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In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes

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Abstract

Tamoxifen is a highly lipophilic drug that is widely used in breast malignancies and also as a prophylactic therapy in women at high risk for the development of this disease. Recently, the terpenes have been reported to show an enhancement effect on percutaneous drug absorption. The effect of terpenes (e.g. carvone, 1,8-cineole, menthol, and thymol) was studied on the in vitro percutaneous absorption of tamoxifen through porcine epidermis. The above terpenes (5% w/v) in combination with 50% ethanol significantly ($P<0.01$) increased the permeability coefficient of tamoxifen in comparison to the control (50% ethanol). The solubility of tamoxifen was determined in the control and enhancer solutions to correct the permeability enhancement by way of fractional solubility adjustment. Binding of tamoxifen to powdered stratum corneum from control and enhancer solutions was also determined. Binding studies reveal that the enhancement in the permeability coefficient of tamoxifen by menthol and thymol is due, at least in part, to improvement in the partitioning of the drug to the stratum corneum. In conclusion, terpenes in combination with ethanol can be used to enhance the percutaneous absorption of the highly lipophilic drug tamoxifen. © 1998 Elsevier Science B.V.

Keywords: Percutaneous absorption; Permeability coefficient; Tamoxifen; Penetration enhancer; Terpenes

1. Introduction

Various studies have demonstrated that the transdermal pathway may be a suitable alternative to the oral route in the administration of drugs with systemic activity. The advantages of transdermal drug delivery systems are well documented [1]. The primary barrier to transdermal diffusion is the stratum corneum, the thin outermost layer of the skin, which is comprised of a regular array of protein-rich cells that are embedded in a multilamellar lipid domain. The lamellar packing of stratum corneum intercellular lipids is established and several experiments have directly implicated these lipoidal domains as the integral components of the transport barrier which must be breached if drugs are to be administered at an appropriate rate. More recently it was found that diffusion barrier reduction may conveniently and elegantly be achieved by the use of chemical [2–4] and physical [5–9] enhancers.

Terpenes are a series of naturally occurring compounds which consist of isoprene (C_5H_8) units. Terpenes are constituents of essential oils, which are the volatile and fragrant substances found mainly in flavorings, perfumes, and medicines. Recently, the terpenes were reported to show an enhancement effect on percutaneous drug absorption [10–12]. 1,8-

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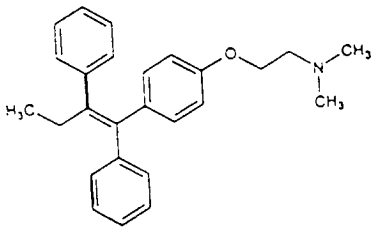
Cineole has been used to promote the percutanous absorption of several lipophilic drugs through hairless mouse skin [13]. Patents exist for the use of 1-carvone and eugenol as skin penetration enhancers [14]. Terpenes containing 50% ethanol increased the total flux of nicotine through the hairless-mouse skin [15]. Some monocyclic monoterpenes such as limonene and menthol enhanced the transport of indomethacin [16] and diazepam [12], respectively, through the rat skin. Thymol is incorporated in some lotions, creams, topical mixtures, and mouth washes. Currently, natural products are receiving considerable interest in the pharmaceutical industry, and terpenes may provide a series of relatively safe, clinically acceptable accelerants for lipophilic and hydrophilic drugs.

Tamoxifen is a highly lipophilic drug [17]. It is a widely used adjuvant therapy following surgery for breast malignancies in postmenopausal women. This agent is also indicated for treatment of estrogen receptor-positive tumors in the premenopausal population [18]. Studies are also in progress to evaluate tamoxifen as a prophylactic therapy in women at high risk for the development of this disease [19,20]. Tamoxifen undergoes extensive hepatic metabolism after oral administration in humans. The usual oral dose of tamoxifen is 10 mg twice daily. The steady-state plasma concentration of 77–274 ng ml^{-1} has been reported for tamoxifen [21]. The chemical structure of tamoxifen is given in Fig. 1.

The basic data for in vitro human percutaneous absorption, with which animal models are compared, were obtained from Feldmann and Maibach [22,23]. The histological characteristics of pig and human skin have been reported to be comparable with similarities existing for epidermal thickness and composition [24,25], dermal structure [26], lipid content [27,28] and general morphology [25,29]. The ranking of skin permeability of different species in vitro has been determined by several investigators [30–32]. Increasing evidence supports the contention that in vitro permeability studies can accurately predict in vivo absorption [33]. Skin from the pig generally approximates the permeability of human skin [34,35]. Thus, the percutaneous absorption of tamoxifen through pig skin can well be used to predict the percutaneous absorption in humans.



Tamoxifen

Fig. 1. Structural formula of tamoxifen, a lipophillc drug of molecular weight 563.65.

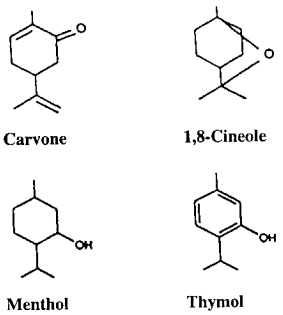


Fig. 2. Structural formula of terpenes used in the in vitro percutaneous absorption of tamoxifen.

In this study, we selected four simple cyclic terpenes (e.g. carvone, 1,8-cineole, menthol, and thymol) from the chemical classes of ketones, oxides, and alcohols (Fig. 2) to investigate their effects on the in vitro percutaneous absorption of tamoxifen through porcine epidermis.

2. Materials and methods

2.1. Materials

[^{3}H]Tamoxifen (specific activity 85.0 Ci $mmol^{-1}$) was obtained from Amersham Life Sci. (Cleveland, OH, USA). Carvone was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA). 1,8-Cineole, menthol, thymol and ammonium acetate (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from CMS (Houston, TX, USA). Acetonitrile (HPLC grade) and Microsorb-MVTM C18 HPLC column

were obtained from EM Science (Gibbstown, NJ, USA) and Rainin Instrument Co. (Woburn, MA, USA), respectively. All other chemicals and reagents used were of analytical grade.

2.2. Preparation of epidermis

Porcine ears were obtained from a local slaughter house. Epidermal membranes were prepared by heat separation technique [36,37]. The whole skin was soaked in water at 60°C for 45 s, followed by careful removal of the epidermis. The epidermis was washed with water and used in the in vitro percutaneous absorption studies.

2.3. Solubility of tamoxifen

Solubility of tamoxifen was determined by agitating excess solute in the control or enhancer solutions for 24 h at 37°C, and then determining the amount of tamoxifen in saturated solution following the HPLC method described by Lim et al. [38] with slight modification. A Hewlett-Packard series 1050 liquid chromatograph (Hewlett-Packard GmbH, Waldbronnz, Germany) was used for tamoxifen analysis. A Microsorb-MVTM C18 column (5 μm, 250×4.6 mm) was used. The mobile phase consisted of a mixture of 0.5 M ammonium acetate (pH 6.4)–acetonitrile (50:50). The flow rate was 2 ml/min. A 20-μl sample size was injected. Tamoxifen was detected using a variable wavelength UV detector at 238 nm. Table 1 depicts the solubility of tamoxifen in control and enhancer solutions.

2.4. Binding to powdered stratum corneum

The binding behavior of tamoxifen in control and enhancer systems to powdered stratum corneum was determined following the method of Wester et al. [39]. Stratum corneum was pulverized in a mortar and pestle containing dry ice. Particles of stratum corneum that were passed through a 48-mesh sieve but retained by an 80-mesh sieve were used. In a centrifuge tube, 10 mg of powdered stratum corneum was mixed with 1 ml of either control or enhancer solution containing 0.2 μCi of [^{3}H]tamoxifen by vortexing. After 10 h of contact time, the mixture was separated by centrifugation and the supernate removed. The stratum corneum pellet was again resuspended in 1 ml of the control or enhancer solution and immediately centrifuged to remove material adsorbed on the surface. The amount of radioactivity was determined in the supernates by liquid scintillation counting. The amount of tamoxifen that bound to the stratum corneum was obtained by substracting the amount of tamoxifen recovered in supernates from the amount of tamoxifen originally added (0.2 μCi) to the control or enhancer solution.

Table 1
Solubility of tamoxifen in control and enhancer solutions

Enhancer solutions	Solubility (μg/ml)
Control (50% ethanol)	9.19
5% carvone/50% ethanol	55.52
5% cineole/50% ethanol	13.03
5% menthol/50% ethanol	1.01
5% thymol/50% ethanol	3.68
Water	0.04

2.5. In vitro percutaneous absorption

Franz diffusion cells were used in the in vitro percutaneous absorption studies. The epidermis was sandwiched between the cells with the stratum corneum facing the donor compartment. The maximum capacity of each of the donor and receiver compartments was 2 and 5 ml, respectively. The surface area of epidermis exposed to the solution was 0.785 cm^2. The donor compartment contained 1 ml of tamoxifen solution (0.2 μCi of tamoxifen contained in 1 ml enhancer solution), and the receiver compartment contained 5 ml of phosphate-buffered saline, pH 7.4. Thus, the donor concentrations of tamoxifen used was 2.35×10^{-3} nmol ml^{-1}. The donor compartment was capped with a glass cap that snuggly fits to the neck of donor compartment to prevent evaporation of the solvents. We used 50% ethanol in water in donor solution to solubilize the 5% of terpenes. The cells were maintained at 37±0.5°C by a PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc., Somerville, NJ, USA). The content of the receiver compartment was stirred with the help of a magnetic bar at 100 rpm. At specified intervals, 0.5-ml samples were withdrawn from the receiver compartment, and an equivalent amount of phosphate-buffered saline (0.5

ml) was added to maintain the constant volume. Control experiments were also performed using 50% ethanol in water without terpenes. All the experiments were run for 10 h.

The samples were assayed for tamoxifen contents by liquid scintillation counting. Each sample was mixed with 10 ml of scintillation cocktail (ECONOSAFE®, Research Products International Corp., Mount Prospect, IL, USA), and counted in a liquid scintillation counter (Packard, Tri Carb® 2100 TR, Downers Grove, IL, USA). The instrument was programmed to give counts for 10 min. The results were expressed as the mean ±S.D. of three experiments.

2.6. Data analysis and statistics

The tamoxifen concentration was corrected for sampling effects according to the equation described by Hayton and Chen [40]:

$$C_n^1 = C_n(V_T/V_T - V_S)(C_{n-1}^1/C_{n-1}) \quad (1)$$

where C_n^1 is the corrected concentration of the nth sample, C_n the measured concentration of tamoxifen in the nth sample, C_{n-1} the measured concentration of the tamoxifen in the $(n-1)$th sample, V_T the total volume of the receiver fluid, and V_S the volume of the sample drawn.

The cumulative amount of tamoxifen permeated per unit skin surface area was plotted against time, and slope of the linear portion of the plot was estimated as steady-state flux (J_{ss}). The permeability coefficient (K_p) was calculated as [41]:

$$K_p = J_{ss}/C_v$$

where C_v is the total donor concentration of the tamoxifen.

We also corrected the K_p from fractional solubility adjustment as [42]:

$$\text{Corrected } K_p = J_{ss}/(C_v/C_s)$$

Where C_s is the saturated solubility of tamoxifen in control/enhancer solutions.

Statistical comparisons were made using analysis of variance procedure (ANOVA) and Duncan's multiple range test with the help of an SAS program. The level of significance was taken as $P<0.05$.

3. Results and discussion

The effect of terpenes (e.g. carvone, 1,8-cineole, menthol, and thymol) on the in vitro percutaneous absorption profiles of tamoxifen through porcine epidermis is shown in Fig. 3. Five percent terpenes in combination with 50% ethanol in water increased the in vitro transport of tamoxifen as compared to the control (50% ethanol in water). The permeability coefficient and enhancement factors of tamoxifen through the epidermis are shown in Table 2. The permeability coefficient of tamoxifen in the presence of terpenes was significantly greater ($P<0.01$) than the control. However, the permeability coefficients of tamoxifen were not significantly different ($P>0.05$) among the terpene-treated groups.

The drug flux through the skin should be directly proportional to drug activity in the vehicle, provided the membrane is unaltered. In all vehicles that contain saturated solutions of the drug, the thermodynamic activity of the drug is also maximal. We can also note that the chemical potential and the thermodynamic activity must remain the same in all saturated vehicles. In this study, we used unsaturated solutions of terpenes in 50% ethanol; therefore, relative activities and chemical potential of terpenes would vary. Table 2 shows the corrected enhancement of tamoxifen by terpenes in comparison to the control. The results show that carvone is a more effective terpene in enhancing the permeability of

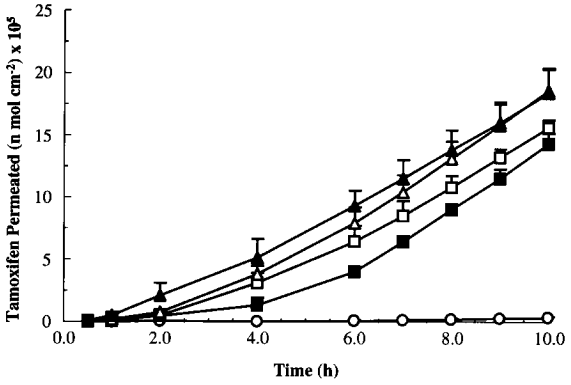


Fig. 3. Effect of terpenes on the in vitro percutaneous absorption of tamoxifen through porcine epidermis. Each data point is the mean±S.D. of three determinations. Key: (○) control; (■) carvone; (□) menthol; (▲) cineole; (△) thymol.

Table 2
Effect of terpenes on the permeability coefficient, and enhancement factors (E_f) of tamoxifen through porcine epidermis

Treatment	Permeability coefficient (cm/h) (mean±S.D.)×10^3	E_f^a	Corrected E_f^b
Control	0.27±0.02	—	
Carvone	10.91±0.72 ($P<0.01$)	40.41	244.13
Cineole	9.78±0.69 ($P<0.01$)	36.22	51.35
Menthol	9.78±0.69 ($P<0.01$)	36.22	3.98
Thymol	11.48±1.25 ($P<0.01$)	42.52	16.75
Water	0.42±0.12 ($P>0.05$)	1.56	0.01

Control is 50% ethanol in water.
$E_f^a = K_p$ with enhancer/K_p with control.
Corrected E_f^b = corrected K_p with enhancer/corrected K_p with control.

tamoxifen in comparison with control. On the basis of corrected enhancement factors, the rank and order of the effectiveness of terpenes are carvone> cineole>thymol>menthol.

Fifty percent ethanol in water was used as control to study the effect of 5% terpenes on the in vitro percutaneous absorption of tamoxifen. The effect of 50% ethanol was also investigated on the permeability of tamoxifen with respect to water alone. The in vitro percutaneous absorption profiles are given in Fig. 4. The transport of tamoxifen was less with 50% ethanol in water than with water alone. Tamoxifen, being highly lipophilic, should be transported through the non-polar pathway (i.e. intercellular lipids of the stratum corneum). When ethanol was used beyond 50%, the solute and concomitant ethanol fluxes started to decrease, probably due to ethanol's dehydrating effect on the skin tissue [43].

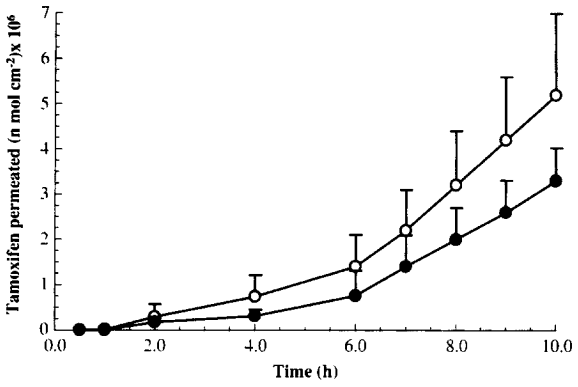


Fig. 4. Effect of 50% ethanol on the in vitro percutaneous absorption of tamoxifen through porcine epidermis. Each data point is the mean±S.D. of three determinations. Key: (○) water; (●) 50% ethanol in water.

Table 3 shows the binding of tamoxifen to stratum corneum from water and 50% ethanol. The partitioning of tamoxifen to stratum corneum is greater from water than 50% ethanol. The above findings explain the greater permeability coefficient of tamoxifen from water than 50% ethanol. An another explanation for the above findings can be offered based on the thermodynamic activity of tamoxifen in water and 50% ethanol. The flux is actually proportional to a gradient of thermodynamic activity rather than concentration. The drug activity will change in different solvents at a definite concentration. The solubility of tamoxifen is less in water than in 50% ethanol (Table 1). At a constant drug concentration, drug activity will be reduced as solubility in a solvent increased. The solvent where the drug is least soluble should provide the highest drug permeation, provided solvent does not alter the membrane. This explains the greater tamoxifen permeability in water than in 50% ethanol.

Penetration enhancers improve drug permeation by interacting with the stratum corneum. The lipid

Table 3
Partition coefficient of tamoxifen in powdered stratum corneum/enhancer solutions

Enhancer solutions	Partition coefficient[a] (×10^2)
Control (50% ethanol)	1.35±0.01
5% carvone/50% ethanol	1.35±0.01
5% cineole/50% ethanol	1.34±0.03
5% menthol/50% ethanol	1.51±0.02
5% thymol/50% ethanol	1.65±0.01
Water	1.61±0.03

[a]Partition coefficient = concentration of tamoxifen in 1000 mg of powdered stratum corneum/concentration of tamoxifen in 1000 mg of control or enhancer solution.

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