Feasibility of In Vitro Permeation Testing for Cleocin T® (Clindamycin Phosphate) Topical Lotion to Support the Demonstration of Bioequivalence

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Abstract

Background: Cleocin T® (clindamycin phosphate) topical lotion, EQ 1% Base ("Cleocin T®") is indicated for the treatment of acne vulgaris and contains clindamycin phosphate at a concentration equivalent to 10 mg/mL of clindamycin free base. Clindamycin phosphate is an ester prodrug that hydrolyzes to its pharmacologically active moiety, clindamycin base, potentially both on the skin surface and within the skin. There is no clear understanding of the relative amounts of clindamycin base versus clindamycin phosphate that permeate across the skin. The objective of this study was to evaluate the feasibility of an in vitro permeation test (IVPT) for Cleocin T® and to identify the appropriate clindamycin analyte(s) to monitor to support a demonstration of bioequivalence (BE).

Methods: IVPT studies across dermatomed human cadaver skin was conducted with the Phoenix dry heat diffusion cell system (Teledyne, Hanson, Chatsworth CA) at three doses (25 and 50 mg/cm^2). An un-occluded dose of Cleocin T® was applied to skin samples (n=6). The sampling schedule was done over 48 hours. Mass balance of clindamycin and clindamycin phosphate were assessed after the IVPT study to determine the relative distribution of the two moieties in the skin, and in the donor and receptor compartments. Simultaneous quantification of clindamycin phosphate and clindamycin base in IVPT samples was done using an in-house developed and validated ultra-performance liquid chromatography equipped with Ultraviolet detector (UPLC-UV) method.

Results: The UPLC method successfully separated clindamycin phosphate (retention time: 4.3 min) and clindamycin base (retention time: 4.9 min). The average recovery of clindamycin phosphate in the drug product was 112.3% ± 2.6% (mean ± RSD, n=6); however, the content of clindamycin base did not exceed 0.01% w/w in the drug product. The IVPT results showed that both clindamycin phosphate and clindamycin base permeated across the skin. The cumulative amounts (mean ± SD, n=6) of clindamycin phosphate and clindamycin base permeated to the receptor solution over 48 h were 5.17 ± 2.1 µg/cm^2 and 28.7 ± 0.21 µg/cm^2, respectively for the 50 mg/cm^2 dose (Figure 1), and 0.31 ± 0.09 µg/cm^2 and 6.75 ± 1.87 µg/cm^2, respectively for the 25 mg/cm^2 dose (Figure 2). The results of the mass balance studies indicated that the recovery of clindamycin phosphate and clindamycin base were ~62.5% and ~31%, respectively, for the 50 mg/cm^2 dose, suggesting that the conversion of clindamycin phosphate to clindamycin base occurs, potentially in the residual formulation, on the surface of the skin, and/or during permeation into or through the skin.

Conclusion: The results of these exploratory IVPT studies suggests that both clindamycin phosphate and clindamycin base permeate across the skin, and that it may be feasible to utilize one or both analytes to evaluate the cutaneous pharmacokinetics of clindamycin from Cleocin T® lotion.

Introduction

Cleocin T® (clindamycin phosphate) topical lotion, EQ 1% Base ("Cleocin T®") is indicated for the treatment of acne vulgaris and contains clindamycin phosphate at a concentration equivalent to 10 mg/mL of clindamycin free base. Clindamycin phosphate is an ester prodrug that hydrolyzes to its pharmacologically active moiety, clindamycin base, potentially both on the skin surface and within the skin. There has been little evidence about the relative amounts of clindamycin base versus clindamycin phosphate that permeate across the skin.

The objective of this study was 1) to evaluate the feasibility of an in vitro permeation test (IVPT) for Cleocin T® and 2) to identify the appropriate clindamycin analyte(s) to monitor to support a demonstration of bioequivalence (BE).

Materials and Methods

Table 1. IVPT protocol parameters

Treatment	Cleocin T® topical lotion, EQ 1% clindamycin base
Product Doses	• 50 mg/cm^2 (n=6) • 25 mg/cm^2 (n=6)
Skin Type	Dermatomed human cadaver skin from different donors (~450 µm thickness)
Skin Surface Temperature	32.5°C ± 1°C
Diffusion Cells	Phoenix dry heat vertical diffusion cell system
Permeation Area	1.76 cm^2
Donor Chamber Condition	Un-occluded
Receptor Volume	16 mL
Receptor Fluid	PBS (pH 7.4 ± 0.2) + 0.02% w/v Oleth 20 + 0.02% sodium azide
Receptor Sampling time points	1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 48 h
Receptor Sampling volume	500 µL (aliquot sampling)

- Mass balance of clindamycin base and clindamycin phosphate were assessed after the IVPT study to determine the relative distribution of the two moieties in the skin, and in the donor and receptor compartments. Recovery values for clindamycin base and clindamycin phosphate were calculated based on their initial concentrations in the drug product prior to conducting the IVPT study.
- Simultaneous quantification of clindamycin phosphate and clindamycin base in IVPT samples was done using an in-house developed and validated Sample analysis: In house developed and validated UPLC-UV method

Results and Discussion

- The UPLC method successfully separated clindamycin phosphate (retention time: 4.3 min) and clindamycin base (retention time: 4.9 min).
- The average recovery of clindamycin phosphate in the drug product was 112.3% ± 2.6% (mean ± RSD, n=6); however, the content of clindamycin base did not exceed 0.01% w/w in the drug product.
- The IVPT results showed that both clindamycin phosphate and clindamycin base permeated across the skin at the 50 mg/cm^2 and 25 mg/cm^2 doses. IVPT profiles and recovery results are shown in Figures 1 and 2, and Tables 2 and 3, respectively.

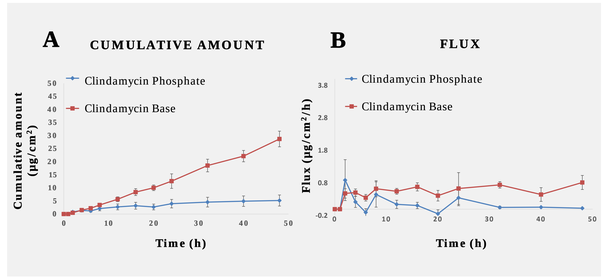

Figure 1. Cumulative amount permeated (A) and flux (B) of clindamycin phosphate and clindamycin base across dermatomed human cadaver skin (**50 mg/cm^2 dose**) at the end of 48 h. Data are represented as mean ± SD (n=6)

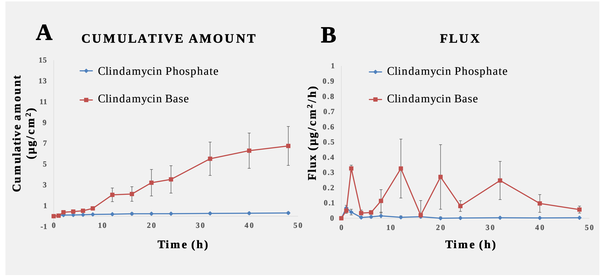

Figure 2. Cumulative amount permeated (A) and flux (B) of clindamycin phosphate and clindamycin base across dermatomed human cadaver skin (**25 mg/cm^2 dose**) at the end of 48 h. Data are represented as mean ± SD (n=6)

Table 2. Recovery (%) of clindamycin phosphate and clindamycin base (recovery based on conversion of clindamycin base from clindamycin phosphate) at the end of 48 h from mass balance studies (**50 mg/cm^2 Dose**). Data are represented as mean ± SD (n=6)

	Clindamycin phosphate (%)	Clindamycin base (%)
Receptor	0.6 ± 1.0	4.2 ± 1.13
Skin	3.6 ± 0.9	1.07 ± 0.48
Donor	58.2 ± 6.1	25.61 ± 1.82
Total	62.5 ± 5.36	30.88 ± 2.56

Table 3. Recovery (%) of clindamycin phosphate and clindamycin base (recovery based on conversion of clindamycin base from clindamycin phosphate) at the end of 48 h from mass balance studies (**25 mg/cm^2 Dose**). Data are represented as mean ± SD (n=6)

	Clindamycin phosphate (%)	Clindamycin base (%)
Receptor	0.09 ± 0.02	2.0 ± 0.68
Skin	0.18 ± 0.05	1.07 ± 0.32
Donor	38.2 ± 4.1	0.44 ± 0.26
Total	38.48 ± 4.13	3.51 ± 1.15

The results of the mass balance studies for 50 mg/cm^2 dose indicated that the recovery of clindamycin phosphate and clindamycin base were ~62.5% and ~31%, respectively, suggesting that the conversion of clindamycin phosphate to clindamycin base occurs, potentially in the residual formulation, on the surface of the skin, and/or during permeation into or through the skin.

It is important to note that the studies conducted with Cleocin T® lotion were exploratory studies that involved method conditions that are not aligned with the recommendations within the Draft Guidance on Acyclovir (for acyclovir topical cream, 5%) for the design and conduct of an IVPT study for establishing BE. For example, relatively high doses were used to alleviate analytical challenges for a better mechanistic understanding of the permeation of the two (anticipated) analytes. Despite the relatively high doses used, there were analytical challenges with the 25 mg/cm^2 dose group using the currently available analytical method in-house, which may be the reason for the higher variability in the data observed; However, the challenges may be expected to be addressed by a more sensitive mass spectroscopy-based method. Additionally, the study also used the partial sampling technique, which may explain the negative flux values within the flux profiles. IVPT studies involving conditions where lower doses and full receptor sampling would be used (which better align with the Draft guidance on acyclovir), are underway to identify the suitable analyte(s) for BE analysis.

Conclusion

The results of these exploratory IVPT studies suggests that both clindamycin phosphate and clindamycin base permeate across the skin, and that it may be feasible to utilize one or both analytes to evaluate the cutaneous pharmacokinetics of clindamycin from Cleocin T® lotion.

Acknowledgement and Disclaimer

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