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The Potential of Metabolic Interventions to Enhance Transdermal Drug Delivery

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The stratum corneum is a complex tissue that is metabolically active, and undergoes dynamic structural modifications due to the presence of several self-regulating enzymatic systems. A large number of defensive (protective) functions are embodied in this tissue, each with its own structural and biochemical basis. Moreover, the stratum corneum is responsive to external perturbations to the permeability barrier, upregulating a variety of metabolic processes aimed at restoring normal barrier function. Traditional drug delivery methods, which are of limited effectiveness, view the stratum corneum as a static, but semipermeable membrane. In contrast, newer metabolically based methods, which can be deployed alone, or in conjunction with standard methods, have been shown to expand the spectrum of drugs that can be delivered transdermally in hairless mouse epidermis. Yet, while these new approaches hold great promise, if equally effective in human skin, they pose new questions about the risks of a highly permeabilized stratum corneum. ***Key words:*** ***Stratum corneum/barrier function/transdermal/lipid metabolism***
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UNIQUE ORGANIZATION AND PROPERTIES OF STRATUM CORNEUM (SC)

The paper-thin SC is a composite material made of proteins and lipids that are crucial for life in a terrestrial environment. In the traditional view, the SC is regarded as impermeable, but inert and highly resilient, analogous to a sheet of plastic wrap. According to this model, transdermal permeation is governed solely by the physical-chemical properties of this supposedly homogenous tissue (Scheuplein and Blank, 1971), and barrier properties can be assessed readily *in vitro*, in either devitalized or fresh epidermal sheets. Site-related variations in the number of SC cell layers, which govern the diffusion path length, again can be integrated into kinetics predicted by the plastic-wrap model.

The first development to cast doubt upon both the plastic-wrap model and its suppositions, was the discovery of the unique structural heterogeneity of the SC; i.e., its "bricks and mortar" organization (Elias, 1983). Rather than being uniformly dispersed, the lipids in normal SC are sequestered within the extracellular spaces, where they are organized into lamellar bilayers that surround the corneocytes (Elias, 1983; Elias and Menon, 1991). Hence, instead of thickness of the SC "membrane", variations in lamellar membrane structure and in total lipid content provide the structural and biochemical basis for site-related variations in permeability (Lampe *et al*, 1983). It follows, then, that the extracellular, lipid-enriched matrix of the SC comprises not only the structure that limits transdermal delivery of hydrophilic drugs, but also the so-called SC "reservoir" (Nemanic and Elias, 1980).

Human SC typically comprises about 20 corneocyte cell layers, which differ in their thickness, packing of keratin filaments, filaggrin content, and number of corneodesmosomes, depending on body site. Corneocytes are surrounded by a highly cross-linked, resilient sheath, the cornified envelope (CE), whereas the cell interior is packed with keratin filaments surrounded by a matrix composed mainly of filaggrin and its breakdown products. Individual corneocytes, in turn, are surrounded by a lipid-enriched extracellular matrix, organized largely into lamellar bilayers, which derive from secreted lamellar body (LB) precursor lipids. Following secretion, LB contents fuse end-to-end, forming progressively elongated membrane bilayers (Elias and Menon, 1991), a sequence mediated by a battery of lipolytic "processing" enzymes (see below). Yet, despite the clear importance of corneocytes both as spacers and as a scaffold for the extracellular matrix, transdermal drug development has focused primarily on manipulations of the extracellular lipid milieu (Flynn, 1989; Schaefer and Redelmeier, 1996). The existence of aqueous pores (Menon and Elias, 1997) not only adds further complexity to the extracellular pathway, but also additional opportunities for novel delivery strategies.

The exceptionally low permeability of normal SC to water-soluble drugs is the consequence of several characteristics of the lipid-enriched, extracellular matrix (**Table I**), including the highly

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Abbreviations: ACC, acetyl coenzyme A carboxylase; aSMase, acidic sphingomyelinase; β-GlcCer'ase, β-glucocerebrosidase; Cer, ceramides; Chol, cholesterol; Chol SO_4, cholesterol sulfate; ECP, extracellular processing; FFA, free fatty acids; GD, Gaucher disease; HMGCoA reductase, hydroxymethylglutaryl Coenzyme A reductase; LB, lamellar bodies; RXLI, recessive x-linked ichthyosis; SC, stratum corneum; SG, stratum granulosum; $sPLA_2$, secretory phospholipase; SPT, serine palmitoyl transferase; TEWL, transepidermal water loss.

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Table I. How stratum corneum lipids mediate barrier function

1.	Extracellular localization (only intercellular lipids play role)
2.	Amount of lipid (lipid wt %)
3.	Elongated, tortuous pathway (increases diffusion path length)
4.	Organization into lamellar membrane structures
5.	Hydrophobic composition (absence of polar lipids and presence of very long chain, saturated fatty acids)
6.	Correct molar ratio (approximately 1:1:1 of three key lipids: ceramides, cholesterol, and free fatty acids)
7.	Unique molecular structures (e.g., acylceramides)

convoluted and tortuous extracellular pathway created by corneocyte "spacers" (Potts and Francoeur, 1991). Moreover, not only the bilayered arrangement of extracellular lipids, but also their extreme hydrophobicity, and their occurence in a critical (1:1:1) molar ratio of the three key species, ceramides (Cer), cholesterol (Chol), and free fatty acids (FFA) (Man *et al*, 1993), are further characteristics that provide for barrier function.

Ceramides account for about 50% of total SC lipid mass (Schurer and Elias, 1991; Wertz and Downing, 1991), and are crucial for the lamellar organization of the SC barrier (Bouwstra *et al*, 1996). Of the seven Cer classes, acylceramides or Cer 1, which contains ω-hydroxy-linked, essential fatty acids in an amide linkage, are believed to be uniquely important for the barrier (Wertz and Downing, 1983). Chol, the second most abundant lipid by weight in the SC, promotes the intermixing of different lipid species, and regulates its "phase" behavoir (Norlen *et al*, 1999). FFA, which account for 10%–15% of SC lipids, consist predominantly of very long-chain, saturated species with $>$ 20 carbon atoms (Wertz and Downing, 1991). A decrease in the concentrations of any of these critical lipid species compromises barrier integrity, by altering the molar ratio required for normal barrier function (Man *et al*, 1995).

The "domain-mosaic model" advocates a meandering, polar (pore) pathway for water transport through grain boundaries within the lipid mosaic (Forslind, 1994), adding potential complexity to the already tortuous, extracellular pathway. The morphologic basis of the aqueous pore pathway (Flynn, 1989), however, instead appears to be lacunar domains embedded within the lipid bilayers (Menon and Elias, 1997). These lacunae correspond to sites of subjacent corneodesmosome degradation (Haftek *et al*, 1998). Whereas these lacunae are scattered and discontinuous under basal conditions, following certain types of permeabilization (e.g., occlusion, prolonged hydration, sonophoresis, iontophoresis), they expand until they interconnect, forming a continuous "pore pathway". The pore pathway reverts back to its original, discontinuous state once the permeabilizing stimulus disappears. Such a lacunar system, then, does not correspond to the grain boundaries of the "domain mosaic model", but instead it forms an "extended macrodomain mosaic" within the SC interstices (Menon *et al*, 1998).

EPIDERMAL LIPID SYNTHESIS, SECRETION, AND PROCESSING

Biosynthetic activities Epidermal differentiation is a vectorial process that is accompanied by dramatic changes in lipid composition, including loss of phospholipids with the emergence of Cer, Chol, and FFA in the SC (Schurer and Elias, 1991; Wertz and Downing, 1991). Although epidermal lipid synthesis is both highly active and largely autonomous from systemic influences, it can be modified by external influences; i.e., changes in permeability barrier status (Feingold, 1991). Acute perturbations of the permeability barrier in mice stimulate a characteristic recovery sequence that leads to restoration of normal function over about 72 h in young skin (the cutaneous stress test). This sequence includes an increase in Chol, FA, and Cer synthesis that is restricted to the underlying epidermis, and attributable to a prior increase in mRNA and enzyme activity/mass for each of the key synthetic enzymes. Furthermore, synthesis of each of the three key lipids is required for normal barrier homeostasis; i.e., topically applied inhibitors of the key enzymes in each pathway produce abnormalities in permeability barrier homeostasis (cited in Feingold, 1991). These experiments provided the seminal observations, as well as the model ("stress test") that led to development of a biochemical strategy to enhance transdermal drug delivery (see below).

Lamellar body secretion The unique two-compartment organization of the SC is attributable to the secretion of LB-derived lipids and colocalized hydrolases at the stratum granulosum (SG)–SC interface (Elias and Menon, 1991). Under basal conditions, LB secretion is slow, but sufficient to provide for barrier integrity. Following acute barrier disruption, calcium is lost from the outer epidermis, and much of the preformed pool of LB in the outermost SG cell is quickly secreted (Menon *et al*, 1992a, 1994). Calcium (Ca^{++}) is an important regulator of LB secretion, with the high levels of Ca^{++} in the SG restricting LB secretion to low, maintenance levels (Lee *et al*, 1992). Although exposure to high Ca^{++} (and K^{+}) delays barrier recovery following acute perturbations, this delay is reversible by coapplications of L-type Ca^{++} channel or calmodulin inhibitors (Lee *et al*, 1992). Finally, barrier homeostasis and LB secretion are regulated not only by changes in Ca^{++} concentrations, but also by agents that block organellogenesis and secretion; e.g., monensin and brefeldin A (Man *et al*, 1995) (see also below). These experiments provide further potential, biochemical approaches to enhance drug delivery.

Extracellular processing Extrusion of LB contents at the SG/SC interface is followed by processing into mature, lamellar membrane unit structures (Elias and Menon, 1991). As noted above, marked alterations in lipid composition occur, including depletion of glucosylCer and phospholipids, and cholesterol sulfate with accumulation of Cer, FFA, and Chol in the SC. This sequence, called extracellular processing (ECP), is attributable to the secretion of hydrolytic enzymes that convert cosecreted LB-derived lipid precursors into the nonpolar species that form the membrane bilayer system (Elias and Menon, 1991). Direct evidence for the central role of ECP in barrier homeostasis came first from studies on glucosylCer-to-Cer processing. For example, applications of specific, conduritol-type inhibitors of β-glucocerebrosidase (β-GlcCer'ase) both delayed barrier recovery after acute perturbations, and produced a progressive abnormality in barrier function when applied to intact skin (Holleran *et al*, 1993). Moreover, both in a transgenic murine model of Gaucher disease (GD), produced by targeted disruption of the *β-GlcCer'ase* gene (Holleran *et al*, 1994), and in the severe, type 2 neuronopathic form of GD, infants present with a barrier abnormality (Sidransky *et al*, 1996). The functional deficit in all three models (inhibitor, transgenic murine, type 2 GD) was attributable to accumulation of glucosylCer, depletion of Cer, and persistence of immature LB-derived membrane structures within the SC interstices.

Phospholipid hydrolysis, catalyzed by one or more 14 kDa secretory phospholipases ($sPLA_2$), generates a family of nonessential FFA, which are required for barrier homeostasis (Mao-Qiang *et al*, 1995; 1996). As applications of either bromphenacylbromide (BPB) or MJ33 (chemically unrelated $sPLA_2$ inhibitors) modulate barrier function in intact murine skin, $sPLA_2$ appears to play a critical role in barrier homeostasis (Mao-Qiang *et al*, 1995; 1996). Moreover, applications of either inhibitor to perturbed skin sites delays barrier recovery.

Sphingomyelin hydrolysis by acidic sphingomyelinase (aSMase) generates two of the seven members of the Cer family required for normal barrier homeostasis. Moreover, patients with mutations in the gene encoding aSMase (Tay-Sachs, Type A) that

lead to low enzyme activity, display an ichthyosiform dermatosis, and transgenic mice with an absence of aSMase demonstrate a barrier abnormality. Finally, applications of nonspecific inhibitors of aSMase to perturbed murine skin sites lead to a delay in barrier recovery (Schmuth *et al*, 2000). Hence, aSMase represents another key ECP enzyme that in theory, could be manipulated to enhance drug delivery.

Just as with glucosylCer and sphingomyelin, Chol SO_4 content increases during epidermal differentiation, and then decreases progressively as Chol SO_4 is desulfated during passage from the inner to the outer SC (Elias *et al*, 1984). Both Chol sulfate and its processing enzyme, steroid sulfatase, are concentrated in SC membrane domains, and the content of Chol sulfate in these sites increases by approximately 10-fold (Elias *et al*, 1984) in recessive X-linked ichthyosis (RXLI). Not only is RXLI characterized by a barrier defect (Zettersten *et al*, 1998), but also repeated applications of Chol SO_4 to intact murine skin produce a barrier abnormality (Maloney *et al*, 1984). In both cases, the barrier abnormality is attributable to Chol SO_4-induced phase separation in lamellar membrane domains (Zettersten *et al*, 1998). But the barrier defect may also be, in part, attributed to the fact that Chol SO_4 is a potent inhibitor of HMGCoA reductase (Williams *et al*, 1992). In summary, manipulation of a variety of ECP enzymes represents a cohort of potential biochemical methods that can be employed to manipulate drug delivery.

Acidification That the SC displays an acidic external pH ("acid mantle") is well documented, but its origin is not known. Extra-epidermal mechanisms, including both surface-deposited eccrine and sebaceous gland-derived products, and metabolites of microbial metabolism, as well as endogenous catabolic processes, such as phospholipid-to-free FFA hydrolysis, and deimination of histidine to urocanic acid have been proposed to influence SC acidity. Protons can also be generated locally in the lower SC by sodium-proton antiporters inserted into the plasma membrane (Behne *et al*, in press). Moreover, if the limiting membrane of the LB contains energy-dependent proton pumps (Chapman and Walsh, 1989), then active acidification of the extracellular space (ECS) could also accompany insertion of such pumps coincident with LB secretion. Thus, ongoing proton secretion at the SG/SC interface, coupled with one or more of the other mechanisms described above, could explain not only the pH gradient across the SC interstices, but also selective acidification of membrane microdomains.

The concept that acidification is required for permeability barrier homeostasis is supported by the observation that barrier recovery is delayed when acutely perturbed skin sites are immersed in neutral pH buffers (Mauro *et al*, 1998), or when either the sodium-proton antiporter (NHE1) or $sPLA_2$-mediated, phospholipid catabolism to FFA (Fluhr *et al*, 2001; Behne *et al*, in press) is blocked. Acidification appears to impact barrier homeostasis through regulation of ECP enzymes, such as β-GlcCer'ase and aSMase, which exhibit acidic pH optima. Whether altering pH alone could facilitate transdermal drug delivery, and serve as an independent or additive-enhancing method remains unknown (see below).

OVERVIEW OF STRATEGIES TO ENHANCE TRANSDERMAL DRUG DELIVERY

Because of its theoretical advantages, enormous efforts have been expended on the development of new approaches to enhance transdermal drug delivery. Yet, despite these efforts, the current list of drugs that have been delivered transdermally for systemic applications is small (< 10), and limited to highly lipophilic compounds of both low molecular weight, and low total absorbed-dose (e.g., nitroglycerin, clonidine, sex steroids, scopolamine, and nicotinic acid). We will now provide a brief overview of current transdermal technology, before proceeding to a discussion of biochemical/metabolic approaches.

The strategies that have been devised to enhance transdermal drug delivery can be classified as either physical, chemical, mechanical, or biochemical approaches. Combinations of these strategies can also be employed to increase efficacy (Johnson *et al*, 1996; Tsai *et al*, 1996; Choi *et al*, 1999), or for extending the time available for transdermal delivery (see below). Physical techniques vary from straightforward approaches, such as occlusion and tape stripping, to highly sophisticated instrumentation and miniaturization (e.g., iontophoresis, electroporation). The most straightforward of physical methods is prolonged occlusion, which alters the barrier properties of SC (Van Den Merwe and Ackermann, 1987; Mikulowska, 1992). Following 24–48 h of occlusion with resultant hydration, corneocytes swell, the intercellular spaces become distended, and the lacunar network becomes dilated. Distention of lacunae eventually leads to connections within an otherwise discontinuous system, creating "pores" in the SC interstices through which polar and nonpolar substances can penetrate more readily.

Another straightforward physical method to abrogate the barrier is removal of portions of the SC by stripping with either adhesive tapes or cyanoacrylate glue. Sequential stripping increases transepidermal water loss (TEWL), an indicator of a barrier defect, which correlates well with enhanced transdermal drug delivery (Spruit and Malten, 1966). Tape stripping removes both corneocytes and extracellular lipids, thereby reducing the tortuous path length that these substances would otherwise need to traverse. Moreover, stripping mechanically disrupts lamellar bilayers, even in retained, lower SC layers. Whereas barrier disruption with stripping is accomplished readily in animal skin, however, human skin requires many more strippings to obtain comparable results, which can result in mast cell degranulation and inflammation, leading to discomfort and pain. Moreover, tape stripping in humans, with or without attendant irritation, is complicated by the tendency for more pigmented skin to develop postinflammatory hyperpigmentation, and more strippings are required to disrupt the barrier in Type 5 and 6 pigmented skin (Reed *et al*, 1995).

Iontophoresis and electroportation represent electrically assisted, physical approaches to enhance delivery of drugs/macromolecules across the SC (Banga *et al*, 1999). Iontophoresis uses low currents from an externally placed electrode, with the same charge as the net polarity of the drug, to drive these molecules across the SC. Whereas the predominant pathway of iontophoretic transport reportedly is transappendageal (hair follicles, sweat glands), extracellular routes across the SC are also traversed (Monteiro-Riviere *et al*, 1994). Iontophoretic delivery through the SC interstices occurs via aqueous pores; and thus it operates at both a macro- (appendegeal) and a micro- (extracellular, lacunar) level. As drug delivery is proportionate to the amount of applied current, iontophoresis offers an opportunity for programmable drug delivery (Green, 1996), especially with the recent development of both miniaturized microprocessor systems and disposable hydrogel pads. Electroporation (electropermeabilization) is a relatively new electrical, nonthermal method, which employs ultrashort pulses with large transmembrane voltages ≈ 100 V to induce structural rearrangement and conductance changes in membranes, again leading to pore formation (Banga *et al*, 1999). Most effective for single bilayer cell membranes, electroporation also permeabilizes human SC (Prausnitz *et al*, 1993; Weaver and Chizmadzev, 1996). Although pore formation again is considered to be the subcellular mechanism, the actual pathway across SC has not yet been visualized.

Ultrasound, which is employed extensively in both medical diagnostics and physical therapy, is considered safe, with no known short- or long-term side-effects. Upon encountering the SC, ultrasound waves generate defects in SC structure (Wu *et al*, 1998), leading to permeabilization of the SC. Although frequency ranges of 1–3 MHz are minimally effective, higher frequencies (10–20 MHz) significantly enhance drug delivery (Bommannan

et al, 1992a). During sonophoresis, electron-dense tracers, such as lanthanum and FITC-conjugated dextrans, penetrate across the SC into the epidermis and dermis within 5 min with no apparent damage to the keratinocytes (Bommannan *et al*, 1992b; Menon *et al*, 1994). Moreover, tracer movement again occurs through lacunae, which become dilated and transiently continuous, followed by collapse of the pore pathway with cessation of applied energy (Menon *et al*, 1997b).

A recently developed technique utilizes pulsed laser beams to generate photomechanical (stress) waves that interact directly with the SC in ways that are different from ultrasound (Lee *et al*, 1992; 1999). These waves are generated by ablation of a target material (polystyrene) that covers the drug-containing solution that is to be delivered. The target first absorbs the laser radiation, and the solution then serves as a coupling medium for stress waves to propagate across the SC. As with sonophoresis and iontophoresis, the pathway of permeation is thought to be extracellular, but morphologic studies are lacking. In murine models, 40 kDa dextran molecules and 20 nm latex particles were delivered across the SC by a single photomechanical wave, generated using a 23-ns Q-switched Ruby laser. As with sonophoresis and iontophoresis, single photomechanical compression waves modulate the permeability of human SC only transiently, and barrier function recovers almost immediately. Recently, this method has been used to deliver small molecules (e.g., 5-aminolevulenic acid) into human skin without discomfort, and without adverse effects on skin structure or viability (Lee *et al*, 1999).

A variety of solvents (ethanol, methanol, chloroform, acetone) and detergents can extract SC barrier lipids and permeabilize the SC. Morphologic changes in human SC following exposure to solvents (Menon *et al*, 1998) show extraction, phase separation, derangement of lamellar bilayers, and often the creation of defects in corneocytes. Moreover, surfactants, such as sodium lauryl sulfate (SDS), and vehicles (e.g., propylene glycol) extract lipids and create extensive expansion of pre-existing lacunar domains. Solvent-based penetration enhancers, such as azone, sulfoxides, urea, and FFA, not only extract extracellular lipids, but they also alter SC lipid organization (phase behavior), thereby increasing transdermal delivery (Santus and Baker, 1993). Finally, liposomes represent yet another "chemical" method, frequently employed to enhance drug delivery; however, liposomes appear to enhance transdermal delivery solely via the appendegeal pathway (Yarosh *et al*, 1994; Domashenko and Cotsarelis, 1999), as yet there is no convincing evidence that they penetrate intact SC (Lasch *et al*, 1991; Korting *et al*, 1995).

METABOLIC APPROACHES TO ENHANCE TRANSDERMAL DRUG DELIVERY

As noted above, a wide variety of methods have been deployed to enhance transdermal drug delivery. Yet, despite their apparent efficacy *in vitro*, or in animal models, they have not yet been shown to be effective without attendant toxicity in humans. A major problem with most of these approaches is their standard assessment *in vitro*, using devitalized human skin. Non-viable samples do not mount a metabolic response against barrier perturbations, and such *in vivo* responses inevitably restrict the efficacy of any enhancing method, i.e., they "slam the window shut". An alternate or concurrent approach aims to enhance the efficacy of standard enhancers by interfering with the repair (metabolic) response in murine skin *in vivo* (Tsai *et al*, 1996) (**Fig 1**). Some of these methods can also abrogate the barrier in intact skin by "opening the window", thereby obviating the requirement for pretreatment or cotreatment with a primary enhancer.

The concept of a biochemical approach to enhance transdermal drug delivery came from pharmacologic studies in mice, where key metabolic sequences that restore and maintain barrier function were inhibited, i.e., epidermal lipid synthesis, LB secretion, ECP, and maintenance of lamellar bilayers (**Table 2**). All of these methods have the net effect of either altering the critical molar ratio of the three key SC lipids, or inducing discontinuities in the lamellar bilayer system. The first pharmacologic study in support of this concept came from experiments in adult hairless mice where topical HMGCoA reductase inhibitors, such as lovastatin and fluvastatin, caused both a delay in barrier recovery (Feingold *et al*, 1990) and a barrier defect following repeated applications to intact skin (Feingold, 1992). As the ability of the inhibitors to alter barrier homeostasis could be reversed by coapplications of either mevalonate (the immediate product of HMGCoA reductase) or Chol (a distal product), the inhibitor effect could not be ascribed to nonspecific toxicity. Likewise, application of specific pharmacologic inhibitors of ACC (Mao-Qiang *et al*, 1993), serine palmitoyl transferase (SPT) (Holleran *et al*, 1991), and glucosylCer synthase (Chujor *et al*, 1998), key enzymes of FA and Cer synthesis, respectively, also provoked a delay in barrier recovery, as measured by TEWL. Yet, whereas the HMGCoA reductase and ACC inhibitors are additive in their capacity to alter barrier recovery, coapplications of HMGCoA reductase and SPT inhibitors instead paradoxically normalize the kinetics of barrier recovery (Mao-Qiang *et al*, 1993). Whereas these studies

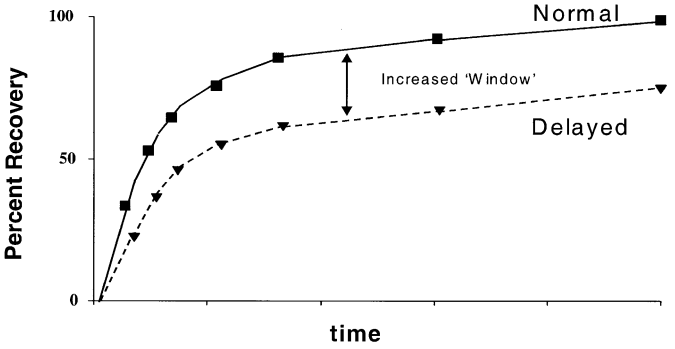

Figure 1. Barrier recovery following acute perturbations. Application of metabolic enhancer causes a delay in barrier recovery, creating a potential "window" for enhanced transdermal drug delivery.

Table II. Mechanistic classification of various biochemical enhancers

Lipid Synthesis Inhibitors
HMGCoA reductase
Acetyl CoA carboxylase
Serine palmitoyl transferase
Glucosylceramide synthase
Lamellar Body Secretion Inhibitors
Organellogenesis
Golgi processing
Exocytosis
Extracellular Acidification Inhibitors
Protonophores
Proton pumps
Extracellular Processing
β-Glucocerebrosidase
Secretory phospholipase A2
Acidic sphingomyelinase
Steroid sulfatase
Ion pump/channel inhibitors
Membrane Bilayer Assembly
Lipids analogues
Complex lipids
Lipid hydrolases

Table III. Lipid synthetic and processing inhibitors that modulate barrier homeostasis

Required lipid	Enzyme targets	Examples
Cholesterol	HMGCoA Reductase	Lovastatin, fluvastatin
Free Fatty Acids	Acetyl CoA carboxylase	TOFA
	Secretory phospholipase A_2	Bromphenacyl bromide, MJ33
Ceramides	Serine palmitoyl transferase	β-chlorolanine
	Glucosylceramide synthase	Morpholino agents (e.g., P4)
	β-Glucocerebrosidase	Conduritols
	Acidic sphingomyelinase	Desipramine

Abbreviations: TOFA: 5-(tetradecyloxy)-2-furancarboxylic acid; P4: d,1-threo-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol; MJ33: 1-hexadecyl-3-trifluoroethylglycero-sn-2-phosphomethanol.

determined metabolic events required for permeability barrier homeostasis, we noted that these inhibitors also caused the "window to remain open longer", and/or they could "open the window" for transdermal drug delivery. Thus, all of the pharmacologic "knockout" studies support the concept that interference with the biosynthesis of any of the key SC lipids can lead to a temporary increase in TEWL, with obvious implications for transdermal drug delivery.

In addition to lipid synthesis inhibitors, agents that interfere with LB assembly, secretion, or ECP delay barrier recovery after acute perturbations, and in some cases, create defects in intact skin (**Table III**). Examples include: (a) brefeldin A, which blocks LB assembly by disorganizing preformed Golgi structures; (b) monensin or chloroquin, which inhibit the apical translocation and secretion of LB; (c) high Ca^{++}/K^{+} levels, which inhibit LB secretion; (d) inhibitors of β-GlcCer'ase, aSMase, and sPLA, which are required for normal ECP; and (e) neutral pH buffers, which impede barrier recovery after acute perturbations, presumably by inactivating pH-dependent, ECP enzymes.

Still another category of biochemical enhancers utilizes approaches that alter either the formation of lamellar bilayers or the supramolecular organization of preformed lamellar bilayers. These include: (a) synthetic analogs of Chol, Cer, and FFA, such as *trans*-vaccenic acid and epicholesterol, which induce abnormalities in lamellar membrane organization; (b) complex precursors of Chol, Cer, and FFA, such as sterol esters, which are not metabolized efficiently to their respective products in the SC; (c) supraphysiologic concentrations of physiologic lipids, such as Chol SO_4, which induce phase separation in preformed membrane bilayers; and (d) hydrolytic enzymes, such as acid ceramidase, which degrade one or more of the three key SC species. Finally, it should be noted that any single- or double-component mixture of the three key lipids, or any mixture of all three species, which includes a greater than 3-fold excess of one of the three key lipid species, will delay barrier repair after acute perturbations. Together, these strategies induce the formation of separate lamellar and nonlamellar domains within the SC interstices. In most cases, the basis for such domain separation relates to changes in the critical mole ratio, i.e., with deletion or excess of any one of the three key lipids, a portion of the excess species no longer can remain in a well-organized lamellar phase. For example, a 50% reduction in Chol would result in an excess of both Cer and FFA, with a portion of the excess forming a nonlamellar phase. The result of phase separation is more permeable SC interstices, due not only to deletion of a key hydrophobic lipid, but also to the creation of additional penetration pathways, distinct from the primary, lamellar membrane route.

Based upon these studies in murine skin, then, strategies that interfere with the synthesis, assembly, secretion, activation, processing, or assembly/disassembly of the extracellular lamellar membranes, could increase drug delivery by interfering with permeability barrier homeostasis. These biochemical/metabolic approaches can also be viewed vectorially, i.e., as operative within different layers of the epidermis. For example, most lipid synthesis occurs within the basal layer, whereas LB formation, acidification, and secretion occur in suprabasal, nucleated cell layers. Finally, ECP and membrane assembly occur even more distally, i.e., within the SC interstices. Ultimately, strategies could be deployed not only to target specific biochemical mechanisms, but also to take advantage of the localization and relative importance of those steps that lead to the generation and maintenance of functional SC extracellular lamellae.

EFFICACY, LIMITATIONS, AND POTENTIAL PITFALLS OF BIOCHEMICAL APPROACH

To date the effectiveness of these biochemical approaches for transdermal drug delivery has been assessed primarily in adult hairless mouse epidermis. In our initial studies, caffeine and lidocaine were used as model permeants to assess whether their penetration characteristics paralleled changes in TEWL produced by metabolic inhibitors. The biochemical approaches here consisted of the topical application of either drug plus either a Chol and/or FA synthesis inhibitor in two different, conventional enhancer/vehicle systems, dimethylsulfoxide or propylene glycol:ethanol (7:3 vols), followed by assessment of both TEWL and drug delivery. These studies showed that the biochemical enhancers accelerated lidocaine and caffeine delivery several-fold across intact skin above levels achieved with either of the chemical enhancer/vehicle systems alone (Tsai *et al*, 1996). Moreover, the extent of changes in TEWL correlated linearly with transdermal delivery of both drugs. This study showed that biochemical enhancers can increase transdermal drug delivery in a widely employed animal model. Additional work will be needed to explore whether TEWL serves as a universal, accurate, and reproducible predictor for transdermal delivery of drugs, with a broad range of physical-chemical properties, and the ability of studies in animals to predict increased drug delivery in humans. Nevertheless, these preliminary studies suggest significant potential for the biochemical/metabolic approach to increase transdermal drug delivery.

As noted above, many of the above approaches employ drugs as the metabolic enhancer, which can impose substantial regulatory issues, unless (a) the enhancing drug is also a potential therapeutic agent (e.g., statins to decrease lipid production in acne vulgaris), or (b) the drug is already approved for systemic use (e.g., the aSMase inhibitor, desipramine; statins, antimalarials, calcium channel blockers). Furthermore, some metabolic enhancers (e.g., monensin) though not approved for use in humans, are already approved for veterinary use. In addition, the prior section describes several approaches that do not use drugs (e.g., increased Ca^{++}, neutral pH buffers, complex lipids, lipid metabolites), which should not impose regulatory hurdles.

For those approaches that do not produce a barrier defect in intact skin, there is an implicit requirement for a "primary" enhancer to provide the initial barrier defect. Such an enhancing system can be a combination of any of the standard physical or chemical methods described above, coupled with one or more of the biochemical/metabolic enhancers. The feasibility/efficiency of such a combined approach has been demonstrated for iontophoresis, coupled with the application of a single-component FFA (oleic acid) and/or an unrelated chemical enhancer (propylene glycol), as well as for chemical enhancers, coupled with lipid synthesis inhibitors, as described above. Thus, it should be possible to devise, and even customize combinations of delivery methods, without the imposition of additional regulatory hurdles.

In summary, based upon extensive studies in mice, we have proposed a number of biochemical/metabolic interventions that could enhance transdermal drug delivery in humans. As these approaches become not only increasingly complex, but also more effective, they could impose not only significant regulatory hurdles, but also raise issues about increased xenobiote or microbial

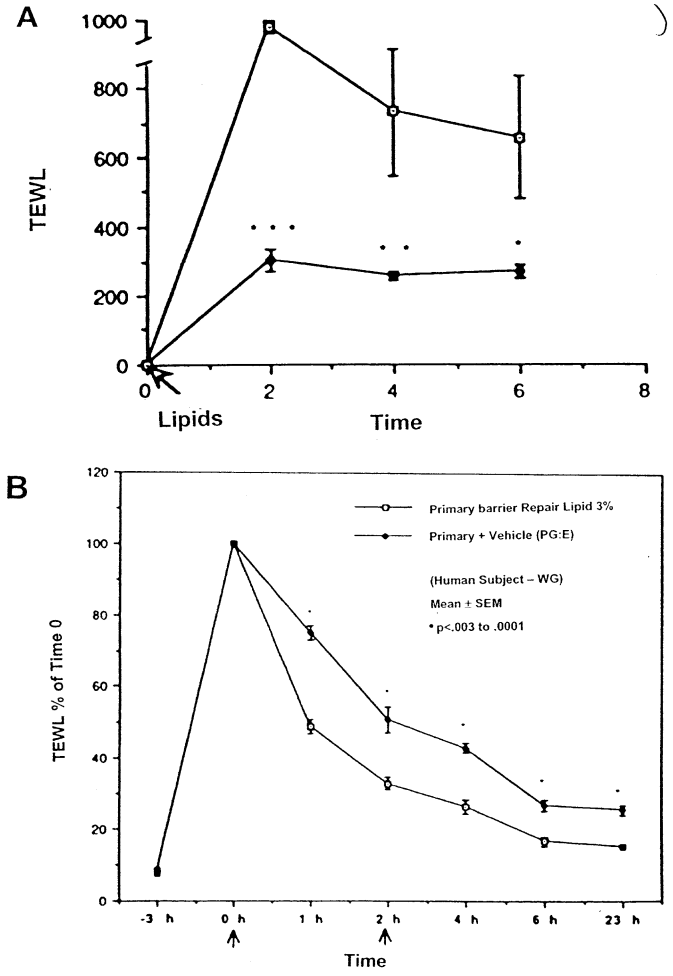

Figure 2. Application of barrier repair lipids decreases barrier abnormality. (***A***) Barrier repair mixture (cholesterol: ceramides: free fatty acids, 3%) or vehicle alone was applied at same time as metabolic enhancer. Note substantial prevention of emergence of abnormality. (***B***) Barrier repair lipids or vehicle were applied at time of maximum abnormality (*vertical arrows*). Note more rapid normalization of abnormality.

access. Obviously, a more patent "window" across the SC raises the theoretical risk of penetration not only of desirable entities, but also of toxic substances or pathogenic microbes. One potential, salutory approach would be to utilize barrier repair technology to "close the window" after a finite period of patency. Indeed, as seen in **Fig 2**, this strategy can be used either (a) with coapplications of physiologic lipids, to modify the extent of the original window, or (b) to arbitrarily terminate the enhancing sequence, by application of the physiologic lipids at a desirable point after enhancer/drug applications.

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