Use of Minipig Skin Biopsy Model as an Innovative Tool to Design Topical Formulation to Achieve Desired Pharmacokinetics in Humans

AMITAVA MITRA,[1] AQUILES LEYES,[1] KIMBERLY MANSER,[1] BRAD ROADCAP,[2] CHRISTINE MESTRE,[3] DANIEL TATOSIAN,[2] LAN JIN,[1] NAOTO UEMURA[4]

[1]Pharmaceutical Sciences and Clinical Supply, Merck & Co. Inc.
[2]Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck & Co. Inc.
[3]Safety Assessment and Laboratory Animal Resources, Merck & Co. Inc.
[4]Clinical Pharmacology, Merck & Co. Inc.

Received 14 November 2014; revised 14 January 2015; accepted 20 January 2015

Published online 17 February 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24383

ABSTRACT: *In vitro* cadaver skin permeation studies are often conducted to characterize the permeation profile of compounds for dermal delivery. However, its utility could be limited in the case of topical products because of lack of reliable prediction of *in vivo* skin kinetics. In this paper, the use of *in vivo* skin biopsy data to guide topical formulation development is described. A formulation was developed by compounding MK-0873, a phosphodiesterase 4 (PDE4) inhibitor, into a commercially available cream base. The cream was characterized by skin pharmacokinetic studies in minipigs, which demonstrated that MK-0873 concentrations in the epidermis and dermis were substantially higher than the IC80 for human whole blood PDE4 inhibition of ~200 nM, suggesting that cream should provide sufficient skin exposure to assess clinical efficacy. In toxicological studies, after 1 month repeat application in minipigs minor dermal irritation and minimal systemic exposure were observed. Based on these preclinical data, the cream formulation was chosen for single rising dose clinical studies, where plasma levels of MK-0873 were mostly below the LOQ, whereas skin biopsy concentrations ranged from 6.5 to 25.1 μM. These data suggested that minipig skin biopsy model can be a valuable tool to assess performance of topical formulations and guide formulation development. J Pharm Sci 104:1701–1708, 2015
Keywords: skin penetration; biopsy; minipig; topical; pharmacokinetics; human; absorption; disposition; skin; clinical pharmacokinetics

INTRODUCTION

Phosphodiesterase 4 (PDE4) is the predominant cyclic AMP degrading enzyme present in a variety of inflammatory cells including eosinophils, neutrophils, macrophages, T cells, and monocytes.[1] In addition, this enzyme is expressed in nonimmune cells such as keratinocytes and fibroblasts. Because of the broad anti-inflammatory and immunomodulatory action of PDE4 inhibitors, it has been proposed that PDE4 inhibitors might also be efficacious for skin disorders such as psoriasis and atopic dermatitis.[2,3] These PDE4 inhibitors displayed strong anti-inflammatory action in models of allergic contact dermatitis in mice, in the arachidonic acid induced skin inflammation in mice, and in ovalbumin-sensitized guinea pigs. The determination of cytokines in skin homogenates revealed that both Th1 as well as Th2 cytokines are suppressed by PDE4 inhibitors, indicating an anti-inflammatory activity in both the Th2-dominated acute phase as well as the Th1-dominated chronic phase of atopic dermatitis. Because of the suppression of Th1 cytokines, activity can also be expected in psoriasis.[2] Consequently, several PDE4 inhibitors are currently in clinical development for the treatment of psoriasis by several companies.[4,5] However, the clinical utility of PDE4 inhibitors can be limited by the occurrence of mechanism-associated side effects such as headache, nausea, and emesis, which often limits the maximally tolerated dose.[6,7]

MK-0873 is a Merck PDE4 inhibitor,[8] which was selected for development as an antipsoriatic agent. It was decided that a topical formulation was to be developed with the goal of delivering adequate levels of the drug to the epidermis and dermis, whereas minimizing systemic exposure and consequently the risk of systemic side effects. Because the formulation development was aimed at increasing MK-0873 concentration in the skin with little or no systemic absorption, it was important to develop preclinical *in vitro* and *in vivo* tools that could provide detailed information on MK-0873 uptake and distribution in the skin after topical administration. Hence, method to reliably determine concentration of MK-0873 in the epidermis and dermis after *in vitro* skin permeation studies was developed. In addition, punch biopsy and skin sample analysis methodologies were developed to accurately quantitate MK-0873 concentration in the skin, during pharmacokinetic (PK) studies in minipigs. These tools could then be used to guide development of a robust topical formulation for clinical evaluation. Furthermore, the clinical study was designed such that skin biopsy samples could be collected and local skin concentrations could be measured in human, in addition to plasma concentration, so as to assess whether MK-0873 levels in the skin were adequate to achieve efficacy. These data could also be used in further refinement of the formulation, if it was thought that MK-0873 concentrations in the skin were not adequate. It should be noted that the use of biopsy to assess skin PKs

Correspondence to: Amitava Mitra (Telephone: +1-215-652 8551; Fax: +1-215-993-1245; E-mail: amitava_mitra@merck.com)
Naoto Uemura present address is Department of Clinical Pharmacology and Therapeutics, Oita University Faculty of Medicine, Japan.

Journal of Pharmaceutical Sciences, Vol. 104, 1701–1708 (2015)

for topical[9–11] and transdermal[12] products has been published previously. However, in this report, we demonstrate the application of skin biopsy data in preclinical and clinical PK studies for rational design of topical formulation.

In this paper, we report the development of a cream formulation, *in vitro* MK-0873 penetration in human cadaver skin, and PK studies in minipigs evaluating drug disposition in the skin and systemic exposure of MK-0873 after topical application of the cream. Additionally, skin toxicity and toxicokinetics data in minipigs are described. Finally, local skin concentration and systemic PK data after topical application of the cream to healthy human volunteers are summarized. The data presented here, along with a previously published report,[13] could be helpful in providing an integrated view on preclinical and clinical models to assess skin uptake and distribution of topically applied drugs.

MATERIALS AND METHOD

Formulation Preparation and Characterization

The topical formulation utilized in these studies was prepared by adding appropriate amounts of a concentrated solution of MK-0873 in benzyl alcohol (BA) (200 mg/mL) to a commercially available topical base (Dermabase™; Perrigo (Allegan, Michigan)).[14] Creams at 0.5%, 1.0%, and 2.0% potency were prepared for *in vitro* and *in vivo* testing. Preparations at 20 g scale or less were made by geometric dilution of the Active Pharmaceutical Ingredient (API) solution into Dermabase with manual mixing in a mortar and pestle. Supplies to support toxicology studies in minipigs were prepared using a bench-top mixer with a whisk attachment. current Good Manufacturing Practices (cGMP) drug product for skin and systemic PK studies in humans were prepared in potencies of 0.05%, 0.5%, and 2% using a Becomix mixer. The maximum feasible concentration (MFC) of MK-0873 was dictated by the capacity of the Dermabase to incorporate BA with no visible base/diluent phase separation and adequate viscosity to allow application in the required quantities to achieve the desired dose. Chemical and physical stability of the formulations was evaluated under accelerated conditions (30°C and 40°C) and at 5°C as a control.

In vitro Skin Permeation Study

The permeation of MK-0873 from the cream formulations across human cadaver skin was assessed in an automated Franz diffusion system (Logan Instruments (Somerset, New Jersey); receptor fluid volume of 12 mL, donor cell capacity of 4 mL, and diffusion area of 1.77 cm^2). The general procedure for *in vitro* skin penetration studies has been described previously.[15,16] Briefly, the cadaver skin (NY Firefighters Skin Bank (New York, New York)) of 200 μm thickness was thawed at room temperature and cut into circular pieces in Phosphate buffered saline (PBS) buffer. A stir bar was added to each receptor chamber. The donor and receptor chambers were clamped together and filled up with 1× PBS buffer with 0.02% sodium azide. The temperature of the Franz diffusion cells were controlled by a circulating water jacket such that the surface temperature of the skin was maintained at 32.5 ± 1°C.[17] The skin was then positioned, mounted, and clamped between the donor and the receptor chambers. The skin was first equilibrated and hydrated with the receptor buffer for 1 h. Tritiated (H^3) water (500 μL/cell, at 0.4 μCi/mL) was then applied onto the skin for 5 min and then removed. One milliliter samples were withdrawn and replaced by fresh buffer by auto sampler. The skin integrity was considered acceptable if the H^3 water permeation was <2 μL/cm^2. All skin samples used in the experiment had to meet this criterion to be considered to have acceptable integrity. Then, the Franz cell system was washed three times, by replacing 2 mL buffer in donor chamber and 13 mL in the receptor chamber each time, for 15 min. Following these washes, the skin was further equilibrated for 24 h before dosing. The formulation was dosed on the skin at 10 μL/cm^2 using a displacement pipette. Samples (1.5 mL/time point) were withdrawn for analysis and replaced with fresh medium at predetermined time intervals up to 72 h. At the end of the study, the skin surface was washed three times with 10% soap, and the wash samples were collected in vials. Subsequently, the epidermis and dermis were separated mechanically by gently pulling off the epidermis with forceps, with the dermis exposed underneath. The epidermis and dermis samples were then collected in preweighed glass vials and weighed again to determine the weight of the samples. Receptor fluid, wash samples, epidermis, and dermis were stored at –70 °C until analysis by liquid chromatography-tandem mass spectrometry (LC–MS/MS).

Single Application Dermal and Systemic PK Studies in Minipigs

The dermal and systemic PK profile of MK-0873 was evaluated in three female Gottingen minipigs after application of the 2% potency cream at a dose of 2 mg/cm^2. Prior to start of the study, the hair on the application site was trimmed. On the day of study, the application site was washed with soap and water. Subsequently, preweighed amount of the 2% cream needed to deliver a dose of 2 mg/cm^2 was applied on 10% of body surface area, using premarked area on each minipig's back. The application area was then covered with cotton sheet and bandaged. After 24 h postapplication, the application area was washed with soap and water to remove the excess cream. A prespecified area within the application site was wiped with alcohol and ten successive tape strippings were conducted to remove the stratum corneum, using 22 mm skin sampling discs (D-Squame® from CuDerm Corporation (Dallas, Texas)). Each tape strip was collected individual tubes, weighed and frozen at –70°C until analysis. Subsequently two 4-mm biopsy punches were taken from the tape stripped area. Hence, these biopsy samples represented viable epidermis and dermis. The biopsy punches were washed with normal saline, weighed and frozen at –70°C until analysis by LC–MS/MS. The biopsy sites were sutured. The minipigs were anesthetized with 8 mg/kg of telazol prior to the biopsy procedure. The tape strip and biopsy samples were collected at predetermined time points up to 7 days. Plasma samples were also taken at prespecified time intervals during the study to assess systemic exposure. All studies were conducted under a protocol approved by the Merck IACUC.

Area under the curve ($AUC_{0-168\,h}$), observed maximum plasma concentration (C_{max}), and time of C_{max} (T_{max}) were calculated using the linear trapezoidal, noncompartmental model in WinNonLin. Plasma concentration values below LOQ were set at 0 for PK calculation purposes.

Five Days Repeat Application Dermal and Systemic PK Studies in Minipigs

To evaluate dermal and systemic PK profile of MK-0873 after multiple dose, a 5-day repeat application study was conducted