Comparative studies of QuSome, liposome, oil-water emulsion and other non-liposomal delivery vehicles

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1. Comparative Study of *In Vitro* Cutaneous Dispositions of Topical Caffeine Formulations in Human Skin: Enhanced Penetration Using Novel QuSomal Caffeine Formulation

INTRODUCTION

Only few studies have been reported on human skin models dealing with the effects of vehicle on percutaneous penetration. Apart from a comparative study carried out on a simple emulsion versus a multiple emulsion containing caffeine applied at infinite dose, most reports relate to the influence of simple vehicles such as water or petrolatum on percutaneous absorption. To our knowledge, no investigation has been published on the effects of a cosmetic formulation on percutaneous absorption using human skin models when the formulation is applied at a finite dose and under realistic exposure conditions.

MATERIALS

[C^{14}]-caffeine was purchased from Sigma Chemical Co. Specific activity was 55 mCi/mmol and radiochemical purity was > 98.5%%. Three formulations were made for caffeine, with different delivery vehicles, a QuSomal® cream, a non-liposomal cream and an aqueous solution. The non-liposomal cream was made with standard commercially available surfactants. The caffeine creams were formulated prior to experiment. All other chemicals were of analytical grade from standard suppliers.

METHODS

Dose Preparation

i) To make Qusome® cream, 246mg of aqueous phase containing 30μCi of ^{14}[C]-caffeine was heated to 60°C. 54.79 mg of oil phase containing QuSomal® lipids was also heated to 60°C. The oil phase was added to the aqueous phase and sonicated for 60 minutes to yield the cream. 3 mg of benzyl alcohol was further added to the cream at room temperature and the mixture sonicated for 15 mins.

ii) non-liposomal cream was made similar to QuSomal® cream. Instead of QuSomal® lipids, commercial surfactants were used.

iii) Aqueous Caffeine solution: 231μl (30μCi) of aliquot of an ethanolic solution of ^{14}[C]-caffeine(containing 3.53μg/μCi) was taken in a vial. The solvent was gradually evaporated using a constant flow of nitrogen gas. 300μl of water was added to the vial and sonicated for 30 min.

Test System

Human cadaver skin was dermatomed prior to delivery, using a Padgett Electrodermatome, to a target thickness of 500 μm. The skin samples were placed in Eagles Minimum Essential Media (MEM) and stored in the refrigerator at 4°C, until use. Wester *et al.* has shown skin to be viable using this storage regimen.[i]

Dose Application

The formulations are identified as A, B and C. Distinction among these are as follows:

10 μl of non-liposomal [C^{14}]-caffeine cream (containing 1.0 μCi) to 0.78 cm^2 skin area

10 μl of QuSomal® [C^{14}]-caffeine cream (containing 1.0 μCi) to 0.78 cm^2 skin area

10 μl of aqueous [C^{14}]-caffeine solution (containing 1.0 μCi) to 0.78 cm^2 skin area

Diffusion Study

The human skin was clamped into a continuous flow-through diffusion cell system.[ii] The glass cell had a 0.78 cm^2 surface area of exposed skin, which was maintained at 32 °C using a Lauda Heating Circulator (Lauda, West Germany). The receptor fluid, MEM, with 50 μg/ml gentamicin sulfate added to ensure sterility during the running of the experiment, was pumped at a rate of 3 ml/hr. Receptor fluid samples were collected every 4 h up to 48 h.

Forty eight hours after dose application, skin surface wash was performed on all skin samples (washed once with 50 % Ivory™ liquid soap/Nanopure water, and then rinsed twice with Nanopure water). Wash and rinse samples for individual skin surfaces were combined.

After skin surface washing, ten consecutive tape strips were performed on all skin samples and added to a separate vial. The remaining skin was separated between epidermis and dermis by putting the skin sample in water at 60 °C for 1 min. The two layers of skin separated out. The two separated layers of skin were placed in separate scintillation vials and digested by adding 5 ml of Packard Soluene-350 for 72 h at room temperature.

Duplicate aliquots (1 ml) of the receptor fluid fractions were taken and assayed for radioactivity by liquid scintillation spectrometry (Packard 1500 counter), after addition of 19 ml scintillation cocktail (Universol, ICN). All other samples (tape strips, skin digest solutions and skin surface washes) were also counted for radioactivity after addition of 15 ml Universol.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA test on SigmaStat (SPSS Inc., Chicago, IL, USA).

RESULTS

The *in vitro* percutaneous absorption (receptor fluid + stratum corneum + epidermis + dermis) of caffeine as mass absorbed (μg) for single dose is given in Table 1. as well as Figures 1 and 2.

Dose Accountability

The human skin surface was washed at the end of the experiment (48 h) to remove any residual radioactivity that did not penetrate the stratum corneum. Table 1. gives the data as percent of applied dose, including dermal reservoir, skin surface wash and dose accountability. Total recovery of radioactivity was good for single dose study for Qusomal and placebo formulations and ranged from 83.9 ± 5.31% to 87.9 ± 4.46%. The recovery of radioactivity was 80.7 ± 24.5% when aqueous caffeine was applied.

Steady State Flux and Kp Values

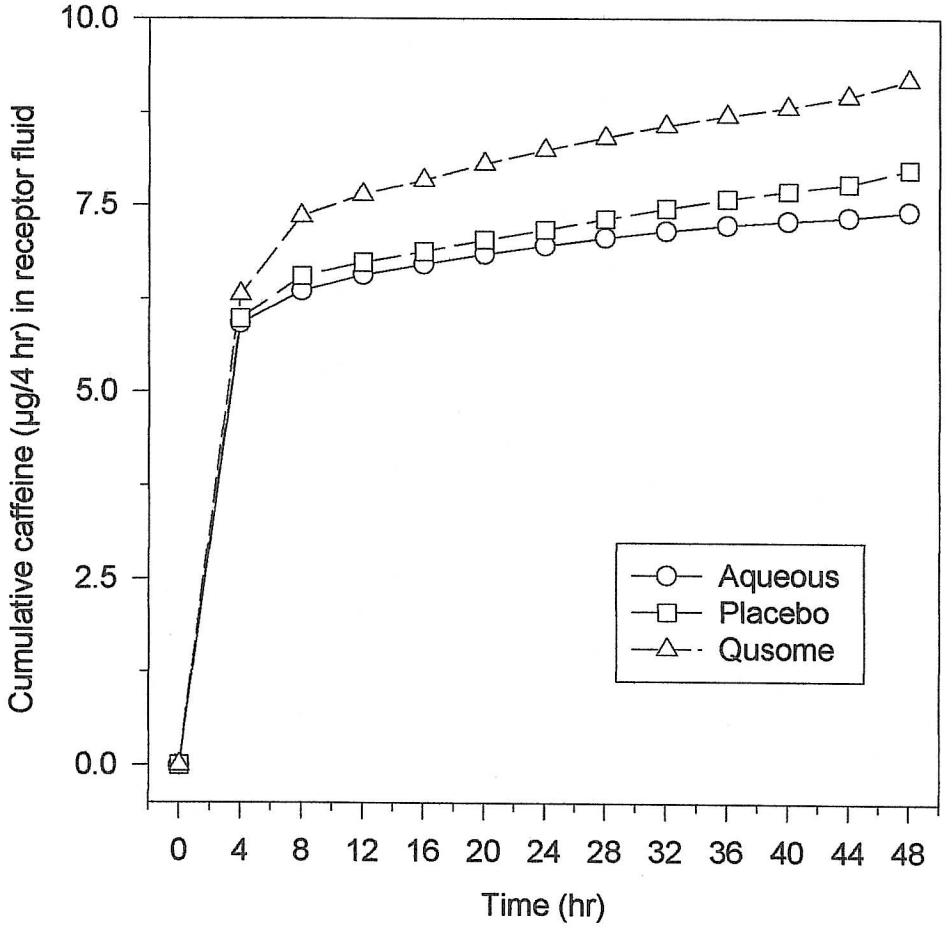
Flux values are calculated during steady state absorption into the receptor fluid and are expressed as mass transfer/unit area of skin/unit time (namely, $\mu g/cm^2/h$). The apparent permeability coefficient (Kp) is an important factor as it is independent of the applied dose. It is calculated from the following equation: Kp = absorption (μg)/ (surface area x concentration ($\mu g/cm^3$) x time). Table 2. shows the data calculated for all the formulations. It was observed that the flux for caffeine in QuSomes was the maximum being 0.06 $\mu g/hr/cm^2$, follwed by non-liposomal emulsion and then aqueous caffeine solution.Cmax, which represents the amount of caffeine in the receptor fluid at Tmax was also maximum for QuSome formulation (0.43μg) and followed the order Qusome > non-liposomal emulsion > aqueous caffeine solution.

DISCUSSION

The *in vitro* absorption of caffeine applied as QuSomal®, non-liposomal and aqueous formulations have been studied in human viable skin. Penetration of caffeine from the QuSomal® and non-liposomal vehicles, into and through human skin was extensive after a single dose. A high proportion of the QuSome® caffeine cream actually penetrated through the skin, demonstrating good transdermal delivery of caffeine to the deeper layers of skin. After a single dose, absorption kinetics were

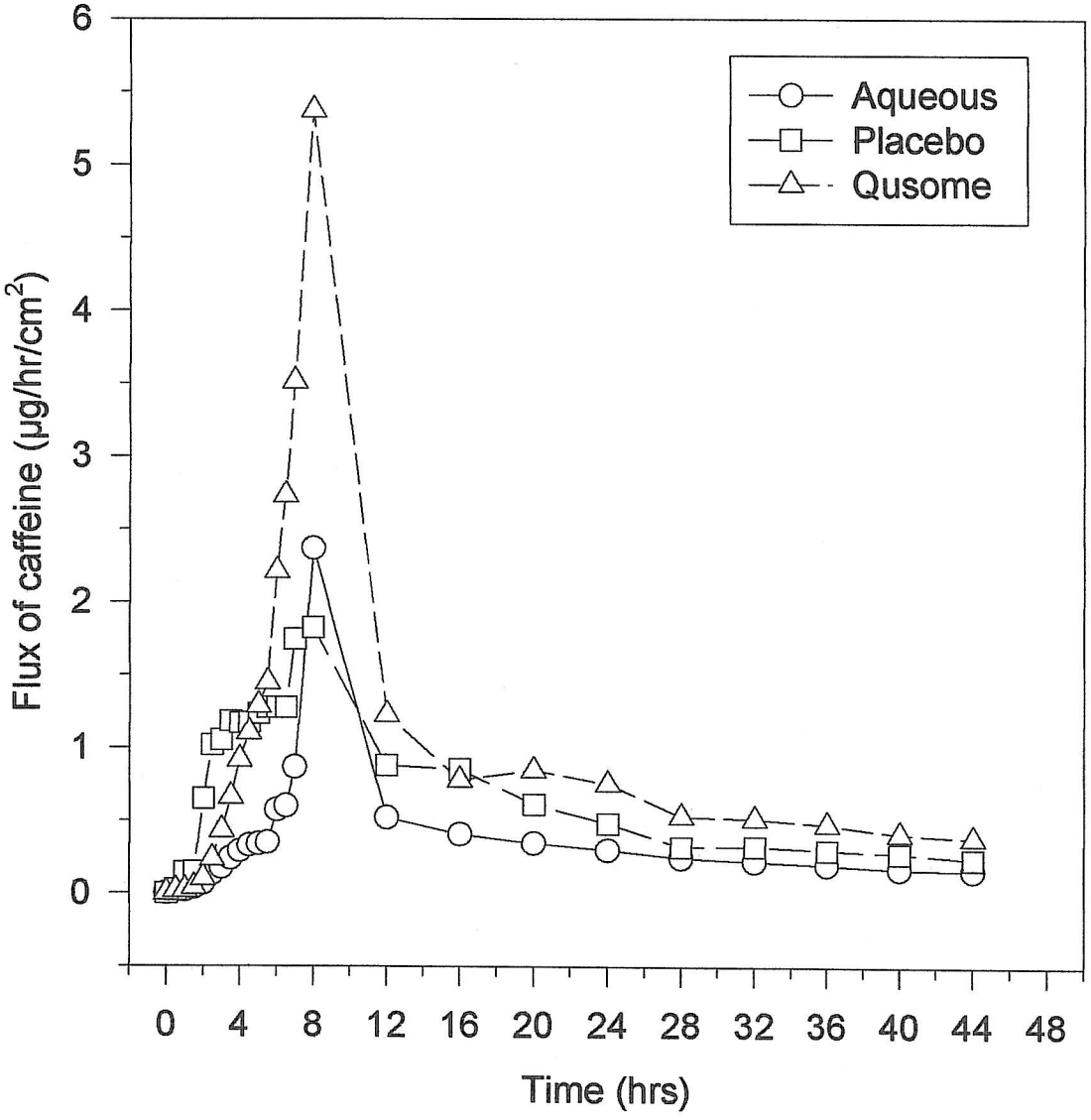
relatively rapid over the first 8 h, after which time the rate slowed up to 48 h. Moreover, the concentration of caffeine in the receptor fluid at T_{max} followed the order [QuSome®] > [non-liposomal placebo] > [aqueous solution]. The area under the curves (AUC) directly measures the amount of caffeine present in the receptor fluid after the dosing period. The AUC values also follow the trend [QuSome®] > [non-liposomal placebo] > [aqueous solution]. The deeper the active ingredient penetrates into the skin and to the receptor the fluid, the more would be the amount of caffeine present in the receptor fluid and hence a higher value of the AUC.

Cumulative Caffeine in Receptor Fluid



Flux of Caffeine

Receptor fluid cumulation of [^{14}C]-caffeine



LEGEND

Table 1. Mass balance of radioactive recovery of [^{14}C]-caffeine as percent administered
dose in three topical formulations. Each number represents the mean ± S.E. of 10 samples. Statistically significant (P > 0.05)

Table 2. Pharmacokinetic analysis of in vitro skin penetration of [^{14}C]-caffeine in three formulations

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[i] R.C. Wester, J. Christoffel, T. Hartway, N. Poblete, H.I. Maibach. Cadaver human skin viability for *in vivo* percutaneous absorption: Storage and detrimental effects of heat-separation and freezing. Pharm. Res. **1998**, *15*, 82-84

[ii] R.C. Wester, P. K. Noonan, H.I. Maibach.Variations in percutaneous absorption of testesterone in the rhesus monkey due to anatomic site of application and frequency of application. *Arch. Dermatol. Res.* **1980**, *116*, 186-188