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Brief/Technical Note

Drug Targeting to the Hair Follicles: A Cyclodextrin-Based Drug Delivery

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INTRODUCTION

Topical drug delivery systems that selectively target hair follicles and sweat glands are of interest to both the pharmaceutical and the cosmetic industry not only to treat dermal complications but also for systemic drug delivery (1,2). Possible dermal applications include treatment of acne, inflammation, and hair growth disorders. Hair follicles are tube-like pockets of the epidermis that extend through most or all of the depth of the skin and enclose a small papilla of dermis in their base. The hair bulb, which lies at the base of the hair follicle, is a structure of actively growing cells that eventually produce the long fine cylinder of a hair. Attached to the follicle are one or more sebaceous glands. The hair shaft is enveloped in an inner root sheath that consists of keratin-rich cells that have migrated from the growing cells that line the inside of the hair follicles (Fig. 1). Microparticulate vehicles, like liposomes (2,3) and nanoparticles (4), have been shown to deliver drug molecules much deeper into the hair follicles than conventional formulations like creams and ointments. It has been shown that liposomes and nanoparticles with diameter between 300 and 750 nm penetrate preferentially into the hair follicles (5) and that titanium dioxide particles with a diameter of about 100 nm penetrate into the hair follicles (6).

Cyclodextrins (CDs) are cyclic oligosaccharides that have in recent years been introduced to the pharmaceutical industry as novel enabling excipients, mainly as solubilizing complexing agents for enhanced drug bioavailability (7,8). CDs consist of six (αCD), seven (βCD), eight (γCD), or more α-1,4-linked α-D-glucopyranose units forming a somewhat truncated cone. The hydroxy groups are oriented towards the cone exterior, making the external surface hydrophilic, while the central cavity is lined by the carbons and ethereal oxygens of the carbohydrate skeleton, making it somewhat hydrophobic. CDs form inclusion complexes by taking up a lipophilic drug molecule, or more frequently some lipophilic moiety on the drug molecule, into the lipophilic central cavity. Although such inclusion complexes are probably the most common form of drug/CD complexes, the hydroxy groups on the outer surface of the CD molecule are able to form hydrogen bonds with other molecules, and CDs can, like non-cyclic oligosaccharides and polysaccharides, form water-soluble non-inclusion complexes with lipophilic water-insoluble drugs (9–11). In saturated aqueous solutions, drug/CD complexes frequently consist of a mixture of inclusion and non-inclusion complexes. CDs and CD complexes are also known to self-associate to form nanoscale aggregates (12–14), and these aggregates are thought to be able to solubilize lipophilic drug molecules in micellar-like fashion (15). The diameter of these cyclodextrin aggregates has been determined to be about 100 to 600 nm (13,14,16). Thus, it is possible that drug/CD complexes will, like liposomes and nanoparticles, selectively target the hair follicles. The purpose of this study was to evaluate follicular targeting potential of drug/CD complexes using curcumin as a sample compound. Curcumin, a lipophilic fluorescent dye, is a non-toxic natural compound with very limited solubility in water. Curcumin forms water-soluble cyclodextrin complexes (17). Previously, curcumin has been used to detect follicular penetration from liposome formulations by laser scanning microscopy using pig skin as a model tissue (3,18,19).

MATERIALS AND METHODS

Materials

2-Hydroxypropyl-γ-cyclodextrin (HPγCD) with molar substitution of 0.6, MW 1,576 Da, was purchased from Wacker Chemie (Burghausen, Germany). Mineral oil (liquid paraffin) was purchased from Bufa (Utgeest, Holland). Pure curcumin was synthesized using the procedure given by Pabon (17,20). All other chemicals were commercially available substances of reagent or analytical grade.

Preparation of the Sample Solutions

Curcumin (1.5 mg/ml) was dissolved in aqueous 10% (*w*/*v*) HPγCD solution. Curcumin (5 mg/ml) solution in mineral oil was used as a positive control (19). Both solutions were placed on an orbital shaker overnight protected from

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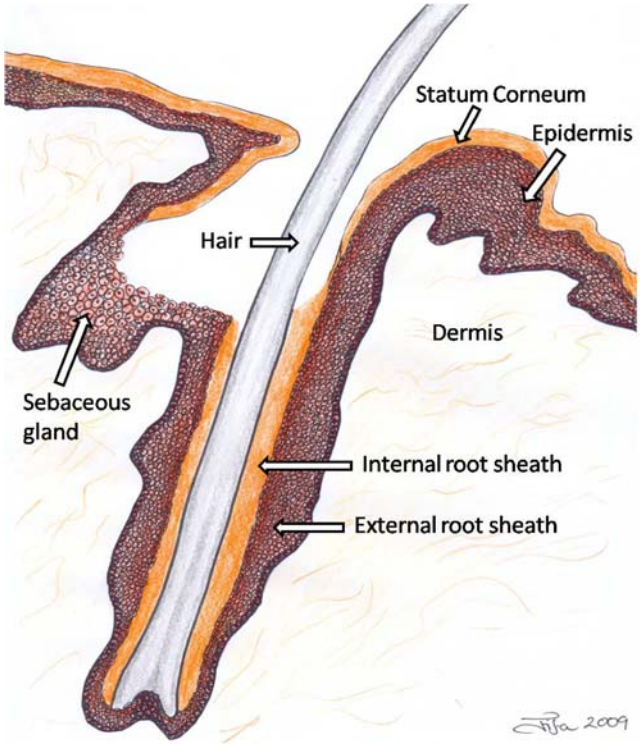

Fig. 1. A schematic drawing of a hair follicle

UV-induced degradation before they were applied to the porcine skin.

Preparation of Porcine Skin and Microscopical Examinations

Fresh porcine skin was excised from the front of the neck of adult female pigs (2 years old, weight 120 to 200 kg) in a slaughterhouse at Grisabol (Saltvik, Iceland) by a veterinarian. The skin was cleaned with running water and then cut into pieces approximately 1.5×1.5 cm in size. The hairs were shortened by cutting with scissors and some of the subcutaneous tissue was removed with surgical scissors, leaving skin pieces with average thickness of 0.5±0.06 cm. The sample solution (3 μl), aqueous curcumin/HPγCD solution (test solution), or curcumin solution in mineral oil (positive control) were distributed on top of the epidermis and left on for 5 min before the skin pieces were frozen in liquid nitrogen and stored at −70°C. The negative control was untreated porcine skin. The frozen samples were placed on a small metal plate, embedded in tissue-tek freezing medium, and left to freeze on to the small metal plate. Then, the sample was cut vertically through the horny layer and dermis into 10-μm-thick slices by a microtome. The samples were placed on a microscopic slide and treated with hematoxylin (nuclear staining) for 30 s, washed, and left to dry. The slices were viewed with fluorescence microscopy (Leica DMLB with attached Leica camera, Bensheim, Germany) (3,18,19).

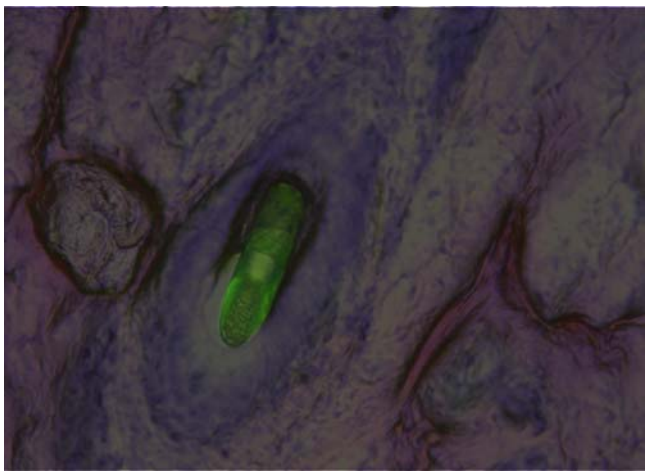

Fig. 2. Cross-section of untreated porcine skin from the negative control sample. The porcine hair has autofluorescence (*green*)

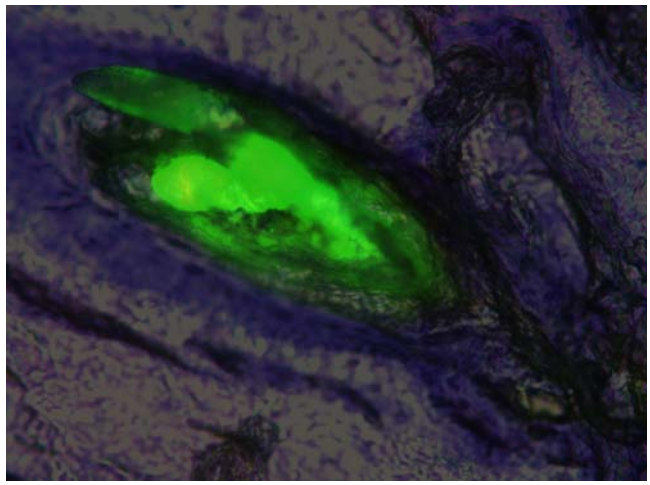

Fig. 3. Cross-section of porcine skin treated with solution of curcumin in mineral oil (the positive control) shown in the cross-section of a hair follicle

RESULTS AND DISCUSSION

The ability of aqueous cyclodextrin solution to deliver lipophilic molecules to hair follicles was investigated using

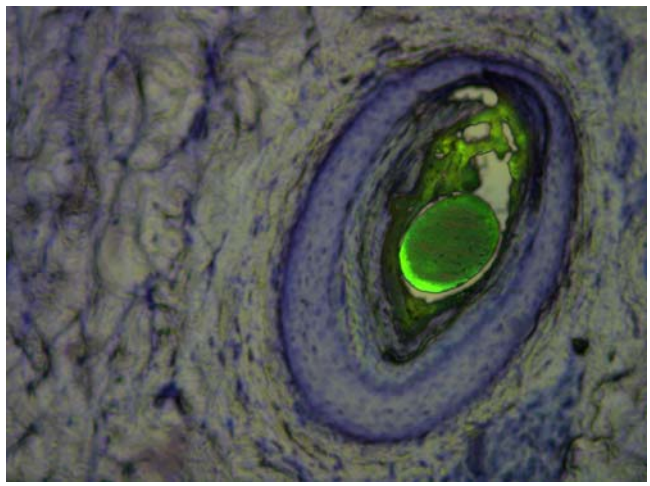

Fig. 4. Cross-section of porcine skin treated with solution of curcumin in aqueous HPγCD solution (the test solution) shown in the horizontal cross-section of a hair follicle. Curcumin causes green fluorescence in the epidermal cells that form the internal root sheath around the cross-section of the porcine hair

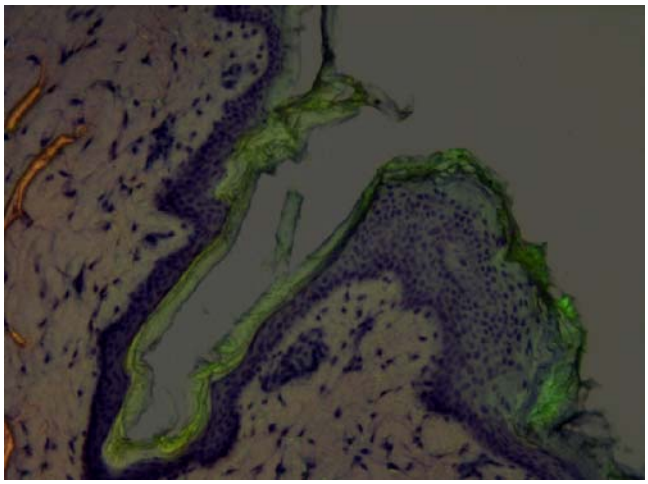

Fig. 5. Vertical cross-section of porcine hair follicle treated with curcumin dye from an aqueous HPγCD vehicle. Curcumin green fluorescence is detected in the internal root sheath all the way down to the base of the hair follicle

curcumin as a sample compound dissolved in pure aqueous HPγCD solution. Curcumin solution in mineral oil was used as a positive control and untreated skin as negative control. The untreated skin, the negative control, did not display fluorescence except some autofluorescence from the porcine hairs (Fig. 2), and the mineral oil solution, the positive control, showed strong fluorescent signal along the stratum corneum (Fig. 3). On the other hand, curcumin dissolved in the aqueous HPγCD solution was detected around the autofluorescent hair (Fig. 4) and deep in the hair follicle (Fig. 5). Curcumin/HPγCD was both located in the subcutis of the porcine skin and also in the hair sheath all the way down to the base of the hair (i.e., hair bulb). The results show that it is possible to transfer curcumin into the hair follicle with the hydrophilic HPγCD. Most of the curcumin seems to be located in the epidermal cells that form the internal root sheath. Other investigators have shown that curcumin in an oil solution is mainly detected on the surface of the skin, whereas curcumin applied in oil/water (o/w) emulsion penetrates deeper into the stratum corneum (21). Even deeper penetration was observed when curcumin was incorporated into liposomes, especially liposomes with positive or weak negative surface charge, or o/w microemulsions (3,22). These results agree well with our observations. HPγCD is a large (MW 1,576 Da) hydrophilic ($\log P_{\text{octanol/water}} < -10$) molecule that does not permeate healthy skin. Studies have shown that hydrophilic CDs like HPγCD enhance drug delivery through unstirred water layers at membrane surfaces (23,24). Furthermore, studies have shown that in aqueous solutions, CDs and CD complexes spontaneously form nanoparticles that are of optimal size for follicular drug delivery (4). These observations might explain why hydrophilic CDs selectively delivery lipophilic molecules to the hair follicles.

SUMMARY AND CONCLUSIONS

The investigation shows that curcumin, a lipophilic water-insoluble compound, solubilized in an aqueous HPγCD vehicle is delivered deep into the skin hair follicles. The HPγCD complex appears to selectively target the hair follicles. Furthermore, a hydrogel formulation containing a complex of βCD and retinoic acid was found to be very effective in treating acne vulgaris, a site-specific infection with primary location in hair follicles, in patients (25). These results indicate that hydrophilic CDs might be excellent vehicles for targeted drug delivery for lipophilic drugs to hair follicles and other microscopic openings on the skin surface.

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