RESEARCH ARTICLE

Adapalene Microemulsion for Transfollicular Drug Delivery

GAURAV BHATIA, YINGCONG ZHOU, AJAY K. BANGA

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, Georgia 30341

Received 6 March 2013; revised 20 April 2013; accepted 8 May 2013

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23627

ABSTRACT: The aim of this study was to develop a microemulsion formulation of adapalene for transfollicular delivery. A pseudoternary phase diagram was developed for microemulsion consisting of oleic acid as oil phase, tween 20 as surfactant, Transcutol® as cosurfactant, and deionized water. Differential tape stripping and confocal laser scanning microscopy were performed to determine the penetration of microemulsion through hair follicles. Transmission electron microscopy, dynamic light scattering, polarizing light microscopy, and differential scanning calorimetry were performed to characterize the microstructures of microemulsion. The pH and viscosity of the microemulsions were also determined. Permeation studies were carried out *in vitro* on porcine ear skin over a period of 24 h using Franz diffusion cells. The drug penetration in the hair follicles increased from 0.109 ± 0.03 to 0.292 ± 0.094 μg, as the microstructure of microemulsion shifted from oil-in-water to bi-continuous, with increase in water content of microemulsion. Confocal laser scanning microscopy images suggested that hair follicles provided the path for transfollicular permeation of adapalene microemulsion. These results suggest that microemulsion penetrated through hair follicles and are promising for transfollicular drug delivery. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: adapalene; microemulsions; calorimetry (DSC); transfollicular drug delivery; skin; permeability; differential stripping; microscopy

INTRODUCTION

Transdermal drug delivery provides many benefits like enhanced patient compliance and reduced first pass metabolism of drug and, avoids gastric irritation.[1] In recent years, transfollicular pathway has been found to be important for percutaneous absorption of topically applied drugs.[2] Hair follicles (HF) represent an invagination of epidermis extending deep into the dermis and providing a good surface area for potential absorption. The density of HFs on the face and scalp can be as much as 10% of total skin surface, thereby creating a higher local surface area that allows greater absorption of drug through this route[3] also the follicular duct is considered as the target site for adapalene.[4] The HFs can also serve as a reservoir for topically applied drugs.[5] HFs may also provide enhanced absorption as they are surrounded by a network of blood capillaries.[6] Therefore, HFs have great potential for delivery of drug into viable skin layers, and in particular microparticulate systems have been used for follicular drug delivery.[7]

Microemulsion (ME) is defined as a dispersion consisting of oil, surfactant, cosurfactant, and aqueous phase, which is optically isotropic and thermodynamically stable system.[8] MEs are considered as an efficient carrier for transdermal delivery as they offer increased drug solubility potential, enabling a high concentration gradient toward skin. MEs have several advantages such as long-term stability, ease of preparation, and considerable capacity for solubilization of variety of drug molecules, and components of ME can act as permeation enhancer by disrupting the stratum corneum (SC) structure and enhancing permeation of drug through the skin.[9]

Adapalene (6-(3-(1-adamantyl)-4-methoxyphenyl)-2-napthoic acid is a synthetic analog of retinol (vitamin A) used for the treatment of acne. Adapalene (Fig. 1; pKa = 4.23 and log P = 8.04)[4] was used as a model drug for the study as the molecule is inherently fluorescent and stable, which allows the quantification and visualization of the adapalene in the formulation and in the skin.

The aim of the study was to develop and characterize adapalene-loaded ME and to evaluate the

Correspondence to: Ajay K. Banga (Telephone: +678-547-6243; Fax: +678-547-6423; E-mail: banga_ak@mercer.edu)

Journal of Pharmaceutical Sciences

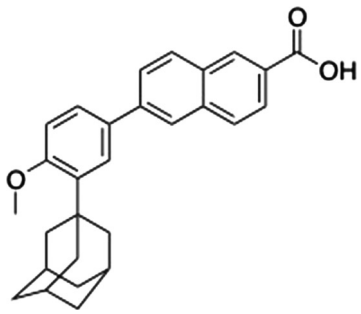

Figure 1. Structure of Adapalene.

permeation of ME through transfollicular route to determine whether the HFs can represent a penetration pathway for the drug into the skin. ME consisting of oleic acid as oil phase and Tween-20 as surfactant are also well tolerated on skin.[10–12] Differential tape stripping was performed to determine the amount of drug that penetrated into the HFs. This technique combines the tape stripping (removal of SC layer by layer), followed by cyanoacrylate skin surface biopsies (removing the content of follicular infundibulum).[13] Confocal laser scanning microscopy (CLSM) was also performed to determine the penetration of adapalene through HFs.

MATERIALS AND METHODS

Materials

Adapalene was purchased from Haorui Pharma Chem-Inc. (California). Polyoxyethylene 20 sorbitan monolaurate (Tween 20®) was obtained from Fisher Scientific (New Jersey), diethylene glycol monoethyl ether (Transcutol®) from Gattefosse (Lyon, France). Deionized water (18.2 MΩ cm) was used. All the other chemicals and solvents were of analytical reagent grade.

Phase Diagram Construction and ME Preparation

Pseudoternary phase diagram was constructed using the water titration method[12] to determine the concentration range of the components used for the formation of ME. Oleic acid was used as the oil phase, Tween 20 was used as the surfactant, and Transcutol was used as cosurfactant. The ratio of oleic acid to the mixture of surfactant and cosurfactant was varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The mixture of oil, surfactant, and cosurfactant was titrated drop-by-drop with deionized water, under moderate stirring. After being equilibrated the mixture was assessed by visual characterization. Samples that remained transparent and homogenous after vigorous vortexing were assigned a monophasic area in phase diagram. Turbidity was considered as indication of phase separation.

After the ME regions in phase diagram were identified, the ME formulations were selected at different component ratios as shown in Table 1. Drug loaded ME was prepared by dissolving adapalene (0.1% w/v) gradually in oil/surfactant–cosurfactant mixture. After complete solubilization, the mixture was titrated with deionized water under moderate stirring. Adapalene (0.1% w/v) dissolved in oleic acid was used as the control for *in vitro* permeation and confocal microscopy study.

Polarized Light Microscopy

Polarized light microscopy (Leica DM 750) [Buffalo Grove, Illinois] was preformed to verify the isotropic nature of ME. A drop of ME sample was taken on a glass slide and covered with coverslip and was observed under cross-polarized light.

Transmission Electron Microscopy

The morphology of adapalene ME was studied using transmission electron microscopy (Hitachi, H-7500, Illinois). The ME sample was deposited on a copper 300 mesh grid, coated with Formvar and carbon (Electron Microscopy Sciences, Fort Washington, Pennsylvania) and allowed to stand for 10 min after which any excess fluid was absorbed in a filter paper. Before examination, one drop of 1% methylamine tungstate was applied and allowed to dry for 5 min.

Droplet Size Measurement

The droplet size and polydispersity index of the ME were determined by dynamic light scattering (DLS; Zetasizer Nano Zs; Malvern Instrument Inc., Malvern, Worcestershire, UK). Samples (1 mL) were loaded into 1 cm^2 cylindrical cuvettes and placed in a thermostated chamber at 25°C. Scattering

Table 1. Composition and Microstructure of Microemulsion Formulation

	Components (% v/v)					
Microemulsion (ME)	Oleic Acid	Tween 20	Transcutol®	Deionized Water	Microstructure	Refractive Index
ME A	19	38	38	5	W/O	1.49
ME B	18	36	36	10	W/O	1.44
ME C	17	34	34	15	W/O	1.41
ME D	15	30	30	25	Bi-continuous	1.36