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Investigation of the Absorption of Allantoin From SD-101 in In Vitro Skin Models to Support Wound Healing

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INTRODUCTION

- Allantoin is a heterocyclic organic compound that has been investigated in wound healing, using formulations with minimal or unknown dermal penetration properties[1,2]
- SD-101 is a novel, proprietary, topical, allantoin-containing cream in development for the daily treatment of wounds caused by all major types of epidermolysis bullosa.[3] SD-101 has received Breakthrough Therapy designation from the US Food and Drug Administration[4]
  - Epidermolysis bullosa is a rare genetic disorder typically manifesting at birth as skin blistering/erosion and, in some cases, the epithelial lining of other organs, in response to minimal friction/trauma[5]
  - In a phase 2b study, patients with epidermolysis bullosa treated with SD-101 6% (SD-101 with 6% allantoin) demonstrated a higher rate of wound closure over a 1-month period than placebo-treated patients[3]
  - SD-101 6% is currently in phase 3 clinical development
- In vitro human cadaver and porcine models are recognized valuable tools to assess the skin absorption and to determine the pharmacokinetics of topically applied drugs[6-8]
- Separate preclinical studies of SD-101 with allantoin concentrations of up to 9% indicated no systemic absorption[9]

OBJECTIVE

- To investigate the skin absorption of 0.5%, 1.5%, 3%, 6%, and 9% concentrations of allantoin, the active ingredient in the SD-101 formulation, in skin models that mimic intact, broken, or blistered human skin

METHODS

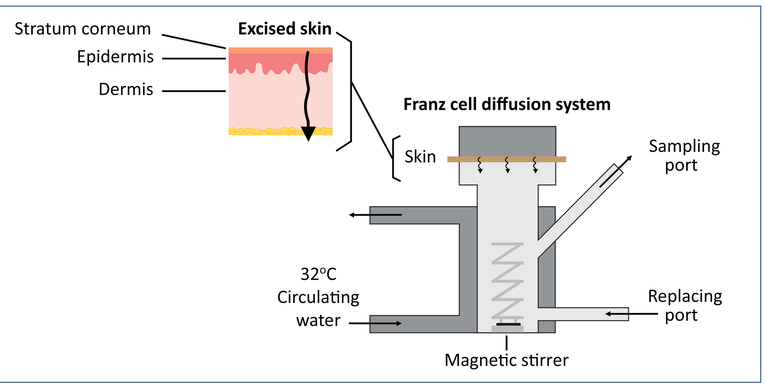
Models

- The skin absorption of 0.5%, 1.5%, 3%, 6%, and 9% concentrations of allantoin, the active ingredient in the SD-101 formulation, was investigated in 5 in vitro models:
  - Barrier-free to simulate delivery directly to the capillary bed
  - Unabraded porcine skin
  - Abraded porcine skin to simulate compromised skin
  - Intact (full thickness) human skin
  - Dermis-only human to mimic loss of skin barrier function due to broken skin

Skin Cadaver Preparation

- All human and porcine cadaver trunk skin without obvious signs of skin disease was stored at less than −70°C within 24 hours of death. On experiment day, the bagged tissue was thawed in 37°C water and rinsed to remove any adherent blood or material from the surface. Approximately 75% of the dermis was removed by dermatome or scalpel visually
  - Donor skin was cut into smaller sections and fitted on 0.8-cm² Franz diffusion cells. The diffusion cells were then placed between the epidermal chamber (route of drug application) and the dermal chamber, which was filled with magnetically stirred phosphate-buffered saline, and sampled at selected time points (**Figure 1**)
  - The permeability to tritiated water was determined prior to experimentation to assure skin integrity[10]

Figure 1. Schematic of the Skin Franz Cell Diffusion System[11]



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Application

- Concentrations of SD-101 or vehicle (SD-101 minus active ingredient) were tested on ≥3 sections from 6 different skin donors (3 cadaver, 3 porcine) that were mounted in chambers designed to maintain skin at a temperature and humidity matching typical in vivo conditions
  - Each test product was applied at a target dose of 100 µL/cm² using a calibrated positive displacement pipette and then covered with 3 layers of medical-grade gauze

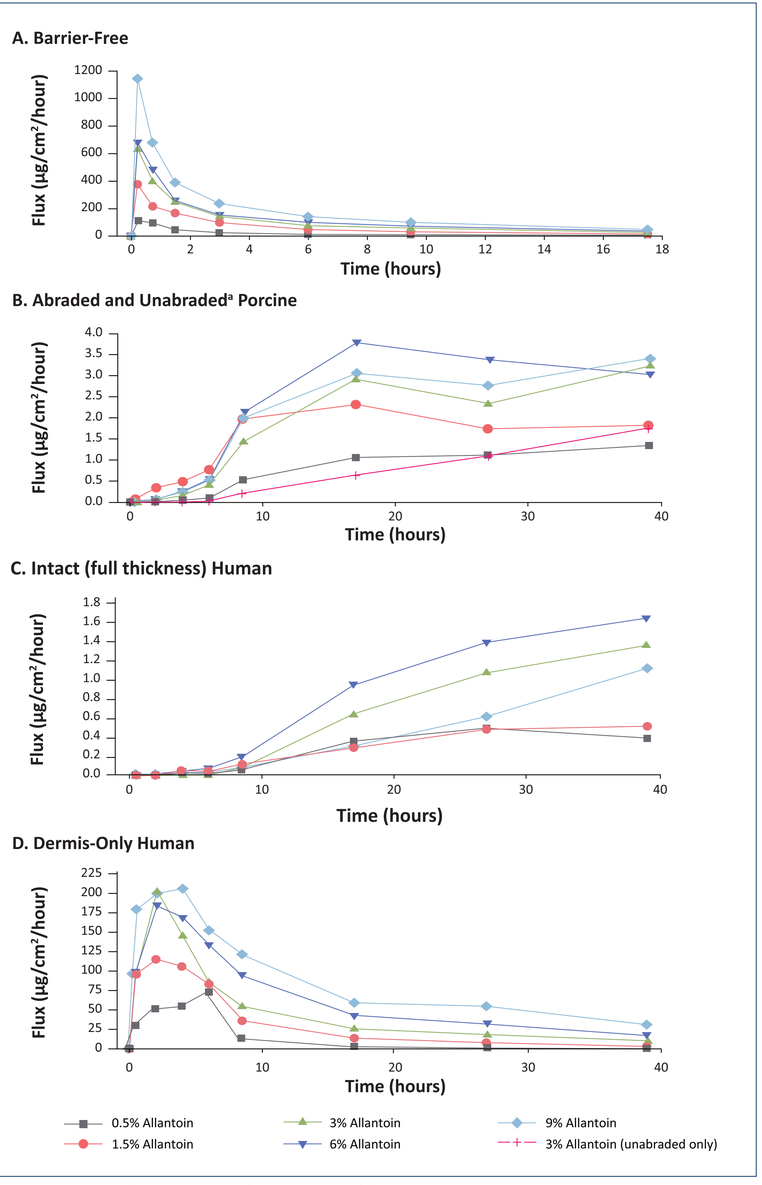
Sampling

- After SD-101 application, skin absorption (total absorption, rate of absorption, and skin content) was measured by monitoring the rate of appearance of drug in the solution bathing the inner surface of the skin
  - Samples were collected roughly 2, 4, 8, 12, 24, 32, and 48 hours after application and analyzed for allantoin using high-performance liquid chromatography with ultraviolet and mass spectrometry detection

RESULTS

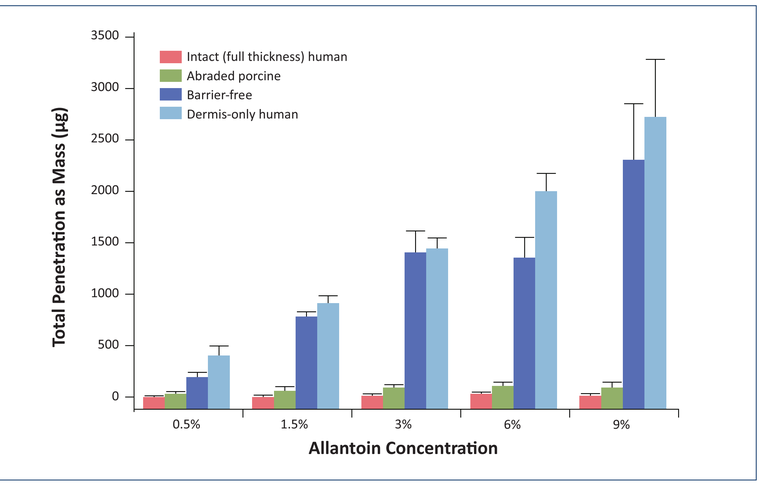
- In the SD-101 formulation:
  - There was evidence of skin absorption of allantoin in all models (**Figures 2** and **3** and **Table 1**)
  - Skin absorption of allantoin was lowest in intact human skin (**Figure 3** and **Table 1**)
  - Skin absorption increased with higher concentrations of allantoin in the dermis-only human model; uptake between the barrier-free and dermis-only human models was similar
  - Allantoin skin absorption in the human skin models was slow (≈8 hours for dermis-only), suggesting a long skin-exposure time (**Figures 2C** and **2D**)

Figure 2. Skin Absorption of Allantoin (0.5% to 9%), a Component of SD-101, Within 40 Hours in Various Skin Models



Data are represented as mean from ≥3 replicates per formulation as µg/cm²/hour.
[a]Only 3% allantoin was tested in the unabraded model.

Figure 3. Total Skin Absorption of Allantoin Over 48 Hours From a Single Application



Data are represented as mean ± standard error from ≥3 replicates per formulation as total mass (µg).

Table 1. Total Skin Absorption of Allantoin (µg) Over 48 hours From a Single Application

Skin Model	Allantoin Concentration				
	0.5%	1.5%	3%	6%	9%
Intact (full thickness) human	12.05 ± 1.88	13.23 ± 5.61	32.04 ± 11.42	41.57 ± 8.43	22.74 ± 12.15
Abraded porcine	38.80 ± 18.34	68.91 ± 31.01	95.74 ± 25.55	109.52 ± 39.31	103.70 ± 34.23
Barrier-free[a]	196.48 ± 45.30	792.25 ± 39.83	1399.49 ± 205.99	1348.30 ± 201.39	2294.42 ± 552.35
Dermis-only human	412.60 ± 96.30	910.51 ± 73.99	1434.19 ± 95.71	1990.40 ± 167.34	2718.74 ± 548.33
Unabraded porcine	Not tested	Not tested	38.64 ± 15.24	Not tested	Not tested

Data are represented as mean ± standard error from ≥3 replicates per formulation as total mass (µg).
[a]Barrier-free study was conducted over 24 hours.

CONCLUSIONS

- Allantoin, the active ingredient of the SD-101 formulation, is absorbed by the intact stratum corneum, or skin, which may reduce wound formation
- In the SD-101 formulation, allantoin skin absorption in the intact (full thickness) human model was slow, suggesting a long skin-exposure time
- In damaged skin models that provide insight into the absorption of wounded skin, absorption of allantoin increased significantly
- Substantial skin absorption of 6% allantoin occurred over several hours in the intact full-thickness human skin model, suggesting that SD-101's formulation, with higher concentrations of allantoin than are currently used, is capable of penetrating human skin
- In summary, these findings further support the therapeutic investigation of SD-101 6% to treat wounds in a clinical setting and the ongoing phase 3 ESSENCE trial in epidermolysis bullosa (NCT02384460)

REFERENCES

1. Becker LC et al. *Int J Toxicol*. 2010;29(suppl 2):84S-97S.
2. Xi H et al. *J Bacteriol*. 2000;182(19):5332-5341.
3. Paller A et al. Presented at: 42nd Annual Meeting of the Society for Pediatric Dermatology; July 14-17, 2016; Minneapolis, MN.
4. Aggarwal SR. *Nature Biotechnol*. 2014;32(4):323-330.
5. El Hachem M et al. *Orphanet J Rare Dis*. 2014;9:76.
6. Franz TJ. *J Invest Derm.* 1975;64(3):190-195.
7. Franz TJ. In: Simon GA et al, eds. *Skin: Drug Application and Evaluation of Environmental Hazards*. Basel, Switzerland: Karger; 1978:58-68. *Current Problems in Dermatology*; vol 7.
8. Schmook FP et al. *Int J Pharm*. 2001;215(1-2):51-56.
9. Amicus Therapeutics Inc. Investigators Brochure, Data on File.
10. Franz TJ, Lehman PA. *J Invest Dermatol.* 1990;94:525.
11. Kim KW et al. *Opt Express*. 2012;20(9):9476-9484.

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DISCLOSURES

Conflicts of Interest

AP is an investigator and a consultant for Amicus Therapeutics. RN and JG are employees of Scioderm - An Amicus Therapeutics Company and own stock in Amicus Therapeutics. HD, AR, CV, HL, JPC, and JAB are employees of and own stock in Amicus Therapeutics.

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