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Note

Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect

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Abstract

The simultaneous skin permeation of drug and penetration enhancer have been studied in vitro. Simple formulations of mefenamic acid in PEG400 incorporating various proportions of ethanol or 1,8-cineole were prepared and applied to porcine ear skin in diffusion cells under infinite conditions. Receptor phases were assayed for mefenamic acid by HPLC and ethanol or 1,8-cineole by GC. Concentration-dependent permeation profiles were obtained for both ethanol or 1,8-cineole, in addition to concentration-dependent enhancement of mefenamic acid. When the steady state flux of mefenamic acid was plotted against ethanol or 1,8-cineole, linear relationships were observed with r^2 values of 0.988 and 0.999, respectively. The close connection between rates of excipient and solute permeation is generally referred to as the 'pull' (or 'drag') effect, where in this case permeation of the enhancer facilitated permeation of the solute. This appears to be sufficient to account for the enhancing activity of ethanol and 1,8-cineole, notwithstanding initial modulations that may occur within the stratum corneum.

Keywords: Mefenamic acid; Skin; Transdermal; Penetration enhancer; Ethanol; 1,8-Cineole; Co-permeation

The skin presents a substantial barrier against drug penetration/permeation and, of the enhancement strategies available, much attention has been focused on the use of chemical penetration enhancers, including ethanol (Sloan et al., 1998) and the terpene 1,8-cineole (Yamane et al., 1995). There is much evidence to prove that such species possess the ability to increase the rate of delivery of a range of drugs and numerous works have been published in order to predict enhancement capability (Pugh et al., 2005) or elucidate the mechanism of action (Touitou et al., 2000; Williams et al., 2006), where processes within the stratum corneum feature predominantly. It is therefore of considerable surprise that the fate of such enhancers once applied to the skin has received scant attention in the literature. One recent paper probed the delivery and elimination kinetics of cyclic terpenes into skin ex vivo, but stopped short of determining transdermal delivery (Cal et al., 2006). Propylene glycol, a further compound with penetration enhancing properties, has been studied and found to behave broadly as any other permeant (Bendas et al., 1995; Bowen and Heard, 2006). In the current work we prepared simple formulations using mefenamic acid as the permeant drug incorporating various proportions of ethanol or 1,8-cineole in polyethylene glycol (PEG) 400, which were applied to excised porcine skin.

Mefenamic acid, 1,8-cineole and potassium phosphate monobasic anhydrous were obtained from Sigma (Poole, UK). Polyethylene glycol 400, propane-2-ol (isopropyl alcohol, IPA), in addition to HPLC grade acetonitrile and ethanol were obtained from Fisher Scientific (Loughborough, UK). Porcine ears were obtained from a local abattoir prior to steam cleaning and stored at −20 °C (∼2 weeks). When required the ears were defrosted naturally to room temperature, cleaned thoroughly under running water and the outer skin liberated from the underlying cartilage by blunt dissection before being cut into 2 cm × 2 cm square sections.

Solutions of 5, 10 and 25% (v/v) enhancer (ethanol or 1,8-cineole) were prepared in PEG 400 base solvent—chosen as it is a typical excipient used in topical formulations. An excess of drug was added to 10 ml of solution in a glass vial and mixed on a rotary blood cell mixer overnight set up in an incubator maintained at 32 °C. The mixtures were centrifuged (3000 × *g*) for 10 min at 32 °C and the supernatants sampled with pipettes and tips also maintained at this temperature. Phosphate buffered

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saline (PBS):IPA (4:1) was selected as receptor phase—the purpose of the IPA was to provide a sink for permeated 1,8-cineole, whilst also being distinguishable from permeated ethanol in the GC analysis. The solution was degassed by vacuum filtering through a 0.45 μm nylon membrane (Whatman, UK) prior to use.

Skin samples were mounted in a Franz diffusion cells (nominally 0.78 cm^2 diffusional area; 3 ml receptor volume), distributed equally between formulations to eliminate donor variability. The receptor chambers were filled with receptor solution using tube-tipped pipettes and micro magnetic stirrer bars added before being placed on a submersible magnetic stirring plate set up in a water bath maintained 37 °C (providing a skin surface temperature of 32 °C by heat dissipation). After 10 min the cells were dosed with 1 ml of the appropriate formulation, the donor chambers occluded and glass caps used to occlude the sampling ports. At standard time points the entire receptor phase was withdrawn and stored at −20 °C; then replaced by fresh temperature-equilibrated receptor phase. Replication was $n = 8$.

Mefenamic acid was determined by HPLC analysis using an Agilent 1100 automated system fitted with a Phenomenex Kingsorb 5 μm C18 (250 mm × 4.60 mm) column. The mobile phase was acetonitrile (60%), 0.01 M potassium phosphate adjusted to pH 3 (40%), vacuum filtered through a 0.45 μm cellulose membrane filter. The flow rate was 1 ml min^{-1}, injection volume was 20 μl, and detection was by UV at 220 nm. The retention time was 8 min. Standard solutions were prepared in the range of 0.4–500 μg ml^{-1} in receptor phase and calibration curves were linear with regressions of ≥0.99. Ethanol, 1,8-cineole and IPA were determined by GC using a Varian CP3800 instrument with flame ionization detection and headspace sampling (CombiPal). Separations were performed on a Restek Rtx-200 column (30 m × 0.53 mm internal diameter) and data acquisition by Varian Star, version 6.30. The internal standard was *n*-propanol and the limit of detection for both analytes was 1 μg ml^{-1}. Cumulative permeation (μmol cm^{-2}) was also plotted against time and the gradient of linear portion of the curve provided steady state flux data. Imprecision was determined as standard error of the mean (S.E.M.) as a consequence of the replicates being split over two or more days.

Fig. 1 shows textbook steady state permeation profiles for both ethanol and 1,8-cineole, which also serve to confirm that sink conditions existed throughout the experiment. It is also clear from this plot that fluxes were concentration dependent and these data demonstrate that even at ∼5% level, commonly used in skin penetration evaluations (Verma and Fahr, 2004), a steady state is established for the transit of ethanol molecules across skin, confirming that although employed as an excipient, the substance in fact behaves like a permeant. Porcine skin was 5–13× more permeable to ethanol than to 1,8-cineole, even though both were used at the same amounts. This can be rationalized in that 1,8-cineole is considerably more lipophilic than ethanol and the more polar domains of the viable epidermis and dermis would relatively hinder its permeation.

The doses applied involved mefenamic acid at saturation. The solubility at 32 °C of mefenamic acid in PEG400 containing 1,8-cineole (as determined by standard techniques) was ∼2.47 mg ml^{-1}, with little difference between the amounts added. A similar picture was observed for ethanol, where the solubility at 32 °C was ∼3.2 mg ml^{-1}. Thus, not only were the thermodynamic activities equal per dose, so were the concentrations. Therefore, the only variable was the concentration of enhancer (or conversely the concentration of bulk solvent, PEG400).

The fate of ethanol from finite doses of formulations across porcine was determined previously to model the doses obtained from aerosol sprays and consumer products (Pendlington et al., 2001). After 24 h, 2.19 mg cm^{-2} was found to permeate, equating to 47.5 μg cm^{-2}, which is in the same order of magnitude as the value observed in the current work at the 5% level (18.7 μmol cm^{-2}), although substantially less than the 10% (203 μmol cm^{-2}) and 25% (404 μmol cm^{-2}) levels (Fig. 1). Under finite conditions surface evaporation was also an important factor. Clearly under infinite conditions, such as when modeling a reservoir-type transdermal patch, the propensity for much greater permeation is much increased.

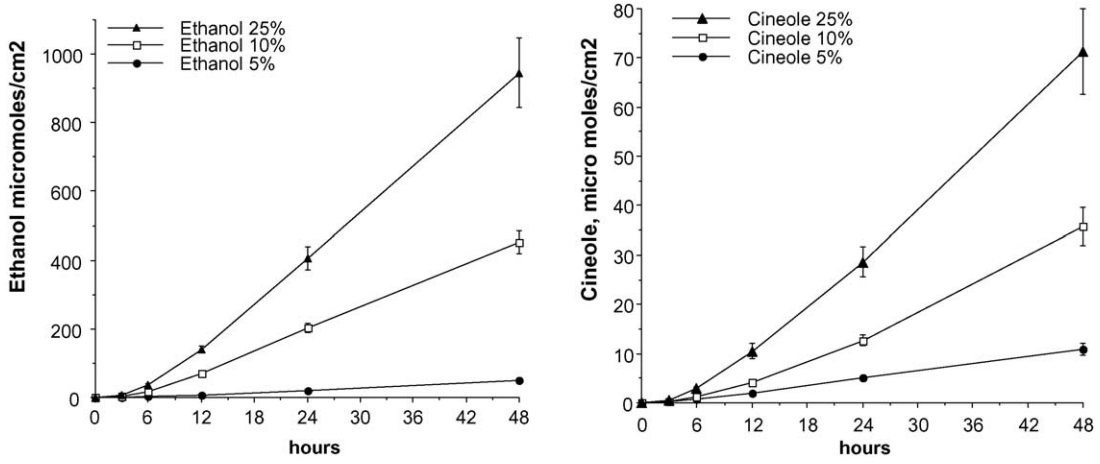

Fig. 1. Concentration-dependent steady state flux of ethanol (left) and 1,8-cineole (right) across porcine ear skin ($n = 8$, ±S.E.M.).

Table 1
Statistical data for IPA present in receptor phase samples (μg ml^{-1})

	Time point (h)				
	3	6	12	24	48
Mean	190.18	192.38	191.03	192.09	188.1
S.D.	9.677	6.908	7.473	7.213	9.72
N	21	21	21	21	21
Lower 95% confidence limit	185.78	189.24	187.62	188.80	183.67
Upper 95% confidence limit	194.59	195.53	194.43	195.37	192.52

Table 1 contains statistical data for the IPA present in samples grouped in sampling times, and a non-parametric Kruskal–Wallis test confirmed that there was no statistical difference between the mean concentrations ($p = 0.5882$, KW statistic = 2.821). This data illustrates that there was no discernible reverse-diffusion of IPA that may have interacted with the stratum corneum thereby modulating permeation results. This is further supported by the linearity of the ethanol and 1,8-cineole permeation profiles (Fig. 1), which would be expected to deviate as a consequence of barrier modulation.

Fig. 2 shows the correlations between the fluxes of mefenamic acid versus ethanol and mefenamic acid versus 1,8-cineole. The correlation coefficients were 0.988 and 0.999, respectively—note that the permeation from PEG400 alone (control) was below the limits of detection. Clearly, the permeation of mefenamic acid was closely linked to the permeation of the enhancer in both cases. In a similar manner it was previously reported that the flux of indomethacin was attained by the control of ethanol permeation from the system during the permeation experiment (Okabe et al., 1994).

If enhancement was the only process occurring one would expect to be able to extrapolate the two plots back to a common value representing the absence of enhancer (i.e. ∼0). Whereas

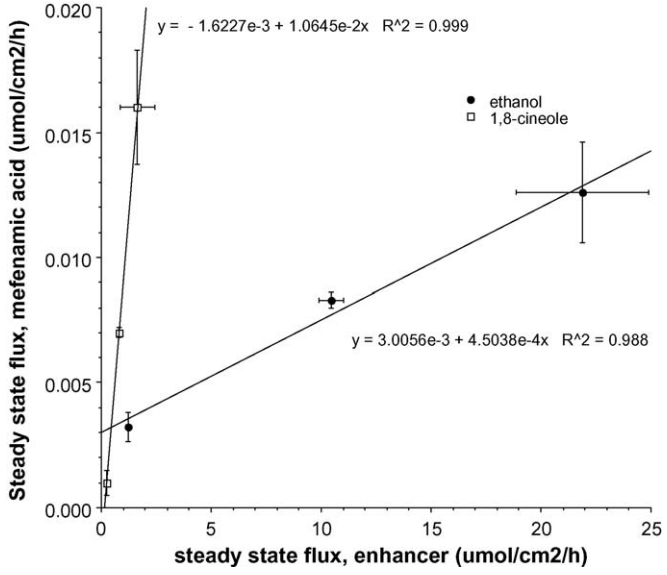

Fig. 2. Steady state flux correlations: mefenamic acid vs. 1,8-cineole (□); mefenamic acid vs. ethanol (●) ($n = 8$, ±S.E.M.).

the plot for 1,8-cineole is generally in the expected direction, the intercept for the ethanol plot is substantially greater. This data suggests that at low levels of ethanol content – between 0 and 5% – a process other than facilitated diffusion is in operation. A similar result was observed previously in the transcutaneous delivery of ketoprofen using fish oil (Thomas and Heard, 2005). Ethanol is a potent solvent for both polar and non-polar species and it is feasible that a small amount of ethanol is capable of leaching significant quantities of non-covalently bound amphiphilic stratum corneum lipids (Bommannan et al., 1991), which would have the effect modulating the skin barrier. Quantification of this effect, which appears not to occur with 1,8-cineole, is currently under investigation.

The undetectable permeation of mefenamic acid from PEG400 alone was indicative of the minimal permeation enhancing/pull effect associated with this excipient.

The close connection between the rates of enhancer permeation and solute is a manifestation of the 'pull' effect (sometimes referred to as the 'drag' effect) whereby the permeation of the enhancer (or appropriate fluid phase) subsequently facilitates that of the solute (Kadir et al., 1987), or more simply, where both species simultaneously permeate skin via a solvation or complexation interaction (Heard et al., 2003). Studying the skin permeability of excipients and enhancers can shed additional light on the complex processes involved in skin permeation enhancement mechanisms. It is proposed that, although simplistic, such processes are sufficient to account for the enhancing activity of ethanol and 1,8-cineole, notwithstanding initial modulations that may occur within the stratum corneum.

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