An Investigation of Cutaneous Absorption of Caffeine through Elderly Female Epidermis

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ABSTRACT SUMMARY
Skin permeation of model compounds in elderly skin was evaluated and their permeability coefficients (K_p) were measured. The differences in stratum corneum thickness of elderly skin compared to young adult skin do not affect overall percutaneous absorption. The observed variability in K_p indicates that a robust assessment of compound absorption requires at least six skin donors.

INTRODUCTION
Percutaneous absorption of various model compounds has been well documented in young adults (20-30 years old). However, due to an increase in the aging population, evaluation of skin permeation in elderly skin merits more attention. In order to standardize an elderly skin permeation model, we sought to determine the minimal number of subjects needed for statistical evaluation.

In this study, penetration of caffeine solubilized in two different vehicles, propylene glycol (PG) and PG/ethanol (PG:OH), was examined using abdominal epidermis obtained from elder female donors. Compound absorption levels in the epidermis and in the receptor fluid were evaluated quantitatively by high performance liquid chromatography (HPLC) and qualitatively by antibody-based immunostaining.

EXPERIMENTAL METHODS
All studies were performed using abdominal human cadaver skin from female donors, age 45 or older regardless of race. Dermis was removed from epidermis by treating with a sodium bromide solution. Saturated caffeine solution (7700 µg/mL) was prepared in propylene glycol or propylene glycol with ethanol co-solvent. Diffusion of caffeine from two test solutions (PG or PG:OH) across human cadaver epidermis was evaluated using the Franz diffusion cells. The effective diffusion area of the Franz cells was 1 cm² and receptor volume was 8 mL. The receptor compartment was filled with degassed PBS containing antibiotics and isolated epidermis was mounted between the donor and receptor compartment. Integrity of epidermal layer was assessed via measurement of trans-epidermal water loss (TEWL). Specimens with TEWL value of 18 or higher were excluded from this study. Once caffeine solution (0.5 mL) was added onto the epidermal surface in the donor compartment, all openings, including donor top and receptor arm, were occluded to prevent evaporation. Quantitative evaluation of caffeine diffused through the epidermis into the receptor fluid was performed using HPLC at 0, 2, 4, 6, 8, 24, 32, and 48 hours.

To measure residual caffeine in treated epidermis and thickness of stratum corneum (SC) and epidermis, skin tissues were recovered after each diffusion experiment. The amount of residual caffeine was analyzed by homogenizing a 6 mm biopsy punch of tissue in 1 mL of PBS and analyzing the supernatant by HPLC. Thickness of SC and epidermis were measured from H&E stained tissue using an image analysis software along with a microscope. In order to reduce the variability of the data and to obtain normal distribution, data were log transformed prior to statistical analysis.

RESULTS AND DISCUSSION
Caffeine in PG:OH solution was found to have greater diffusion through the epidermis than caffeine in PG only. After 48 hours, the concentration of caffeine in the receptor
compartment was 1.5 fold higher in PG:OH compared to PG (n=21). Although there was a significant increase in the amount of caffeine in the receptor fluid when ethanol was added into the formulation, accelerated rate of penetration (flux) due to the presence of ethanol was reduced over time (Figure 1).

Addition of ethanol, a known penetration enhancer, increased $K_p$ and significantly enhanced overall caffeine penetration. $K_p$ of caffeine was $20.02 \pm 1.9 \times 10^{-5}$ cm/h and $28.56 \pm 2.2 \times 10^{-5}$ cm/h for caffeine dissolved in PG and PG:OH, respectively. Although age-related SC thinning was reported, caffeine $K_p$ in PG were similar to previously published data using skin derived from young donors ($22.1 \times 10^{-5}$ cm/h). The average thickness of epidermis was $22.8 \pm 1.1 \, \mu$m and the thickness of SC was $12.8 \pm 2.6 \, \mu$m.

Due to donor to donor permeation variance, all the PG:OH data were normalized by subtracting mass or flux data from donor matched PG data (figure 2). Drug permeability through ex vivo human skin, especially in the initial period, was found to be increased in the presence of ethanol.

Caffeine retained in epidermis was higher ($1.62 \pm 0.31 \, \mu$g/cm$^2$) in PG compared to PG:OH ($1.04 \pm 0.17 \, \mu$g/cm$^2$), however no statistically difference was found (paired t-test; $p = 0.142$). Immunostaining of the caffeine-treated epidermis demonstrated that a pronounced amount of caffeine was retained in the SC and while caffeine penetrated intracellularly through the suprabasal and basal strata.

![Figure 1. Diffusion profile of caffeine in different vehicles.](image1)

![Figure 2. Donor matched mass accumulation differential (a) and flux differential (b) due to effect of ethanol.](image2)

CONCLUSION

These results suggest that differences in SC thickness, $12.8 \pm 2.6 \, \mu$m in elderly skin compared to $18.3 \pm 4.9 \, \mu$m in young adult skin, do not affect overall percutaneous absorption. Further, the observed variability in $K_p$ indicates that a robust assessment of compound absorption requires at least six skin donors.

REFERENCES