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Note

Effect of menthone on the in vitro percutaneous absorption of tamoxifen and skin reversibility

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Abstract

The effect of penetration enhancer (i.e., 1, 2, 3 and 5% menthone in combination with 50% ethanol (EtOH)) was investigated on the in vitro percutaneous absorption of tamoxifen, and post-recovery epidermal permeability after removal of the above enhancer. The flux of tamoxifen with menthone in combination with 50% EtOH was significantly greater ($P < 0.05$) than the control (50% EtOH). The flux of tamoxifen increased with increasing concentrations of menthone. The post-recovery flux through enhancer exposed epidermis was significantly decreased ($P < 0.05$) as compared to pre-recovery. However, post-recovery flux of tamoxifen through the enhancer-exposed epidermis did not completely recover to the baseline (i.e., post-recovery flux through phosphate buffered saline, pH 7.4 treated epidermis).

Keywords: Penetration enhancer; Menthone; Percutaneous absorption; Tamoxifen; Skin reversibility

The transdermal delivery of drugs may offer an attractive possibility because it avoids problems with gastrointestinal intolerance, reduces first-pass liver metabolism, and removes the need to maintain intravenous access (Bhatia et al., 1997). However, the transdermal delivery of moderately large molecules such as tamoxifen (MW 563.65) is often extremely difficult, if not impossible, without the assistance of penetration enhancers. Penetration enhancers partition into, and interact with, skin constituents to induce a temporary reversible increase in skin permeability. Recently, terpenes were reported to show an enhancement in the percutaneous drug absorption (Takayama et al., 1991; Yamane et al., 1995a,b; Williams and Barry, 1991). Menthone has been found to be a potent percutaneous penetration enhancer (Zhao and Singh, 1998). Menthone/50% ethanol (EtOH) enhanced the permeability of propranolol by stratum corneum lipid extraction, and improvement in the partitioning of the drug in the stratum corneum (Zhao and Singh, 1999). An ideal en-

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hancer should have immediate, predictable, and reversible effect. Tamoxifen is a widely used adjuvant therapy following surgery for breast malignancies in postmenopausal women. It is also indicated for treatment of estrogen receptor positive tumors in the premenopausal population. Tamoxifen undergoes extensive hepatic metabolism after oral administration in humans (Jordan and Murphy, 1990). In this study, we investigated the effect of 1–5% menthone in combination with 50% EtOH on the in vitro percutaneous absorption of tamoxifen through porcine epidermis and reversibility of epidermal permeability to baseline after removal of the enhancer.

[^{3}H] Tamoxifen (specific activity 85.0 C_i $mmol^{-1}$) and menthone was obtained from Amersham Life Sci. (Cleveland, OH) and Fluka (Buchs, Switzerland), respectively. Porcine ears were obtained from a local slaughterhouse. The epidermis was prepared by a heat separation technique. The whole skin was soaked in water at 60°C for 45 s, followed by careful removal of the epidermis. The intact epidermis was then teased off from the dermis with forceps, washed with water and used in the in vitro permeability studies (Bhatia et al., 1997). Franz diffusion cells were used in the in vitro percutaneous absorption studies. The epidermis was sandwiched between the cells with the SC facing the donor compartment. The maximum capacity of the donor and receiver compartments was 1 and 5 ml, respectively. The effective diffusional area was 0.785 cm^2. The donor compartment contained 1 ml of tamoxifen solution (0.2 μCi of tamoxifen in 1 ml of enhancer solution, 0.874 ng tamoxifen ml^{-1}) and the receiver compartment was filled with 5 ml of phosphate buffered saline, pH 7.4 (PBS). The cells were maintained at 37 ± 0.5°C by a PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc., Somerville, NJ). The content of the receiver compartment was stirred with the help of a magnetic bar at 100 rpm. At appropriate times, 0.5-ml samples were withdrawn from the receiver compartment and transferred to scintillation vials. After sampling, an equivalent amount of PBS (0.5 ml) was added to the receiver compartment to maintain a constant volume. Appropriate control experiments with PBS and 50% EtOH were also performed.

Reversibility studies were carried out to address the issue of post-enhancer recovery of tamoxifen flux. We designed a three-step experiment (Bhatia and Singh, 1998). During step I as explained above, in vitro transport was carried out for 10 h. In step II, the receiver and donor solutions were withdrawn leaving the epidermis intact in the diffusion cells. The donor and receiver compartments, with intact epidermal membrane, were washed three times with fresh PBS. Thereafter, the donor and receiver compartments were filled with PBS and allowed to recover for the next 18 h. In step III, the donor and receiver compartments contained 0.2 μCi ml^{-1} tamoxifen in 1 and 5 ml PBS, respectively, and the transport of tamoxifen was carried out for an additional 10 h. This experimental strategy allowed each condition to serve as its own control. Introduction of an 18-h recovery period allowed the skin to recover and also helped eliminate the drug retention problem. Following the experiments, flux (post-recovery) through enhancer exposed epidermis was compared with the control (50% EtOH or PBS exposed epidermis).

The samples were assayed for tamoxifen content using liquid scintillation counting. Each sample was mixed with 10 ml of scintillation cocktail (Econosafe®, Research Products International Corp., Mount Prospect, IL) and was counted in a liquid scintillation counter (Packard, Tri Carb® 2100 TR, Downers Grove, IL) for quantification of ^{3}H in disintegrations per minute (dpm). The instrument was programmed to give counts for 10 min. Net dpm for the samples were obtained by subtracting background dpm. All experiments were performed in quadruplicate, and the results were expressed as the mean ± SD of four experiments. The receiver compartment concentration of tamoxifen was corrected for sample removal by using the equation given by Hayton and Chen (1982). The cumulative amount of tamoxifen permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the flux. Statistical comparisons were made using the analysis of variance procedure (ANOVA). The level of significance was taken as $P < 0.05$.

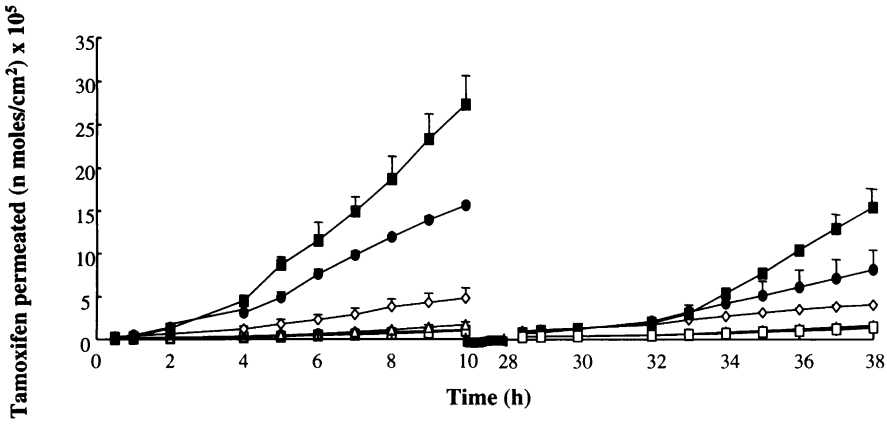

Fig. 1. Effect of menthone/50% EtOH on the in vitro transport of tamoxifen through porcine epidermis (pre- and post-recovery). Key: (△) phosphate buffered saline, pH 7.4; (O) 50% EtOH; (□) 1% menthone/50% EtOH; (◇) 2% menthone /50% EtOH; (●) 3% menthone/50% EtOH; (■) 5% menthone/50% EtOH.

Fig. 1 shows the effect of 1%, 2%, 3%, and 5% menthone in combination with 50% EtOH on the in vitro pre- and post-recovery transport of tamoxifen through porcine epidermis. Table 1 shows the flux of tamoxifen due to above enhancers. The flux of tamoxifen was significantly increased ($P < 0.05$) relative to the control (50% EtOH) with the increasing concentrations of menthone. The flux of tamoxifen through the enhancer-treated epidermis significantly decreased ($P < 0.05$) post-recovery as compared to pre-recovery. However, post-recovery flux of tamoxifen through the enhancer-treated epidermis did not completely recover to the baseline (i.e., post-recovery flux through the PBS treated epidermis).

In general, an ideal enhancer should be a safe compound, which can induce a temporary, reversible increase in skin permeability. The acceptance of skin penetration enhancers as adjuvants will depend on the degree to which their effects are transient. Owing to the dynamics of the SC replacement mechanisms, almost any damage to the SC can eventually be repaired. The human SC is continually shed off and replaced by newly evolved SC cell layers. Thus, the epidermis is a unique tissue with its programmed replacement and constant renewal of its barrier. Therefore, percutaneous absorption enhancement by enhancers should be completely reversible upon removal of the enhancer molecule. We found that the permeability of the epidermis was largely restored after the terpene was removed, although the post-recovery flux of tamoxifen through terpene in combination with 50% EtOH treated epidermis could not fully recover to the baseline flux (Table 1).

Table 1
Pre- and post-recovery flux of tamoxifen with different concentrations of menthone in combination with 50% EtOH[a]

Enhancer	Flux (n moles cm^{-2} h) $\times 10^6$ (mean $\pm$ SD, $n = 4$)	
	Pre-recovery	Post-recovery
50% EtOH	1.30 ± 0.19	1.50 ± 0.10^{c}
1% menthone/50% EtOH	2.70 ± 0.34	$1.80 \pm 0.25^{b,c,d}$
2% menthone/50% EtOH	6.50 ± 1.70	$3.20 \pm 0.19^{b,c,d}$
3% menthone/50% EtOH	20.00 ± 0.95	$9.80 \pm 2.50^{b,c,d}$
5% menthone/50% EtOH	43.00 ± 7.60	$25.02 \pm 1.21^{b,c,d}$
PBS	1.40 ± 0.22	1.60 ± 0.19^{c}

[a] PBS – Phosphate buffered saline, pH 7.4; 50% EtOH = 50% ethanol in normal saline.

[b] Significantly ($P < 0.05$) different from pre-recovery.

[c] Significantly ($P < 0.05$) different from post-recovery flux through PBS treated epidermis

[d] Significantly ($P < 0.05$) different from post-recovery flux through 50% EtOH treated epidermis.

Porcine skin is a good model to predict human skin permeability. When tamoxifen is used alone, as an adjunct to surgery or in radiation therapy in the treatment of breast cancer, the usual oral dosage is 10 mg 2 or 3 times daily. Our previous study (data not given) has shown that there is no metabolic degradation of tamoxifen in the skin; therefore, it is a suitable candidate to deliver transdermally. One of the objectives of this study was to ascertain whether menthone in combination with 50% EtOH could enhance the percutaneous absorption of tamoxifen to an extent that would deliver a clinically relevant dose of the drug. Flux is proportional to the concentration gradient. We had determined solubility of tamoxifen in PBS, 50% EtOH, and 5% menthone/50% EtOH in our laboratory, which were 4×10^{-5}, 9.20, and 4.17 mg/ml, respectively. From the above solubility data, we corrected the flux from fractional solubility adjustment (Baker, 1987; Gao and Singh, 1998). Based on a 20 cm^2 surface area device, 13.06×10^{-6}, 24.4, and 36.6 mg/day of tamoxifen could be delivered with PBS, 50% EtOH, and 5% menthone/50% EtOH, respectively, from the saturated solution of tamoxifen in the donor compartment. It is based on assumption that there is a linear increase in the flux of tamoxifen as a function of concentration provided sink condition is maintained (i.e., tamoxifen is constantly removed from the absorption site into systemic circulation). Thus, we could achieve the target delivery rate (20–30 mg/day) of tamoxifen by varying patch surface area and concentrations of tamoxifen and enhancers in donor solution.

Our experiments also showed that the lower terpene concentrations (e.g., 1% and 2%) induced skin damage was reversible to a greater extent than higher concentrations (e.g., 3% and 5%) (Table 1). Our observation is consistent with the findings of Scheuplein and Ross (1970). These authors treated human epidermis with a sodium dodecyl sulfate solution of concentrations up to 5% and observed that the recovery of the barrier function was almost complete with the 1% solution but not with the 5% solution. Although the mechanism(s) of percutaneous absorption enhancement of sodium dodecyl sulfate may be different than terpenes, the two groups of enhancers have similar recovery of the barrier function. A study on reversibility has demonstrated that the Azone^{R}-induced skin penetration enhancement is not readily reversible (Haberkamp, 1994). In contrast, decylmethyl sufoxide (DCMS) in vivo has been shown to produce an initial high flux of 5-fluorouracil that is reversible as the DCMS is washed out of the skin (Cooper, 1982). It might be true that actively metabolizing skin repairs itself within the given time period or is capable of removing the enhancer through some enzymatic or other neutralizing pathway (Haberkamp, 1994). Our in vitro experiments showed that the skin's ability to recover from enhancer-induced effects is partially impaired. Under these conditions, the skin lacks circulation and is metabolically compromised.

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