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Review

Transfollicular Drug Delivery

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The hair follicle, hair shaft, and sebaceous gland collectively form what is recognized as the pilosebaceous unit. This complex, three-dimensional structure within the skin possesses a unique biochemistry, metabolism and immunology. Recent studies have focused on the hair follicle as a potential pathway for both localized and systemic drug delivery. Greater understanding of the structure and function of the hair follicle may facilitate rational design of drug formulations to target follicular delivery. Targeted drug delivery may enhance current therapeutic approaches to treating diseases of follicular origin. Presented here is a review of follicular drug delivery and a discussion of the feasibility of the pilosebaceous unit as a target site.

KEY WORDS: pilosebaceous unit; hair follicle; sebaceous gland; topical drug delivery.

INTRODUCTION

Although the stratum corneum is widely acknowledged as the main barrier to percutaneous absorption, it is also regarded as the main pathway for penetration. However, recent reports have suggested that in addition to the transepidermal route, hair follicles and sebaceous glands may contribute significantly to topical or transdermal delivery. In the past, doubt has been cast upon the actual significance of the follicular pathway based on the fact that the orifices of hair follicles occupy only about 0.1% of the total skin surface area (1). However, the hair follicle is an invagination of the epidermis extending deep into the dermis, providing a much greater actual area for potential absorption below the skin surface. Release of sebum by sebaceous glands associated with the hair follicle may also influence absorption by providing a lipoidal pathway (2).

The mammalian hair follicle is a complex, dynamic structure in which unique biochemical and immunological reactions dictate cyclic phases of growth, regression and activity throughout life. Several epithelial cell types, specialized structures and immunocompetent cells co-exist within the structure. Hormones, aging, growth factors, ultraviolet radiation and some pharmacological agents are known to exert varied effects upon the hair follicle. Recent new approaches to molecular and cellular biology may be useful in elucidating molecular signals that control the onset and duration of hair follicle growth and development, which still are not fully understood (2–8). Greater understanding of cellular interactions within the structure and the biochemical mechanisms that govern it may enable rational design of targeted delivery systems.

Heightened interest in the pilosebaceous unit as a potential drug delivery target lies in the fact that the etiologies of several dermatological abnormalities relate to the hair follicle. Acne, androgenetic alopecia, alopecia areata and some skin cancers are among these conditions (2,7,9,10). Besides localized delivery, systemic delivery via the hair follicle may also be desirable.

This review provides a basis for the feasibility of follicular drug delivery along with a partial historical review of literature on the topic. Hair follicle structure and biology relevant to follicular drug delivery will be discussed in addition to experimental strategies and models.

DEFINITION OF TERMS

Frequently, the terms "hair follicle" and "pilosebaceous unit" have been used interchangeably, although the pilosebaceous unit actually comprises the hair follicle, hair shaft, and sebaceous glands. In this review, "transfollicular drug delivery" will encompass both the hair follicle itself and the sebaceous glands. However, potential individual contributions from the hair follicle and the sebaceous glands will also be considered.

HISTORICAL REVIEW OF FOLLICULAR DELIVERY

Early Qualitative Studies

Vehicle and Drug Effects

The earliest investigations of the transfollicular delivery route were mostly qualitative, histological studies based on stain and dye localization within the hair follicle. MacKee *et al.* (11) were among the first to investigate the role of hair follicles in percutaneous delivery. In histological studies of

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guinea pig and human skin, preferential staining of hair follicles was observed after topical application of iron, bismuth, sulfonamides and dyes in different vehicles. A vehicle-dependent effect was suggested in that the most intense staining occurred with a vehicle consisting of a mixture of propylene glycol, surface active agents and solubilizers rather than with a lanolin ointment.

Using qualitative fluorescent microscopy methods, Montagna (12) detected *in vivo* follicular deposition of Vitamin A in guinea pig skin. Deposition was determined as a function of solvent type and application time. Penetration into both the follicular duct and the sebaceous gland was detected within ten minutes after application of Vitamin A in ethanol or chloroform vehicles, but was much slower from oleic acid and petrolatum vehicles.

Rutherford and Black (13) found vehicle and drug effects on germicide localization in hair follicles and various strata of guinea pig skin after topical application. A trichlorocarbanalide (TCC) compound incorporated into a soap vehicle was found exclusively in the stratum corneum, whereas TCC incorporated into a synthetic non-soap sodium alkoyl isethionate detergent resulted in distinct germicide deposition into hair follicles and sebaceous glands as well. Topical application of identical shampoo vehicles containing similar germicides, zinc pyridine-2-thione-1-N-oxide (PTO) and zirconium PTO, resulted in germicide deposition into the stratum corneum, hair follicles and sebaceous glands. However, zirconium PTO was visualized in the epidermis and dermis, whereas zinc PTO was not. Since zinc PTO is soluble in sebum, it may remain localized in the hair follicle, whereas zirconium PTO is not soluble in sebum and may thereby be transported into the dermis. These findings suggest that in addition to vehicle effects, the physicochemical properties of the drug itself may be influential in the ultimate transport of the drugs within the skin.

Follicular Density Effects

Shelley and Melton (14) noted the appearance of tiny multiperifollicular wheals on hairy skin of human subjects after topical application of epinephrine and histamine phosphate in propylene glycol. No pharmacological response occurred at areas with less hair density, thereby suggesting follicular involvement. Wahlberg (15) used surface radiation disappearance measurements to quantify *in vitro* and *in vivo* percutaneous absorption in hairy and hair follicle-free guinea pig skin. The skin behind the ears of guinea pigs is unique in that it is completely devoid of hair follicles and sebaceous glands. Topical solutions of $HgCl_2$ and NaCl in water or alkyl aryl sulfonate vehicles were applied to both hairy and follicle-free skin. *In vitro* absorption through hairy skin was highly independent of vehicle, whereas *in vivo* absorption through hairy skin was not significantly different from that through hairless skin, suggesting that absorption was primarily transepidermal. However, the possible contribution of hair follicle-associated systemic absorption was overlooked.

Systemic clearance was considered by Maibach *et al.* (16), who noted regional variations in absorption after *in vivo* topical application of ^{14}C-labeled pesticides to human skin. Greater urinary recovery was obtained after application to hair follicle-rich areas such as the forehead and scalp than after application to less hairy areas such as the forearm. Although the investigators did not explicitly attribute the differences to follicular absorption, the possibility of this route was noted.

Follicular (Shunt) Transport: Theoretical Considerations

Scheuplein (17) was among the first to theoretically consider the follicular, or shunt pathway. Rapid *in vitro* and *in vivo* percutaneous transport of charged dyes was attributed to a transient, shunt-mediated diffusion associated with a lag time of less than one hour. The basis of transient diffusion is that under appropriate conditions, either the transepidermal or transfollicular route may be important. Initially only transient diffusion occurs, but after steady-state diffusion is established, bulk diffusion through the lipid-keratin matrix of the stratum corneum becomes dominant. Besides applying the transient diffusion concept to ions and dyes, Scheuplein also investigated its application to large steroid molecules that ordinarily would not be expected to penetrate the stratum corneum quickly (18). Cortisol in aqueous solution was found to penetrate the skin quickly, thereby suggesting that a wide range of compounds may be transported by transient diffusion. The above interpretation of the mechanism of transient percutaneous absorption encompasses only solution dosage forms, and may not necessarily be extended to other topical vehicles.

Improved Qualitative Methods and Quantitative Studies

Time Dependence Effects

Although early studies provided strong qualitative evidence of follicular deposition, the data were often difficult to interpret and yielded poor quantitative information. Qualitative observations, especially those indicating the importance of time and vehicle dependencies, provided the foundation for more quantitative work in later follicular delivery studies. After Scheuplein delineated the principles of transient diffusion, other investigators incorporated improved qualitative techniques and developed increasingly quantitative methods for assessing follicular delivery. Nicolau *et al.* (19) combined microscopic autoradiography and liquid scintillation counting to assess *in vivo* follicular delivery of viprostol, a synthetic prostaglandin E_2 analogue, in a petrolatum base. After 12 hours, viprostol was found in the stratum corneum, epidermis, and deep in the hair follicle. However, after 72 hours, drug could be found only in hair follicle structures.

Dose and Vehicle Effects

Bidmon (20) found dose and vehicle dependencies after using an improved, dry-mount autoradiography technique to detect ^{3}H-estradiol-17β in hairy rat skin. After a two hour *in vivo* topical application of drug in DMSO, ethylene glycol or sesame oil, radiolabel was found in the epidermis, sebaceous glands and follicular papilla of the hair follicle. In the sebaceous glands and follicular papilla, radioactivity was maintained at the highest concentration for at least 24 hours, suggesting that a drug depot effect may occur within the pilosebaceous unit. Higher doses allowed better visualization of gradients and routes. With DMSO- and ethylene gly-

col-based formulations, a time-dependent gradient was observed from the hair follicle to the dermis that was shorter and less pronounced than the gradient from the stratum corneum to the epidermis. Fast removal by blood vessels surrounding hair follicles or increased penetration within the dermis may explain these observations.

Particle Size Effects

Recent studies have suggested that an optimal particle size may exist for targeted delivery into the hair follicle (1,26). Schaefer *et al.* (1) targeted drug delivery to the hair follicle by using size-characterized fluorescent polystyrene microbeads in aqueous or lipophilic suspensions. After 20 minutes of topical application time to hairless rat skin *in vivo* and to excised human skin, follicular biopsy results indicated that 9 μ- and 10 μ-diameter beads concentrated at the stratum corneum surface and follicular opening, whereas 7 μ-diameter beads were found mostly deep within the follicle. Fluorescence was detected in both the stratum corneum and hair follicle after application of beads with diameters less than 3 μ. Increased deposition was observed with lipophilic vehicles. Similar size dependencies were observed with dansyl chloride-labeled poly-β-alanine microbeads of 2 μ-, 5 μ- and 12 μ-diameter. Greatest follicular deposition was achieved after application of 5 μ-diameter beads.

Rolland *et al.* (26) investigated the effect of microbead size on follicular delivery of adapalene-loaded polymeric microspheres dispersed in an aqueous gel. Size-specific delivery of adapalene, which fluoresces, was visualized by fluorescent microscopy in both *in vitro* and *in vivo* experiments. After 5 hours of topical application to excised human skin, random distribution of 1 μ-diameter beads into the stratum corneum and hair follicle was observed, whereas 20 μ-diameter beads were visualized only at the skin surface. Topical application of 5 μ-diameter beads resulted in optimal deposition in the hair follicle. Comedelytic activity of 5 μ-diameter adapalene-loaded spheres was comparable to that obtained with an aqueous adapalene gel formulation applied at higher doses and longer application times.

Drug Property and Follicular Density Effects

Fabin and Touitou (21) interfaced microcomputer-based image analysis with autoradiography to quantitate follicular deposition in hairless rat skin of two lipophilic compounds, oleic acid and tetrahydrocannabinol (THC), in diethylene glycol monoethyl ether. After 2 hours, twice as much of each compound was found in the epidermis and hair follicle than in the dermis. However, after 24 hours, different localization patterns were observed for both compounds. THC penetrated the skin to a greater extent and a sharper gradient from the epidermis to the hair follicle to the dermis was apparent. The oleic acid diffusion gradient was not distinct between the epidermis and appendages. The conclusion from this study was that a molecule-dependent choice of penetration route may be possible between 2 and 24 hours.

Another recent approach to assessing follicular drug delivery has been the analysis of follicles removed by cyanacrylate adhesive on a glass slide (22,23). Bojar *et al.* (24) used HPLC to detect azelaic acid in the supernatant of follicular casts. After 5 hours of topical application in a 20% w/w cream, greater follicular concentrations were found in back casts than in forehead casts, indicating that circulatory access to the follicle-rich forehead may have resulted in rapid removal from the hair follicle.

The advent of cell, tissue and organ culture systems has led to additional approaches to studying follicular delivery. Kao *et al.* (25) developed an *in vitro* skin culture penetration chamber system to study follicular delivery of two lipophilic compounds with greatly differing permeabilities. Skin was cultured from several hairy and hairless mouse strains. After 16 hours, overall permeability of testosterone (>65%) was much greater than that of benzo[a]pyrene (<10%), regardless of hair follicle presence and mouse strain. However, penetration of benzo[a]pyrene was higher in hairy skin ~10%) than in hairless skin (~2%). In order to lessen the effect of confounding genetic influences, an in-house strain was developed that comprised three phenotypic hair densities: hairy, fuzzy and hairless. Topical drug application of the same formulations to different phenotypic strains resulted in the highest permeability in hairy skin, intermediate permeability in fuzzy skin and lowest permeability in hairless skin. Photomicrographs of hairy skin showed benzo[a]pyrene fluorescence deep within the dermis, which the authors correlated with follicular and sebaceous ducts. Less fluorescence was observed in the dermis of fuzzy skin, whereas no fluorescence was observed in the dermis of hairless rat skin. The combined results suggested that transfollicular diffusion may play a major role for transport of some compounds, depending on their physicochemical properties. The overall extensive absorption of testosterone may be attributed to bulk diffusion via the stratum corneum, whereas transient diffusion via follicular shunts may be more important for benzo[a]pyrene since it is less well absorbed.

Induced Follicle-Free Models

The first systematic, quantitative *in vivo* studies of follicular delivery were reported by Illel and Schaefer *et al.* (1,27). Follicular absorption studies have frequently been based on transport through hairless rodent skin. However, rodents that are genetically altered to a hairless phenotype actually do possess hair follicles along with sparse, fuzzy hair. Compared with hairy rats, the hair follicles of hairless rats are underdeveloped and less dense.

Illel and Schaefer (27) adapted a truly follicle-free rat skin model based on the follicle-free mouse skin model developed by Behl *et al.* (28). After immersion of the dorsal side of the rat in 60°C water for one minute, the epidermis was removed and allowed to redevelop over several weeks into truly follicle-free skin. Upon histological examination, the resulting skin was found to be follicle- and sebaceous gland-free and only minor structural differences were noted after comparing with untreated hairless rat skin. Transepidermal water loss measurements returned to normal baseline values, suggesting that the barrier function was not greatly compromised. Lipid content of the skins were similar except the follicle-free skin lacked some lipids of sebaceous gland origin. Using both follicle-free and intact hairless rat skin, *in vitro* absorption of ^{3}H-hydrocortisone in a hydroalcoholic lotion was then investigated using the Franz diffusion cell method. After 24 hours, the skin was tape-stripped, and the

strips, residual epidermis and dermis were analyzed by liquid scintillation counting. The steady state flux and total diffusion of hydrocortisone through hairless rat skin were almost 50 times greater than that through follicle-free skin. Also, drug concentration within the residual skin was 20 to 30 times greater in normal hairless rat skin than in follicle-free skin, suggesting that the follicular route may be important for transport of some compounds into as well as beyond the skin.

Hueber *et al.* (29) investigated the *in vivo* disposition of ^{3}H-hydrocortisone and ^{3}H-testosterone in normal hairless rat skin and induced follicle-free rat skin after 0.5, 2 and 6 hours of topical application. Model steroid compounds were selected based on their differing lipophilicities (hydrocortisone, log P ~1.61 and testosterone, log P ~3.32), and were dissolved in ethanol:water (95:5, v/v). Greater concentrations of both compounds were found in the epidermis and dermis of intact skin than of follicle-free skin, especially at the depth where sebaceous glands are located. However, absorption differences were less pronounced than in similar *in vitro* studies of hydrocortisone flux through follicle-free skin (27). The amount of hydrocortisone penetrating the skin was unaffected by application time, whereas the amount of testosterone absorbed increased slightly with time. The results suggested that the stratum corneum of follicle-free skin may serve as a drug reservoir, and that the pilosebaceous unit may contribute to the absorption of compounds, depending on their physicochemical properties.

In another study, Illel *et al.* (30) used the induced follicle-free rat skin model to investigate the absorption of compounds with varying alcohol solubility characteristics: caffeine, niflumic acid, and p-aminobenzoic acid. After 48 hours, *in vitro* flux and absorption values for all three compounds were 3 times greater for the normal hairless skin than for the follicle-free skin. The results suggest that the shunt pathway may be important for a large range of substances with varying solubility characteristics.

Targeted Delivery via Liposomes

The most recent approaches to follicular delivery have focused on the development of pilosebaceous unit-specific delivery systems. The Syrian golden hamster ear model, introduced by Plewig and Luderschmidt (31), has been used to study drug delivery to sebaceous glands since the ventral side contains a high density of sebaceous glands similar to that found in human skin. After separation of the anatomical layers of the ear skin structure, sebaceous glands can be scraped away for analysis. Lieb *et al.* (32) adapted the hamster ear model and scraping technique to assess *in vitro* pilosebaceous deposition of liposomal carboxyfluorescein as a function of time. Fluorescence was detected after 12 hours in the follicular openings and even more intensity in the sebaceous glands.

Li *et al.* found that liposomal entrapment of calcein (33), melanin (34), and high molecular weight DNA (35) resulted in specific delivery into the hair follicles of histocultured mouse skin. Aqueous control solutions of calcein, melanin and DNA showed no drug localization within the follicle. Liposomal calcein was detected in hair follicles by laser confocal microscopy after a 20 minute topical application (33). Liposome-mediated targeted delivery of melanin was observed by fluorescent microscopy and histological examination after a 12 hour incubation period (34). Autoradiography showed label localization within the hair follicle after incubation for 44 hours with liposomally entrapped [^{35}S]DNA (35). These findings suggest that liposomes may allow for targeted delivery of a wide range of compounds into the hair follicle. Moreover, delivery of high molecular weight DNA to the hair follicle may lead to new gene therapy approaches to altering hair growth processes or treating hair follicle-associated disorders.

Iontophoresis

Although a comprehensive discussion of iontophoresis is beyond the scope of this review, recognition of this drug delivery method as a potential follicular process is relevant. Application of an electric potential gradient to the skin surface has been found to increase flux of ionic compounds by field-induced migration and neutral compounds by an electroosmotic effect (36). Although the exact routes for ion transport have yet to be fully characterized, hair follicles have been implicated as possible ion channels, which may ultimately be useful in localized or systemic drug delivery into the skin (36–39).

During electrophoresis of excised human skin, Burnette and Ongpipattanakul (37) detected charge flow across pores localized by fluorescein. Microscopically-viewed pore sites were sometimes located at hair follicles. Cullander and Guy (38), using a vibrating probe technique, reproducibly measured site-specific ionic flow across excised hairless mouse skin and superimposed current vectors on video images of microscopically-viewed samples. Sebaceous glands and hair follicles, especially those with small hairs, consistently showed high ionic flow. Scott *et al.* (36) also identified hair follicles as ionic channels during iontophoresis across excised hairless mouse skin. Scanning electrochemical microscopy images of Fe^{2+} and Fe^{3+} flux showed large peaks corresponding to high ionic flux through hair follicles.

Iontophoresis may be particularly important for delivery of ionic, hydrophilic and high molecular weight compounds, which have been problematic in conventional topical and transdermal delivery strategies. Green *et al.* (39) used iontophoresis to enhance delivery of neutral and ionic tripeptides across excised hairless mouse skin. Degree of flux was found to be independent of lipophilicity, but dependent on penetrant charge and size (39), which may be indicative of the environment and diameter of the hair follicle. Despite its great potential for enhancing drug delivery, widespread use of iontophoresis may be impeded by safety concerns. Even with application at clinically acceptable current densities, iontophoresis-induced skin damage may result in passive permeation of undesirable solutes (37).

CONSIDERATIONS IN EXPERIMENTAL DESIGN

Qualitative and quantitative data obtained from past investigations of follicular delivery have indicated that certain factors should be considered in the experimental design of follicular delivery systems:

- Hair follicle anatomy and circulatory proximity
- Specific targets for drug delivery

- Models for follicular delivery
- Quantitative assessment methods
- Formulation design

Hair Follicle Structure and Biology

In order to develop appropriate models for studying follicular and non-follicular delivery routes, an understanding of the structure and function of the pilosebaceous unit is useful. Design of follicular delivery systems necessitates initially defining the desired targeted area within the hair follicle.

Figure 1 (40) illustrates the hair follicle and associated structures. Of the three primary layers of the hair follicle, the outer root sheath is the most important with regard to drug delivery. Continuity of this layer with the epidermis allows increased surface area for absorption beneath the skin's surface. Depending on the hair type, human hair follicles have a mean diameter ranging from 10 to 70 μ. Sebum released from sebaceous glands consists mostly of neutral, nonpolar lipids, which create a lipid-enriched environment in the follicular canal that may provide a lipophilic pathway for potential drug delivery (2,3,41). In order to deposit drugs into the follicular opening or the sebaceous duct, wetting barriers of the lipophilic environment must first be overcome. With regard to hydrophilic drug delivery, the lower portion of the hair follicle that is in proximity to the dermis represents a much less resistant barrier than the stratum corneum, epidermis and sebaceous duct.

The follicular papilla and the hair matrix are located at the base of the hair follicle, in the bulb, where key cellular interactions may play an important role in hair growth regulation (2,5). Close proximity to capillary networks may also be an important consideration in drug transport from the hair follicle to the systemic circulation. A perifollicular network of capillaries associated with the upper dermal vasculature

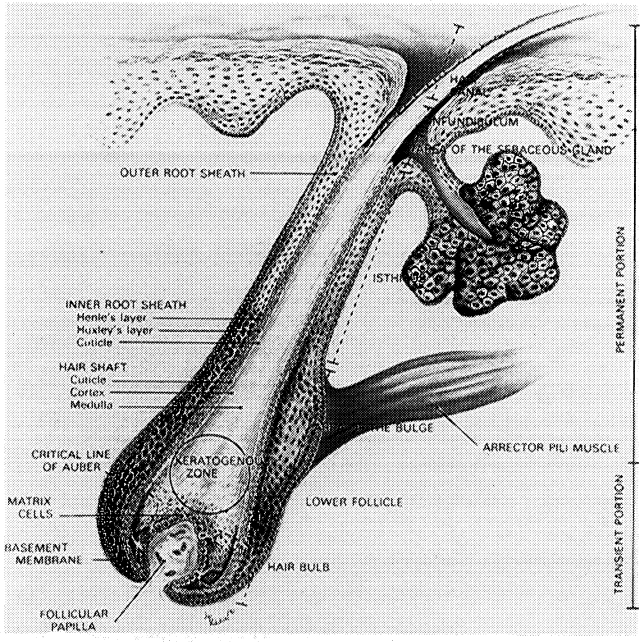

Fig. 1. Hair follicle anatomy (Reprinted from Dermatology in General Medicine, McGraw-Hill).

supplies the upper portion of the hair follicle with blood, whereas the base of the follicle receives its blood supply from the deep dermis and subcutaneous tissue. In humans, sebaceous glands are richly supplied with blood vessels. The density of the capillary network is proportional to the size of the hair follicle and sebaceous gland (2,42).

The Hair Growth Cycle

All hair follicles undergo a species-specific growth cycle of alternating periods of activity and rest, as illustrated in Figure 2 (43). Anagen is the active phase in which the hair matrix cells divide rapidly and migrate upward to form the hair shaft. During the relatively short catagen phase, mitosis ceases and the lower portion of the follicle is largely resorbed through a process of apoptosis (programmed cell death). With completion of this process, the follicle withdraws from the dermal papilla and the follicle passes into the resting phase, telogen. The follicle then returns to anagen, and the hair matrix cells resume division and the lower follicle is reformed. In studying follicular delivery, recognition of a particular stage of hair growth may be important in standardizing experimental conditions (2,4,5,7).

Pilosebaceous Targets for Drug Delivery

Regulatory Receptors

Androgens, in particular testosterone metabolites, exert a dominant hormonal influence on hair growth and sebum secretion by interacting with receptors at several locations, including the follicular papilla and sebaceous glands (2,4,5,7). Androgens act paradoxically in the hair follicle: small, fine-haired follicles gradually transform to large, coarse-haired follicles in axillary, pubic, and beard regions, whereas the opposite process occurs on the scalp of genetically predisposed individuals. Enzymatic conversion of testosterone to more potent target tissue androgens occurs within the hair follicle (4). Manipulation of androgen receptors or blockade of enzymatic reactions within the hair follicle may be a useful strategy in treating androgen-mediated dermatological disorders such as androgenetic alopecia and acne.

The hair follicle growth cycle may be influenced by multiple other factors. Potassium channel openers such as minoxidil and diazoxide have been found to enhance hair follicle growth (44). Receptors for retinoic acid, epidermal growth factor and transforming growth factor have also been identified (45,46).

Bulge Area

Another potentially important site for drug delivery is the mid-follicle bulge area, which has one of the fastest rates of cell division in mammals. Cotsarelis *et al.* (7) found a bulge-like subpopulation of slow-cycling cells at the midportion of the follicle just below the sebaceous gland that share key characteristics of stem cells: they are slow cycling and are located near highly proliferative cells in well-protected, highly vascularized areas. According to the bulge activation hypothesis, which is illustrated in Figure 3, diffusable growth factors from the follicular papilla cause proliferation of the normally slow-cycling bulge cells in early anagen. This

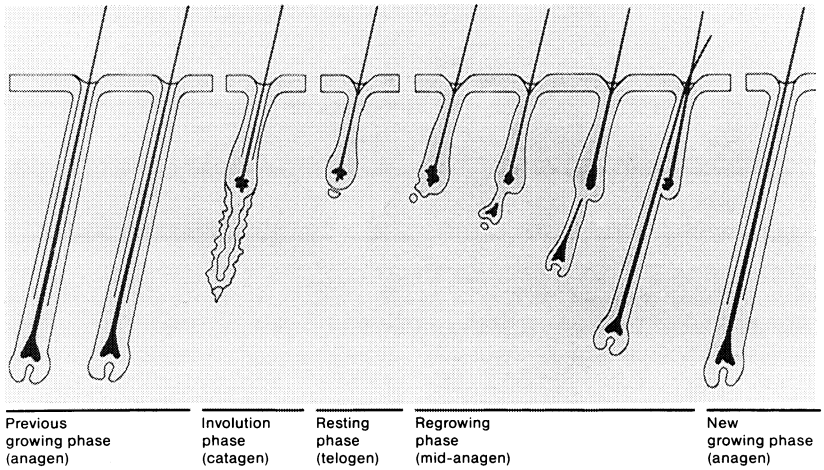

Fig. 2. Growth cycle of hair follicle (Reprinted from Current Concepts, Scope Publications)

highly proliferative process has been speculated to be associated with some forms of skin cancer (7). During telogen, the bulge area is more accessible than follicular papilla to topically applied carcinogens, resulting in greater susceptibility to experimentally induced skin carcinomas. Absence of the inner root sheath results in greater penetration and residence time in telogen follicles as compared with anagen follicles. Recognition of growth cycle phase may be an important consideration in designing follicular delivery systems.

Genes and Immunocompetent Cells

Gibson *et al.* (6) found that the lower part of the follicle, which undergoes resorption during catagen, lacked Class I major histocompatibility complex (MHC) antigen, whereas it was present in the stable upper portion of the follicle. Increased staining for activated macrophages and decreased staining for proteoglycans were also seen in the regressing follicle in catagen, suggesting that proteoglycans may act as protective screens that prevent detection of lack of Class I MHC in anagen. Follicular drug delivery targeting immunocompetent cells may provide potential treatments for immune cell-mediated dermatological disorders such as alopecia areata. Molecular probing within the hair follicle may be useful in delineating regulatory processes, and may ultimately lead to development of powerful targeted follicular delivery systems that incorporate gene therapy (8).

Models for Establishing Delivery Pathways

A major limitation to assessing the distinct contributions of the transepidermal and transfollicular routes has been the lack of a well-characterized pharmacokinetic model. A model is needed that is truly follicle-free, but retains the structural and biochemical properties of normal skin. Genetically-induced hairless rodents that are currently available are not perfect models for a distinct transepidermal route since they are not completely follicle-free. The poorly developed follicles of these animals are very different from the fully developed follicles of hairy animals. The guinea pig

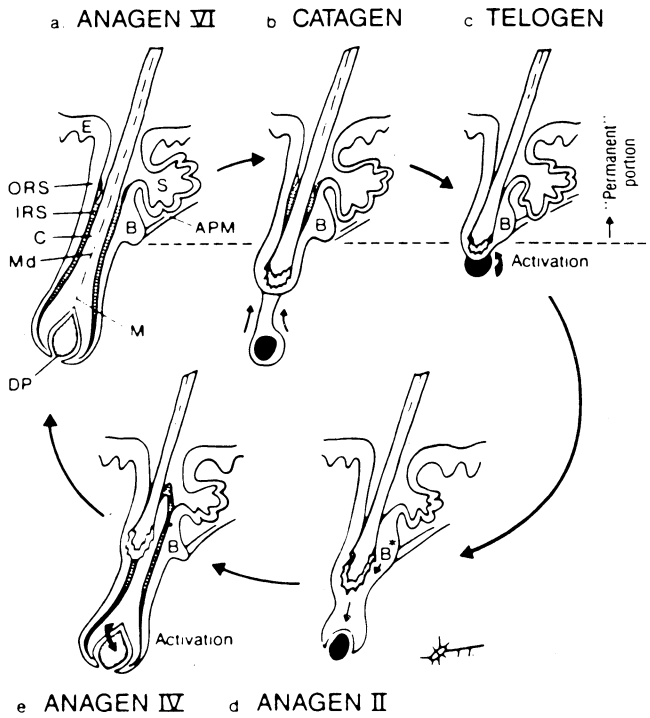

Fig. 3. Hair cycle: The bulge activation hypothesis (Reprinted from Cell, Cell Press) Illustrated are different phases of the hair cycle including (a) anagen VI, (b) catagen, (c) telogen, (d) anagen II, and (e) anagen IV. Different structures are labeled, including the arrector pili muscle (APM), bulge (B), cortex (C), dermal papilla (DP), epidermis (E), inner root sheath (IRS), matrix (M), medulla (MD), outer root sheath (ORS), and sebaceous gland (S). B and B* denote quiescent and activated bulge cells, respectively. Follicular structures above the dashed line form the permanent portion of the follicle; keratinocytes below the bulge degenerate during catagen and telogen and are therefore dispensable. This model defines four major elements involved in controlling the cyclic growth of hair follicles: activation of the bulge by dermal papilla (c), activation of mesenchymal papilla by the growing matrix (e), limited proliferative potential of matrix cells as TA cells (a), and upward migration/movement of the dermal papilla (b).

model used by Wahlberg is also imperfect in that although the area behind the ear of guinea pigs is completely follicle-free, the surface area available is small and impractical for topical delivery studies. Induced follicle-free rodent skin models still must be further characterized to ensure that the resultant skin is not different from untreated skin since even subtle differences may result in different absorption profiles.

Macaque Monkey Model

The stumptailed macaque monkey model may offer some promise in unraveling the mysteries of the human hair follicle. The macaque exhibits a species-specific frontal scalp baldness that coincides with puberty at a rate of nearly 100 percent in both sexes. Interestingly, the hormonal and genetic factors that induce baldness in the macaque seem to parallel those in human androgenetic alopecia, thus making it a relevant model to study (43). Uno (43,47,48) developed quantitative *in vivo* methods for studying hair growth in the stumptailed macaque. Uno and Kurata (47) found that topical application of hypertrichotic drugs, minoxidil and diazoxide, resulted in significant follicular enlargement and hair regrowth in bald macaques. Topical application of an antiandrogen which inhibits 5-alpha-reductase resulted in prevention of baldness in preadolescent macaques (48). Uno (43) studied DNA synthesis *in vitro* in macaque hair follicles by visualizing uptake of tritiated thymidine. Telogen follicles showed no label, whereas anagen follicles showed several labeled cells, suggesting that any compounds that stimulate hair growth act on follicular cells that actively proliferate and produce new anagen follicles. Depending on the desired pharmacological response, recognition of hair growth cycle-dependency may be critical.

Quantitative Assessment Methods

In addition to difficulties in establishing appropriate models, problems associated with follicular skin sectioning and stripping techniques have also made the follicular route difficult to elucidate. Skin must be carefully sectioned to minimize cross-contamination of sections. Incomplete tape stripping may result in the detection of artificially high marker levels in the residual skin. Tape stripping may also result in underestimating follicular deposition if follicular contents are stripped away. Harsh histological fixation techniques and varying visual interpretations have also hindered definition of the transfollicular route. Follicular casting methods are tedious and provide better qualitative than quantitative data.

Formulation Design

Studies have suggested that follicular delivery may be dependent on the physiochemical properties of the drug and/or vehicle (11–13,20,21,25,30). The lipoidal environment of the follicular canal may favor certain drugs and vehicles, and follicular delivery thereby may be enhanced by manipulating formulation factors. Solvents (e.g., ethanol) may be used to delipidize or reorganize the sebum, thereby opening the passageway for drug deposition within the follicle. Wetting agents (e.g., sodium lauryl sulfate) may be useful in decreasing the interfacial tension between hydrophilic drugs and sebum, which may promote mixing in the form of an emulsion, providing a more favorable environment for drug partitioning and absorption. Liposomal systems, upon dehydration, may yield a fluid liquid crystalline state in which bilayers containing drug can partition and pack into the follicular openings. Follicular delivery of hydrophilic molecules may be facilitated via association with polar head groups of liposomal bilayers. Studies have suggested that this mechanism of action is not dependent on the extent of hydrophilic drug entrapment (32). Empty liposomes along with "free" aqueous solution exhibit similar absorption profiles. Following dehydration, extent of entrapment may be inconsequential due to the establishment of a new equilibrium between the drug and bilayers. Recent studies have indicated that particle size of drug carrier systems may also be an important consideration in designing follicular delivery systems (1,26). Delivery systems with optimally sized particles may allow preferential targeting to the hair follicle rather than through the stratum corneum lipid-keratinocyte matrix.

CONCLUSIONS

The contribution of the pilosebaceous unit to localized and percutaneous absorption has probably been underestimated in the past. Greater understanding of the control mechanisms which govern this complex structure may accelerate rational design of hair follicle-specific drug delivery systems. Genetic engineering techniques may enable the generation of a truly follicle-free skin model that would allow for direct comparison between follicular and non-follicular delivery routes. Formulation factors such as drug and vehicle physiochemical properties and particle size may be important considerations in optimizing follicular delivery. Targeted drug delivery to the hair follicle could have important consequences in the treatment of hair follicle-associated dermatological disorders. However, in order to truly ascertain the significance of follicular delivery, appropriate models and quantitative methods must still be developed.

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