Topical minocycline formulations: Evaluation and comparison of dermal uptake efficacy

Diana Lac, Maiko Hermsmeier*, Xin Chen, Noymi Yam, Akira Yamamoto, Susan Huang, Tanvee Sawant, Kin F. Chan, Usha Nagavarapu*

BioPharmX, Inc., 115 Nicholson Lane, San Jose, CA 95134, USA

ARTICLE INFO

ABSTRACT

Acne vulgaris is a clinically distinct skin condition with evidence suggesting that inflammation plays a critical role in the pathogenesis of this disorder. Treatment of severe inflammatory acne often involves the use of oral antibiotics, sometimes in combination with topical products. Oral antibiotics often result in systemic side effects and the risks of antibiotic resistance, but no commercial topical minocycline is currently available. We have developed a unique, stable, hydrophilic topical gel formulation with fully solubilized minocycline (MNC-H). Minocycline delivered in our hydrophilic gel remained more stable *in situ*, resulting in less degradation product (4-epiminocycline) than a lipophilic formulation (MNC-L). The hydrophilic nature of our formulation enabled 2–3 fold increase in delivery into the skin *ex vivo* compared to a lipophilic counterpart, mostly seen in the epidermis and pilosebaceous units. The lipophilic formulation also appeared to be more occlusive, resulting in higher sebum production in minipigs, which may exacerbate acne vulgaris. As our results indicate, a 1, 2% minocycline hydrophilic gel may deliver sufficient drug ($> 15\,\mu g/g$) to potentially demonstrate clinical efficacy. These findings suggest that topical hydrophilic minocycline gel may provide a novel tool for topical acne therapy.

1. Introduction

Acne vulgaris primarily affects individuals during their teenage years although it can persist into adulthood. Globally, acne is the 8th most common disease, which affects approximately 650 million people, or about 9.4% of the population (Vos et al., 2012). In the United States alone, acne affects 40–50 million people each year, with approximately 10 million suffering from moderate-to-severe acne. The commonly known features involved in the pathogenesis of acne are androgen-stimulated seborrhea, hyperkeratinization, obstruction of the follicular epithelium, proliferation of *Propionibacterium acnes (P. acnes)*, and inflammation (Kirschbaum and Kligman, 1963) (Zouboulis, 2004). Acne has a significant negative impact on the quality of life and often results in permanent physical (scarring) and emotional (self-consciousness, depression, and social isolation) trauma to those who suffer from it. Early and effective treatment is important to mitigate the long-term physical and emotional damage associated with acne (Goodman, 2006).

Minocycline is an antibiotic currently available only in standard and extended release oral dosage forms for treating various bacterial infections (Pawelczyk and Matlak, 1982) (Mandaogade et al., 2004). Because of its known anti-inflammatory properties, it has been particularly successful in the treatment of non-nodular, moderate-to-severe inflammatory acne vulgaris (Torok, 2013). The effectiveness of oral minocycline and its safety has previously been well proven in both animals and humans (Fleischer et al., 2006).

Oral minocycline, widely used for many indications besides the treatment of acne, has severe side effects associated with its use (Garner et al., 2012). The most common of these include hyperpigmentation (Pecina and Pittelkow, 2011), dizziness and gastrointestinal side effects. Additionally, the development of bacterial resistance to clindamycin and other antibiotics commonly used to treat acne has become a growing problem as a result of increased use of these drugs in recent years. To alleviate some of these issues, the topical use of low dose minocycline is a potential alternative. Topical minocycline has significantly lower systemic exposure of minocycline in comparison to orally administered minocycline. This lower systemic exposure is expected to reduce the severity or incidence of side effects. Unfortunately, there is no commercially available topical minocycline.

Some challenges in the development of a topical form of minocycline include solubility, maintaining its stability, and efficiently delivering it to the targeted tissue area. Skin penetration of active ingredients is influenced by a continuously changing equilibrium between the active ingredients, vehicle, and skin. With careful selection of the vehicle, the skin penetration of the active ingredient can be optimized (Mbah et al., 2011) (Nair et al., 2013). Most topical systems require prolonged occlusion of the skin by a cream or lotion. This occlusion of the skin has been found to increase the pH and temperature and reduce oxygenation of the skin, as well as trap moisture and sweat, which creates a humid environment. These humid conditions are favorable for bacterial growth, especially *P. acnes*, which could worsen acne vulgaris (Paudel et al., 2010). Attempts have been made to formulate topical lipophilic solutions of minocycline to ensure its stability (Salman et al., 2014). Unfortunately, a lipophilic environment in which minocycline could be kept stable presents limited delivery to target pilosebaceous units in the skin as it results in lower solubility of the minocycline. Consequently, increased dosages are required in order to deliver minocycline at efficacious levels, resulting in unnecessary superficial accumulation that causes cutaneous staining when minocycline degrades. Lipophilic formulations also contain hydrophobic substances, which can produce an occlusive barrier that limits drug penetration and can be counterproductive in the fight against *P. acnes* as previously discussed.

We have developed stable and potent topical hydrophilic minocycline gel formulations (MNC-H) that have the ability to penetrate the skin and directly target the epidermis and the pilosebaceous unit, thereby delivering a local, targeted treatment of acne vulgaris. A phase 2b clinical trial of BPX-01 for the treatment of moderate-to-severe acne vulgaris further confirmed that this formulation is safe and effective (Alexis et al., 2018).

In this article we present studies with evidence that support effective delivery of MNC-H to cutaneous targets in the epidermis and pilosebaceous unit where *P. acnes* typically reside. The topical hydrophilic minocycline gel formulations were non-occlusive and non-irritating to the skin and does not have significant systemic absorption. Together these studies establish that local topical application of a minocycline gel may be clinically efficacious while avoiding systemic side effects and, potentially minimizing systemic antibiotic resistance.

2. Material and methods

2.1. Materials

Minocycline hydrochloride was the active pharmaceutical ingredient (API) and was purchased from Euticals SpA (Lodi, Italy). The excipients were purchased from Spectrum Chemical (New Brunswick, NJ). Deuterated minocycline (minocycline-d6) was purchased from Toronto Research Chemicals (North York, ON, Canada).

2.2. Formulations

Topical lipophilic minocycline formulations (MNC-L) were made as described in literature using common oils (Salman et al., 2014) (Tamarkin, 2016). Soybean, coconut and mineral oils were mixed together and warmed at 60–70 °C and all other excipients (cyclomethicone, cetostearyl alcohol, stearic acid, myristyl alcohol, hydrogenated castor oil, beeswax, stearyl alcohol, benzyl alcohol, silicon dioxide) were then added and let cool to 32 °C. Minocycline-HCl was added and mixed until homogenous. The resulting formulation was stored at room temperature, existing as a solid. For use, the formulation was heated to 32 °C in a water bath to bring it to a liquid state.

Ethanol based hydrophilic minocycline (w/w) gel formulations (MNC-H) were made with ethanol and propylene glycol. Other components include cineole as a co-solvent, and hydroxypropyl cellulose as a stabilizer and thickener, resulting in a viscosity of 100–250 cP and an approximate density of 0.9 g/mL. Minocycline in these formulations is fully soluble and the formulations are stable at room temperature without the need for special handling. The preparation of MNC-H formulations performed here, has been further used to produce GLP and GMP batches.

Formulations, in both lipophilic and hydrophilic forms, were prepared at 0% minocycline (vehicle), 1% minocycline, and 4% minocycline. Additional hydrophilic (MNC-H) formulations were also prepared at 0.5% minocycline and 2% minocycline for *in vivo* dose–response studies.

2.2.1. Physicochemical stability of formulations

Stability of topical minocycline formulations over 28 days was assessed by an HPLC method similar to the described method in Section 2.5.1. Formulations were stored at room temperature (~25 °C/60% RH), protected from light. The analyte was extracted from the formulation with a known quantity of 1% HCl/methanol solution. A 10-fold dilution was used for MNC-H and a 30-fold dilution factor was used for MNC-L, to limit the amount of components (waxes, oils, etc.) that may produce system clogging. The diluted MNC-L formulations were vortexed and shaken, then further centrifuged to remove any solid components from solution. The diluted samples were then injected into the HPLC and the percentage of the parent minocycline and 4-epiminocycline was determined.

2.3. Ex vivo human skin uptake

Excised human periauricular facial skin from facelift patients was obtained and stored at −80 °C. The donors were females with Fitzpatrick skin type III. MNC-H or MNC-L formulations at 1% and 4% minocycline concentrations and their respective vehicles (without minocycline) were applied at 2.5 mg/cm^2. The samples were kept in an opened face petri dish and placed on top of a gauze pad dampened with 0.9% sodium chloride to prevent the tissue from drying out. Samples were then incubated for 2 or 4 h at 32 °C. Human facial skin samples (n = 3 donors, with an n = 2 samples per donor) were cleansed and biopsied at the end of the incubation period. For bioanalytical measurements, minocycline was then extracted from each biopsy with 500 μL of acidified methanol for 24 h at 25 °C and the supernatant was analyzed by HPLC. Parent minocycline as well as the 4-epiminocycline peak area were measured and summed to show total delivery. Data from all facial samples were averaged for each treatment group. Quantification of uptake was calculated based on HPLC standards of minocycline hydrochloride. Statistical tests were performed using Student's T-test (Gilbert, 2018).

Samples were also processed for conventional fluorescence microscopy. The skin surface was cleansed prior to processing. Histological sections were prepared from frozen tissue embedded in optimal cutting temperature (OCT) compound and vertically cross-sectioned (through layers of the skin). Slides were washed once with phosphate buffered saline (PBS) and imaged. Fluorescence microscopy (Axiovert 100 M Fluorescence Microscope, Carl Zeiss Microscopy GmbH, Germany) was used for imaging analysis of the presence of minocycline in *ex vivo* skin samples. Subsequent serial sections were H&E stained.

2.4. In vivo studies

Three *in vivo* studies are presented here.

2.4.1. In vivo rat skin uptake: MNC-H minocycline formulations at 14 and 28 days

To demonstrate absorption of minocycline from the topical administration of our MNC-H formulations, an uptake study was conducted with male CD hairless rats, 6–8 weeks old weighing approximately 0.35 kg, (Charles River Laboratories) according to the Institutional Animal Care and Use Committee (IACUC) approved protocol. Animals were individually housed throughout the duration of the experiment

with free access to food and water. Rats were separated into 4 groups and were given either 2.5 mg/cm^2 or 5.0 mg/cm^2 of vehicle or 4% topical hydrophilic minocycline formulations daily. A sample size of four rats was used for the treatment group, with two rats continuing for the 28-day study. A sample size of two rats were used in the vehicle group, with one rat continuing for the 28-day study. The studies were conducted for 14 or 28 days. In the 28-day study, a recovery period of 7 days was also included. Treatment site was cleaned daily followed by reapplication of the next dose. At the end of the study, animals were humanely euthanized. Three 6-mm skin biopsies were obtained from each treatment site. Minocycline was extracted from each biopsy and analyzed with HPLC and is reported as the sum of the parent and epimerized minocycline to show total delivery. Tissue from one biopsy was also embedded, sectioned, and imaged under fluorescence microscopy. Subsequent serial sections were stained with H&E, cover-slipped, and imaged.

2.4.2. In vivo minipig cutaneous tolerance and uptake: MNC-H formulations for 14 days

In a second study, cutaneous tolerance and irritation due to the minocycline formulations were evaluated. In a pilot dose-selection study in minipigs, multiple doses of different MNC-H formulations were applied to a series of skin test areas of female Göttingen minipigs (12–18 month old, weighing approximately 20–30 kg) for 14 days. A total of four minipigs were included, with a sample size of two minipigs per treatment group. Animal studies were conducted at an external contract research organization. Housing, feed and water conditions were maintained according to the Testing Facility's Standard Operating Procedures which follow general accepted procedures. The protocol was reviewed and approved by the IACUC. The right and left flank areas were clipped, shaved, and demarcated with indelible ink to define multiple application sites. Test and control articles were applied and carefully spread using a spatula. Prior to daily dosing, treatment sites were cleaned with soap and dosed daily for 14 days. Vehicle, 0.5%, 1%, 2%, and 4% MNC-H formulations were applied to specific 10 cm^2 patches of porcine flank skin at 2.5 mg/cm^2, 7.5 mg/cm^2, or 12.5 mg/cm^2. Dosing sites were evaluated daily for erythema and edema based on the Draize scoring system. At necropsy, blood was collected and three 6-mm skin biopsies were obtained from each treatment site. Minocycline was extracted from each biopsy and analyzed by LC/MS/MS, and reported as the sum of parent and epimerized minocycline.

2.4.3. In vivo minipig uptake comparison: MNC-H and MNC-L minocycline formulations at 28 days

In a third study, a 28-day comparison of MNC-H and MNC-L minocycline formulations was conducted with male Göttingen minipigs (~20 weeks old, weighing approximately 8–10 kg). The protocol was reviewed and approved by the IACUC. Minipigs were acclimated for at least 7 days before the first day of dosing. Minipigs had access to water ad libitum, and an approximate 300 g ration of feed was provided daily to each minipig with remaining food left in the cage except during designated procedures (i.e. fasted overnight for clinical pathology sample collections). Minipigs were divided into three treatment groups, MNC-H and MNC-L treated with a sample size of two minipigs per group, and a sham group with a sample size of one minipig. Hair was removed from the treatment area and demarcated. The test articles were applied directly to the skin in a uniform layer over each designated area by gentle inunction with a disposable plastic applicator. Each formulation was applied at 2.5 mg/cm^2 to approximately 15% body surface areas. Prior to daily dosing, treatment sites were cleaned. Dosing was continued for 28 days. Dosing sites were evaluated daily for erythema and edema based on the Draize scoring system. On day 29, at necropsy, blood was collected and skin biopsies were obtained from each treatment site. Minocycline was extracted from plasma and skin samples and analyzed by LC/MS/MS and toxicokinetic parameters were estimated.

In this study, Sebutape$^©$ (CuDerm Corp, Dallas, TX) was used to collect sebum from within cleaned treatment sites at baseline, day 7, day 15 and day 29 on three different sites of each animal. The three sites will be different on each animal and will be around the same site each week. The Sebutapes$^©$ were pre-weighed prior to application, and immediately after the 45-minute application on the skin. The difference was calculated to be the amount of sebum collected from the skin.

2.5. Methods of bioanalysis

2.5.1. HPLC methods: Ex vivo human and in vivo rat skin

For *ex vivo* facial skin and rat skin studies, an HPLC analysis for measuring parent minocycline and 4-epiminocycline was carried out using the method in USP monographs with minor modifications (Gilbert, 2018). A 5-μm C18 LC Column (Phenomenex Inc., USA) was used with the Agilent 1100 HPLC system with UV detector (Agilent Technologies Inc., USA). The detection wavelength was set at 350 nm. The mobile phase was an isocratic elution and its composition was 0.12 M ammonium oxalate monohydrate, 18 mM EDTA-2Na, 8% THF, 12% DMF, pH 7.1–7.2.

2.5.2. LC-MS/MS methods: minipig skin and plasma

Both minipig studies used similar validated LC-MS/MS methods for plasma and qualified LC-MS/MS methods for skin. A Shimadzu UHPLC Nexera Series Pumping System (Shimadzu Corp., Tokyo) equipped with a 5-μm Atlantis T3 column (Waters, USA) and a Sciex API 4000 Mass Spectrometer (Sciex, USA) were used for HPLC-MS/MS analysis. The binary LC mobile phases were used for separation of the analyte. Mobile phase A consists of 10 mM ammonium trifluoroacetate and 0.1%(v/v) formic acid in water, and mobile phase B was methanol. The separation of the analyte was performed using linear gradient elution from 30% of mobile phase A to 50% of mobile phase B in 2.75 min at the flow rate of 1.0 mL/minutes. Subsequently, the column was rinsed out with 90% of mobile phase B. The HPLC column temperature was maintained at 40 °C. The effluent from the HPLC was infused into the ESI source with the positive polarity mode. The MS parameters were: ion spray voltage 5.5 kV, source temperature 700 °C, de-clustering potential 60 V, collision energy 30 V, and dwell time 300 m seconds. The analyte and the internal standard (deuterated minocycline; minocycline-d6) were detected at the ion transitions m/z 458.2 → m/z 441.2 for minocycline, and m/z 464.2 → 447.2 for the internal standard in a multiple-reaction monitoring (MRM) mode.

2.6. Pharmacokinetics and Statistical analysis

Non-compartmental pharmacokinetic parameters were calculated from the mean plasma versus time data using WinNonlin software (Pharsight Corporation). Statistical analysis was carried out by Student's T-test. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

Minocycline is a broad-spectrum tetracycline antibiotic and is the most lipid-soluble of the tetracycline-class antibiotics (Macdonald et al., 1973). Traditionally, lipophilic formulations are used to deliver small molecules that exhibit lipophilic properties. In our studies we developed a hydrophilic minocycline formulation to determine variances in formulation properties and to further understand its effect on skin uptake both *in vitro* and *in vivo*. The MNC-H formulations used in this study were designed to completely solubilize minocycline HCl. In contrast, minocycline in the MNC-L formulations was apparently dispersed within each formulation (data not shown).

Table 1
Stability profile of MNC-L (1%, 4%) and MNC-H (1%, 4%) formulations over 28 days.

Minocycline Stability				
Time (days)	Hydrophilic Formulations		Lipophilic Formulations	
	1% MNC-H	4% MNC-H	1% MNC-L	4% MNC-L
0	98.5%	98.3%	98.3%	98.5%
28	98.6%	98.3%	N/A	98.2%

The formulations used for the *in vivo* repeat dose studies, MNC-H (1%, 4%) and 4% MNC-L, were sampled over the same time course as the *in vivo* studies. The 1% MNC-L was sampled only at baseline to confirm minocycline amounts.

3.1. Physicochemical stability of formulations

The stability of minocycline over 28 days was assessed in the various formulations kept at room temperature and protected from light. As shown in Table 1 presenting parent minocycline, all formulations used remained stable throughout the study period.

3.2. Ex vivo human skin uptake

The uptake of minocycline was compared in an *ex vivo* human facial skin system using 1% and 4% formulations. In this comparison the MNC-H formulation had significantly greater ability to deliver minocycline to human *ex vivo* facial skin when compared to MNC-L formulations (Fig. 1). By 2 h post-application, MNC-H (1% and 4%) gel formulations demonstrate approximately a 2- to 3-fold increase in uptake compared to MNC-L (1% and 4%) formulations. By 4 h post-application, 1% MNC-H formulation demonstrates similar uptake to that of the 4% MNC-L formulation.

It is well known that API stability is a critical component to the efficacy of a successful formulation. In our *ex vivo* uptake studies, minocycline and a degradation product of minocycline, 4-epiminocycline, was measured. The MNC-H formulations demonstrated greater uptake of minocycline in to the skin (Fig. 1) and the minocycline present in the skin apparently remained more stable after application (Fig. 2) compared to MNC-L formulations. As shown in Fig. 2, there was less 4-epiminocycline identified in the skin from the hydrophilic formulation compared to the lipophilic formulations at 2 h and 4 h post application. Epimer formed after 2 h post application was 9.7% and

5.1% for 1% and 4% MNC-H, respectively, and 37.6% and 7.3% for 1% and 4% MNC-L, respectively. At 4 h post application, 10.6% and 4.5% epimer formed with the 1% and 4% MNC-H, respectively, and 29.8% and 11.6% formed with the 1% and 4% MNC-L, respectively. The percent of 4-epiminocycline was significantly less in topical hydrophilic minocycline formulations (1% and 4%) compared to the MNC-L (1% and 4%) formulations (Fig. 2).

In order to understand the deposition of the formulation in the skin, tissue sections, each treated with either MNC-H or MNC-L formulation, were sectioned and further analyzed using conventional fluorescence microscopy. In the human skin samples treated with MNC-H 1% and 4% formulations, minocycline fluorescence (red) was evident in the pilosebaceous unit and epidermis (Fig. 3). For the MNC-L treated samples, minocycline was identified in the stratum corneum and pilosebaceous unit, but the signal was considerably weaker than the fluorescence observed in the epidermis and pilosebaceous units of the samples treated with the hydrophilic composition.

3.3. In vivo uptake and dermal irritation studies

The challenges in developing a topical form of minocycline include maintaining the stability of the drug substance and efficiency in delivering the drug substance to the targeted tissue area. In developing the MNC-H formulations, our goal was to develop a potent hydrophilic topical agent that has the ability to penetrate into the skin and the pilosebaceous unit, thereby delivering local, targeted treatment for acne.

3.3.1. In vivo rat skin uptake: MNC-H minocycline formulations at 14 and 28 days

To further confirm the results from our *ex vivo* findings, and to understand absorption of minocycline, dermal application studies were designed and performed *in vivo* with CD hairless rats. In Fig. 4a, similar to the *ex vivo* uptake studies with human skin, minocycline was found in the skin of CD hairless rats that were treated daily for 14 days with topical hydrophilic minocycline formulations (MNC-H). No minocycline was detected in the vehicle group (0% MNC-H) as expected, and minocycline uptake of 6.4 µg/cm² and 7.4 µg/cm² was detected with 4% MNC-H at application amounts of 2.5 mg/cm² and 5 mg/cm², respectively. Minocycline was not detected in the skin in the recovery group (a recovery phase of 7 days after the in-life period of 28 days). Through fluorescence imaging and H&E stains for morphology
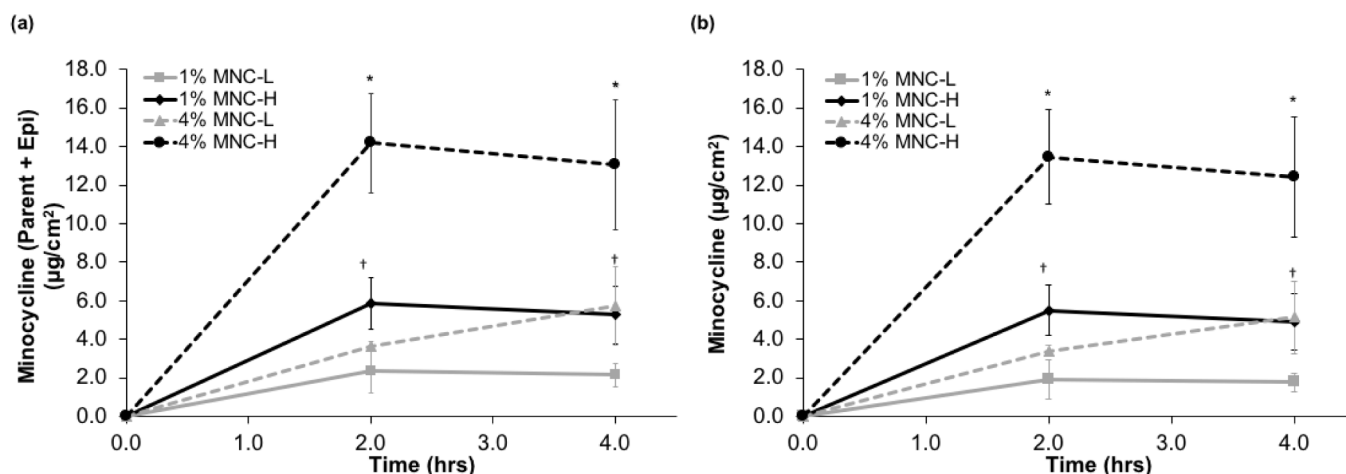

Fig. 1. Comparison of topical minocycline formulations MNC-H (1%, 4%) and MNC-L (1%, 4%) at 2 and 4 h post-application. Uptake is of (a) combined parent minocycline and 4-epiminocycline or (b) parent minocycline only in *ex vivo* human facial skin. Graph presents the mean ± standard error (SE). (*$P \leq 0.05$ between hydrophilic minocycline and the lipophilic minocycline formulations, 4% MNC-H versus 4% MNC-L at 2 and 4 h, and †$P \leq 0.05$ between 1% MNC-H versus 1% MNC-L at 2 and 4 h).
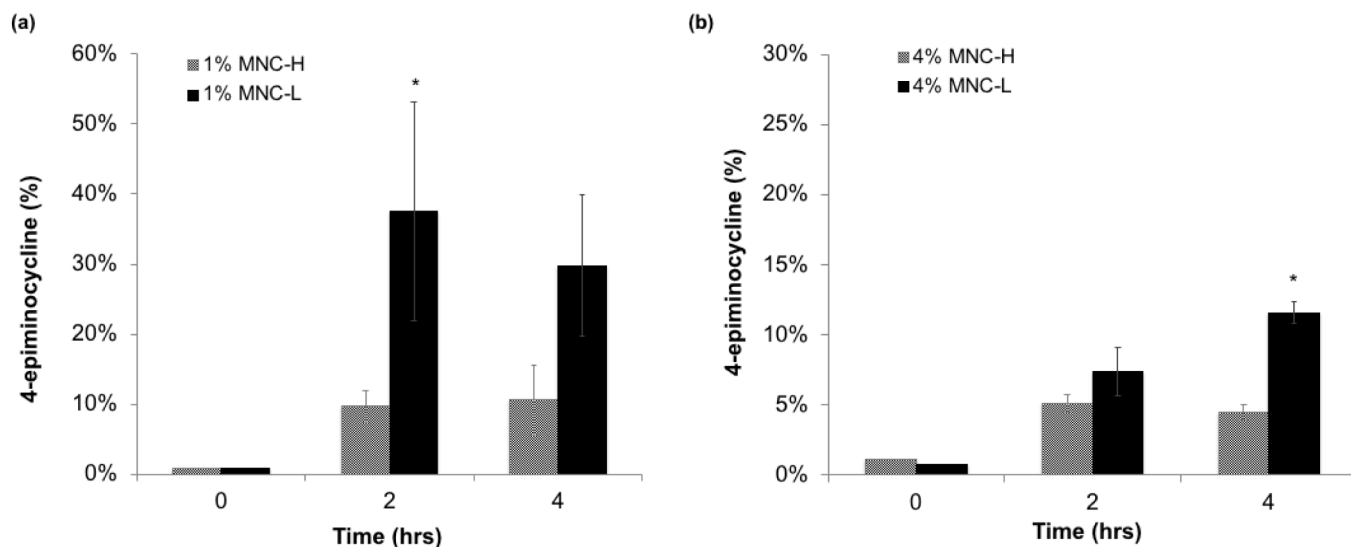
Fig. 2. Minocycline epimerization resulting from topical application of MNC-H or MNC-L formulation in *ex vivo* human facial skin. 4-epiminocycline amount in formulation prior to dosing (0 h) and extracted from skin samples that were processed 2 or 4 h post-application with (a) 1% or (b) 4% formulations. Graphs present the mean ± standard error (SE) ([*]$P \leq 0.05$ between hydrophilic minocycline and the lipophilic minocycline formulations; 1% MNC-H versus 1% MNC-L at 2 h and 4% MNC-H versus 4% MNC-L at 4 h).
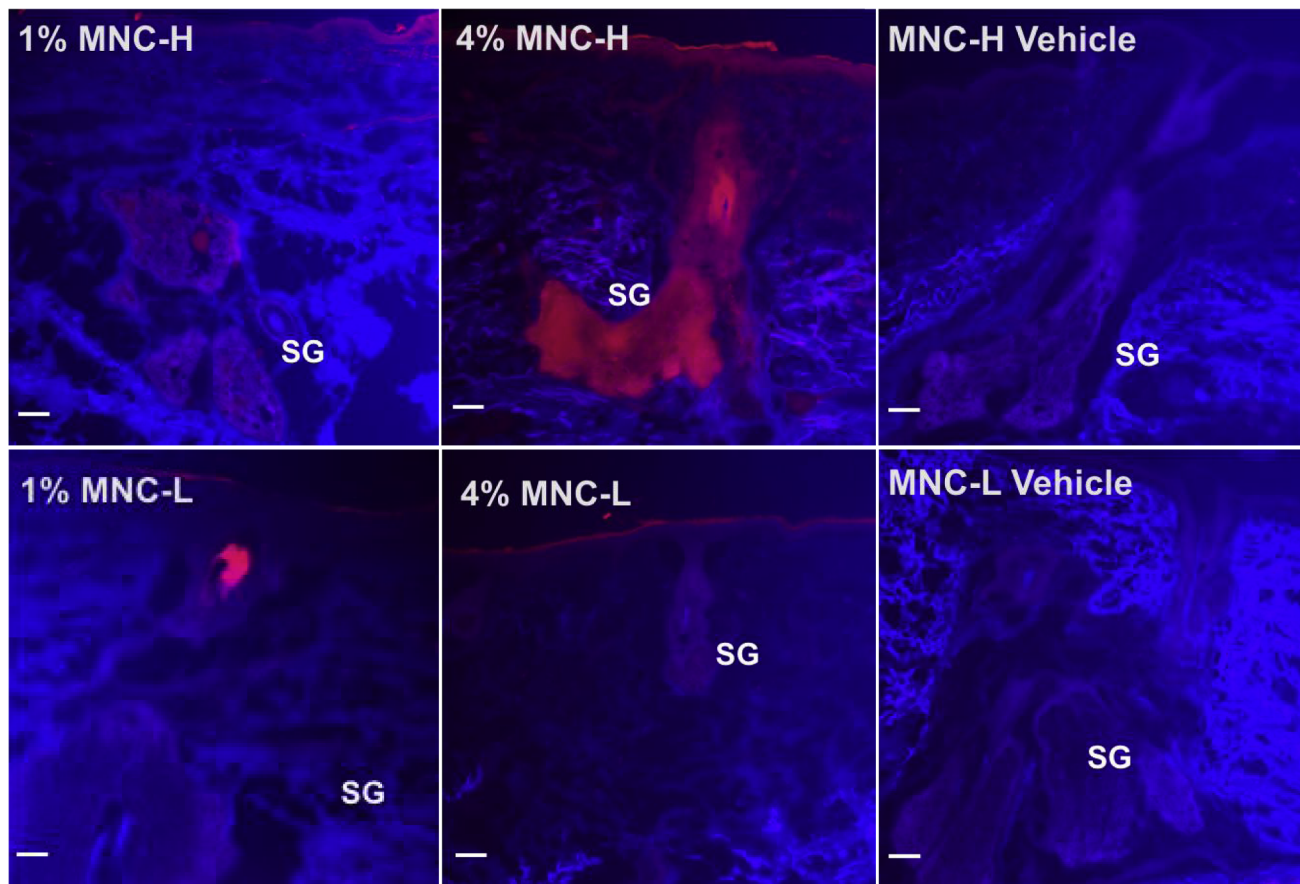

Fig. 3. Detection of minocycline (red fluorescence) in *ex vivo* human facial skin after application of hydrophilic MNC-H or lipophilic MNC-L formulations. Tissue was treated with an excess amount of 1% MNC-H, 1% MNC-L, 4% MNC-H, or 4% MNC-L and its appropriate vehicle control (hydrophilic MNC-H vehicle or lipophilic MNC-L vehicle) for 24 h. Relevant skin structures such as the stratum corneum, epidermis and sebaceous gland (SG) can be observed. Scale bar represents 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
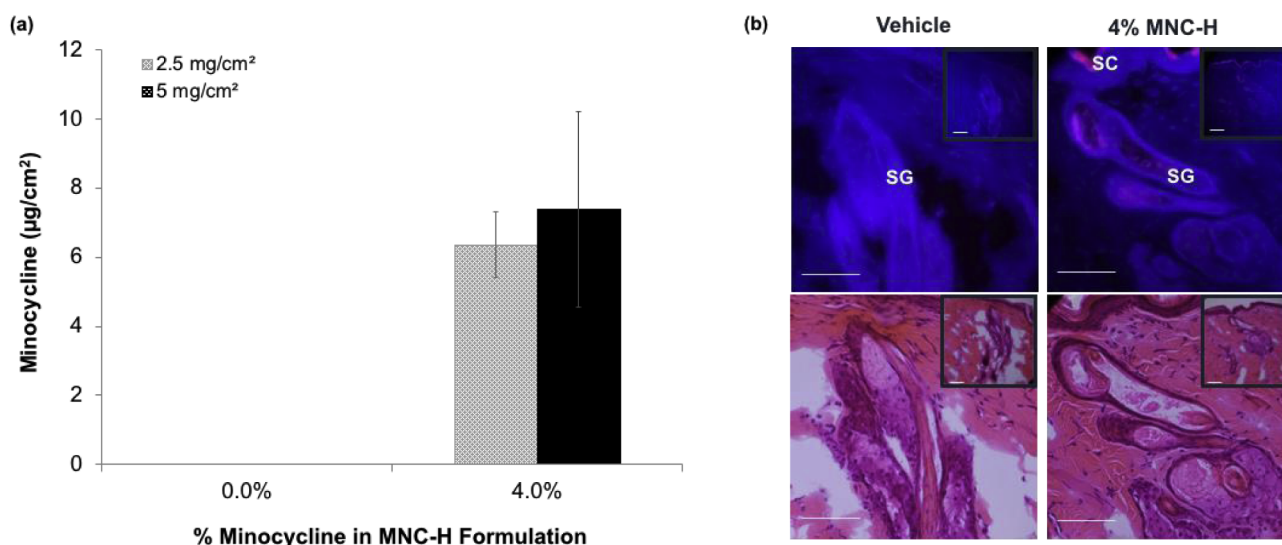
Fig. 4. Minocycline uptake and visualization in rat skin treated with 4% MNC-H or vehicle over 14 days. (a) Uptake of minocycline in rats with daily application of 2.5 mg/cm^2 and 5 mg/cm^2 of 4% MNC-H or hydrophilic vehicle. Graph presents mean ± SE. (b) Visualization of minocycline (red) in the rat skin, specifically the sebaceous gland (SG) and stratum corneum (SC). Serial H&E images are also shown. Scale bar indicates 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Different topical hydrophilic minocycline formulations (MNC-H) and application amounts applied to individual 10 cm^2 test areas (skin) on Göttingen minipigs.

Minocycline Applied Per Test Area (mg)				
		Application Dose Levels of MNC-H		
		2.5 mg/cm^2	7.5 mg/cm^2	12.5 mg/cm^2
MNC-H % Minocycline Formulations	Vehicle MNC-H	0.00	0.00	0.00
	0.5% MNC-H	0.13	0.38	0.63
	1% MNC-H	0.25	0.75	1.25
	2% MNC-H	0.50	1.50	2.50
	4% MNC-H	1.00	3.00	5.00

The amount of minocycline (mg) was estimated based on the level of minocycline in each formulation, applied volume, and application area.

(Fig. 4b), minocycline (red signal) was observed in the stratum corneum and sebaceous glands of the skin after 14 days application with 4% MNC-H. Vehicle treatment presented no signal.

3.3.2. In vivo minipig cutaneous tolerance and uptake: MNC-H formulations for 14 days

A 14-day repeat-dose patch study in minipigs was conducted to further understand the delivery aspects of our topical hydrophilic minocycline formulation. Minipig skin is known to be an excellent representative of human skin for purposes of preclinical testing (Gerd Bode, 2010). The dose–response relationship for minocycline uptake in porcine skin was also evaluated. In this study, multiple doses of 15 different test conditions, including several different MNC-H formulations (0%, 0.5%, 1%, 2%, and 4%) were tested at several different doses (2.5 mg/cm^2/day, 7.5 mg/cm^2/day, and 12.5 mg/cm^2/day), each over a 10 cm^2 area (Table 2).

Analysis of the minocycline uptake in the test area indicated a mainly dose-dependent increase of minocycline in the minipig skin (Fig. 5). Not every formulation is presented in the graph, but the range is given. Minocycline applied at 2.5 mg/cm^2 was detected at 5.4 μg/g, 15.6 μg/g, 45.0 μg/g, 41.9 μg/g with 0.5%, 1%, 2% and 4% MNC-H, respectively. The increased volumes of 7.5 mg/cm^2 and 12.5 mg/cm^2 of 4% MNC-H showed further increased uptake amounts of 103.9 μg/g and 116.7 μg/g, respectively. The *in vivo* skin minocycline

concentrations, ranging from about 5 μg/g to 120 μg/g with different MNC-H formulations, exceeded the minimum inhibitory concentration (MIC) of 0.03 μg/mL, as measured with different strains of *P. acnes* (Gübelin et al., 2006). No side effects such as erythema or edema were observed during the study.

3.4. In vivo minipig comparative study: MNC-H and MNC-L minocycline formulations at 28 days

In a comparative *in vivo* minipig study, topical hydrophilic minocycline gel and lipophilic minocycline formulations were dermally administered daily for 28 days. Minocycline was measured in the plasma during the study and was detected in the MNC-L group, but no minocycline was found with the MNC-H treatment group (Table 3). Interestingly, higher skin levels of minocycline were detected in the MNC-H group than animals treated with MNC-L formulations. Similar to the previous minipig patch study, no side effects, such as erythema or edema, were observed with either MNC-H or the MNC-L formulations.

Sebum was collected and compared between treatment groups. A significant increase in sebum levels was observed in the MNC-L formulation group but not in the MNC-H group when compared to the sham group (no treatment) on Days 7, 15 and 29 (Fig. 6). Furthermore, significantly more sebum was produced from MNC-L treatment on Day 7, 15 and 20 at 2.5 mg, 1.8 mg and 2.1 mg, respectively, compared to
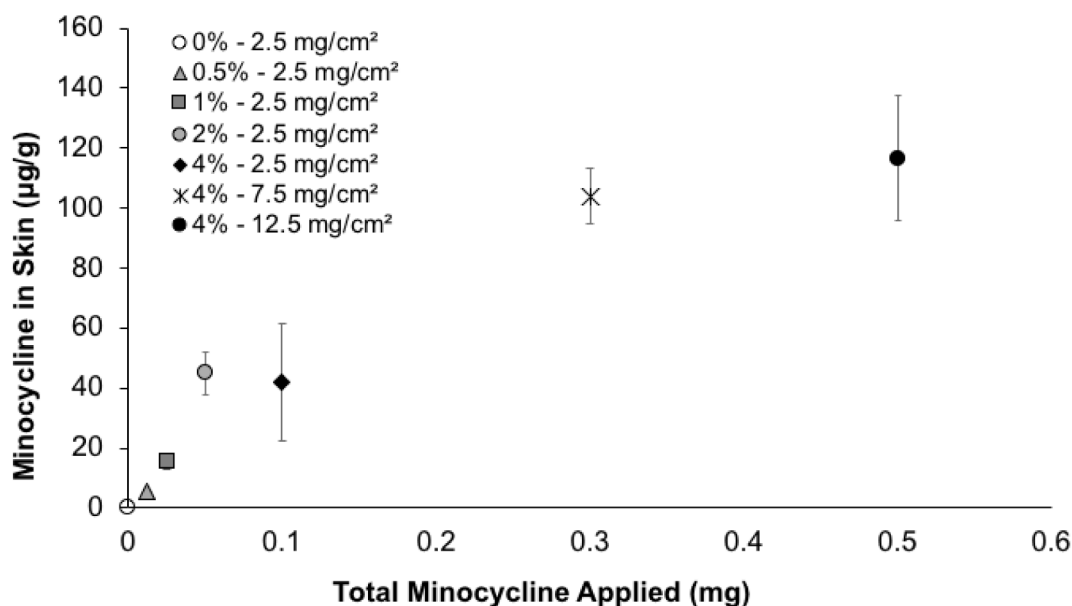
Fig. 5. Minocycline uptake in minipig skin after 14 days of various MNC-H formulations. MNC-H minocycline concentrations (0.5%, 1%, 2%, 4%) and application volumes (2.5, 7.5 and 12.5 mg/cm^2) were applied daily to the skin. Triplicates of each sample were analyzed by LC-MS/MS. Graph presents mean ± SE.

Table 3

Minocycline plasma and skin uptake levels in minipigs treated daily with topical 4% MNC-L or 1% MNC-H.

Minocycline in Plasma	1% BPX-01			4% MNC-L		
	1 d	14 d	28 d	1 d	14 d	28 d
T_{max} (hr)	–	–	–	–	1	2
C_{max} (ng/mL)	0.00	0.00	0.00	0.00	1.96	2.58
AUC_{all} (hr * ng/mL)	0.00	0.00	0.00	0.00	26.00	14.52
Minocycline in Skin (µg/mL)	2.08 ± 0.62			0.86 ± 0.25		

Pharmacokinetics measurements were performed at 1 day, 14 days and 28 days (1 d, 14 d, and 28 d) post treatment. Skin minocycline concentrations were measured after 28 days of application.

MNC-H treatment on Day 7, 15 and 20 at 0.3 mg, 0.9 mg and 0.1 mg, respectively.

4. Discussion

Development of a topical hydrophilic minocycline formulation has been particularly challenging due to the difficulty of stabilizing this active ingredient. Minocycline is generally unstable in aqueous form and is also sensitive to moisture, temperature, and light (Salman et al., 2014). Despite being soluble in an aqueous solution, it is highly unstable, resulting in formulations limited to lipophilic options. Several groups (Vyas et al., 2014) (Tamarkin, 2016) (Shigeyama et al., 2000) have attempted to formulate topical lipophilic minocycline solutions. The limited solubility of minocycline in a lipophilic environment
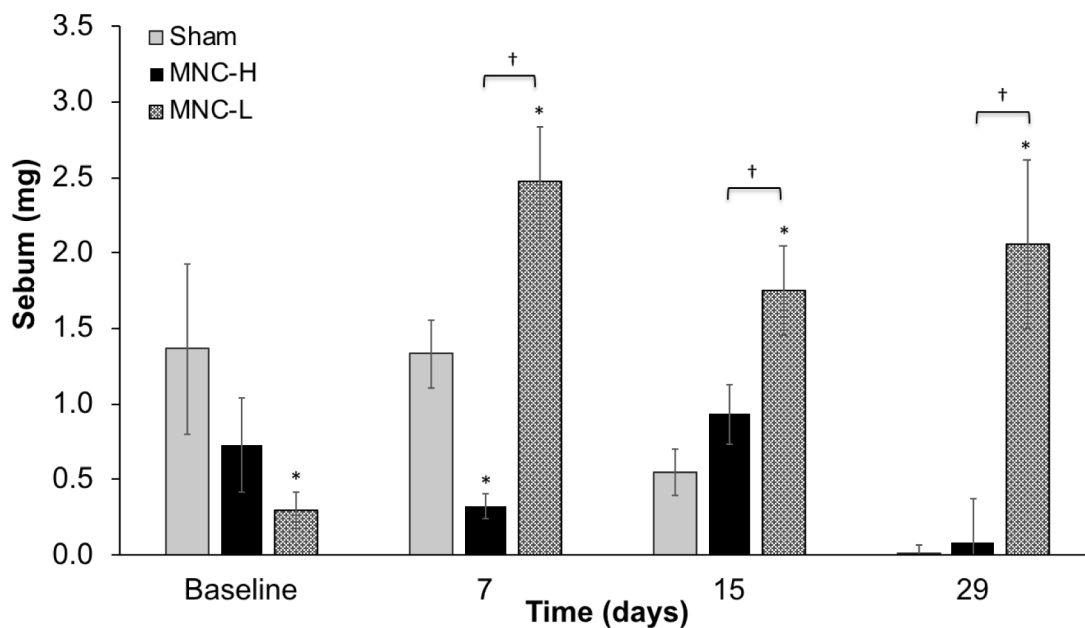

Fig. 6. Sebum production observed from the skin of minipigs treated with 4% MNC-L, 1% MNC-H or non-treated (sham). Sebutape© was used to collect sebum and weighed prior to and post Sebutape© application on the skin. Graph presents mean ± SE (*P ≤ 0.05 between sham and minocycline treated groups at baseline, day 7, 15 and 29, †P ≤ 0.05 between the 1% MNC-H and 4% MNC-L group on day 7, 15 and 29).

presents limited cutaneous uptake (Prausnitz et al., 2017) thereby requiring increased concentrations to deliver sufficient quantities of the active ingredient into the skin to produce clinical efficacy, as indicated in Fig. 1, where fully solubilized formulations of topical hydrophilic minocycline gel formulations demonstrated more than 2 to 3-fold drug penetration compared to topical lipophilic minocycline formulations of equivalent concentration. Interestingly, the kinetics of the epimerization observed in this study does not necessarily follow a first order kinetic, instead, we are observing concentration dependent kinetics (Fig. 2). More studies are currently being conducted to understand the kinetics and behavior of MNC-H more thoroughly.

Furthermore, in our 14-day *in vivo* repeat-dose minipig patch study, dose-dependent uptake of minocycline was observed with various topical hydrophilic minocycline formulations. The cutaneous absorption ranged from 5 μg/g to 120 μg/g, which is higher than the concentration required to inhibit the growth of *P. acnes* as the MIC value is equal to 0.03 μg/mL (Fig. 5). By applying a higher volume of 4% MNC-H formulation amount, the available minocycline on the surface of the skin probably acts as a reservoir resulting in sustained delivery (Prausnitz et al., 2017), potentially driving a higher amount of minocycline into the skin. In other words, applying a higher concentration of solubilized minocycline increases the concentration gradient at the skin surface, as a result driving more drug into the skin.

Absorption of a topical formulation usually happens along the skin appendages such as hair follicles or through the stratum corneum and the underlying layers (Mohd et al., 2016) (Lademann et al., 2008) (Desai et al., 2010). Though skin appendages present only a small portion of the surface, a topical formulation can lead to a direct and targeted delivery and accumulation in these structures (Grice et al., 2009) (Verma et al., 2016), thus leading to faster and more efficient uptake in skin as observed with the topical MNC-H gel in our *ex vivo* studies and *in vivo* studies (Figs. 1, 3 and 4). Generally, the stratum corneum is assumed to be the main barrier for absorption due to the low diffusivity (Prausnitz et al.,2017). Drug delivery occurs either through the corneocytes or along the intercellular spaces through the lipid matrix (Alkilani et al., 2015). A general belief exists that intercellular channels through the lipid matrix provides better diffusivity although this pathway is much longer (Elisa, 1991).

Typically, lipophilic formulations are readily absorbed by the stratum corneum but lose absorption efficacy in the hydrophilic epidermis and epithelial cells lining of the follicle and sebaceous glands (Dash, 2014). In our *ex vivo* study with human excised facial skin samples with 4% MNC-L, minocycline was identified in the stratum corneum and part of the pilosebaceous unit compared to the 1% MNC-H, which was found to be present specifically in the sebaceous glands, epidermis, and stratum corneum (Fig. 3). The minocycline signal exhibited as red color, was much weaker from MNC-L compared to MNC-H composition which further corroborates the bioanalytical measurements of low amounts of minocycline in the lipophilic treated samples such that the 1% MNC-H formulation exhibited a similar level of absorption as the 4% MNC-L formulation in an *ex vivo* situation (Fig. 1). These data further suggest that the topical hydrophilic minocycline gel formulation is superior to the topical lipophilic minocycline formulation in delivering minocycline to the pilosebaceous unit where *P. acnes* typically reside.

Moreover, in the comparative 28-day *in vivo* minipig study, a higher minocycline amount was absorbed in the skin with the 1% MNC-H group compared to the 4% MNC-L group (Table 3). Further evaluation of these formulations also revealed that minocycline was present in the plasma when applied daily for 28 days in the 4% MNC-L treated group while no minocycline was detected in the 1% MNC-H treated group. It is hypothesized then that these formulations influence the drug delivery and tissue pharmacokinetics differently. Apparently, the hydrophilic formulation resulted in more minocycline retained in the skin compared to the lipophilic formulation where minocycline reached the bloodstream at detectable amounts. Such phenomena is suggested by

Desai *et al.*, which reports that the nature of the formulations and the presence of several excipients could drive the absorption into the deeper skin layers and even into systemic circulation. Such formulation characteristics could explain the different behaviors of MNC-H and MNC-L. Further studies focusing on minocycline distribution and pharmacokinetics in skin tissue as a function of the vehicle are needed.

It should be noted that some lipophilic formulations are made with auxiliary mechanisms to aid in the delivery of the oily formulation and to minimize the greasy feeling on the skin. To overcome this, we applied a very thin layer (2.5 mg/cm^2) of MNC-H and MNC-L formulations in order to minimize the unwanted effects. Additionally, mild detergent was used in the *in vivo* experiments prior to daily dosage in order to remove residual formulation. However, an increase in sebum was seen in the skin in the MNC-L formulation (Fig. 6) when compared to the sham control and the MNC-H formulation in the *in vivo* minipig 28-day comparative study. This increase in sebum from the MNC-L formulation could create a barrier to the skin, which may exacerbate *P. acnes* as suggested and reported by Grice and Serge (2011), and the progression of acne vulgaris. Such negative reported observations with a lipophilic formulation support the development of a stable, hydrophilic topical minocycline gel formulation to treat acne vulgaris. Since minocycline has a MIC value equal to 0.03 μg/mL for *P acnes* and based in the data presented above, it is highly possible that 2.5 mg/cm^2 application doses of 1 or 2% of MNC-H formulation is likely sufficient for delivering efficacious amounts of minocycline as compared to 4% MNC-L formulations, as shown in Fig. 1.

Traditionally, topical formulations for dermal diseases are designed to enhance the uptake of the active ingredient to the dermis (Paudel et al., 2010). Efforts have been placed in developing penetration enhancers that promote active ingredient uptake to this portion of the skin (Pathan and Setty, 2009). Even in the treatment of acne vulgaris, many formulation studies have been designed to help for maximal dermal uptake (OECD, 2011). However, *P. acnes* typically reside in the pilosebaceous unit due to the favorable anaerobic environment that is conducive for its growth. In order to effectively treat acne vulgaris, an effective formulation should be able to deliver the minocycline directly to the affected anatomy. Our data suggest the ability to deliver minocycline directly to the epidermis and pilosebaceous unit in both *ex vivo* human skin and *in vivo* rodents (as shown in Figs. 3 and 4). In addition, our studies propose a dose-dependent increase of minocycline in the minipig skin. Recently, the delivery of a single daily dose of minocycline with various MNC-H formulations in *ex vivo* human face skin has been further evaluated by more selective methods with higher minocycline specificity for visualization within human skin tissue by utilizing two-photon excitation fluorescence (TPEF) microscopy and fluorescence lifetime imaging microscopy (FLIM). Based on these results the drug local distribution visualization method using TPEF and FLIM with phasor analysis can play an important role in studying the pharmacokinetics and pharmacodynamics of a topical or transdermal drug (Jeong et al., 2018).

5. Conclusions

Our reported data have demonstrated that topical application of the topical hydrophilic minocycline gel formulation can limit systemic exposure while delivering sufficient minocycline directly to the targeted skin anatomy to potentially treat acne vulgaris. Minocycline from the hydrophilic formulation was found to have significantly higher presence within the targeted areas in the skin but not detectable in the systemic circulation after a 28-day repeat application in minipigs when compared to a lipophilic formulation. We were able to demonstrate that 1% MNC-H delivers minocycline in the *ex vivo* human skin at least as effectively as 4% MNC-L. This is particularly exciting because a lower percentage of minocycline formulation translates into a reduction in systemic exposure and can decrease the severity of side effects, which is commonly seen with the use of oral minocycline. These results also

suggest that both systemic exposure and peak plasma concentrations of minocycline are minimized by dermal administration of a topical hydrophilic minocycline gel formulation. The lack of skin irritation and the absence of treatment-related toxicity further suggest that MNC-H formulations may be a safe and targeted treatment approach for acne vulgaris. We anticipate that MNC-H formulations will have an increased efficacy while providing a safer alternative to oral minocycline.

CRediT authorship contribution statement

Diana Lac: Conceptualization, Methodology, Project administration, Visualization, Investigation, Writing - original draft. **Maiko Hermsmeier:** Conceptualization, Methodology, Data curation, Investigation, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. **Xin Chen:** Conceptualization, Writing - review & editing, Resources, Resources. **Noymi Yam:** Conceptualization, Methodology. **Akira Yamamoto:** Conceptualization, Methodology, Writing - review & editing, Resources, Software. **Susan Huang:** Data curation. **Tanvee Sawant:** Formal analysis. **Kin F. Chan:** Supervision, Conceptualization, Writing - review & editing. **Usha Nagavarapu:** Supervision, Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing.

Acknowledgement

Potential conflict of interest statement/disclosure

• All the authors of this paper are or were employees of BioPharmX.
• None of the authors of this paper have a personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

Alexis, A., Del Rosso, J.Q., Desai, S.R., Downie, J.B., Draelos, Z.D., Feser, C., Forconi, R., Fowler Jr., J.F., Gold, M., Kaufman-Janette, J., Lain, E., Lee, M., Ling, M., Shambran, A.T., Werschler, P., Daniels, A.M., 2018. BPX-01 minocycline topical gel shows promise for the treatment of moderate-to-severe inflammatory acne vulgaris. J. Clin. Aesthetic Dermatol. 11 (11), 25–35.

Alkilani, A.Z., McCrudden, M.T., Donnelly, R.F., 2015. Transdermal drug delivery: innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. Pharmaceutics 7 (4), 438–470. https://doi.org/10.3390/pharmaceutics7040438.

Bode, G., Clausing, P., Gervais, F., Loegsted, J., Luft, J., Nogues, V., Sims, J., 2010. Steering group of the RETHINK Project The utility of the minipig as an animal model in regulatory toxicology. J. Pharmacol. Toxicol. Meth. 62 (3), 196–220. https://doi.org/10.1016/j.vascn.2010.05.009.

Dash, A., Somnath, S., Tolman, J., 2014. Pharmaceutics: Basic Principles and Application to Pharmacy Practice. Academic Press, Boston MA.

Desai, P., Patlolla, R.R., Singh, M., 2010. Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol. Membr. Biol. 27 (7), 247–259. https://doi.org/10.3109/09687688.2010.522203.

Elisa, P.M., 1991. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. J. Controlled Relaease 15 (3), 199–208.

Fleischer Jr., A.B., Dinehart, S., Stough, D., Plott, R.T., 2006. Solodyn phase 2 study group; solodyn phase 3 study group safety and efficacy of a new extended-release formulation of minocycline. Cutis 78, 21–31.

Garner, S.E., Eady, A., Bennett, C., Newton, J.N., Thomas, K., Popescu, C.M., 2012. Minocycline for acne vulgaris: efficacy and safety. Cochrane Database Syst. Rev. 15 (8), 1–5. https://doi.org/10.1002/14651858.CD002086.pub2.

Gilbert, B.D., 2018. Pharmacopeial Forum: Volume No. 28(3) Pg770. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m54170.html. (Accessed 22 August 2018).

Goodman, G., 2006. Acne and acne scarring – The ace for active and early intervention. Aust. Fam. Physician 35 (7), 503–504.

Grice, E.A., Serge, J.A., 2011. The skin microbiome. Nat. Rev. Microbiol. 9 (4), 244–253 10.1038%2Fnrmicro2537.

Grice, E.J., Ciotti, S., Weiner, N., Lockwood, P., Cross, S.E., Roberts, M.S., 2009. Relative uptake of minoxidil into appendages and stratum corneum and permeation through human skin *in vitro*. J. Pharm. Sci. 99 (2), 712–718. https://doi.org/10.1002/jps.21856.

Gübelin, W., Martinex, M.A., Molina, M.T., Zapata, S., Valenzuela, M.E., 2006. Antimicrobial susceptibility of strains of *Propionibacterium acnes* isolated from inflammatory acne. Rev. Latinoam. Microbiol. 48 (1), 14–16.

Jeong, S., Hermsmeier, M., Osseiran, S., Yamamoto, A., Nagavarapu, U., Chan, K.F., Evans, C.L., 2018. Visualization of drug distribution of a topical minocycline gel in human facial skin. Biomed. Opt. Exp. 9 (7), 3434–3448. https://doi.org/10.1364/BOE.9.003434.

Kirschbaum, J.O., Kligman, A.M., 1963. The pathogenic role of Corynebacterium acnes in Acne Vulgaris. Arch. Dermatol. 88 (6), 832–833. https://doi.org/10.1001/archderm.1963.01590240156026.

Lademann, J., Knorr, F., Richter, H., Blume-Peytavi, U., Vogt, A., Antoniou, C., Sterry, W., Patzelt, A., 2008. Skin Pharmacol Physiol 21 (3), 150–155. https://doi.org/10.1159/000131079.

Macdonald, H., Kelly, R.G., Allen, E.S., Noble, J.F., Kanegis, L.A., 1973. Pharmacokinetic studies on minocycline in man. Clin. Pharmacol. Ther. 14, 852–861. https://doi.org/10.1002/cpt1973145852.

Mandaogade, P.M., Raghuvanshi, R.S., Rampal, A., 2004. Extended release minocycline compositions and processes for their preparation. PCT Patent Application No. WO2004078111 A2.

Mbah, C.J., Uzor, P.F., Omeje, E.O., 2011. Perspectives on transdermal drug delivery. J. Chem. Pharm. Res. 3 (3), 680–700.

Mohd, F., Todo, H., Yoshimoto, M., Yusuf, E., Sugibayashi, K., 2016. Contribution of the hair follicular pathway to total skin permeation of topically applied and exposed chemicals. Pharmaceutics 8 (4), 32. https://doi.org/10.3390/pharmaceutics8040032.

Nair, A., Jacob, S., Al-Dhubiab, B., Attimarad, M., Harsha, S., 2013. Basic considerations in the dermatokinetics of topical formulations. Braz. J. Pharm. Sci. 49 (3), 423–434. https://doi.org/10.1590/S1984-82502013000300004.

Pathan, I.B., Setty, C.M., 2009. Chemical penetration enhancers for transdermal drug delivery systems. Tropical J. Pharm Res. 8 (2), 173–179. https://doi.org/10.4314/tjpr.v8i2.44527.

Pecina, J.L., Pittelkow, M.R., 2011. Hyperpigmentation - a case study. Aust. Fam. Physician 40 (9), 710–712.

Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P., Stinchcomb, A.L., 2010. Challenges and opportunities in dermal/transdermal delivery. Therapeutic Deliv. 1 (1), 109–131. https://doi.org/10.4155/tde.10.16.

Pawelczyk, E., Matlak, B., 1982. Kinetics of drug decomposition. Part 74. Kinetics of degradation of minocycline in aqueous solution. Pol. J. Pharmacol. Pharm. 34 (5–6), 409–421.

Prausnitz, M.R., Elias, P.M., Franz, T.J., Schumth, M., Tsau, J.C., Menon, G.K., Holleran, W., Feinglod, K.R., 2017. Skin barrier and transdermal delivery. In: Bolognia, Schaffer and Cerroni. Dermatology: 2-Volume set, fourth ed. Elsevier Publishing, pp. 2065–2073.

Salman, M., Angel, A., Swaminathan, V., 2014. Tetracycline topical formulations, preparation and uses thereof. United States Patent Application No. US20140147504 A.

Shigeyama, M., Ohgaya, T., Kawashima, Y., Takeuchi, H., Hino, T., 2000. Modificarion of the physicochemical properties of Minocycline hydrochloride ointment with cyclodextrines for optimum treatment of bedsore. Chem. Pharm. Bull. 48 (5), 617–622.

OECD: Organisation for Economic Co-operation and Development, 2011. Guidance Notes on Dermal Absorption. OECD Environment, Health and Safety Publications series on Testing and Assessment No.156. Paris, France, France: OECD.

Tamarkin, D., 2016. Foam: A unique delivery vehicle for topically applied formulations. In: Dayan, N. (Ed.), Apply Topically: A practical Guide to Formulating topical Applications. Allured Pub Corp., New York, pp. 233–260. https://doi.org/10.1002/9781119364221.ch9.

Torok, H.M., 2013. Extended-release formulation of minocycline in the treatment of moderate-to-severe acne vulgaris in patients over age of 12 years. J. Clin. Aesthetic Dermatol. 6 (7), 19–22.

Verma, A., Jain, A., Hurkat, P., Jain, S.K., 2016. Transfollicular drug delivery: current perspectives. Res. Rep. Transdermal Drug Deliv. 5, 1–17. https://doi.org/10.2147/RRTD.S75809.

Vos, T., Flaxman, A.D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380 (9859), 2163–2196. https://doi.org/10.1016/S0140-6736(12)61729-2.

Vyas, A., Kumar Sonker, A., Gidwani, B., 2014. Carrier-based drug delivery system for treatment of acne. Sci. World J. https://doi.org/10.1155/2014/276260.

Zouboulis, C.C., 2004. Acne and sebaceous gland function. Clinical Dermatol. 22 (5), 360–366. https://doi.org/10.1016/j.clindermatol.2004.03.004.