Investigation of Reversible Protein Binding of Solutes in Human Stratum Corneum for Validation of Reservoir Effect

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Introduction

The stratum corneum reservoir is an effect that occurs when a chemical binds to the proteins in the stratum corneum which therefore prolongs its presence and potentially also prolongs its effects. The reservoir was first recognized by Vickers in the 1960's when a study on topical hydrocortisone revealed that the typical skin blanching effects could be exhibited days beyond initial exposure.[1] Initial application and occlusion causes blanching of the skin, due to the vasoconstrictive properties of the steroid. Even with typical skin washing and no reapplication, when the test site was occluded 6 days later, the blanching was again observed. Therefore, the hydrocortisone was stored within the skin somehow and later re-emerged. This same study proved that this storage occurs in the stratum corneum.

The reservoir effect has been observed in many chemicals, not just corticosteroids. The working theory is that increased keratin binding of the solute favors the formation of the reservoir.[2] Additionally, reservoir formation will depend on the partitioning and diffusion of the solute.[3]

While widely recognized, little research exists on reservoir formation and quantification. This project aims to remedy this. Studying the reversible binding of solutes from the stratum corneum's primary protein-keratin, will allow for better understanding of reservoir formation. Reversible binding of solutes from keratin will provide valuable kinetic data such as partition coefficients and binding rates.

Objective

Understanding the stratum corneum reservoir is necessary for risk assessments of topical products as well as accidental exposures to hazardous chemicals. If a chemical can bind to the stratum corneum and remain present for extended periods of time, this may increase the length of adverse effects, or may prolong the onset of beneficial effects of the product due to it being withheld in the stratum corneum. By quantifying and understanding how the stratum corneum reservoir functions, then more efficacious topical products can be developed. Additionally, if one is exposed to hazardous material, knowing how long that chemical may be present in the stratum corneum will allow for better treatment options.

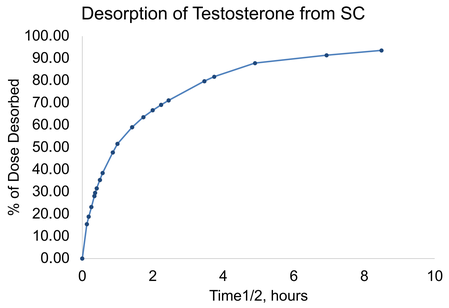
Desorption

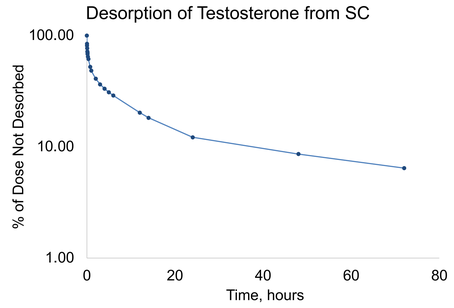
The stratum corneum is equilibrated in the solute-buffer solution for 24 hours. Equilibration allows for binding of the solute to the keratin in the stratum corneum.

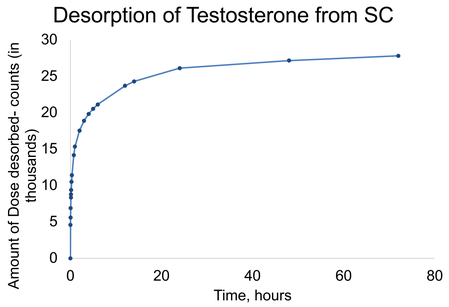
Desorption is the process in which the bound solute unbinds from keratin. By measuring how much solute desorbs, we can quantify how much solute was originally bound to the keratin. To facilitate desorption, the stratum corneum is removed from the equilibration solution, rinsed, and then placed into fresh buffer containing no solute. This process is then repeated at various time points. By desorbing the solute in steps, each time point provides information about the amount of solute desorbing from the stratum corneum at a specific time. This will allow for determination of desorption rate.

Equilibrium binding and desorption are carried out under constant agitation and are kept at a temperature near that of the skin surface.

Results







Desorption of Radiolabeled Testosterone from Isolated Stratum Corneum

% normalized to amount of testosterone present in samples

Stratum Corneum Characteristics	Kinetics	
Isolated SC	PC_{pro}	84.22
5.4 mg	$K_{sc/w}$	23.94
262.5 mm^2	k_{off}	0.02

Sample Preparation

Tissue Preparation

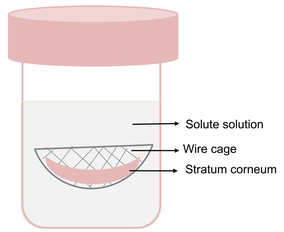
- Split thickness skin processed with heat separation and trypsin soak to isolate stratum corneum
- Isolated SC dried and weighed
- Isolated SC then treated with 2:1 chloroform:methanol to delipidize
- Delipidized SC dried and weighed to quantify lipids removed

Solute Preparation

- Sodium citrate buffer
  - Formulated to skin pH (5-5.5)
  - Receptor solution for desorption
  - Solute solution for equilibrium
- Radiolabeled chemicals of interest
- Solute solutions
  - Contain low chemical concentration of solute
  - May contain some amount of unlabeled solute

Desorption Vial

- 5 ml of equilibrium solution/10 ml receptor solution
- SC held within wire cage
  - Prevents tissue from sticking to the side of vial



Kinetics

Partition Coefficient[3]	PC= (mass of solute in the hydrated SC per mass of the original dry SC) / (mass of solute per unit mass of water in the adjacent solution)
Correction for Hydrated SC[4]	$K_{SC/w}$=PC/3.518
Relationship between forward and reverse binding rates[2]	Rate of binding= $k_{on}c_u$ - $k_{off}c_b$
Fick's Law (to determine diffusion)[5]	$\frac{\partial C_m}{\partial t} = D_m \frac{\partial^2 C_m}{\partial x^2}$
Relationship between PC and k_{off}[2]	$1/k_{off}$ = 25.75 + 0.459K

Data Analysis

Analysis of all samples is achieved using scintillation counting. All desorption samples, equilibrium solutions, rinses, and tissue are counted to quantify solute. The number of disintegrations per minute (DPM) of each sample quantifies the amount of solute in the sample. The equilibration solution is counted prior to equilibration and after. This difference is the amount of solute that should be bound to the stratum corneum. Compiling the results of the desorption samples will provide the cumulative amount of solute desorbed from keratin. By comparing the amount of solute that should have bound to the keratin, and the amount that is desorbed at each time point, we can determine specific kinetics from the system.

Conclusions

This project is just beginning and will expand to include many other solutes. In addition to the experimental work with isolated and delipidized stratum corneum, computer models will help to further advance our understanding of keratin binding kinetics and the stratum corneum reservoir.

Our existing data show that desorption as a tool to measure keratin binding kinetics will be successful in proving our hypothesis.

Acknowledgements

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