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Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers

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Summary

Delivery systems for caffeine have been designed for two different purposes: (1) enhancing drug permeation through the skin for systemic delivery and (2) accumulating drug reservoir in the skin for local delivery. Caffeine exhibited concentration-dependent growth inhibition of normal and psoriatic human fibroblasts, as well as 3T3 mouse fibroblasts. High flux of caffeine through the skin was obtained from an aqueous solution containing an enhancing mixture of 20% Transcutol and 10% oleic acid. The presence of the enhancers resulted in caffeine flux 40 times greater than in their absence. The high flux of caffeine through the skin in vitro which was obtained using the enhancing composition was shown to be parallel to increased serum concentrations of drug in rats in vivo. Application of caffeine in aqueous solution containing enhancing mixture resulted in high serum concentrations of 50–60 μg/ml after 1 h, which remained high for at least 12 h following. The greatest caffeine accumulation in the skin was measured from small liposomal vesicles, 2260 $\mu g/cm^2$, this being 3 times greater than from aqueous solution containing enhancers, the system which exhibited the second largest accumulation of drug in the skin. Using quantitative skin autoradiography, it was found that after 24 h, the greatest concentration of caffeine (280 μg/g tissue) was localized in the epidermis and the lowest amount (50 μg/g tissue) in the dermis. In addition, a relatively high concentration of caffeine was found in the appendages.

Introduction

Current uses of xanthines are in treatment of asthma and as respiratory stimulants. Systemic levels are achieved either by injection or by oral administration. In neonates, xanthines are administered parenterally for treatment of respiratory disorders and apnoea. Amato et al. (1991) have shown percutaneous absorption to be a safe and useful approach for treatement of apnoea in infants. A new potential indication for xanthines which we are currently investigating is in treatment of hyperproliferative skin diseases (Levi-Schaffer and Touitou, 1991; Touitou et al., 1991). Their effect is thought to be achieved by increasing intracellular levels of cAMP, a compound involved in cell differentiation and inhibition of

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cell proliferation (Kaplan et al., 1977; Iancu et al., 1979).

Through the use of carrier systems, it is possible to modulate the delivery of a drug for specific treatment. Using enhancer systems, it is possible to increase the skin permeation of drugs, increasing their concentration in the systemic circulation, while other carrier systems such as liposomes can be used to concentrate the drug in the skin. The goal of the present study was two-fold: (1) to design a system which would allow for enhanced permeation of caffeine through the skin in order to achieve systemic concentrations of the drug for treatment of neonates; and (2) to design a delivery system characterized by enhanced caffeine skin penetration, in which the drug remains within the skin layers, building a large reservoir for local treatment in hyperproliferative skin diseases.

With these two goals in mind, we investigated an enhancing carrier for increasing systemic concentrations of caffeine and a liposomal carrier for obtaining a reservoir of the drug in the skin.

Materials and Methods

Materials

Caffeine, PEG 400, PEG 4000 and oleic acid (OA) were purchased from Sigma and were of pharmacopoeial grade. Transcutol™ (diethylene glycol monoethyl ether) was a gift from Gattefosse (France). Other materials and reagents were of analytical grade. Soya phosphatidylcholine (Phospholipon 90) was a gift from Natterman Phospholipid GmbH (Germany). Cholesterol (99%) was purchased from Sigma. Tritiated caffeine with a specific activity of 17.8 Ci/mmol and a concentration of 1 mCi/ml was prepared by Kamag (Dimona, Israel).

Methods

Liposome preparation

Phospholipon 90, 50 mg/ml, and cholesterol, 1 mg/ml, were dissolved in $CHCl_3$ and the solvent was vacuum evaporated. After adding a solution of caffeine (30 mg/ml), the mixture was shaken gently for 1 h and then sonicated (while nitrogen was slowly bubbled) for 40 min to form SV. Before use, the liposome dispersion was filtered through a 1.2 μm filter (Acrodiscs). Before and after filtration, the vesicle dispersion was tested for phospholipid concentration in order to ensure that no phospholipid remained on the filter. Sonication was carried out with a Soniprep 150 (MSE, Crowley, U.K.) equipped with a 19 mm probe, operating at 23 kHz and an amplitude of 6 μm. The temperature was maintained at 15–20°C.

The concentration of caffeine entrapped in liposomes was determined by dialysis. Cellulose dialysis tubing (Spectrum Medical Industries) was used. The caffeine concentration in the external medium was measured at fixed time intervals. Equilibrium was reached within 48 h at a temperature of 5°C. No phospholipid was detected in the outer solution using the phospholipid B Test (Wako Chemicals GmbH, Germany). The entrapment efficiency of caffeine was 16% (w/w).

Assay of caffeine

Caffeine was determined at 274 nm using an HPLC system (Merck-Hitachi) equipped with a variable UV detector (Merck-Hitachi). A reverse-phase C_{18} column was used. The mobile phase was 12% acetonitrile in water, at a flow rate of 1.0 ml/min (Touitou et al., 1991).

Caffeine systemic absorption in rats after application of enhancing aqueous systems

The amount of caffeine absorbed into the systemic circulation was measured at 1, 2, 4, 6, 8 and 12 h after application of the two systems, aqueous, or aqueous containing Transcutol and oleic acid. Six rats were tested at each time point for each system. Blood samples were taken from the jugular vein, and caffeine was extracted from the blood. Caffeine was determined by HPLC.

Assay of fibroblast proliferation

Culture of human dermal fibroblasts and the assay for their proliferation have been previously described (Levi-Schaffer and Touitou, 1991; Touitou et al., 1991). Briefly, the fibroblasts were

seeded in multiwell plates, and after 3 days, at subconfluency, growth medium was replaced with medium containing caffeine. Cultures were incubated for an additional 3 days. On the last day of the experiment, cells were washed with HBSS, detached using trypsin, and counted under a light microscope.

Skin permeation

Skin permeation experiments were carried out in Valia-Chien or Franz diffusion cells for 24 h through abdominal skin from 5–7 week old male hairless mice (Touitou et al., 1991). The experiment was conducted at 37°C using distilled water as the receiver medium. At the end of the experiment, the skin was washed and homogenized. Drug remaining in the skin was extracted in distilled water and assayed. Untreated skin was treated as above and used as a control. Each system was tested in four cells and duplicated.

Skin quantitative autoradiography

Caffeine liposomes contained 50 μCi [^{3}H]caffeine/ml liposomal system and 30 mg/ml unlabelled caffeine in 700 μl liposome system SV2.

This SV2 system was applied to a 3.2 cm^2 area of dorsal skin of six male rats (weight, 220–250 g). After 24 h, the rats were killed and the treated area was excised. Quantitative measurements of the amount of drug localized in various regions of the skin were performed by a computerized image analysis system as previously described (Fabin and Touitou, 1991).

Data analysis

Kinetic parameters were calculated using the computer program for data analysis of transdermal delivery (Touitou and Wartenfeld, 1987). Statistical significance was determined by the two-tailed Wilcoxon test using the 'Balance' (IBM) computer program.

Results and Discussion

Effect of caffeine on fibroblast proliferation

The effect of three concentrations of caffeine (10^{-2}, 10^{-1} and 1 mM) was measured on three

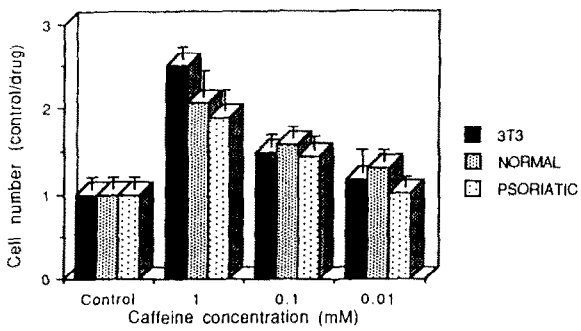

Fig. 1. Effect of caffeine concentrations on the proliferation of 3T3, normal human fibroblasts and psoriatic human fibroblasts.

cell types: normal and psoriatic human fibroblasts, and mouse 3T3 fibroblasts. Fig. 1 depicts the effect of caffeine on fibroblast proliferation. Results are presented as the ratio of control cells to drug-treated cells. Caffeine inhibited growth of all fibroblasts types. Inhibition was concentration dependent and observed at all concentrations of caffeine tested. The greatest response was seen in 3T3 and normal cells at the highest caffeine concentration tested. At the lower concentrations, no significant differences between the cell types were seen in their response to caffeine.

From the above results it can be concluded that caffeine effectively inhibits fibroblast proliferation.

Delivery of caffeine to the skin

Five different carrier systems containing 3% caffeine were used for delivering caffeine. Transcutol (20%) and oleic acid (10%) were tested as enhancers for caffeine delivery. The enhancers were added to a PEG base and an aqueous system. Transcutol and OA were chosen as enhancers based on our previous work (Katz and Touitou, 1991; Touitou and Katz, 1991; Touitou et al., 1991), where we showed this combination to be an effective enhancer of skin permeation in vitro. The liposomal system was composed of small liposome vesicles (SV) (40 nm average diameter) with a phosphatidylcholine/cholesterol ratio of 50:1. These vesicles were found to be the most efficient among four liposome systems tested in localizing drug to the skin layers (Touitou et

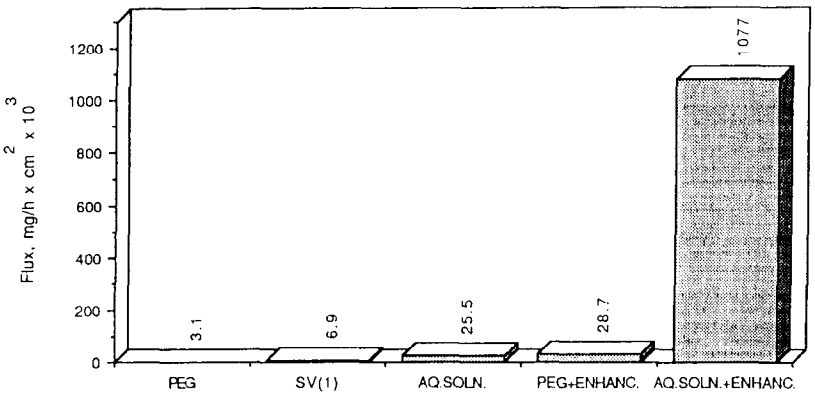

Fig. 2. Effect of different formulations on the flux of caffeine through hairless mouse skin (3% caffeine in each system).

al., 1992). The flux of caffeine through the skin from the above five systems is shown in Fig. 2. The flux of caffeine from aqueous solution containing enhancers was very high (1.1 mg/h per cm^2), being 40 times greater than from aqueous solution alone, and 37 times greater than from PEG-containing enhancers. A PEG-enhancer combination gave a flux 9 times greater than PEG alone, similar to the value for aqueous solution without enhancer. Skin permeation from an aqueous solution containing the enhancing combination is clearly much greater than for any of the other systems tested.

The small vesicle liposomes gave a very low skin permeation flux of 6.9×10^{-3} mg/h per cm^2.

On the other hand, Q_s, the quantity of caffeine accumulated in the skin after 24 h, was greatest for the small liposome vesicles (2260 $\mu g/cm^2$), as seen in Fig. 3. This value is over 3 times greater than for aqueous solution with enhancers, which exhibited the second largest accumulation of the systems tested. Addition of enhancers to the aqueous solution induced only a relatively small increase (1.7 times) over aqueous solution alone. PEG and PEG containing enhancers showed the lowest drug accumulation, 122 and 128 $\mu g/cm^2$, respectively.

Systemic absorption

In order to determine the in vivo significance of the high flux measured in vitro, the serum

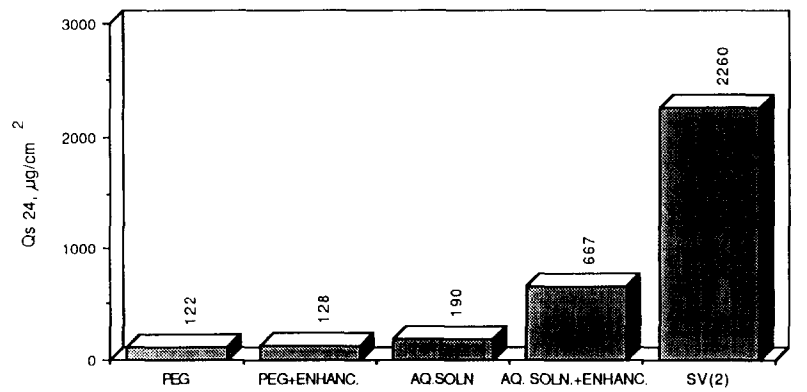

Fig. 3. Effect of different formulations on the quantity (Q_s) of caffeine found in the hairless mouse skin after 24 h application (3% caffeine in each system)

concentration of caffeine was measured after application of the enhancing aqueous system. The results presented in Fig. 4 show that the concentration of caffeine in the blood after application of this enhancing system reached a plateau of 50–60 μg/ml after about 1 h, and remained high for at least 12 h. When the aqueous system without enhancers was applied, barely detectable levels were measured after 1, 4, and 6 h (20, 4, and 6 μg/ml, respectively). In short, enhancing combinations can be used to achieve effective permeation of the drug through the skin resulting in high systemic concentrations.

Localization of caffeine in the skin

The liposomal system showed the greatest accumulation of caffeine in the skin after 24 h in vitro. It was therefore interesting to determine in which layers of skin the caffeine was concentrated after being applied in liposomes. Quantitative audioradiography was used to determine where the drug was localized in the skin. After 24 h, the greatest concentration of caffeine was found in the epidermis and the lowest amount in the dermis, 280 and 50 μg/g tissue, respectively (Fig. 5). It is interesting to note that even in the appendages, a relatively high concentration of caffeine was measured after 24 h. The results of quantitative autoradiography do not give any indication as to the state of the caffeine measured, whether free or entrapped in liposomes. They do show, however, that liposomal systems can be used to obtain a large reservoir of drug in the skin.

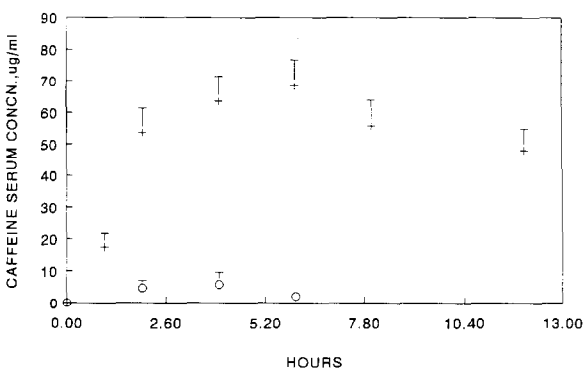

Fig. 4. Serum concentration of caffeine in rats after application to the skin (+) in the presence or (○) in the absence of enhancers.

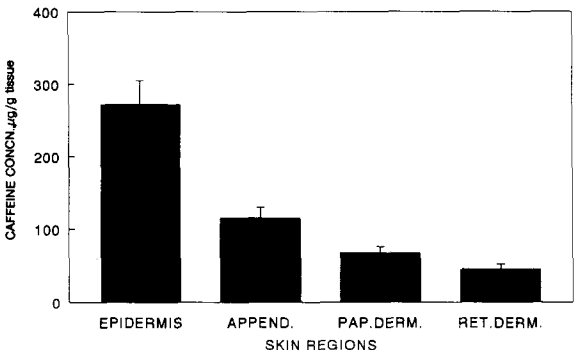

Fig. 5. Caffeine concentration in rat skin layers after application of liposomal system (measured by skin autoradiography).

Conclusion

In this work, we have shown that carrier systems can be designed to deliver drug to specific areas. We were able to achieve high skin permeation of caffeine using an oleic acid/Transcutol enhancer in an aqueous solution. The high skin permeation in vitro was correlated with high serum levels of the drug. Using a system of small liposomal vesicles, a large reservoir of caffeine in the skin was obtained after 24 h and this was further visualized and quantitatively measured by autoradiography.

These results show that it is possible to modulate drug delivery for either enhanced permeation of caffeine through the skin with potential for transdermal treatment of neonatal respiratory disorders or for building a large reservoir for local treatment in hyperproliferative skin diseases.

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