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The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs

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Summary

A series of cyclic monoterpenes has been assessed as skin penetration enhancers towards a model lipophilic drug, oestradiol. In vitro permeation experiments on human epidermal membranes showed that the terpenes varied in their activities; hydrocarbon (e.g., limonene) and cyclic ether (e.g., 1,8-cineole) terpenes were effective accelerants providing approximately 4-fold increases in the permeability coefficient of aqueous oestradiol, whereas alcohols (e.g., carveol), ketones (e.g., menthone) and epoxides (e.g., pinene oxide) were ineffective. The results of this study are compared with terpene activities towards a model hydrophilic drug, 5-fluorouracil. A novel concept, the enhancement index (EI), is introduced to compare differences in terpene activities towards the two permeants; EI provides information as to the partition coefficient and maximum achievable permeation enhancement for a drug, together with a measure of a penetration enhancer's activity towards that drug expressed as a percentage of the maximum effect. This approach permits useful comparisons between the activities of various enhancers towards different drugs.

Introduction

The rate determining step for transdermal delivery of most drugs is provided by the stratum corneum (Scheuplein, 1965). Its structure has been depicted in the brick and mortar model (Michaels et al., 1975; Elias, 1981) in which anucleate keratinised cells are embedded in a lipid mortar. The stratum corneum lipids are arranged in multiple bilayers providing alternate hydrophobic and hydrophilic barriers. Drugs must diffuse through the intercellular lipid matrix, and to reduce reversibly the resistance of this pathway researchers employ penetration enhancers (or accelerants). These materials interact reversibly with stratum corneum constituents to disrupt the highly ordered structure and hence facilitate drug diffusion. Many established penetration enhancers are synthetic chemicals which are not yet approved by regulatory authorities for use with drugs. Recently, a novel series of penetration enhancers, classed as terpenes or terpenoids, has been described (Williams and Barry, 1989, 1990). These

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chemicals may provide a series of safe, naturally occurring penetration enhancers whose toxicities are well documented (e.g., Opdyke, 1974–1976). Several terpenes were shown to be effective accelerants for the hydrophilic cytotoxic drug, 5-fluorouracil (Williams and Barry, 1991). The present study extends the investigation to the effects of some terpenes on transdermal permeation of a model lipophilic drug, oestradiol (ES).

Topical oestrogens are employed when endogenous hormones are lacking, such as in postmenopausal women. A transdermal oestradiol patch, Estraderm TTS, has recently been developed to treat menopausal symptoms. Clinical trials have indicated that transdermal delivery holds many advantages over oral oestrogen administration, including reduced variation in serum hormone concentrations, a more normal oestrone : oestradiol ratio and minimal pharmacological effects on hepatic proteins (Powers et al., 1985; Crust et al., 1989; Yum, 1989).

Our report also compares the activities of terpene penetration enhancers towards the model hydrophobic drug (oestradiol) and the model hydrophilic drug (5-fluorouracil). A novel concept, the enhancement index (EI), is used to compare accelerant actions for the two drugs; it is hoped that such an approach may be of value with a wide variety of drugs and enhancers. A significant advantage of the method is that it allows an assessment of the maximum benefit which can be expected in chemically enhancing the skin permeation of a particular drug.

Materials and Methods

The terpenes used as received were α-pinene, 3-carene, terpinen-4-ol, carveol, carvone, pulegone, menthone, α-pinene oxide, limonene oxide, cyclohexene oxide, cyclopentene oxide and 7-oxabicyclo[2.2.1]heptane supplied by Aldrich Chemical Company, *d*-limonene and 1,8-cineole provided by Sigma Chemical Company, α-terpineol obtained from BDH Chemicals Limited and piperitone from Field and Co. Ascaridole, the main constituent of oil of chenopodium was isolated from the oil (supplied by Field and Co.) by fractional distillation in vacuo (Pinder, 1960). The chemical formulae of these terpenes are given in Fig. 1.

An assessment of the terpene purities has been published (Williams and Barry, 1991); no single impurity was present in each terpene at greater than 2%, and such traces were considered to be at sufficiently low thermodynamic activities that their effects on skin permeability would be negligible compared with that of the main terpene. As an initial assessment of accelerant activity, all terpenes were employed as neat liquids.

The model lipophilic permeant was [2,4,6,7-^{3}H(N)]oestradiol (NEN Research Products), radiochemical purity 99%. Unlabelled oestradiol (Sigma Chemical Company) was used to prepare a saturated aqueous drug solution (0.003 mg/ml at 30°C, Michaels et al., 1975).

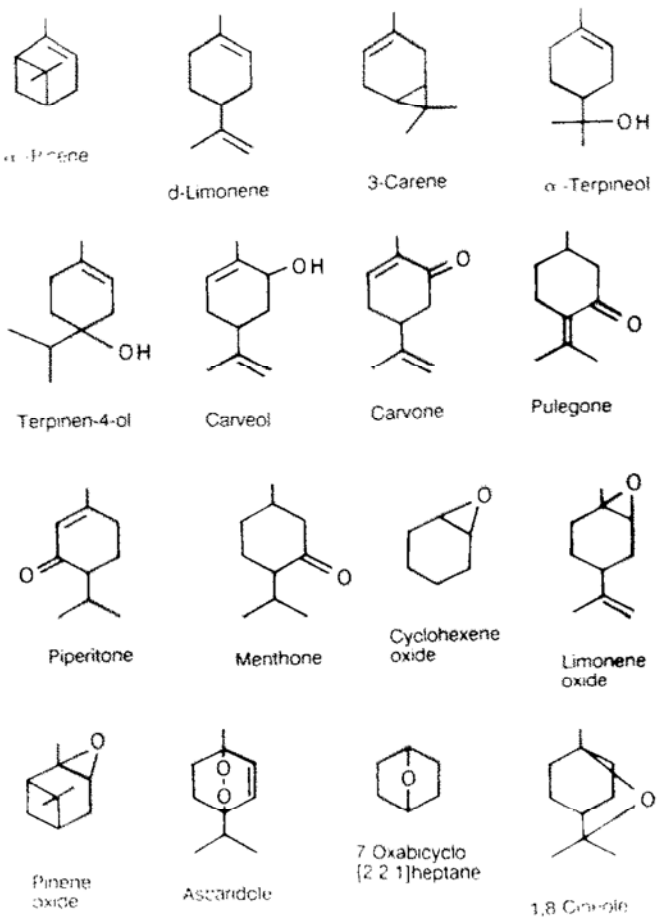

Fig. 1. The structural formulae of terpenes used in this study.

Preparation of human skin membranes

Caucasian abdominal skin (male and female, age 17–89) was obtained postmortem. Excess fatty and connective tissues were removed and the samples stored at −20°C (Harrison et al., 1984).

Full thickness membranes. Skin samples were trimmed of fatty material to provide tissue approximately 1 cm thick and essentially flat. The samples were clamped between stainless steel plates with a polythene sheet covering the stratum corneum and the membrane refrozen to adhere the fatty layer to the metal. The upper plate and polythene sheet were removed and the stratum corneum surface of the tissue was thawed slightly before a membrane, approximately 430 μm, was cut using a Duplex Electro Dermatome 7, providing a sample of full thickness skin comprising stratum corneum, nucleate epidermis and some dermal tissue.

Stripped full thickness membranes. Samples of full thickness skin with the stratum corneum removed were prepared by tape stripping (Clipper tape). Typically 25–30 strippings were required to remove the stratum corneum from full thickness skin membranes. Fully hydrated stratum corneum comprises approximately 30 μm of the membrane, hence the resulting stripped full thickness tissue provided a sample approximately 400 μm thick.

Epidermal membranes. Epidermal membranes, incorporating the anucleate stratum corneum and nucleate epidermal tissue, were prepared by a heat separation technique (Kligman and Christophers, 1963). Skin samples trimmed of fatty tissue were immersed in water at 60°C for 45 s, after which the epidermal membranes were teased off the underlying dermis. The membranes were floated on an aqueous solution of 0.002% sodium azide for 36 h to ensure full hydration of the stratum corneum.

Stratum corneum membranes. Stratum corneum samples were prepared from epidermal membranes (Kligman and Christophers, 1963); epidermal membranes were floated overnight on an aqueous solution of trypsin (0.0001% w/v) and sodium hydrogen carbonate (0.5% w/v) at 37°C. The enzyme digests the nucleate epidermal tissue allowing the remnants to be removed by swabbing. The stratum corneum membranes were floated on water before use to ensure full tissue hydration.

A diagrammatical representation of the skin membranes used in this study is shown in Fig. 2.

Permeation experiments

Experiments at 32 ± 1°C used an automated diffusion apparatus with 24 stainless-steel diffusion cells (diffusional area 0.126 cm^2) and 0.002% aqueous sodium azide as flow-through receptor solution (Akhter et al., 1984).

Fully hydrated epidermal membrane samples were mounted in the cells and treated with 150 μl aliquots of saturated radiolabelled ES. To ensure saturation of the donor solution a crystal of ES, with the same radioactivity as the drug solution, was placed in each donor compartment, and the donor drug solution was replenished every 8 h. Under these conditions, ES is at maximal

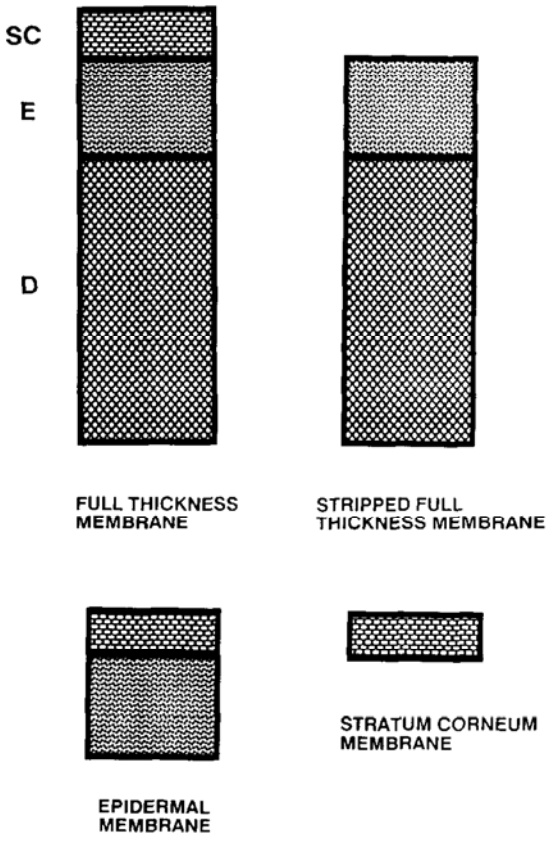

Fig. 2. A diagrammatical representation of skin membranes employed in this study (not to scale). SC, stratum corneum; E, nucleate epidermis; D, dermis.

thermodynamic activity throughout the diffusion experiment with negligible donor depletion. Samples of the receptor solution (2 ml) were collected every hour for 24 h, to which 5 ml OptiPhase HiSafe II scintillation fluid (Pharmacia) was added, and the radiolabelled drug determined by liquid scintillation counting (Packard Tri-Carb 460). The permeant solution was washed from the membrane with 0.002% aqueous sodium azide and replaced with 150 μl of a terpene. After 12 h treatment, the terpene was washed from the membrane and ES permeation was redetermined as above. Linear regression analysis of the pseudo steady-state diffusion results after the lag time allows evaluation of the permeability coefficient (Kp) of the drug in the membrane before and after terpene treatment. As a measure of the penetration enhancing activity of the terpenes, the enhancement ratio (ER) was calculated as (Goodman and Barry, 1988):

$$ER = \frac{\text{Kp after terpene treatment}}{\text{Kp before terpene treatment}} \quad (1)$$

Values reported are mean ratios from 4–13 replicates.

Permeation across the epidermis and dermis and clearance into the aqueous receptor fluid may provide the rate determining step in the permeation of very highly lipophilic drugs. The barrier to ES diffusion was therefore investigated using skin membranes composed of a variety of layers: stratum corneum alone, epidermis (including stratum corneum), full thickness skin (stratum corneum, nucleate epidermis and some dermal tissue) and tape stripped full thickness skin (stratum corneum removed). Oestradiol is not a very highly lipophilic drug (log P octanol/water = 2.29) and has a small but significant aqueous solubility (0.003 mg/ml at 30°C). Thus, removal of the drug into the flow through receptor fluid is unlikely to provide a significant resistance to drug permeation, but passage across epidermal/dermal tissue may.

Partitioning experiments

The method used to assess the effects of terpenes on ES partitioning into stratum corneum membranes was as described previously (Williams and Barry, 1991). Fully hydrated stratum corneum samples were equilibrated in a terpene for 12 h. The tissues were blotted dry and placed in a saturated radiolabelled aqueous solution of ES for 4 h. The samples were blotted dry, solubilised and the drug determined by liquid scintillation counting. Triplicate partition coefficients (stratum corneum/water) were determined using tissue samples from three different human sources. Controls were stratum corneum samples untreated with terpene. The results were expressed as a partition ratio P_R where:

$$P_R = \frac{\text{partition coefficient after terpene treatment}}{\text{partition coefficient with untreated membrane}} \quad (2)$$

Solubility studies

The solubility of ES in the terpenes was determined by the method of Williams and Barry (1991). Terpenes were saturated with radiolabelled crystals of ES and the saturated drug concentration determined in triplicate by liquid scintillation counting.

Results and Discussion

The experimental design for permeation studies of determining Kp, treating the membranes with a terpene and then redetermining Kp, allows each piece of tissue to act as its own control, thereby reducing errors due to the biological variability of human skin. The conditions for drug delivery were optimised by the use of saturated drug solutions, replenished every 8 h, which maintains the permeant at near maximal thermodynamic activity. Typical permeation profiles under these conditions are in Fig. 3. From the diffusion experiments, the mean permeability coefficient of aqueous ES through normal (untreated) human epidermal membranes at 32°C is $3.68 \pm 0.36 \times 10^{-3}$ cm/h ($n = 144$). This result shows good agreement with literature values of 3.2×10^{-3} cm/h (Goodman and Barry, 1988) and 5.2×10^{-3} cm/h (Michaels et al., 1975; Flynn and Stewart, 1988).

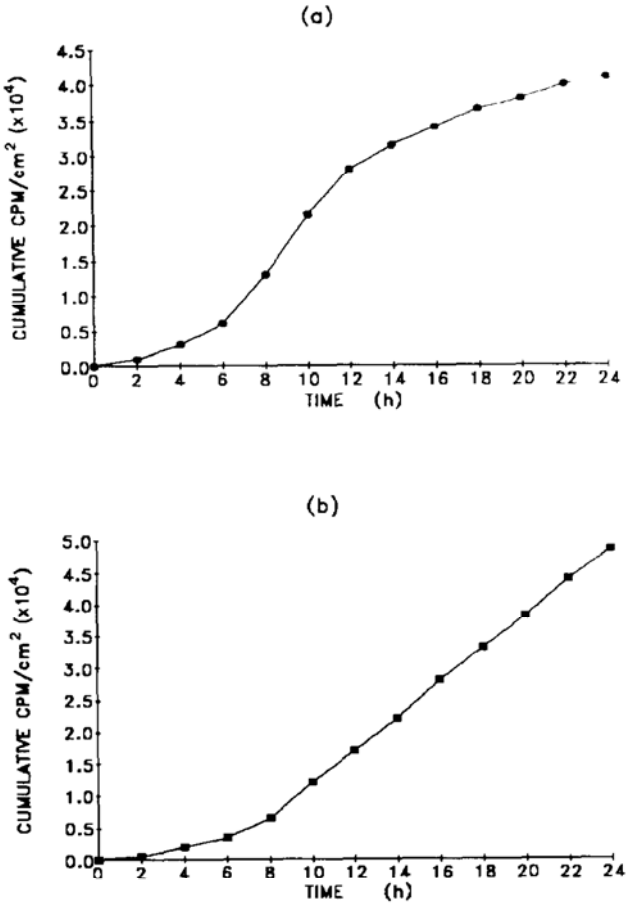

Fig. 3. Typical permeation profiles of aqueous oestradiol through human epidermal membranes showing the effects of donor replenishment. a. Donor not replenished. b. Donor replenished. Every second experimental value plotted.

For highly lipophilic drugs, the rate determining step in transdermal permeation may reside in the partitioning process into the essentially aqueous nucleate epidermis and dermis, and/or in clearance from the skin into the systemic circulation. To investigate the rate limiting step for ES permeation, a variety of skin membranes were employed in diffusion experiments. Relevant data are summarised in Table 1.

These results illustrate no significant difference in ES permeation through stratum corneum, epidermal or full thickness membrane with an aqueous receptor fluid ($P = 0.05$). This clearly illustrates that intact stratum corneum is the main barrier to oestradiol permeation. The resistance to drug permeation provided by various skin layers may be calculated as the reciprocal of the drug permeability coefficient. It should be noted that the resistance of each membrane includes those contributions arising from donor and receptor stationary layers. Due to the natural variability of human skin, no significant difference exists between the resistances of stratum corneum, epidermal and full thickness membranes ($P = 0.05$). However, the stratum corneum provides significantly greater resistance to drug permeation than stripped full thickness skin (E/D in Table 1; $P = 0.05$). After removal of the stratum corneum the permeability coefficient of ES increases by a factor of 5 compared with full thickness skin with the stratum corneum intact. This result implies that the *maximum* enhancement effect that an accelerant such as a terpene may induce is a 5-fold increase in permeability corresponding to full removal of the barrier resistance of the stratum corneum. Clearly, if the barrier nature of the stratum corneum is diminished by the use of penetration enhancers, then the resistance provided by epidermal/dermal tissue will exert a proportionally greater influence on oestradiol permeation.

Table 2 shows data on penetration enhancing activities of the terpenes. These results demonstrate that the hydrocarbon terpenes are acceler-

TABLE 1

The permeability coefficient (Kp) of aqueous oestradiol permeating through various layers of human skin, with SE

Membrane	Description	Kp (cm/h, $\times 10^3$)	Tissue resistance (h/cm)	*n*
SC	Stratum corneum	4.47 ± 0.77	224 ± 39	3
SC/E	Epidermal	3.68 ± 0.36	272 ± 27	144
SC/E/D	Full thickness	2.45 ± 0.42	408 ± 89	6
E/D	Stripped full thickness	13.0 ± 1.23	76.9 ± 7.8	8

SC, stratum corneum; E, epidermis; D, dermis; *n*, number of replicates.

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