic steps: a rapid, non-specific, high-capacity association to the cell membrane followed by a slower process leading to strong binding of glucocorticoid within the cell [Ponec, 1983]. The total uptake of steroid by fibroblasts and keratinocytes was related to drug lipophilicity. Although, as stated, steroids are generally thought to be transported across the cell

membrane by passive diffusion, there is some evidence that certain target cells possess a specific transport system for these compounds [Rao, 1981]. The anti-inflammatory and immunosuppressive effects of TG seem to be mediated largely by regulation of corticosteroid- responsive genes. At the cellular level, corticosteroids bind with cytoplasmic corticosteroid (or glucocorticoid) receptors to form a steroid-receptor complex that then translocates into the nucleus. Once inside the nucleus, the steroid-receptor complex binds as a homodimer to the glucocorticoid-responsive element in corticosteroid-responsive target genes to either stimulate or inhibit transcription and thus protein synthesis. In addition to this direct regulatory effect on gene transcription, corticosteroids can also indirectly regulate transcription by blocking the effects of other transcription factors. In particular, corticosteroids have been shown to increase cellular levels of inhibitory nuclear factor-κ Bα (IκBα) by stimulating expression of the IκBα gene. IκBα protein, in turn, inhibits transcription by binding with nuclear factor-κ B (NF-κB), another transcription regulator, to prevent the latter's translocation to the nucleus. In this manner, corticosteroids may affect the transcription of genes that do not contain a glucocorticoid- responsive receptor. Fig 2.8 illustrates this double-pronged inhibitory effect of corticosteroidson NF-κB [Hughes and Rustin, 1997, Scheinman et al, 1995, Auphan et al, 1995].

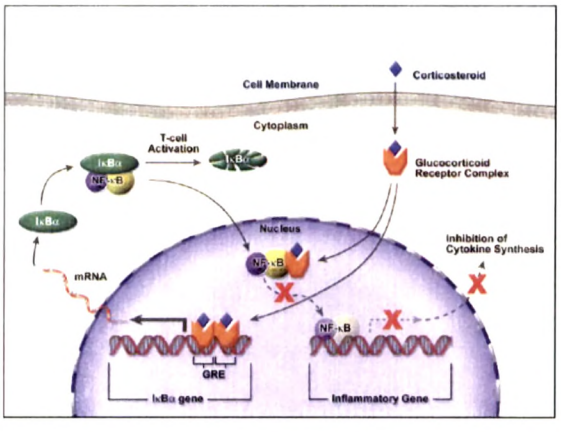


Fig 2.8: Mechanism of action of topical corticosteroids

Topical corticosteroids may inhibit the transcription of proinflammatory cytokine genes (including the interleukins IL-1, IL-2, IL-6, interferon c, and tumour necrosis factor-a genes), T-cell proliferation, and T-cell dependent immunity. In fibroblasts, IL-1a is responsible for proliferation, collagenase induction, and IL-6 synthesis, which control skin thickness [Lange et al, 2000]. The inhibition of IL-1a in keratinocytes has anti-inflammatory effects, whereas the same inhibition in fibroblasts has anti-proliferative and atrophogenic effects. Corticosteroids also appear to stimulate lymphocyte expression of genes for anti-inflammatory cytokines, like transforming growth factor-b and IL-10. Through regulation of cytokine production, corticosteroids probably play a role in rebalancing the T-helper cell type 1 (Th1) to Th2 lymphocyte ratio in skin lesions. Corticosteroids have also been demonstrated to inhibit the proliferation of various cell types, including T lymphocytes. The antiproliferative effects of corticosteroids have not been clearly defined but probably involve both blockade of cytokine expression and suppression of cytokine effects. At least some of the antiproliferative effects of corticosteroids are mediated through lipocortins, which act as second messengers for corticosteroids. IL-10 and transforming growth factor-b1 have been shown to potentiate the inhibitory effects of corticosteroids on T-lymphocyte proliferation (Norris, 2005).

The vasoconstrictive effect of topical corticosteroids may contribute to their anti-inflammatory activity, diminishing erythema at the lesion site. It may also reduce their local clearance. However, the exact mechanism is not completely clear. Finally, clinical efficacy is self-evidently and significantly influenced by corticosteroid structure, the formulation and to some extent the applied concentration of the drug (Mckenzie and Atkinson, 1964). Because

dose applied is very loosely related to dose absorbed, the applied concentration is much less a factor than the formulation.

Side-effects

Despite their clear benefit in the therapy of inflammatory disease, topical corticosteroids are associated with a number of side effects that limit their use (Hengge et al, 2006). One particularly important local side-effect is epidermal thinning or atrophy. This effect can start after 3 to 14 days of corticosteroid treatment with microscopic degenerative changes in the epidermis, including reduction in cell size and number of cell layers [Sheu et al, 1997]. Topical corticosteroids inhibit epidermal cell differentiation by inhibition of keratinocyte proliferation and acceleration of keratinocyte maturation [Woodbury and Kligman, 1992]. Moreover, prolonged topical corticosteroids therapy increases basal transepidermal water loss, indicating an effect on permeability barrier function. This change has been associated with a decrease in SC thickness, a reduction in lipid content , a decrease in the number of lamellar bodies and in the number of intercellular lamellae (although the structure of these lipid bilayers appeared normal [Sheu et al, 1997]). Topical corticosteroids also exerted negative effects on the integrity and cohesion of the SC owing to a reduction in the number of corneodesmosomes in the SC.

Furthermore, histological changes are observed in the dermis. Dermal atrophy results from the direct anti-proliferative action of topical corticosteroids on fibroblasts; in turn, this leads to a reduction in the synthesis of collagen and mucopolysaccharides and a loss of dermal support. The elastin fibers in the upper layers of the dermis become thin and fragmented, while the deeper fibers collapse to form a compact and dense network. As a

result of this thin and brittle skin, there is local vascular dilatation, which is responsible for striae, telangiectasia, and purpura [Kerscher and Korting, 1992]. These side effects are seen mainly on the (permeable) face and are (over) dose related.

Systemic side-effects of topical corticosteroids, such as pituitary–adrenal axis suppression, are rare but have to be seriously considered when treating children because of the potential for growth retardation. The degree of adrenal suppression increases with the potency and concentration of the topical corticosteroids, application area, occlusion and degree of inflamed skin. Other systemic side-effects include Cushing's syndrome, the aggravation of diabetes mellitus, and increasing or causing hypertension and osteonecrosis (Hengge et al, 2006.)

Glucocorticoid chemistry

The majority of topical corticosteroids that are used therapeutically are synthetic derivatives of hydrocortisone. The lipophilicity of the steroid and the duration of action are greatly increased by fluorination of the B ring at the C-9 and/or C-6 position. Moreover the lipophilicity and metabolic resistance of topical corticosteroids may also be increased by adding ester or acetal groups to the D-ring (e.g., betamethasone 17-valerate) (Weidersberg et al, 2008).

Potency/classification

The efficacy of a topical corticosteroid is related to its pharmacological potency and to its ability to be absorbed into the target cells within the viable epidermis and dermis. Potency is a complex function of the physical and chemical properties of both the drug and its vehicle [Pershing et al, 1994]. For

the topical corticosteroids, a ranking of drugs and vehicles has been evolved using the skin blanching assay. The American classification includes seven potency groups, while the British National Formulary recommends only four The British classification system is made irrespective of the topical vehicle used. That is, the greater the potency, the greater the therapeutic efficacy, but also the greater the adverse effects. Low-potency formulations are considered acceptable for long-term treatments while the more potent products should be reserved for shorter regimes and for use at sites, such as the palms and soles.

Vehicles and formulations

Based on the Higuchi model, in the early 1970s, Katz and Poulsen (1972) described the significance of both release (diffusion out of the vehicle) and penetration (diffusion into the skin barrier) for topical product design based on corticosteroids. These two processes are dependent upon the physico-chemical properties of both the drug and the vehicle [Katz and Shaikh, 1965]. Topical corticosteroids are formulated in a variety of vehicles, including ointments, creams, lotions, gels and, more recently, foams. As mentioned above, the vehicle has a great influence on penetration into the SC and consequently on the bioavailability bioavailability and potency of the glucocorticoid [Ayres and Hooper, 1978]. Ointment formulations are generally more potent than creams containing the same drug presumably due to their occlusive effect on the skin which may increase SC hydration and enhance drug transport [Stoughton, 1972, Poulsen and Rorsman, 1980]. Ointments are preferred for infiltrated, lichenified lesions, whereas creams are preferred for acute and subacute dermatoses. Lotions and gels are suitable for the treatment of scalp psoriasis. The novel, thermolabile, low-residue foam formulations are safe and effective in the treatment of psoriasis affecting scalp and non-scalp regions of the body. The foam formulations are associated with

better patient compliance and improvements in quality of life [Stein, 2005]. The activity of a topical corticosteroids formulation can be enhanced by adding a chemical penetration enhancer which may result in an increase of drug delivery into and through the SC Various studies, using a vasoconstrictor assay, have shown that huge differences existed between generic and original formulations containing the same glucocorticoid in the same concentration in different vehicles [Woodford and Barry, 1974]. With suspensions the rate of penetration should be independent of the vehicle However, in the case of solution-type formulations; the vehicle has an enormous influence on the rate of penetration. High drug solubility in the vehicle and a low partition coefficient between the SC and the vehicle lead to poor penetration of the drug into the SC and low bioavailability [Katz and Poulsen, 1972]. Therefore, it is important to know and control, where possible, the thermodynamic activity of the drug in the vehicle. In the case of damaged skin, the release of drug from the formulation will determine uptake, and will be controlled by the characteristics of the vehicle.

Bioavailability/bioequivalence testing

Typical bioavailabilities are only a few percent of the applied dose. Several in vivo and in vitro methods have been employed to assess the BA/BE of topical corticosteroids, and are summarized in Fig. 2.9.

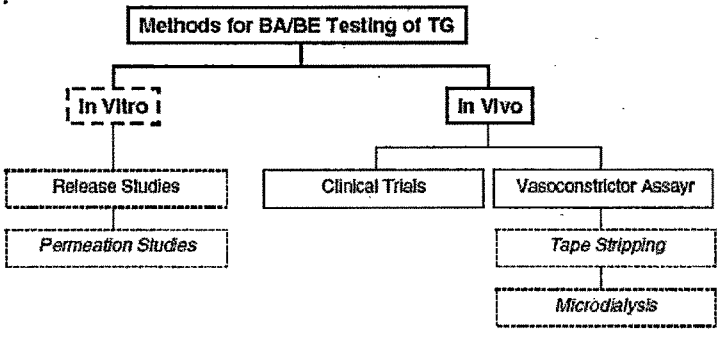


Fig 2.9: Methods for BA/BE testing of topical glucocorticoids

For the moment, the only acceptable methods to assess BA/BE of topically applied drug formulations are clinical trials between generic and original products and pharmacodynamic response studies. Comparative clinical trials are considered to be the 'gold standard', but these studies are relatively insensitive, costly, time-consuming and require large numbers of subjects [Shah et al, 1998]. In contrast, pharmacodynamic response studies are relatively easy to perform, expose the subjects to only a small amount of the formulation for a short period of time, are fairly reproducible, and require a relatively small number of subjects.

The topical corticosteroids pharmacodynamic response is the ability to produce vasoconstriction of the microvasculature of the skin, leading to skin blanching (whitening) at the site of application. This "vasoconstrictor assay" was first described by McKenzie and Stoughton in 1962. Since that time, the method has been modified and extended to provide a reliable means to test topical corticosteroids and their formulations. The intensity of skin blanching has been correlated with drug potency and the degree of drug delivery through the SC [Haigh and kanfer, 1984]. The formulation is applied for various times (dose durations) up to 6 h to manipulate the amount of steroid delivered. At the end of the treatment period, the skin blanching response is measured with a chromameter over the next 24–28 h. From the resulting response versus time profiles, the areas above the response curves (AARC) are calculated and plotted. The situation becomes complex for preparations in which the drug is in solution and may therefore deplete over time, and their comparison with suspension-type formulations [Leopold, 1998]. Other pharmacodynamic effects that may be quantified are the vasodilatation (erythema) and skin temperature increase induced by nicotinic acid esters, and the response to local anaesthetic bases [Weidersberg et al, 2008].

In summary, therefore, apart from the vasoconstrictor assay, which is clearly restricted, at this time, to topical corticosteroids, there are currently no non-invasive or minimally invasive techniques for the assessment of BA/BE of topically applied drugs that are acceptable to the regulatory bodies. In an effort to address this situation and to provide viable alternatives for BE determination, significant efforts are being directed to the dermatopharmacokinetic (DPK) approach, microdialysis and the use of in vitro experiments (Shah, 2004).

2.4.7.2 Topical Calcineurin Inhibitors

Systemic or topical glucocorticosteroids exert good therapeutic efficacy in the management of inflammatory skin diseases. However, there is a long list of severe side effects which limits their clinical use. Consequently, the search for potent alternative drugs has resulted in a new generation of immunosuppressants, the macrolide lactones. The leading compound of this class is cyclosporine which has revolutionized immunosuppression in transplantation as well as the therapy of severe inflammatory cutaneous disorders (Schreiber and Crabtree, 1992). However, its lack of topical activity combined with side effects including nephro- and neurotoxicity have limited its use in dermatology (Lauerma and Maibach, 1994). Another calcineurin inhibitor used to prevent transplant rejection, FK506, is 10 to 100 times more potent than cyclosporine in inhibiting T-cell activation.

Two TCIs, tacrolimus and pimecrolimus, have recently become available in the United States to treat AD. Both agents are complex macrocyclic compounds that bind to the intracellular protein 12-kd macrophilin-12 (previously known as FK506- binding proteins) and function as macrolactam immunomodulators, thereby inhibiting the activity of the phosphorylase

enzyme, calcium-dependent serine/threonine phosphatase calcineurin. (Nakagawa et al, 1994) Although the actions of the two agents are likely similar, tacrolimus has been more extensively studied. These structurally distinct compounds all bind to unrelated cytosolic binding proteins, known as immunophilins, but have a common result: the formation of inhibitory complexes that block calcineurin phosphatase activity, which, in turn, inhibits initiation of cytokine transcription and activation of T cells. Most of the therapeutic effects of tacrolimus are assumed to be related to the activation of T lymphocytes. Normally, interaction of an antigen/allergen with the T-lymphocyte receptor results in an increase in cytosolic calcium, which then forms a complex with the calcium-binding protein calmodulin that activates calcineurin (Bornhovd et al, 2001, and Crabtree, 2001). Activated calcineurin catalyzes the dephosphorylation of various substrates within the cell, including NF-AT. Dephosphorylated NF-AT moves into the nucleus to regulate the transcription of various genes. Tacrolimus disrupts this intracellular signaling by forming a complex with macrophilin-12 and calcium-calmodulin. This complex inhibits activity of calcineurin, thereby preventing the dephosphorylation of NF-AT and interfering with its ability to regulate gene transcription (Fig. 2.10). Tacrolimus also blocks calcineurin-stimulated activation of NF-kB and inhibits the transcription of several genes, the products of which are important in the pathology of AD, including IL-2, IL-3, IL-4, IL-5, IL-12, IFN-g, tumor necrosis factor-alpha, and GMCSF (Bornhovd et al, 2001, Panhans-Gross, 2001). In addition to its effects on T lymphocytes, tacrolimus down-regulates IL-8 receptors on the cell surface of human keratinocytes and changes the structure and function of epidermal dendritic antigen-presenting cells, including decreasing expression of Fc epsilon receptor I in Langerhans cells and inflammatory dendritic epidermal cells from lesional skin and reducing the ability of antigenpresenting cells to

stimulate T lymphocytes. Anti-inflammatory effects of tacrolimus may also include inhibition of histamine release from skin mast cells. The main inhibitory mechanism of FK506 ointment was thought to be inhibition of Th2 and Th1 type allergic reactions. Its topical application has been shown to be effective in animal models of inflammation as well as in short-term clinical trials in AD (Michel et al, 1993, Wollenberg et al, 2001 and De Paulis, 1992). Additionally, their size and lipophilicity limit systemic absorption, affording nonsystemic, localized, skin-selective treatment.

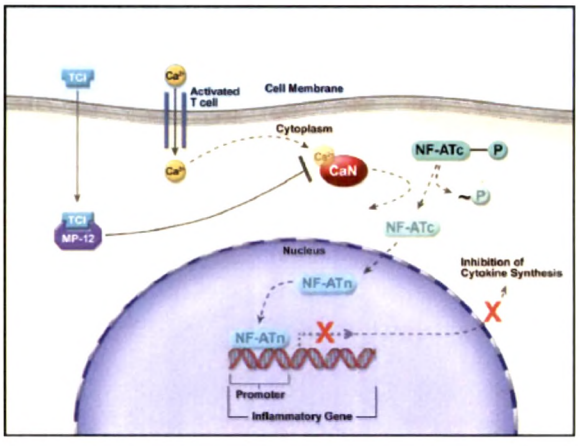


Fig. 2.10: Mechanism of action of topical calcineurin inhibitors

Topical calcineurin inhibitors can have advantages over topical corticosteroids in some circumstances, such as in patients who do not respond well to topical corticosteroids, those who are steroid phobic, and those who have areas of AD on their face and neck, where low-potency steroids still have a risk of skin thinning. The potential use of topical calcineurin inhibitors as maintenance therapy is also intriguing for prevention of AD flares and progression of the atopic march (Boguniewicz et al, 2003). However, although systemic absorption of these compounds is low, there is a need for careful surveillance to rule out the possibility that skin cancers and increased viral skin infections will appear when such agents are used long-term (Leung and Bieber, 2003).

The most frequently reported adverse events with TCI therapy are skin irritation at the application site, including transient burning sensations, erythema, and pruritus. Although there is a theoretical risk of nephrotoxicity, hypertension, neurotoxicity, and cancer—as have been reported with systemic calcineurin inhibitors—TCIs were not associated with these events in clinical trials, even with extensive body surface area coverage (Fleischer, 1999).

2.4.7.3 Coal tar preparations

Coal tar preparations also are used to treat AD. However, these agents are infrequently used in the current environment for a variety of reasons. First, they usually are less effective as monotherapy than other topical treatments, such as corticosteroids. Moreover, coal tar preparations have an offensive odor, are messy to use, and cause staining. Additional side effects associated with coal tar include folliculitis and photosensitivity. Coal tar has been shown to be carcinogenic in animal models (Thami, 2002).

2.4.7.4 PDE inhibition

PDE inhibitors target the cyclic nucleotide abnormalities characteristic of AD (Fig 1). Drugs such as theophylline and other agents have been used in asthma. However, theophylline lacks sufficient potency to have much effect in AD, and systemic treatment with newer, more potent PDE inhibitors has been associated with a high incidence of nausea and vomiting.35 Newer type IV PDE inhibitors have evolved through the years and are coming under increased research scrutiny for asthma and AD (Hanifin et al, 1996).

2.4.7.5 Systemic therapies

Antihistamines

Oral antihistamines are sometimes prescribed to relieve the pruritus associated with AD. The rationale for the use of antihistamines is that activation of histamine H1 receptors is involved in the pathogenesis of pruritus, and blocking these receptors should ameliorate this symptom. However, histamine is only one of several mediators of pruritus in AD. Many antihistamines have anxiolytic properties that may mitigate stress-related exacerbation of disease and sedative properties that may help patients with AD to sleep. The results from studies of nonsedative antihistamines for pruritus in patients with AD are mixed (Doherty et al, 1989 and Krause and Schuster, 1983).

Systemic corticosteroids.

Systemic corticosteroids usually are not useful for the treatment of AD, although they are sometimes used to provide rapid relief of severe refractory disease while transitioning to other therapies and appear to be used more commonly than cyclosporine in general practice. However, the use of systemic corticosteroids is susceptible to rebound flare upon discontinuation, and the safety and dosing of systemic corticosteroids have not been evaluated systematically in well designed clinical trials of patients with AD. Systemic corticosteroids are associated with a number of potential side effects, including suppression of the hypothalamic-pituitary-adrenal axis and the possibility of Cushing's syndrome, growth retardation in children, development of cataracts or glaucoma, glucose intolerance, fluid and sodium retention, weight gain, and changes in mood and sleep patterns, among others. The risk of significant adverse events increases with the duration of

therapy, and prolonged use in children is not recommended (Leung and Bieber, 2003 and Knowles, 2002).

Cyclosporine.

Although systemic corticosteroids are used much more commonly than cyclosporine in general practice, the American Academy of Dermatology guidelines recommend the use of cyclosporine for the treatment of severe AD, on the basis of the results of well-designed randomized controlled trials, although the agent is not approved by the FDA for treating AD. In general, cyclosporine should be used only on a short-term basis in either adults or children with severe refractory AD. Longterm treatment consisting of multiple 12-week courses for up to a year may be considered in exceptional cases. Maximal efficacy of cyclosporine for AD lesions is typically observed within about 2 weeks, but relapse is relatively rapid after cessation of therapy (Naeyaert et al, 1999 and Granlund et al, 1995).

Mycophenolate mofetil.

This purine biosynthesis inhibitor is approved as an immunosuppressant in organ transplantation. It is the ester of mycophenolic acid, which was introduced in the early 1970s as a psoriasis treatment, but discontinued because of its acute gastrointestinal side effects and potential carcinogenicity. Results from two small open reports suggest mycophenolate mofetil may offer potential benefit in the treatment of severe or recalcitrant AD (Grundmann et al, 2001 and Neuber et al, 2000).

Azathioprine.

This inhibitor of DNA and RNA synthesis helps in refractory cases through its immunosuppressive and cytotoxic effects. A few studies support its use in

AD although they consist of case series, retrospective analyses, or small trials. Azathioprine is associated with significant side effects that limit its use, including hypersensitivity reactions (eg, fever, rigors, myalgia, arthralgia, and occasionally pancreatitis) as well as dose-dependent toxicities, the most commonly serious of which are hepatotoxicity and myelotoxicity. Long-term use is not recommended because of the potential for development of malignancy, predominantly squamous cell skin cancer and non-Hodgkin's lymphoma (Meggitt and Reynolds, 2001)

Recombinant interferon gamma.

Interferons are a family of naturally occurring glycoproteins that have broad-spectrum antiviral, antitumor, and immunomodulatory activity. Patients with AD often exhibit decreased levels of interferon gamma (IFN-g). Although IFN-g is not approved by the FDA for treating AD, several studies suggest that systemic IFN-g therapy may provide relief to patients with AD. It should be noted, however, that the incidence of side effects associated with IFN-g therapy is high, particularly for headache, myalgia, and chills. Long-term IFN-g therapy is associated with decreased eosinophil counts and increased immunoglobulin E (IgE) levels; clinical improvement correlates more closely with changes in eosinophil counts. It has been recommended that a higher dosage of IFN-g be used in the early phase of therapy and then switched to a lower dosage for maintenance therapy (Stevens et al, 1998 and Jang et al, 2000).

Other systemic therapies.

Other systemic therapies include leukotriene inhibitors, oral pimecrolimus, methotrexate, biologic agents, PDE inhibitors and intravenous immunoglobulin. However, none have been approved by the FDA for

treatment of AD. Leukotrienes are inflammatory mediators derived from arachidonic acid and appear to play an important role in the late-phase reactions in AD; studies demonstrate that montelukast and zileuton, two inhibitors of leukotrienes, may be effective for reducing symptom severity in AD. Clinical trial reports regarding the possible use of oral pimecrolimus, methotrexate, and biologic agents such as adalimumab, alefacept, efalizumab, etanercept, and infliximab in the treatment of AD are not currently available. The biologic omalizumab, a recombinant, humanized, monoclonal antibody against IgE that was recently approved for the treatment of asthma, may offer some hope ; although its use in the treatment of AD has yet to be clearly defined, planning for a clinical trial is expected. Finally, a few case reports and uncontrolled studies suggest intravenous immunoglobulin may provide some benefit in severe refractory AD; the data indicated immunoglobulin was more effective as monotherapy in children (\6 years of age) and as adjunctive therapy in adults (Yanase and David, 2001, Woodmansee and Simon, 1999, Jolles, 2002 and Hanifin et al, 2004).

2.4.7.6 Supportive Treatments

Anti-infectious disease management

Patients with AD are at increased risk for bacterial, viral, or fungal infections. S aureus, in particular, appears to be involved in the pathogenesis of AD and may aggravate or maintain skin inflammation. S aureus has been found in more than 90% of AD skin lesions, compared with less than 5% colonization in normal skin. S aureus involvement is also suggested by a report that antistaphylococcal treatment reduces clinical severity of skin lesions in patients with AD. S aureus in AD lesions may secrete various toxins that act as allergens or "superantigens," causing marked activation of T lymphocytes and macrophages and induction of IgE-mediated histamine release from mast

cells or basophils. Antistaphylococcal treatment may be useful in patients heavily colonized or infected with S aureus. A topical agent, such as mupirocin or fusidic acid, or systemic agents may be considered, depending on lesion spread and the degree of superinfection. However, current guidelines from the American Academy of Dermatology indicate that emergence of resistant bacteria is a concern, and use of antibiotics should be short term (Lever et al, 1988 and Hofer et al, 1999).

Fungal infections are more prevalent in patients with AD and may warrant treatment with antifungal agents such as ketoconazole to reduce the severity of skin lesions. The scalp and seborrheic regions of the skin may be particularly susceptible to infection with Malassezia furfur, and antibodies against this fungus are often found in patients with head or neck dermatitis. Patients with AD are also susceptible to complications due to recurrent viral infections, probably because of local impairment of T-lymphocyte function. Life-threatening dissemination of cutaneous herpes simplex virus may occur in patients with widespread AD, and antiviral therapy is critical in these patients (Leung and Bieber, 2003).

Phototherapy

Some investigators believe that phototherapy is the treatment of choice for patients with severe AD. There is growing support for the effectiveness of narrowband ultraviolet B (UVB) light and high or medium-dose ultraviolet A (UVA) light, although such studies typically have involved relatively small numbers of patients. Phototherapy has been shown to have immunosuppressive effects that involve disruption of key cells associated with the pathogenesis of AD or psoriasis, or both. Photons from UV light reduce T lymphocytes by inducing apoptosis. In addition, the number of

Langerhans cells (antigen-presenting cells) is directly reduced, and cytokines and adhesion molecules are indirectly reduced or their functioning altered by UV light.64 The exact mechanisms by which these effects are brought about are not clear, but may involve UV light-induced alterations in DNA molecules within the cells. Narrowband UVB is preferred for most patients because of its better safety profile compared with PUVA, which has been associated with squamous cell carcinomas and malignant melanomas. Support for the role of psoralen plus UVA (PUVA) in AD is generally weaker An increased cancer risk with UVB has not been as firmly established, but remains an area of concern, Phototherapy has been used in combination with topical corticosteroids for many years; its safety in combination with TCIs is not yet supported by adequate documentation (Grundmann et al, 1999, Abeck et al, 2000, Morris and Saihan, 2000).

2.4.7.7 Developing a treatment algorithm with combinational, rotational and sequential treatment

The ideal algorithm or paradigm for treatment of this disorder should be permutational, that is, the order of its components should be interchangeable (easily individualized) and centered around preventive measures. (Abramovits et al, 2003)

An example of a permutation paradigm for the treatment of AD

- TCI
- High-potency topical corticosteroids
- Lowest effective potency topical corticosteroids
- TCI and high-potency topical corticosteroids
- TCI and lowest effective potency topical corticosteroids

This paradigm permits treatment to progress from a chosen induction therapy to maintenance therapy. The clinician should bear in mind that TCI and lowest effective potency topical corticosteroid treatments (both monotherapy and in combination) can be sustained safely for a full 12-week course. During the patient's induction therapy, as soon as an acceptable level of clearance (defined as a >75% reduction of disease severity as judged by global assessment, Eczema Area and Severity Index, or Severity Scoring of Atopic Dermatitis index) is achieved, therapy should be moved to a maintenance regimen, such as monotherapy with either a TCI or a lowest effective potency topical corticosteroid (the latter used intermittently) or an alternation of the two agents (Abramovits, 2005).

Studies support use of TCIs as maintenance therapy to prevent flares. If the initial high-potency corticosteroid/TCI combination is unsuccessful, strong consideration should be given to the addition of systemic drugs, physical treatment approaches, other topical agents, and supportive treatment (Kyllonen et al, 2004).

More specific approaches to minimize side effects associated with topical corticosteroids (and other drugs) have included combination, rotational, and sequential therapy. The concept behind combination therapy is to use agents with different or nonoverlapping mechanisms of actions and possibly reduced dosages of the individual agents to achieve additive or synergistic efficacy and reduced side effects. They possess a commonality: all interfere with the transcriptional activation of cytokine genes that control inflammation and immune response. This effect and the different ways through which it is achieved provide a useful rationale for combination therapy. The possibility

of additive toxicity and incompatibility between the combined agents must be considered (Norris, 2005).

With rotational therapy, patients are rotated or switched from different monotherapies or combination therapies over time to minimize cumulative toxicities. Rotational therapy usually does not include topical corticosteroids. With sequential therapy, two agents are administered in different sequences in order to maximize efficacy and reduce side effects. The aim with this approach is to produce rapid clearance with a more powerful agent before switching to a second agent with reduced toxicity for maintenance. In related approaches, rather than transition from one drug to another, the two agents are administered on alternate days, either from the beginning of therapy or after an initial treatment period designed to maximize and expedite effectiveness. In sequential therapy, the use of specific therapeutic agents in a specific and deliberate sequence will maximize the rate of initial improvement (eg, induction therapy), minimize long-term toxicity, and improve the overall outcome of treatment.

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. 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. Few studies have evaluated this combination. In a smallscale (N = 57), randomized clinical trial lasting 21 days, (Torok et al, 2003) evaluated concomitant use of tacrolimus ointment and a midpotency corticosteroid cream (clocortolone pivalate 0.1%) in combination compared with either drug alone for the treatment of AD. The researchers found statistically significant

improvements in various assessments of efficacy (eg, excoriation, induration, erythema, oozing and crusting, lichenification, pruritus, stinging/burning) with the combination therapy when compared with either drug alone.

In a separate uncontrolled observational pilot study evaluating 12 pediatric and adult patients with a flare of AD, BMV foam and pimecrolimus 1% cream applied twice daily resulted in rapid clearance or marked improvement of clinical signs of the disease in 83% of patients and of pruritus in 91% of patients within 7 days (Del-rosso, 2004). In addition, in vitro studies using fresh human dermatomed skin showed that coapplication of BMV foam and tacrolimus ointment or pimecrolimus cream on the same site may positively affect penetration of the calcineurin inhibitors (Lenn et al, 2004). Such enhanced penetration may be particularly relevant for tacrolimus, as this agent is less able to penetrate thick skin than are corticosteroids. 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. Although it is unlikely that the increased penetration of a TCI resulting from any combination with other therapeutic agents would lead to a clinically significant increase in systemic absorption and higher rates of adverse events, additional controlled clinical trials would be of value in further establishing the safety profiles of these combination therapies. With this background, the combination of topical calcineurin inhibitors and topical steroids becomes an appealing choice.

Another option is use of calcineurin inhibitors as first-line pharmacologic therapy to treat AD and steroids to be administered for short courses as rescue therapy. The advantage of this strategy is 2-fold; first, the exposure to topical steroids is limited, markedly reducing the risk of side effects, and

second, when topical steroids are used, they are applied on steroid naive skin, maximizing their efficacy and avoiding tachyphylaxis. Using a topical calcineurin inhibitor as a first-line pharmacological agent for the treatment of early signs and symptoms of AD, as opposed to treating only more severe exacerbations, necessitates an excellent safety and tolerability profile to ensure practicability and compliance. In this respect, neither skin atrophy nor hypothalamic-pituitary-adrenal axis suppression has been observed with topical calcineurin inhibitors, making them more suitable than topical steroids for frequent or prolonged use, especially on larger body surfaces or on areas especially prone to atrophy with steroid use (Singh and Singh, 1986).

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).

The corticosteroid/TCI combination for AD is favored by many practitioners because the two drug classes have different and possibly complementary mechanisms of action. 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).Until they occur, dermatologists must continue to rely on their clinical experience and empirical findings when making treatment decisions regarding combination therapy.

2.5 Drug Profiles

2.5.1 Halobetasol Propionate

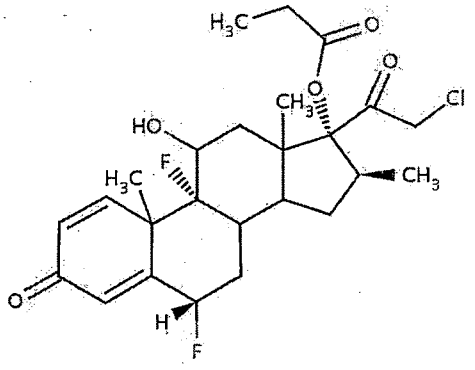
CAS number : 66852-54-8

Molecular weight : 484.96

Chemical name : (1R,8S,13S,14R)-14-(2-chloroacetyl)-1,8-difluoro-17-hydroxy-2,13,15-trimethyl-5-oxotetracyclo heptadeca - 3,6-dien-14-yl propanoate.

Moecular formula : $C_{25}H_{31}ClF_2O_5$

Molecular structure :



Appearance : white to off-white powder.

Water solubility : Mostly insoluble

Dissociation constant pK_a : 14.41

Partition co-efficient : About 2.9

Melting range : About 200°C

Solubility: halobetasol propionate is practically insoluble in water. It also dissolves freely in methanol.

Dosage available : available as 0.05% cream and ointment

Indication: Halobetasol topical is used to treat the inflammation and itching caused by a number of skin conditions such as allergic reactions, eczema, and

psoriasis. is a super-high potency corticosteroid indicated for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses. Treatment beyond two consecutive weeks is not recommended, and the total dosage should not exceed 50 g/week because of the potential for the drug to suppress the hypothalamic-pituitary-adrenal (HPA) axis. Use in children under 12 years of age is not recommended. As with other highly active corticosteroids, therapy should be discontinued when control has been achieved, If no improvement is seen within 2 weeks.

Dose: Apply a thin layer of cream to the affected skin once or twice daily,as directed by your physician,and rub in gently and completely.
halobetasol propionate cream is a super-high potency topical corticosteroid; therefore,treatment should be limited to two weeks,and amounts greater than 50 g/wk should not be used. As with other corticosteroids, therapy should be discontinued when control is achieved. If no improvement is seen within 2 weeks,reassessment of diagnosis may be necessary. It should not be used with occlusive dressings

Side effects: In controlled clinical trials, the most frequent adverse events reported included stinging or burning in 1.6% of the patients. Less frequently reported adverse reactions were pustulation, erythema, skin atrophy, leukoderma, acne, itching, secondary infection, telangiectasia, urticaria, dry skin, miliaria, paresthesia, and rash The following additional local adverse reactions are reported infrequently with topical corticosteroids, and they may occur more frequently with high potency corticosteroids. These reactions are listed in an approximate decreasing order of occurrence: folliculitis, hypertrichosis, acneiform eruptions, hypopigmentation, perioral dermatitis, allergic contact dermatitis, secondary infection, striae.

Systemic absorption of topical corticosteroids can produce reversible hypothalamic-pituitary-adrenal (HPA) axis suppression with the potential for glucocorticosteroid insufficiency after withdrawal of treatment. Manifestations of Cushing's syndrome,hyperglycemia, and glucosuria can also be produced in some patients by systemic absorption of topical corticosteroids while on treatment.

Mechanism of action: Like other topical corticosteroids,halobetasol propionate has anti-inflammatory,antipruritic and vasoconstrictive actions. The mechanism of the anti-inflammatory activity of the topical corticosteroids, in general, is unclear. However,corticosteroids are thought to act by the induction of phospholipase A_2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A_2.

Pharmacokinetics:

The extent of percutaneous absorption of topical corticosteroids is determined by many factors including the vehicle and the integrity of the epidermal barrier. Topical corticosteroids can be absorbed from normal intact skin. Inflammation and/or other disease processes in the skin may increase percutaneous absorption. Human and animal studies indicate that less than 6% of the applied dose of halobetasol propionate enters the circulation within 96 hours following topical administration of the ointment.

2.5.2 Tacrolimus

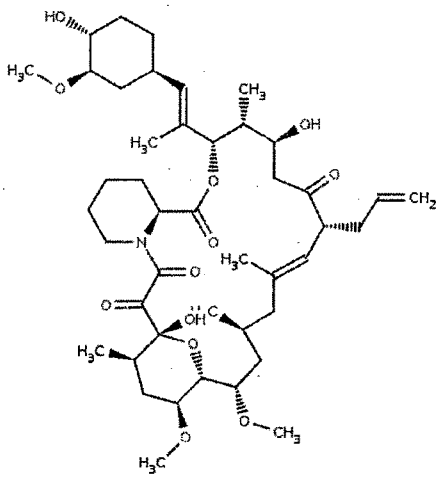
CAS number : 104987-11-3

Molecular weight : 822.03

Chemical name : [3*S*- [3*R** [*E*(1*S**,3*S**,4*S**)] ,4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]] - 5,6,8,11,12,13,14,15,16,17, 18,19,24,25,26,26a– hexadecahydro - 5,19-dihydroxy-3-[2-(4-hydroxy-3- methoxy cyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10, 12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-*c*][1,4] oxaazacyclotricosine - 1,7,20,21 (4H,23H)- tetrone,monohydrate

Moecular formula : $C_{44}H_{69}NO_{12}.H_2O$

Molecular structure :



Appearance : white to off-white powder.

Water solubility : Mostly insoluble

Dissociation constant pK_a : 14.07

Partition co-efficient : About 3.3

Melting range : 126° C

Solubility: Tacrolimus is practically insoluble in water. It dissolves freely in methanol.

Dosage available : available as 0.03% and 0.1% ointment

Indication: Tacrolimus ointment is indicated as second-line therapy for the short-term and non-continuous chronic treatment of moderate to severe atopic dermatitis in non-immunocompromised adults and children who have failed to respond adequately to other topical prescription treatments for atopic dermatitis, or when those treatments are not advisable.

Dose: Apply a thin layer of tacrolimus ointment to the affected skin twice daily. The minimum amount should be rubbed in gently and completely to control signs and symptoms of atopic dermatitis. Stop using when signs and symptoms of atopic dermatitis resolve. Ointment should not be used with occlusive dressings

Side effects: One out of 198 normal volunteers showed evidence of sensitization in a contact sensitization study. The use of tacrolimus ointment may cause local symptoms such as skin burning (burning sensation, stinging, soreness) or pruritus. The most common side effects of tacrolimus ointment at the skin application site are stinging, burning, or itching of the skin treated with tacrolimus. These side effects are usually mild to moderate, are most common during the first few days of treatment, and usually go away as skin heals.

Other side effects include acne, swollen or infected hair follicles, headache, increased sensitivity of the skin to hot or cold temperatures, or flu-like symptoms such as the common cold and stuffy nose, skin tingling, upset stomach, muscle pain, swollen glands enlarged lymph nodes), or skin infections including cold sores, chicken pox or shingles.

Prolonged systemic use of calcineurin inhibitors for sustained immunosuppression in animal studies and transplant patients following systemic administration has been associated with an increased risk of infections, lymphomas, and skin malignancies. These risks are associated with the intensity and duration of immunosuppression.

Mechanism of action: The mechanism of action of tacrolimus in atopic dermatitis is not known. While the following have been observed, the clinical significance of these observations in atopic dermatitis is not known. It has been demonstrated that tacrolimus inhibits T-lymphocyte activation by first binding to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin is inhibited. This effect has been shown to prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). Tacrolimus also inhibits the transcription for genes which encode IL-3, IL-4, IL-5, GM-CSF, and TNF-α, all of which are involved in the early stages of T-cell activation. Additionally, tacrolimus has been shown to inhibit the release of pre-formed mediators from skin mast cells and basophils, and to down regulate the expression of Fcε RI on Langerhans cells.

Pharmacokinetics: tacrolimus is minimally absorbed after the topical application of ointment. Peak tacrolimus blood concentrations ranged from undetectable to 20 ng/mL after single or multiple doses of 0.03% and 0.1% tacrolimus ointment, with 85% (75/88) of the patients having peak blood concentrations less than 2 ng/mL. In general as treatment continued, systemic exposure declined as the skin returned to normal. In clinical studies with

periodic blood sampling, a similar distribution of tacrolimus blood levels was also observed in adult patients, with 90% (1253/1391) of patients having a blood concentration less than 2 ng/mL. The absolute bioavailability of tacrolimus from ointment in atopic dermatitis patients is approximately 0.5%. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes There was no evidence based on blood concentrations that tacrolimus accumulates systemically upon intermittent topical application for periods of up to 1 year.

Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A). A metabolic pathway leading to the formation of 8 possible metabolites has been proposed. Demethylation and hydroxylation were identified as the primary mechanisms of biotransformation in vitro.

2.6 References

Anderson RL, Cassidy JM. Variations in physical dimensions and chemical composition of human stratum corneum, J. Invest. Dermatol. (1973) 61, 30–32.

Menon GK. New insights into skin structure: scratching the surface, Adv. Drug Deliv. Rev. (2002) 54, S3–S17.

Marekov LN, Steinert PM, Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope, J. Biol. Chem. (1998) 273, 17763–17770.

Elias PM, Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism, J. Control. Release (1991) 15, 199–208.

Wertz PW, Downing DT, Stratum corneum: biological and biochemical considerations, In Transdermal Drug Delivery, J. Hadgraft, R.H. Guy (Eds.), Marcel Dekker, New York, 1989, pp. 1–22.

Grubauer G, Feingold K, Harris R, Elias P, Lipid content and lipid type as determinants of the epidermal permeability barrier, J. Lipid Res. (1989) 30, 89–96.

Cevc G. Lipid vesicles and other colloids as drug carriers on the skin. . Advanced Drug Delivery Reviews (2004) 56, 675– 711

Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM, Urtti A. Microemulsions for topical delivery of estradiol. Int J Pharm (2003) 254, 99.

Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev (2004) 56, 603.

Higuchi, W.I. Analysis of data on the medicament release from ointments. J. Pharm. Sci. (1962) 51, 802-4

Wertz, PW, Downing DT, In Transdermal drug delivery: developmental issues and research iniatives J. Hadgraft, R.H. Guy (Eds.), Marcel Dekker, New York, 1989, pp. 1–22.

Bouwstra JA, Gooris GS, van der Spek, JA, Bras W. Structural investigations of human stratum corneum by small-angle X-ray scattering J. Invest Dermatol., (1991) 97, 1005-12.

Scheuplein RJ, Blank IH. Permeability of the skin. Physiol. Rev., (1971) 51, 702-747.

Potts RO, Francoeur ML. The influence of stratum corneum morphology on water permeability. J. Invest Dermatol., (1991) 96, 495-499.

Potts RO, Guy RH. Predicting skin permeability. Pharm. Res., (1992) 9, 663-669.

Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp. Dermatol., (2000) 9, 165-9.

Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev (2002) 54, S77.

Hadgraft J. Skin, the final frontier. Int J Pharm (2001) 224, 1.

Kemken J, Ziegler A, Muller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm. Res., (1992) 9, 554-558.

Dias MM, Raghavan SL, Pellett MA, Hadgraft J. The effect of beta-cyclodextrins on the permeation of diclofenac from supersaturated solutions. Int. J. Pharm. (2003) 263, 173-81.

Scheuplein RJ, Blank IH. Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. J. Invest Dermatol. (1973) 60, 286-96.

Roberts MS, Walker M. In Pharmaceutical Skin Penetration Enhancement, Walters KA, Hadgraft J., Eds. Marcel Dekker: New York. 1993, pp. 1-30.

Walters KA, Walker M, Olejnik O. Non-ionic surfactant effects on hairless mouse skin permeability characteristics J. Pharm. Pharmacol. (1988), 40, 525-9

Walters KA, In Transdermal drug delivery, Hadgraft J.; Guy RH, Eds., Marcel Dekker: New York. 1988, pp. 197-246.

Liron Z, Cohen S. Percutaneous absorption of alkanoic acids II: Application of regular solution theory. J. Pharm. Sci. (1984) 73, 538-542.

Adams WP and Singh GJP. Guidance: Topical dermatology corticosteroids: In vivo bioequivalents, Division of Bioequivalence, Office of Generic Drugs, Food and Drug Administration, 1995

Smith EW, Haigh JM, and Walker RB. Analysis of chromameter results obtained from corticosteroid-induced skin blanching I: Manipulation of data. Pharm. Res. (1998) 15, 280–285

Klimisch HM and Chandra G. Use of Fourier transform infrared spectroscopy with attenuated total reflectance for in vivo quantitation of polydimethylsiloxanes on human skin. J. Soc. Cosmet. Chem. Jpn. (1986) 37, 73–87

Reddy MB, Stinchcomb AL, Guy RH, and Bunge AL. Determining dermal absorption parameters in vivo from tape strip data. Pharm. Res. (2002) 19, 292–298

McCleverty D, Lyons R, and Henry B. Microdialysis sampling and the clinical determination of topical dermal bioequivalence. Int. J. Pharm. (2006) 308, 1–7

Davies MI. A review of microdialysis sampling for pharmacokinetic applications. Anal. Chim. Acta (1999) 379, 227–249

Kreilgaard M. Assessment of cutaneous drug delivery using microdialysis. Adv. Drug Deliv. Rev. (2002) 54, S99–S121

Riviere JE. Perfused skin models. In Dermal Absorption Models in Toxicology and Pharmacology, Riviere JE (Ed.), Taylor & Francis Group, Boca Raton, 2006.

Wagner H, Kostka KH, Lehr CM, and Schaefer UF. Drug distribution in human skin using two different in vitro test systems: Comparison with in vivo data. Pharm. Res. (2000) 17, 1475–1481

Netzlaff F, Kostka KH, Lehr CM, and Schaefer UF. TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz type diffusion cells in vitro. Limitations shown by transport data testing. Eur. J. Pharm. Biopharm. (2006) 63: 44–50

Haigh JM and Smith EW. The selection and use of natural and synthetic membranes for in vitro diffusion experiments. Eur. J. Pharm. Sci. (1994) 2, 311–330

Bronaugh RL, Stewart RF, and Congdon ER. Methods for in vitro percutaneous absorption studies. II. Animal models for human skin. Toxicol. Appl. Pharmacol. (1982) 62, 481–488

Hatanaka T, Inuma M, Sugibayashi K, and Morimoto Y. Prediction of skin permeability of drugs. II. Development of composite membrane as a skin alternative. Int. J. Pharm. (1992) 79, 21–28.

Hoar TP and Schulman JH. Transparent Water-in-Oil Dispersions: the Oleopathic Hydro-Micelle. Nature (1943) 152, 102-103.

Schulman JH, Strokenius W and Prince LM. Mechanism of Formation and Structure of Microemulsions by Electronmicroscopy. Phys. Chem., (1959) 63, 1677

Tadros Th F. In surfactants and in solution, Mittal KL and Lindman B, Eds., Vol. III, 1501-1532, 1984 New York, Plenum Press.

Shinoda K and Friberg SE. 1986. Stability of emulsion. In. Emulsion and Solubilization, 125-158, New York, Wiley.

Shinoda K and Kuneida H. Conditions to produce so called microemulsions: Factors to increase the mutual solubility of oil and water by solubilizer. J Colloid Interf Sci (1973) 42, 381.

Friberg S, Lapczynska L, Gillberg G. Microemulsions containing non ionic surfactants- The impotance of PIT value. J Colloid Interface Sci (1976) 56, 19-32.

Ruckenstein E and Krishnan R. Swellon micellar for solubilisation model. J Colloid Interface Sci (1979) 71, 321-335.

Ruckenstein E and Krishnan R.1980. The equilibrium radius of microemulsions formed with ionic surfactants. J Colloid Interface Sci (1980) 75, 476-492.

Ruckenstein E, Chi JC. Stability of Microemulsion, J Chem Soc Faraday Trans 2. (1975) 71, 1690-1707.

Friberg SE, Buraczewska I, Ravery JC. 1977. in Micellization, Solubilization and Microemulsion, Mittal KL Ed., New York, Plenum Press, 901.

Friberg SE, Venable RL. 1983 In Encyclopedia of Emulsion Technology (Becher, P Eds) Vol-I., New York, Marcel Dekker, 287-336.

Israclachvii JN, Mitchell DJ. and Ninham BW. Phase Behaviour of Amphiphile-Water Binary Mixtures. J Chem Soc Faraday Trans 1. (1976) 72, 1525.

Mitchell DJ and Ninham BW Micelles, Vesicles and Microemulsions. J Chem Soc. Faraday Trans 2. (1981) 77, 601-629.

Winsor PA 1954. Solvent properties of amphiphilic compounds, London, Butterworth.

Winsor PA. Binary and multi component solutions of amphiphilic compounds. Solubilisation and the formation, structure and theoretical significance of liquid crystalline solutions. Chem Rev (1968) 68, 1-40

Kogan, A. and Garti, N. Microemulsions as transdermal drug delivery vehicles. Adv. Colloid Interface Sci., (2006) 123–126, 369–385.

Bowcott JE and Schulman JH. Elektrochem (1955) 59, 283-288.

Sheikh S and Faiyaz S. Nano emulsions as vehicles for transdermal delivery of Accelofenac. AAPS pharmscitech (2007) 8, 104, E1-E9.

Nornoo AO, Chow DS. Cremophor-free intravenous microemulsions for paclitaxel II. Stability, in vitro release and pharmacokinetics._Int J Pharm (2008) 12, 349,117-23

Gloor M, Haus G, and Keipert S. Keratolytic activity of microemulsions. Skin Pharmacol. Physiol. (2003) 16, 151–155.

Changez M, Varshney M, Chander J, and Dinda AK. Effect of the composition of lecithin/n-propanol/isopropyl myristate/water microemulsions on barrier properties of mice skin for transdermal permeation of tetracaine hydrochloride: In vitro. Colloid Surf. B (2006) 50, 18–25.

Kemken J, Ziegler A, and Mueller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm. Res., (1992) 9, 554–558.

Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM and Urtti A. Microemulsions for topical delivery of estradiol. Int. J. Pharm. (2003) 254, 99–107.

Biju SS., Ahuja A. and Khar RK. Tea tree oil concentration in follicular casts after topical delivery: Determination by high-performance thin layer chromatography using a perfused bovine udder model. J. Pharm. Sci. (2005) 94, 240–245

Sintov AC. and Shapiro L. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. J. Control. Release (2004) 95, 73–183

Kweon JH, Chi SC, Park ES. Transdermal delivery of diclofenac using microemulsions. Arch Pharm Res (2004) 27, 351–356.

Hua L, Weisan P, Jiayu L, Hongfei L. Preparation and evaluation of microemulsion of vinpocetine for transdermal delivery. Pharmazie (2004) 59, 274–278

Kantarci G, Ozgungey I, Karasulu HY, Guneri T, Basdemir G. In vitro permeation of diclofenac sodium from novel microemulsion formulations through rabbit skin. Drug Dev Res (2005) 65, 17–25

Chen H, Chang X, Weng T, Zhao X, Gao Z, Yang Y, Xu H, Yang X. A study of microemulsion systems for transdermal delivery of triptolide. J Control Rel (2004) 98, 427–436.

Schmalfuss U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Rel (1997) 46, 279–285.

Lehmann L, Keipert S. and Gloor M. Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur. J. Pharm. Biopharm. (2001) 52, 129–136

Changez M. and Varshney M. Aerosol-OT microemulsions as transdermal carriers of tetracaine hydrochloride. Drug Dev. Ind. Pharm. (2000) 26, 507–512.

Changez M, Chander J. and Dinda AK. Transdermal permeation of tetracaine hydrochloride by lecithin microemulsion: In vivo. Colloid Surf. B (2006) 48, 58–66

Leung DY, and Bieber T. Atopic dermatitis. Lancet (2003) 361, 151–160

Novak N, Bieber T. and Leung DY. Immune mechanisms leading to atopic dermatitis. J. Allergy Clin. Immunol. (2003) 112, S128–139.

Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol. Today (1998) 19, 359–361.

Michiko K, Oyoshi, Rui He, Lalit Kumar, Juhan Yoon, and Raif S. Geha. Cellular and Molecular Mechanisms in Atopic Dermatitis. Advances in Immunology, (2009) 102, pp. 135, Elsevier Inc.

Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol (2000) 105, 860–76

Beck LA, Leung DY. Allergen sensitization through the skin induces systemic allergic responses. J Allergy Clin Immunol (2000) 106, S258–263.

Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest (1998) 101, 1614–22.

Langeveld-Wildschut EG, Bruijnzeel PL, et al. Clinical and immunologic variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions. J Allergy Clin Immunol (2000) 105, 1008–16.

Reich K, Hugo S, Middel P, Blaschke V, et al. Evidence for a role of Langerhans cell-derived IL-16 in atopic dermatitis. J Allergy Clin Immunol (2002), 109, 681–87.

Jasna Lipozen_cic´ Ronni Wolf. Atopic Dermatitis: an Update and Review of the Literature. Dermatol Clin (2007) 25, 605–612

von Bubnoff D, Geiger E, Bieber T. Antigen-presenting cells in allergy. J Allergy Clin Immunol (2001) 108, 329–39.

Taha RA, Minshall EM, Leung DY, et al. Evidence for increased expression of eotaxin and monocyte chemotactic protein-4 in atopic dermatitis. J Allergy Clin Immunol (2000) 105, 1002–07.

Yawalkar N, Uguccioni M, Scharer J, et al. Enhanced expression of eotaxin and CCR3 in atopic dermatitis. J Invest Dermatol (1999) 113, 43–48.

Galli G, Chantry D, Annunziato F, et al. Macrophage-derived chemokine production by activated human T cells in vitro and invivo: preferential association with the production of type 2 cytokines. Eur J Immunol (2000) 30, 204–10.

Bratton DL, Hamid Q, Boguniewicz M, Doherty DE, Kailey JM, Leung DY. Granulocyte macrophage colony-stimulating factor contributes to enhanced monocyte survival in chronic atopic dermatitis. J Clin Invest (1995) 95, 211–18

Forrest S, Dunn K, Elliott K, et al. Identifying genes predisposing to atopic eczema. J Allergy Clin Immunol (1999) 104, 1066–70

Cookson WO, Ubhi B, Lawrence R, et al. Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. Nat Genet (2001) 27, 372–73.

Leung DYM. Cellular and immunologic mechanisms in atopic dermatitis. J Am Acad Dermatol (2001) 44, S1-12,

Sator PG, Schmidt JB, Honigsmann H. Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. J Am Acad Dermatol (2003) 48, 352–8.

Irvine AD, Mc Lean WHI. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. J Invest Dermatol (2006) 126, 200–202.

Di Nardo A, Wertz P, Giannetti A, Seidenari S. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. Acta Derm Venereol (1998) 78, 27-30.

Murata Y, Ogata J, Higaki Y, et al. Abnormal expression of sphingomyelin acylase in atopic dermatitis: an etiologic factor for ceramide deficiency? J Invest Dermatol (1996) 106, 1242-9

Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. J Allergy Clin Immunol (1999) 103, 717–28.

Tupker RA, De Monchy JG, Coenraads PJ, Homan A, van der Meer JB. Induction of atopic dermatitis by inhalation of house dust mite. J Allergy Clin Immunol (1996) 97, 1064–70

Leung DY, Harbeck R, Bina P, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J Clin Invest (1993) 92, 1374–80.

Loden, M., Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders, Am J Clin Dermatol. (2003) 4, 771-88,

Chamlin SL, Kao J, Frieden IJ, Sheu MY, Fowler AT, Fluhr JW, et al. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. J Am Acad Dermatol. (2002) 47, 198-208.

Marks R, Barlow JW, Funder JW. Steroid-induced vasoconstriction: glucocorticoid antagonist studies, J. Clin. Endocrinol. Metab. (1982) 54, 1075–1077

Ponec M, Kempenaar JA. Biphasic entry of glucocorticoids into cultured human skin keratinocytes and fibroblasts, Arch. Dermatol. Res. (1983), 275, 334–344.

Rao GS. Mode of entry of steroid and thyroid hormones into cells, Mol. Cell. Endocrinol. (1981) 21, 97–108.

Hughes J, Rustin M. Corticosteroids, Clin. Dermatol. (1997) 15, 715–721.

Scheinman RI, Cogswell PC, Lofquist AK, Baldwin ASJ. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids, Science (1995) 270, 283–286.

Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis, Science (1995) 270, 286–290.

Lange K, Kleuser B, Gysler A, Bader M, Maia C, Scheidereit C, et al., Cutaneous inflammation and proliferation in vitro: differential effects and mode of action of topical glucocorticoids, Skin Pharmacol. Appl. Skin Physiol. (2000) 13, 93–103.

Norris DA. Mechanisms of action of topical therapies and the rationale for combination therapy, J. Am. Acad. Dermatol. (2005) 53, S17–S25

McKenzie AW, Atkinson RM. Topical activities of betamethasone esters in man, Arch. Dermatol. (1964) 89, 741–746

Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. J Am Acad Dermatol. (2006) 54, 1-15

Sheu HM, Lee JYY, Chai CY, Kuo KW. Depletion of stratum corneum intercellular lipid lamellae and barrier function abnormalities after long-term topical corticosteroids, Br. J. Dermatol. (1997) 136, 884–890

Woodbury R, Kligman AM. The hairless mouse model for assaying the atrophogenicity of topical corticosteroids, Acta Derm. Venereol. (1992) 72, 403–406.

Kerscher MJ, Korting HC. Comparative atrophogenicity potential of medium and highly potent topical glucocorticoids in cream and ointment according to ultrasound analysis, Skin Pharmacol. (1992) 5, 77–80.

Wiedersberg S, Leopold CS, Guy RH. Bioavailability and bioequivalence of topical glucocorticoids. European Journal of Pharmaceutics and Biopharmaceutics (2008) 68, 453–466

Pershing LK, Corlett JL, Lambert LD, Poncelet CE. Circadian activity of topical 0.05% betamethasone dipropionate in human skin in vivo, J. Invest. Dermatol. (1994) 102, 734–739

Katz M, Poulsen BJ. Corticoid, vehicle, and skin interaction in percutaneous absorption, J. Soc. Cosmet. Chem. (1972) 23, 565–590.

Katz M, Shaikh ZI. Percutaneous corticosteroid absorption correlated to partition coefficient, J. Pharm. Sci. (1965) 54, 591–594

Ayres JW, Hooper G. Assessment of the skin penetration properties of different carrier vehicles for topically applied cortisol, Br. J. Dermatol. (1978) 99, 307–317.

Poulsen J, Rorsman H. Ranking of glucocorticoid creams and ointments, Acta Derm. Venereol. (1980) 60, 57–62.

Stoughton RB. Bioassay system for formulations of topically applied glucocorticosteroids, Arch. Dermatol. (1972) 106, 825–827.

Stein L. Clinical studies of a new vehicle formulation for topical corticosteroids in the treatment of psoriasis, J. Am. Acad. Dermatol. (2005) 53, S39–S49.

Woodford R, Barry BW. Bioavailability and activity of betamethasone 17-benzoate in gel and cream formulations: Comparison with proprietary topical corticosteroid preparations in the vasoconstrictor assay, Curr. Ther. Res. (1974) 16, 338–345

Shah VP, Flynn GL, Yacobi A, Maibach HI, Bon C, Fleischer NM, et al., Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence, Pharm. Res. (1998) 15, 167–171.

Haigh JM, Kanfer I. Assessment of topical corticosteroid preparations: the human skin blanching assay, Int. J. Pharm. (1984) 19, 245–262.

McKenzie AW, Stoughton RB. A method for comparing percutaneous absorption of steroids, Arch. Dermatol. (1962) 86, 608–610.

Leopold CS. Quantification of depletion in solution-type topical preparations in vivo, J. Cosmet. Sci. (1998) 49, 165–174.

Shah VP. Topical drug products – microdialysis: regulatory perspectives, Int. J. Clin. Pharmacol. Ther. (2004) 42, 379–381.

Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. Immunol. Today 13, (1992) 136–142.

Lauerma AI, Maibach HI. Topical FK506 clinical potential or laboratory curiosity? Dermatology (1994) 188, 173–176.

Nakagawa H, Etoh T, Ishibashi Y, et al. Tacrolimus ointment for atopic dermatitis. Lancet (1994) 344, 8926.

Bornhovd E, Burgdorf WH, Wollenberg A. Macrolactam immunomodulators for topical treatment of inflammatory skin diseases. J Am Acad Dermatol (2001), 45, 736-43.

Crabtree GR. Calcium, calcineurin, and the control of transcription. J Biol Chem (2001) 276, 2313-6.

Panhans-Gross A, Novak N, Kraft S, Bieber T. Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). J Allergy Clin Immunol (2001) 107, 345-52.

Michel G, Ried C, Beetz A. FK-506 blocks expression of interleukin-8 receptor in normal human keratinocytes. J Invest Dermatol (1993) 100, 449A

Wollenberg A, Sharma S, von Bubnoff D, Geiger E, Haberstok J, Bieber T. Topical tacrolimus (FK506) leads to profound phenotypic and functional alterations of epidermal antigenpresenting dendritic cells in atopic dermatitis. J Allergy Clin Immunol (2001) 107, 519-25.

de Paulis A, Stellato C, Cirillo R, Ciccarelli A, Oriente A, Marone G. Anti-inflammatory effect of FK-506 on human skin mast cells. J Invest Dermatol (1992) 99, 723-8

Boguniewicz M, Eichenfield LF, and Hultsch T. Current management of atopic dermatitis and interruption of the atopic march. J. Allergy Clin. Immunol. (2003) 112, S140–S150.

Leung DY, Bieber T. Atopic dermatitis. Lancet (2003) 361, 151-160.

Fleischer AB Jr. Treatment of atopic dermatitis: role of tacrolimus ointment as a topical noncorticosteroidal therapy. J Allergy Clin Immunol (1999) 104, S126-30.

Hanifin JM, Chan SC, Cheng JB, Toffe SJ, Henderson WR Jr, Kirby DS, et al. Type 4 phosphodiesterase inhibitors have clinical and in vitro anti-inflammatory effects in atopic dermatitis. J Invest Dermatol (1996) 107, 51-6.

Doherty V, Sylvester DG, Kennedy CT, Harvey SG, Calthrop JG, Gibson JR. Treatment of itching in atopic eczema with antihistamines with a low sedative profile. BMJ (1989) 298, 96.

Krause L, Shuster S. Mechanism of action of antipruritic drugs. Br Med J (1983) 287, 1199-1200

Knowles S. Atopic dermatitis. Pharm Pract (2002) July,1-8.

Naeyaert JM, Lachapelle JM, Degreef H, de la Brassinne M, Heene M, Lambert J. Cyclosporin in atopic dermatitis: review of the literature and outline of a Belgian consensus. Dermatology (1999) 198,145-52.

Granlund H, Erkko P, Sinisalo M, Reitamo S. Cyclosporin in atopic dermatitis: time to relapse and effect of intermittent therapy. Br J Dermatol (1995) 132, 106-12.

Grundmann-Kollmann M, Podda M, Ochsendorf F, Boehncke WH, Kaufmann R, Zollner TM. Mycophenolate mofetil is effective in the treatment of atopic dermatitis. Arch Dermatol (2001) 137, 870-3.

Neuber K, Schwartz I, Itschert G, Dieck AT. Treatment of atopic eczema with oral mycophenolate mofetil. Br J Dermatol (2000) 143, 385-91.

Stevens SR, Hanifin JM, Hamilton T, Tofte SJ, Cooper KD. Long-term effectiveness and safety of recombinant human interferon gamma therapy for atopic dermatitis despite unchanged serum IgE levels. Arch Dermatol (1998) 134, 799-804.

Jang IG, Yang JK, Lee HJ, Yi JK, Kim HO, Kim CW, et al. Clinical improvement and immunohistochemical findings in severe atopic dermatitis treated with interferon gamma. J Am Acad Dermatol (2000) 42, 1033-40.

Yanase DJ, David-Bajar K. The leukotriene antagonist montelukast as a therapeutic agent for atopic dermatitis. J Am Acad Dermatol (2001) 44, 89-93.

Woodmansee DP, Simon RA. A pilot study examining the role of zileuton in atopic dermatitis. Ann Allergy Asthma Immunol (1999) 83, 548-52

Jolles S. A review of high-dose intravenous immunoglobulin treatment for atopic dermatitis. Clin Exp Dermatol (2002) 27, 3-7.

Hanifin JM, Cooper KD, Ho VC, Kang S, Krafchik BR, Margolis DJ, et al. Guidelines of care for atopic dermatitis, developed in accordance with the American Academy of Dermatology (AAD)/American Academy of Dermatology Association "Administrative Regulations for Evidence-Based Clinical Practice Guidelines." J Am Acad Dermatol (2004) 50, 391-404.

Lever R, Hadley K, Downey D, Mackie R. Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. Br J Dermatol (1988) 119, 189-98.

Hofer MF, Harbeck RJ, Schlievert PM, Leung DYM. Staphylococcus toxins augment specific IgE responses by atopic patients exposed to allergen. J Invest Dermatol (1999) 112, 171-6.

Grundmann-Kollmann M, Behrens S, Podda M, Peter RU, Kaufmann R, Kerscher M. Phototherapy for atopic eczema with narrow-band UVB. J Am Acad Dermatol (1999) 40, 995-7.

Abeck D, Schmidt T, Fesq H, Strom K, Mempel M, Brockow K, et al. Long-term efficacy of medium-dose UVA1 phototherapy in atopic dermatitis. J Am Acad Dermatol (2000) 42, 254-7.

Morris AD, Saihan EM. Maintenance psoralen plus ultraviolet A therapy: does it have a role in the treatment of severe atopic eczema? Br J Dermatol (2002) 146, 705-7.

Abramovits W, Goldstein AM, Stevenson LC. Changing paradigms in dermatology: topical immunomodulators within a permutational paradigm for the treatment of atopic and eczematous dermatitis. Clin Dermatol (2003) 21, 383-91.

Kyllonen H, Remitz A, Mandelin JM, Elg P, Reitamo S. Effects of 1-year intermittent treatment with topical tacrolimus monotherapy on skin collagen synthesis in patients with atopic dermatitis. Br J Dermatol (2004) 150, 1174-81.

Torok HM, Maas-Irslinger R, Slayton RM. Clocortolone pivalate cream 0.1% used concomitantly with tacrolimus ointment 0.1% in atopic dermatitis. Cutis (2003) 72, 161-6.

Del Rosso JQ. The use of foam vehicle topical corticosteroid formulations in combination with other topical agents: a clinical perspective based on

available studies and observational use. Poster presented at the 62nd Annual Meeting of the American Academy of Dermatology, Washington, DC, 2004 Feb 6-11.

Lenn J, Tanojo H, Huang X. Effects of co-application of betamethasone 17-valerate, from different formulations, with tacrolimus ointment on in vitro skin permeation. Poster presented at the 62nd Annual Meeting of the American Academy of Dermatology, Washington, DC, , 2004, Feb 6-11

Singh G, Singh PK. Tachyphylaxis to topical steroid measured by histamine-induced wheal suppression. Int J Dermatol (1986) 25, 324-6

Nakahara T, Koga T, Fukagawa S, Uchi H, Furue M. Intermittent topical corticosteroid/tacrolimus sequential therapy improves lichenification and chronic papules more efficiently than intermittent topical corticosteroid/emollient sequential therapy in patients with atopic dermatitis. J Dermatol (2004) 31, 524-8.

Imokawa G. Lipid abnormalities in atopic dermatitis. J Am Acad Dermatol (2001) 45, S29-32.

Jones SM. Triggers of atopic dermatitis. Immunol Allergy Clin North Am (2002) 22, 55-72.

Thami GP, Sarkar R. Coal tar: past, present and future. Clin Exp Dermatol (2002) 27, 99-103.

Meggitt SJ, Reynolds NJ. Azathioprine for atopic dermatitis. Clin Exp Dermatol (2001) 26, 369-75.

Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol (2004) 5, 752–60

Yamanaka K, Tanaka M, Tsutsui H, Kupper TS, Asahi K, Okamura H et al. Skinspecific caspase-1-transgenic mice show cutaneous apoptosis and pre-endotoxin shock condition with a high serum level of IL-18. J Immunol (2000) 165, 997–1003

Jin H, He R, Oyoshi M and Geha RS. Animal Models of Atopic Dermatitis. Journal of Investigative Dermatology (2009) 129, 31–40

Shiohara T, Hayakawa J, Mizukawa Y. Animal models for atopic dermatitis: are they relevant to human disease? Journal of Dermatological Science (2004) 36, 1—9

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Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol (1997) 9, 461-466.

Kondo K, Nagami T, Tadokoro S. Differences in haematopoietic death among inbred strains of mice. In: Bond P, Sugawara S, editors. Comparative cellular and species radiosensitivity. Tokyo, Japan: Igakushoin; 1969. p. 20.

Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T. Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol (1995) 105, 749—55.

Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol (1997) 159, 2484-91.

Thomas WR, Watkins MC, Jouhal SS, Asherson GL. Induction and modification of anti-TNP reaginic and IgG antibody response by reactive trinitrophenyl derivatives. Immunology (1978) 35, 673—82.

Spergel J, Mizoguchi E, Brewer J, Martin T, Bhan A, Geha R. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to metacholine after single exposure to aerosolized antigen in mice. J Clin Invest (1998) 101, 1614–22

Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. J Clin Invest (1999) 103, 1103–11

Huang CH, Kuo IC, Xu H, Lee YS, Chua KY. Mite allergen induces allergic dermatitis with concomitant neurogenic inflammation in mouse. J Invest Dermatol (2003) 121, 289–93

Hoshino T, Kawase Y, Okamoto M, Yokota K, Yoshino K, Yamanura K, et al. IL-18-transgenic mice: in vivo evidence of a broad role for IL-18 in modulating immune function. J Immunol (2001) 166, 7014-8.

Tanaka T, Tsutsui H, Yoshimoto T, Kotani M, Matsumoto M, Fujita A, et al. Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga. Int Arch Allergy Immunol (2001) 125, 236-40.

Xu D, Trajkovic V, Hunter D, Leung BP, Schultz K, Gracie JA, et al. IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background. Eur J Immunol (2000) 30, 3147-56.

Kawase Y, Hoshino T, Yokota K, Kuzuhara A, Kirii Y, Nishiwaki E, et al. Exacerbated and prolonged allergic and non-allergic inflammatory cutaneous reaction in mice with targeted interleukin-18 expression in the skin. J Invest Dermatol (2003) 121, 502-9.

Barton D, HogenEsch H, Weih F. Mice lacking the transcription factor RelB develop T cell-dependent skin lesions similar to human atopic dermatitis. Eur J Immunol (2000) 30, 2323-32.

Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol (2001), 117, 977–83