Comparative Analysis of Human and Pig Skin Permeation of Metformin Lotion Formulations

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INTRODUCTION

The skin serves as a vital barrier against the external environment and regulates key physiological functions. The outermost epidermis contains the stratum corneum, whose "brick and mortar" composition effectively limits water loss and drug permeation [1]. Transdermal drug delivery systems (TDDS) offer a non-invasive route for medication administration but must overcome this barrier [2]. Metformin lotion (ML) was developed as a potential treatment for tendon injuries, with three formulations — containing glycerol (Gly), propylene glycol (PG), and/or Transcutol (T) — tested on human skin (HS) and pig ear skin (PS), a common surrogate model due to structural similarities.

METHODS

A 6% ML formulated with Gly only, a 6% ML with additional PG, and a 10% ML with PG and T were developed in house. Human abdominal and thigh tissue obtained from the Skin Wound Healing and Tissue Regeneration Lab (Department of Plastic Surgery, University of Pittsburgh) were freshly excised during plastic surgery and otherwise intended for disposal; thus, patient consent was not required. The HS samples were sourced from nondiabetic, Caucasian female donors, aged 35–50. *Ex vivo* pig ears were freshly butchered and purchased ethically from a local farm with skin and hair intact. HS and PS were prepared by removing all fat and adipose tissue until approximately 0.5 or 1.0 mm thick, respectively, and sectioned into 2 cm² squares. The skin sections were then placed in sterile gauze soaked with 1x PBS and stored at 4 °C overnight. Permeation testing was conducted on a 5 mL Franz diffusion cell system, maintained at 37 °C. Skin sections were submerged in PBS at 35 °C on a hot plate for 30 min to restore hydration and bring to temperature, before applying about 5 mg of lotion, and mounting onto the Franz cell. Samples from the receptor were collected over 48 h. After 48 h, the residual lotion was collected from the skin surface, and the epidermis and dermis were mechanically separated for Met quantification. Samples were analyzed using HPLC, and the cumulative permeation, flux, percent diffused, and percent recovered in each compartment were calculated. A Kruskal-Wallis test, followed by a pairwise Dunn's test, was performed to determine significant group differences between lotion formulations.

RESULTS

In HS, the PG+T lotion exhibited significantly greater permeation compared to both Gly and PG formulations, reaching 23.7 ± 16.4% diffusion into the receptor after 48 h (**Fig. 1, Table 1**). No significant difference was observed between Gly and PG lotions in HS, which had less than 5% of total Met diffused into the receptor. In PS, overall mean permeation was greatest in PS treated with the PG+T lotion, followed by PG and then Gly, however, variation was large, and the differences were not statistically significant (**Fig. 2**). Peak flux in PS and HS treated with PG+T were over 4x greater than the Gly and PG groups (**Fig. 1B, 2B**). Met permeation rates were greater in PS compared to HS for Gly and PG formulations but were similar for the PG+T lotion (**Table 1**). A substantial amount of Met was recovered within the epidermis and dermis for all groups, accounting for about 22-48% of the total Met.

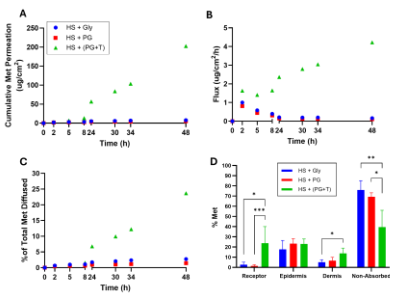

Fig. 1 Metformin permeation profiles of ML in human skin (HS): (**A**) cumulative permeation over time (μg/cm²); (**B**) flux (μg/cm²/h); (**C**) percentage of total Met diffused into the receptor over time; and (**D**) distribution of Met in the receptor, epidermis, dermis, and unabsorbed fraction after 48 h.

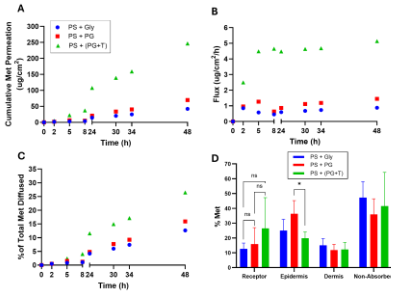

Fig. 2. Metformin permeation profiles of ML in pig skin (PS): (**A**) cumulative permeation over time (μg/cm²); (**B**) flux (μg/cm²/h); (**C**) percentage of total Met diffused into the receptor over time; and (**D**) distribution of Met in the receptor, epidermis, dermis, and unabsorbed fraction after 48 h.

DISCUSSION

Transcutol substantially enhanced Met permeation in HS, supporting its utility in TDDS development. While Gly and PG provided minimal receptor diffusion, both facilitated substantial dermal retention, suggesting value for topical applications. Species-specific differences — particularly with Gly and PG formulations — underscore the importance of model selection in permeation studies. Variability may reflect donor differences and skin preparation. Further optimization of Met dose and enhancer ratios may improve permeation efficiency.

SIGNIFICANCE/CLINICAL RELEVANCE: The addition of Transcutol to ML formulations supports development of metformin-based TDDS for tendinopathy, skin wounds, and related conditions through regulating cellular metabolic and anti-inflammatory pathways.

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[2] Naik, A., *et al.* Pharmaceutical Science & Technology Today, 2000. **3**(9): p. 318-326.

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Table 1. Summary of mean Met permeation profiles in human skin and pig ear skin.

Test Group	Receptor (%)	Epidermis (%)	Dermis (%)	Non-absorbed (%)	J_{max} (μg/cm²/h)	J_{steady} (μg/cm²/h)
HS + Gly	2.7 ± 2.4	17.6 ± 8.9	5.1 ± 2.5	76.0 ± 9.2	1.0 ± 0.5	0.2 ± 0.08
HS + PG	1.5 ± 1.2	23.3 ± 5.0	6.5 ± 3.4	69.3 ± 3.9	0.8 ± 0.9	0.13 ± 0.07
HS + (PG+T)	23.7 ± 16.4	23.0 ± 4.9	13.8 ± 5.2	39.5 ± 16.5	4.2 ± 2.8	n/a*
PS + Gly	12.6 ± 4.0	25.0 ± 7.6	15.1 ± 4.6	47.3 ± 10.8	0.7 ± 0.3	0.7 ± 0.3
PS + PG	15.9 ± 11.1	36.3 ± 8.9	11.9 ± 3.9	35.9 ± 10.6	1.1 ± 1.0	1.1 ± 1.0
PS + (PG+T)	26.5 ± 20.7	19.8 ± 4.2	12.2 ± 4.8	41.5 ± 22.8	4.7 ± 3.4	4.7 ± 3.4

* Steady-state flux was not reached within 48 h.