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Effect of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis

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Abstract

Transdermal delivery of most drugs is precluded due to the impervious nature of the stratum corneum. Chemical penetration enhancers offer an approach to enhance the transdermal transport of drugs by partitioning into and interacting with skin constituents, inducing a temporary reversible increase in skin permeability. The effect of penetration enhancers (e.g. oleic acid/ethanol and oleic acid/propylene glycol) was investigated on the in vitro percutaneous absorption of a hydrophilic (5-fluorouracil) and a lipophilic (tamoxifen) anticancer drug through porcine epidermis. In vitro transepidermal water loss (TEWL) studies were undertaken to investigate the effect of the above enhancers on the macroscopic barrier properties of the epidermis. Oleic acid/ethanol and oleic acid/propylene glycol significantly enhanced ($P < 0.05$) the permeability coefficient of 5-fluorouracil (5-FU) and tamoxifen in comparison to their controls. In vitro TEWL was significantly greater ($P < 0.01$) through epidermis treated with the above enhancers in comparison with control (epidermis that was not treated). However, neither oleic acid/ethanol nor oleic acid/propylene glycol enhanced ($P > 0.05$) TEWL in comparison with ethanol and propylene glycol alone. Thus, changes in the permeability of 5-FU and tamoxifen caused by oleic acid/ethanol or oleic acid/propylene glycol could not be correlated with the in vitro TEWL.

Keywords: Percutaneous absorption; 5-Fluorouracil; Tamoxifen; Oleic acid; Transepidermal water loss

1. Introduction

The advantages of transdermal drug delivery systems are well-documented (Wester and Maibach, 1992). An increasing number of drugs

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are being added to the list of therapeutic agents that can be delivered into systemic circulation, in clinically effective concentrations, via the skin portal (Smith and Maibach, 1995). The bilayer domains of the intercellular lipid matrices within the stratum corneum, coupled with its hydrophilic and lipophilic regions, form an excellent penetration barrier which must be breached if poorly penetrating drugs are to be administered at an appropriate rate. More recently it was found that diffusion barrier reduction may conveniently, and more elegantly be achieved in a reversible manner by the use of chemical (Williams and Barry, 1992; Gao and Singh, 1997) or physical enhancers such as iontophoresis (Singh and Bhatia, 1996; Bhatia et al., 1997b), phonophoresis (Singh and Singh, 1990), and electroporation (Prausnitz et al., 1993).

It has been found that oleic acid/ethanol and oleic acid/ propylene glycol increased the percutaneous absorption of drugs (Bennett et al., 1985; Yamane et al., 1995). In a comparison of the effects of various enhancers on 5-FU permeation through hairless mouse or human skin pretreated with aqueous or propylene glycol vehicles, oleic acid was as effective or more effective than dodecylazocycloheptanone or decylmethylsulfoxide (Bond and Barry, 1988; Goodman and Barry, 1989). Turunen et al. (1993) reported enhanced delivery of 5-FU with several enhancers including oleic acid. However, the above reported that dodecyl 2-methyl-2 (*N*,*N*-dimethyl-lamino) acetate (DDAIP) increased the skin permeability of 5-FU greater than oleic acid. Wang et al. (1994) demonstrated that oleic acid and some other fatty acids could induce permeability changes without detectable cellular damage. Some in vivo studies have shown that oleic acid /propylene glycol is an effective penetration enhancer for nicardipine and ketorolac acid in rhesus monkeys (Yu et al., 1988) and for azidothymidine in rats (Seki et al., 1989).

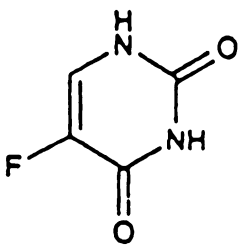
Although the mechanism by which fatty acids enhance the permeation of drugs through the skin is not clearly understood, oleic acid can interact with stratum corneum lipids and disrupt their structures, increasing their fluidity and consequently, increasing the flux (Barry, 1991). Studies show that oleic acid provides a pathway of diminished resistance for drug transport by disrupting the intercellular lipid domain of stratum corneum or coexisting as pools in the ordered stratum corneum lipid structure (lipid phase separation) (Ongpipattanakul et al., 1991; Williams and Barry, 1992).

TEWL provides a robust method for assessing macroscopic changes in the barrier properties of the skin (Abrams et al., 1993). TEWL can be considered a determinant indicative of the functional state of the cutaneous barrier (Wilson and Maibach, 1982; Maibach et al., 1984; Rougier et al., 1989). TEWL is widely used to characterize the water barrier function of skin, both in physiological and pathological conditions to perform predictive irritancy test, and to evaluate the efficacy of therapeutic treatment (Distante and Berardesca, 1995). Rougier et al. (1989) observed a linear relationship between TEWL and percutaneous absorption of molecules. Measurement of TEWL is a relevant parameter for the prediction of percutaneous penetration of substances (Lotte et al., 1987). Biophysical evidence suggests that stratum corneum lipid domains are the primary barrier to water loss and to penetration of compounds into the skin (Van Duzee, 1971). In addition, removal of lipids from the stratum corneum by solvent extraction leads to a 100-fold increase in water permeability (Scheuplein and Blank, 1971). Thus, the role of stratum corneum lipids in regulating water loss is well-established (Knutson et al., 1985). The mechanism by which water passes through the stratum corneum, or any other lamellar lipid phase, is not well-characterized. It has been suggested that water permeates through lipid lamellae via free-volume voids created due to random fluctuations in alkyl chain packing (Golden et al., 1987).

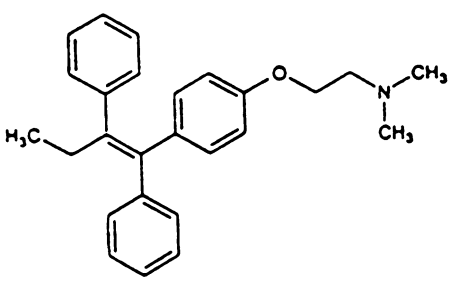
5-FU is indicated for palliative treatment of various carcinomas. 5-FU is a hydrophilic drug with a short elimination half-life. It is extensively metabolized in the liver after oral administration (USP DI, 1997). Tamoxifen is a highly lipophilic drug (Cotreau-Bibbo et al., 1996) that is widely used as 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 (Jordan and Murphy,

1990). Studies are also in progress to evaluate tamoxifen as a prophylactic therapy in women at high risk for the development of this disease (Nayfield et al., 1991; Jordan et al., 1993). Tamoxifen undergoes extensive hepatic metabolism after oral administration in humans. The chemical structures of 5-FU and tamoxifen are given in Fig. 1.

In this study, we investigated the effects of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of a hydrophilic (5-FU) and a lipophilic (tamoxifen) anticancer drug and on in vitro TEWL.



A. 5-Fluorouracil



B. Tamoxifen

Fig. 1. Structure formula of drugs used in the in vitro percutaneous absorption: A, 5-fluorouracil, a hydrophilic drug with molecular weight 130.08; B, tamoxifen, a lipophilic drug of molecular weight 563.65.

2. Materials and methods

2.1. Materials

[^{3}H]5-Fluorouracil (specific activity 23.1 Ci mmol^{-1}) was obtained from Moravek Biochemicals, Brea, USA. [^{3}H]Tamoxifen (specific activity 85.0 Ci mmol^{-1}) was purchased from Amersham Life Sci., Cleveland, USA. Oleic acid, propylene glycol and ethanol were obtained from E.M. Sciences, Gibbstown, USA; Sigma Chemical Co., St. Louis, USA; and CMS, Houston, USA; respectively.

2.2. Preparation of epidermis

Porcine ears were obtained from a local slaughter house. The epidermal membrane was prepared by soaking the whole skin 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. In vitro percutaneous absorption

Franz diffusion cells were used in the in vitro percutaneous absorption studies. The epidermis was sandwiched between the cells with 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 of epidermis exposed to the solution was 0.785 cm^2. The donor compartment contained 1 ml of 5-FU or tamoxifen solution (0.2 μCi of 5-FU or tamoxifen contained in 1 ml enhancer(s) solution), and the receiver compartment contained 5 ml of phosphate buffered saline, pH 7.4. Thus, the donor concentrations of 5-FU and tamoxifen used were 8.66×10^{-3} and 2.35×10^{-3} nmol ml^{-1}, respectively. The cells were maintained at 37 ± 0.5°C by PMC Dataplate® stirring digital dry block heater (Crown Bioscientific, Somerville, USA). The content of the receiver compartment was stirred with the help of 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 main-

tain the constant volume. Control experiments were also performed without enhancer.

The samples were assayed for 5-FU and tamoxifen contents by liquid scintillation counting. Each sample was mixed with 10 ml of scintillation cocktail (Econosafe®, biodegradable counting cocktail, Research Products International Corp., Mount Prospect, USA), and counted in liquid scintillation counter (Packard, Tri Carb® 2100 TR, Downers Grove, USA). The instrument was programmed to give counts for 10 min. The results were expressed as the mean ± S.D. of three experiments.

2.4. In vitro transepidermal water loss (tewl) through epidermis

Franz type diffusion cells were used for in vitro TEWL studies. The epidermis was soaked in the enhancer solution for 2 h. The epidermis was sandwiched between the diffusion cells with the stratum corneum side up and the dermal side exposed to the receiver compartment containing isotonic saline (0.9% sodium chloride solution). The surface area of the epidermis exposed for TEWL was 0.785 cm^2. The temperature of the diffusion cells was maintained at 37 ± 0.5°C. The epidermis was allowed to equilibrate in the in vitro system for 4 h before TEWL measurements were taken with Tewameter™ (Courage and Khazaka, Colgne and Acaderm, Menlo Park, CA, USA). TEWL measurements were performed by holding the Tewameter™ probe over the donor cell opening until a stable TEWL value achieved. The experiments were performed in a room with the ambient temperature in the range 20–26°C and relative humidity between 46 and 58%. All experiments were performed in triplicate, and the results expressed as mean ± S.D. Experiments were performed in the same manner without enhancer treatment of the epidermis to serve as control.

2.5. Data analysis and statistics

The cumulative amount of 5-FU or tamoxifen permeated per unit skin surface area was plotted against time, and slope of the linear portion of the plot (over 6–10 h) was estimated as steady state flux (J_{ss}). The permeability coefficient (K_p) was calculated as (Scheuplein, 1978):

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

where C_v is the total donor concentration of the 5-FU or tamoxifen.

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

3. Results

The effect of ethanol and oleic acid/ethanol on the in vitro percutaneous absorption profiles of 5-FU through porcine epidermis is shown in Fig. 2. Oleic acid/ethanol increased the 5-FU transport in comparison to ethanol alone. The permeability coefficient, and enhancement factors of 5-FU (i.e. ratio of the permeability of 5-FU with enhancers to the permeability without enhancers) through the porcine epidermis are shown in Table 1. The permeability coefficient of 5-FU in the presence of oleic acid/ethanol was significantly higher ($P < 0.05$) than the control. There was also a significant increase in the permeability ($P < 0.05$) of 5-FU by oleic acid/ethanol when compared with ethanol alone. However, ethanol alone could not enhance the permeability coefficient of 5-FU ($P > 0.05$) in comparison to the control.

The effect of propylene glycol and oleic acid/propylene glycol on the in vitro percutaneous absorption profiles of 5-FU through porcine epidermis is shown in Fig. 3. Oleic acid/propylene glycol increased the 5-FU transport, where as propylene glycol alone did not increase the transport as compared to the control. The permeability coefficient of 5-FU in presence of oleic acid/propylene glycol was significantly higher ($P < 0.05$) in comparison to the control (Table 1). Oleic acid/propylene glycol significantly increased ($P < 0.05$) the permeability coefficient of 5-FU in comparison with propylene glycol. The enhancement factors for propylene glycol and oleic acid/propylene glycol were 1.61 and 14.58, respectively.

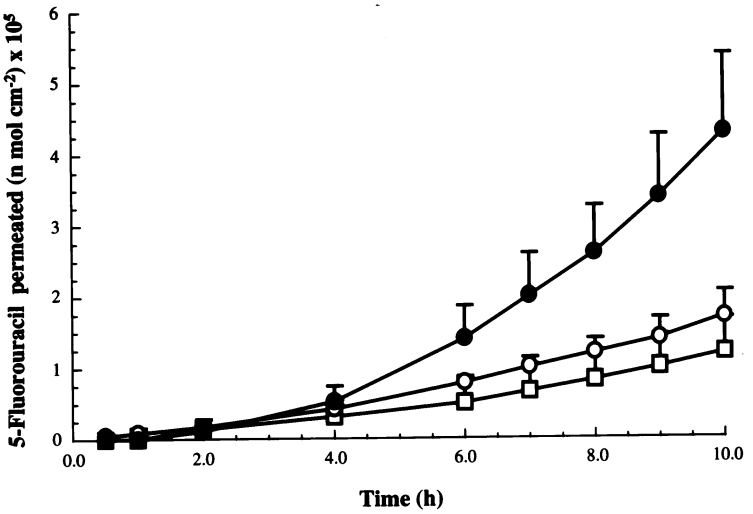


Fig. 2. The effect of ethanol and oleic acid on the in vitro transport of 5-fluorouracil through porcine epidermis. Each data point is the mean ± S.D. of three determinations. (●), 10% OA/ETH; (○), ETH; (□), control.

The effect of ethanol and oleic acid/ethanol on the in vitro percutaneous absorption profiles of tamoxifen through porcine epidermis is shown in Fig. 4. Oleic acid/ethanol increased tamoxifen transport, however, ethanol alone decreased the transport compared to the control. Oleic acid/propylene glycol increased the tamoxifen transport; however, propylene glycol alone decreased the transport compared with control (Fig. 5). The permeability coefficient and enhancement factor of tamoxifen through the epidermis are shown in Table 2. The permeability coefficient of tamoxifen in the presence of oleic acid/ethanol was significantly higher ($P < 0.05$) than the control. However, ethanol alone significantly decreased the permeability coefficient of tamoxifen ($P < 0.05$) in comparison to the control. The permeability coefficient of tamoxifen in the presence of oleic acid /propylene glycol was significantly higher ($P < 0.05$) in comparison with the control (Table 2). The enhancement in the permeability of tamoxifen with oleic acid /propylene glycol was 2.60 in comparison with control. Table 3 provides solubility data of 5-FU and tamoxifen in phosphate buffered saline (pH 7.4) and enhancers used in this study.

Table 1
Effect of enhancers on the permeability coefficient and enhancement factor of 5-fluororuracil through porcine epidermis

Treatment	Permeability coefficient (cm h^{-1}) (Mean ± S.D.) × 10^4	Enhancement factor
Control	2.08 ± 0.53	—
ETH	2.66 ± 0.94 ($P > 0.05$)	1.28
PG	3.34 ± 3.09 ($P > 0.05$)	1.61
10% OA in ETH	8.39 ± 1.75 ($P < 0.05$)	4.03
10% OA in PG	30.33 ± 2.89 ($P < 0.05$)	14.58

Control, phosphate buffered saline; ETH, ethanol; PG, propylene glycol; OA, oleic acid.

Transepidermal water loss (TEWL) through the epidermis treated with phosphate buffered saline, ethanol, oleic acid/ethanol, propylene glycol, and oleic acid/propylene glycol are given in Table 4. Treatments of the epidermis with the above test enhancers have significantly enhanced the in vitro TEWL ($P < 0.01$) in comparison with the control (phosphate buffered saline, pH 7.4).

4. Discussion

The stratum corneum lipids are arranged in multiple bilayers providing alternate hydrophobic and hydrophilic barriers. In general, routes of

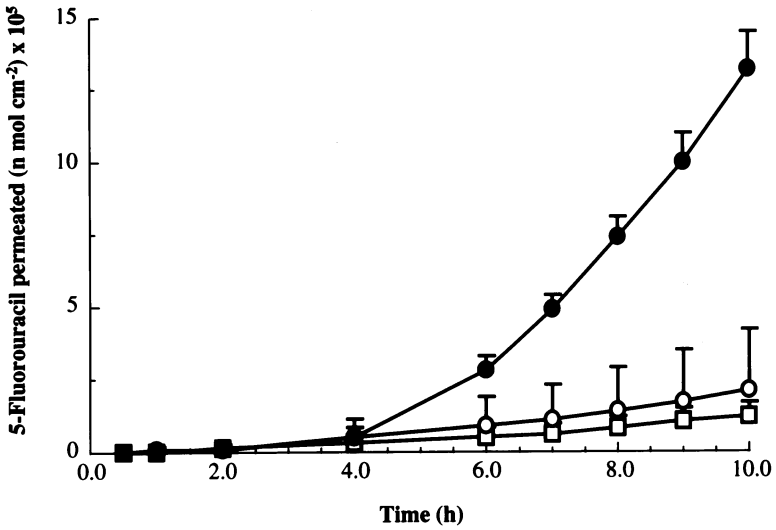


Fig. 3. The effect of propylene glycol and oleic acid on the in vitro transport of 5-fluorouracil through porcine epidermis. Each data point is the mean ± S.D. of three determinations. (●), 10% OA/PG; (○), PG; (□), control.

skin penetration are classified into two pathways, i.e. polar and non-polar in the intercellular domain. Drug permeation through nucleate epidermis should be rapid for hydrophilic drug such as 5-FU compared with passage through the stratum corneum. However, for the highly lipophilic drug (tamoxifen), the barrier may reside in the partitioning process into the aqueous nucleate epidermis. In this study, the permeability coefficients of 5-FU and tamoxifen in the control (phosphate buffered saline, pH 7.4) were 2.08×10^4 and 4.96×10^4 cm h^{-1}, respectively. The permeability of lipophilic solute is expected to be far greater than hydrophilic solute (Flynn and Stewart, 1988). It is worth considering here a study by Flynn (1971), where vidarabine and its 5′-octanoate ester differ in ether/water partition coefficient by roughly a factor of 10000, the far more hydrophobic ester exhibits only a 4-fold higher permeability coefficient. Barry and Bennett (1987) studied the transport of a hydrophilic (mannitol) and a lipophilic (progesterone) solute through skin. The above reported transport of 1.20 ± 0.52 and 1.56 ± 0.41 μg cm^{-2} in 60 h for mannitol and progesterone, respectively. For a highly lipophilic drug, permeation generally becomes diffusion layer-controlled (Roberts et al., 1978; Flynn, 1989). Tamoxifen is a highly lipophilic drug, therefore, clearance from the stratum corneum into the nucleate epidermis could affect permeation so that the process may be modified by diffusion layers (i.e. nucleate epidermis and aqueous stationary layers).

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 5-FU is greater in the control (phosphate buffered saline, pH 7.4) followed by ethanol and propylene glycol. The permeability coefficient of 5-FU is lesser in control followed by ethanol and propylene glycol. At a constant drug concentration, drug activity will be reduced as solubility in the solvent is increased. However, difference in permeabilities of 5-FU in the above solvents are not significant ($P > 0.05$).

Tamoxifen solubility is greater in alcohol and propylene glycol than phosphate buffered saline (pH 7.4). Tamoxifen activity will be reduced in ethanol and propylene glycol in comparison with phosphate buffered saline (pH 7.4). Therefore, tamoxifen permeability in phosphate buffered saline (pH 7.4) was greater than ethanol or propylene glycol. The solvent where the drug is least

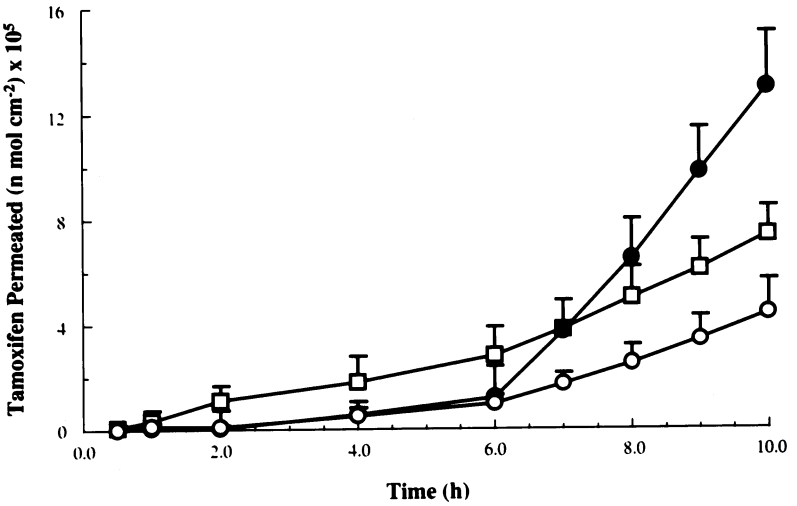


Fig. 4. The effect of ethanol and oleic acid on the in vitro transport of tamoxifen through porcine epidermis. Each data point is the mean ± S.D. of three determinations. (●), 10% OA/ETH; (○), ETH; (□), control.

soluble should provide the highest drug permeation. This assumption is only valid when the solvent and solvent systems do not alter the membrane. Ethanol, oleic acid/ethanol and oleic acid/propylene glycol change non-polar pathway for tamoxifen transport, therefore, thermodynamic activity of tamoxifen could not be correlated with its permeability. Megrab et al. (1995) reported that the decreased permeation of a lipophilic drug (estradiol) from vehicles with higher ethanol concentrations was due to dehydration of the stratum corneum. The decrease in the permeability of tamoxifen through the epidermis with ethanol may also be due to the dehydration of the lamellar layers of the stratum corneum.

The activity of other enhancers, however, can be significantly increased when applied in combination with propylene glycol (Aungst et al., 1986). In this study, oleic acid/ethanol and oleic acid/propylene glycol enhanced the permeability coefficient of 5-FU and tamoxifen through porcine epidermis. It is reported that oleic acid can interact with stratum corneum lipids and disrupt their structures, increasing fluidity of the stratum corneum lipids and the skin permeability (Barry, 1991). Oleic acid increased the drug transport by coexisting as pools in the stratum corneum lipids structure (Williams and Barry, 1992). Ongpipattanakul et al. (1991) suggested that oleic acid existed as a liquid within the stratum corneum lipids. The coexistence of fluid oleic acid and ordered stratum corneum lipids, at physiological temperatures, is consistent with the phase-separation transport mechanism for enhanced diffusion. The enhanced transport of polar molecules across the stratum corneum can be explained by the formation of permeable interfacial defects within the stratum corneum lipid bilayers which effectively decrease the resistance, without necessarily invoking the formation of pores.

We investigated the in vitro transport of 5-FU and tamoxifen through epidermis by applying enhancers in the donor compartment of the diffusion cells followed by monitoring of transport through epidermis into an aqueous receiver fluid (phosphate buffered saline, pH 7.4). The above is a typical classical in vitro evaluation technique (i.e. non-equilibrium) of enhancer efficacy (Aungst et al., 1986; Catz and Friend, 1989). This methodology is useful for the development and optimization of transdermal drug delivery systems. However, the skin, donor phase, and receiver phase are not at equilibrium with respect to enhancer in this method. In addition to enhancer/vehicle concentration gradients, water gradients in the opposite direction will be established that may

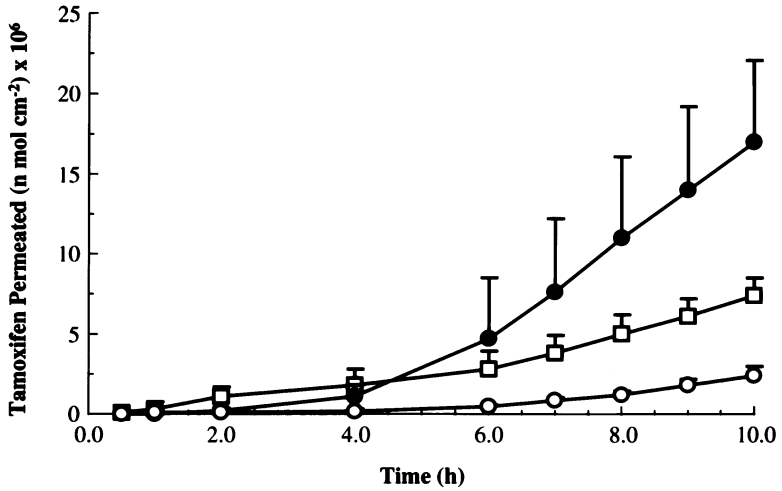


Fig. 5. The effect of propylene glycol and oleic acid on the in vitro transport of tamoxifen through porcine epidermis. Each data point is the mean ± S.D. of three determinations. (●), 10% OA/PG; (○), PG; (□), control.

or may not reach a steady state during the course of an experiment. An elegant in vitro method has been developed to investigate the enhancement of solute across the skin by a model enhancer under equilibrium conditions (Smith and Anderson, 1995).

Water is an important permeant that can be measured bidirectionally across skin (i.e. transdermal transport and TEWL). Of low molecular volume, it is capable of only weak polarizable interactions (London forces) but has the capacity of forming substantial interactions with amide linkages and some protein side groups via strong acceptor and donor hydrogen bonds. Several investigators (Wertz and Downing, 1982; Elias, 1983) have suggested that the high resistance of the stratum corneum to water flux is due to the extended multilamellar lipid domains present intercellularly in the stratum corneum. According to this hypothesis, water molecules must traverse the hydrocarbon regions of these lamellae to diffuse across this barrier. The diffusion of water molecules through hydrocarbon domains has been measured in a variety of lipid bilayers and liposomes (Boehler et al., 1978; Worman et al., 1986), yielding activation energies for water flux similar

Table 2
Effect of enhancers on the permeability coefficient and enhancement factor of tamoxifen through porcine epidermis

Treatment	Permeability Coefficient (cm h^{-1}) (Mean ± S.D.) × 10^4	Enhancement factor
Control	4.96 ± 0.2	—
ETH	3.44 ± 0.75 ($P<0.05$)	0.69
PG	2.01 ± 0.54 ($P<0.05$)	0.46
10% OA in ETH	12.89 ± 1.31 ($P<0.05$)	2.60
10% OA in PG	12.89 ± 2.23 ($P<0.05$)	2.60

Control, phosphate buffered saline; ETH, ethanol; PG, propylene glycol; OA, oleic acid.

Table 3
Solubility[a] of 5-FU and tamoxifen in solvents

Solvent	Solubility (μg/ml)	
	5-FU	Tamoxifen
Phosphate buffered saline (pH 7.4)	7521.60	0.04
ETH	1782.98	1154.00
PG	1304.62	53.40
10% OA in ETH	171.53	488.00
10% OA in PG	80.09	189.20

ETH, ethanol; PG, propylene glycol; OA, oleic acid.
[a] Solubility of solute (5-FU or tamoxifen) was determined by agitating excess solute in the solvent for 24 h at 37°C, and then determining the amount of solute in the saturated solution using a UV spectrophotometer.

Table 4
Effect of enhancers on in vitro transepidermal water loss (TEWL) through porcine epidermis

Treatment	TEWL(g m^{-2} h^{-1}) (Mean ± S.D.)
Control	6.77 ± 0.34
ETH	12.57 ± 0.69 ($P<0.01$)
PG	14.37 ± 1.00 ($P<0.01$)
10% OA in ETH	12.70 ± 0.43 ($P<0.01$)
10% OA in PG	13.80 ± 1.00 ($P<0.01$)

Control, epidermis without enhancer treatment; PG, propylene glycol; OA, oleic acid.
The results are given as mean ± S.D. of three determinations.

to the value reported through the stratum corneum (Golden et al., 1987). The biophysics of water loss through the skin has been studied by Potts and Francoeur (1990), who concluded that water loss also depends on conformational disorder in the lipid hydrocarbon chains. We believe that ethanol and propylene glycol enhanced the TEWL by reducing interaction of water molecule with protein domain of polar routes. The ability of the above solvents (ethanol and propylene glycol) in presence of oleic acid to decrease interaction of water molecules with protein domain will be reduced leading to a decrease in TEWL through polar routes. Therefore, oleic acid/ethanol and oleic acid/propylene glycol did not alter ($P > 0.05$) the in vitro TEWL values in comparison with either ethanol or propylene glycol alone. However, oleic acid/ethanol and oleic acid/propylene glycol significantly enhanced ($P < 0.05$) the permeability coefficient of 5-FU and tamoxifen in comparison with ethanol or propylene glycol alone, possibly by fluidizing the stratum corneum lipids (Potts et al., 1991; Naik et al., 1995; Bhatia et al., 1997a).

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