Assessing Barrier Integrity and Normality of Skin for In Vitro Permeation Tests (IVPT) using both ^{3}H-Water and ^{14}C-Octanol

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INTRODUCTION

In order to properly evaluate the percutaneous absorption of compounds dosed upon excised skin *in vitro*, the barrier integrity of the skin specimen must first be confirmed as a validation of the test system. As skin permeability to water (H_2O) has been extensively characterized, $^{3}H_2O$ permeability is frequently used to verify skin barrier integrity for IVPT studies. To better understand skin barrier integrity the absorption of $^{3}H_2O$ and ^{14}C-octanol were characterized simultaneously in a combined test with 245 excised trunk skin sections from 31 human donors. The goals were to 1) assess the normal range of absorption for each model compound, 2) to establish boundary limits for the normal absorption of each in the population that may support exclusion criteria, 3) to compare the results of water absorption with octanol absorption, and 4) to better differentiate the concepts of barrier integrity and/or barrier function in relation to hydrophilic and hydrophobic molecules.

MATERIALS AND METHODS

A test solution of ^{14}C-octanol (0.3 Ci/mL; 0.016 mg/mL) in $^{3}H_2O$-water (0.6 Ci/mL) was dosed to cryopreserved, dermatomed *ex vivo* human skin mounted onto static 1 cm^2 Franz diffusion cells. Skin surface temperature was maintained at 32 ± 1°C, and ambient laboratory conditions were 21-24°C with 40-55% RH. The receptor solution, stirred at 600 RPM, was comprised of normal phosphate buffered saline supplemented with 0.1% oleth-20 and 0.008% gentamicin. A 200 - 300 µL dose of the test solution was administered to fully cover the skin surface area. Five minutes after application, the dose was removed by blotting until visibly dry. Thirty minutes after dose application the receptor solution was removed and an aliquot saved for analysis. ^{3}H and ^{14}C were quantified by mixing 1 mL of the receptor sample with 6 mL of scintillation fluid, and analyzed in a scintillation counter using dual-channel isotope discrimination with external standard quench correction.

Figure 1. Frequency distribution of $^{3}H_2O$ and ^{14}C-octanol absorption. Data from 31 donors with all sections included

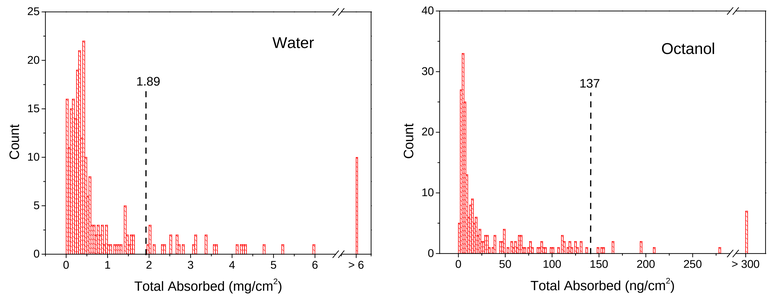


Figure 2. $^{3}H_2O$ absorption (mg/cm^2) from all sections and donors. Line at 1.89 mg/cm^2 is the determined acceptance threshold.

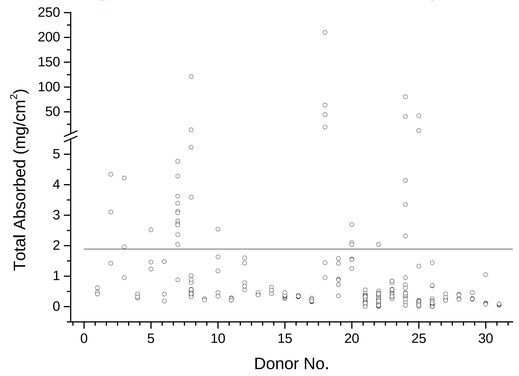
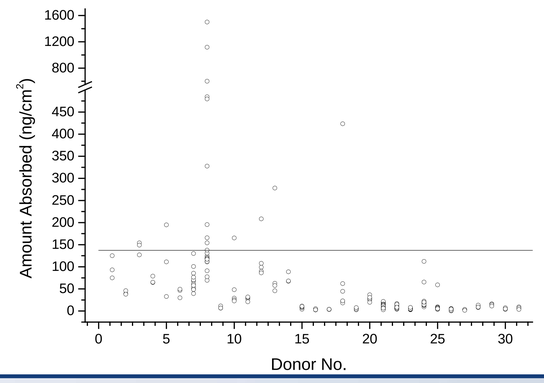


Figure 3. ^{14}C-octanol absorption (ng/cm^2) from all sections and donors. Line at 137 ng/cm^2 is the determined acceptance threshold.



RESULTS

It was observed that $^{3}H_2O$ and ^{14}C-octanol absorption did not correlate to each other except when gross skin barrier integrity damage was apparent. Of several statistical methods used, the Tukey outlier method on the median amount permeated was found to be the most conservative. The permeability acceptance limit for normal human skin was determined to be ≤ 1.89 mg/cm^2 for $^{3}H_2O$, and ≤ 137.2 ng/cm^2 for ^{14}C-octanol. The mean permeation of $^{3}H_2O$ for all sections was 3.35 ± 17.29 mg/cm^2; and after outlier removal, 0.42 ± 0.37 mg/cm^2. The mean permeation of ^{14}C-Octanol for all sections was 44.48 ± 143.98 ng/cm^2; and after outlier removal, 27.88 ± 34.57 ng/cm^2.

CONCLUSIONS

Either $^{3}H_2O$ or ^{14}C-octanol, as a single marker, can be useful for barrier integrity testing to identify grossly damaged skin sections. However, it is challenging to interpret the results when both markers are used simultaneously. A large number of skin sections identified as outliers for $^{3}H_2O$ were not identified as outliers with ^{14}C-octanol (15%), and vice versa. This suggests that local anomalies or imperfections in barrier function may influence the percutaneous absorption of compounds differently based on their hydrophobic or hydrophilic properties. When there is a discrepancy in absorption between the two using the combined test, one may opt to reject the skin section based upon abnormal permeation for either marker, or to retain that skin section with the caveat that it may possess a potentially abnormal barrier function. Further research is warranted to determine how $^{3}H_2O$ and ^{14}C-octanol absorption, in a combined skin barrier integrity test, may provide information about the physiology of the stratum corneum and its permeability barrier function.

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