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Note

Probing the permeation enhancement of mefenamic acid by ethanol across full-thickness skin, heat-separated epidermal membrane and heat-separated dermal membrane

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Abstract

The permeation enhancement of mefenamic acid by ethanol across full-thickness porcine skin, heat-separated epidermal membrane and heat-separated dermal membrane has been probed. Two donor phases saturated with mefenamic acid were used: (1) PEG400; (2) PEG400 with 10% ethanol: these were applied to membranes mounted in Franz diffusion-type cells with 30 mg ml^{-1} cetrimide as receptor phase ($n \geq 5$). Across full-thickness skin, the flux was below the limit of detection from PEG400, but with the inclusion of 10% ethanol was 0.83 μg cm^{-2} h^{-1}. Across heat-separated epidermal membrane the flux from PEG was 11.9 ± 2.4 μg cm^{-2} h^{-1} with a 2.42× increase in flux observed when 10% ethanol was present ($p = 0.0095$). Across heat-separated dermal membrane the flux from PEG400 was 0.62 ± 0.13 μg cm^{-2} h^{-1}, with a 2.34× increase in flux observed when 10% ethanol was present ($p = 0.0027$). To conclude, complexation and co-permeation with ethanol via a pull effect was confirmed as the mechanism of enhanced skin permeation of mefenamic acid. Full thickness skin provides a more effective barrier than either isolated dermis or epidermis, casting doubt over the use of heat-separated epidermal membranes to model skin permeation and penetration.

Keywords: Mefenamic acid; Skin; Penetration enhancer; Ethanol; Mechanism; Complexation; Pull effect; Barrier; Stratum corneum; Epidermal membrane; Dermal membrane; Full-thickness skin

The fate of excipients from topically applied formulations is rarely examined. We recently reported on the facilitated transcutaneous permeation of mefenamic across skin in vitro using ethanol and 1,8-cineole as enhancer (Heard et al., 2006), where a close connection between rates of enhancer and solute was observed. This was attributed to the 'pull' (or 'drag') effect and was proposed to be sufficient to account for the enhancing activity of ethanol and 1,8-cineole. However, the topical drug delivery establishment considers (reversible) interactions with stratum corneum lipid domains to be the predominant enhancement mechanism, also that the main barrier to permeation is the stratum corneum. The main aims of the current short program of work were to establish whether enhanced skin permeation was only observed across full-thickness or heat-separated skin membranes where lipid domains are present compared to heat-separated dermal membranes where they are absent, in addition to establishing the primary barrier for this drug.

Mefenamic acid and potassium phosphate monobasic anhydrous were obtained form Sigma (Poole, UK). Polyethylene glycol (PEG) 400, HPLC grade acetonitrile and ethanol (absolute—analytical grade reagent) were obtained from Fisher Scientific (Loughborough, UK). Phosphoric acid (85% aqueous solution) and cetrimide were obtained from Acros Organics (Geel, Belgium). Freshly excised porcine ears were obtained from a local abattoir prior to steam cleaning, thoroughly cleaned under running water, and stored at −20 °C overnight before being thoroughly defrosted. The dorsal layer of skin was removed from the underlying cartilage by blunt dissection and electric clippers were used to remove hairs before the skin was cut into 2 cm × 2 cm sections. Heat-separated membrane separation was achieved using a previously reported method (Christophers and Kligman, 1963). The epidermal membranes were checked for integrity using a magnifying lens and only

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Table 1
The solubility of mefenamic acid at 32 °C (mean$_3$ ± S.D.) and solubility factor relative to de-ionised water

Solvent	Solubility ($\mu g\,ml^{-1}$)	Solubility factor
De-ionised water	64.4 ± 0.49	1
Water and 10% ethanol	0.818 ± 0.02	0.013
Water and cetrimide 30 mg/ml	337.9 ± 2.97	5.25
PEG 400	21461 ± 127	333
PEG 400 with 10% ethanol	26363 ± 64.5	409

those that were intact were used. Samples of full-thickness skin, dermal and epidermal membranes were then stored at 2–4 °C for ∼24 h.

Three donor phases were examined: (1) saturated solution of mefenamic acid in PEG 400; (2) saturated solution of mefenamic acid in PEG 400 with 10% ethanol; (Table 1). These were prepared by adding an excess of drug to 20 ml of the respective vehicle in 50 ml glass jars which were shaken thoroughly and placed in an incubator maintained at 32 °C for 24 h and shaken at regular intervals. The mixtures were centrifuged at 15,000 rpm for 15 min at 32 °C and supernatants sampled with pipette tips also maintained at this temperature. The receptor phase was 30 mg ml^{-1} cetrimide which was degassed by vacuum filtration using a 0.45 μm nylon filter prior to use.

The skin samples were mounted in glass Franz-type diffusion cells, the flanges of which had been smeared beforehand with high vacuum grease (Dow Corning, UK) and the donor compartments were then clamped into position. The receptor chambers were inverted to ensure that no air bubbles became trapped, and filled with receptor phase using a 10 ml syringe and flexible tube attachment. Micro-magnetic stirrer bars were added to the receptor chambers and the cells were placed on a submersible magnetic stirring plate in a water bath maintained at 37 °C in order to achieve a skin temperature of 32 °C by heat dissipation. The cells were left to equilibrate for 30 min before being dosed with 0.5 ml of the appropriate donor solution, the donor chambers occluded using lab film and glass caps placed over the sampling ports. At pre-determined time points the entire receptor phase was withdrawn using a dedicated pipette and a 1 ml aliquot transferred to a 2 ml auto-sampler vial and stored at −20 °C prior to HPLC analysis. The receptor phase was replaced with fresh solution maintained at 32 °C. Each set of experiments was performed with replicates of $n \geq 5$. Mefenamic acid was determined by HPLC analysis as previously described (Heard et al., 2006). Typical permeation profiles were obtained and cumulative permeation ($\mu g\,cm^{-2}$) was plotted against time and steady state flux, J_{ss}, obtained from the linear, post-lag phase. Permeability coefficient, k_p, was obtained by dividing flux by donor concentration. Statistical analyses were carried out using Instat3 for Macintosh, GraphPad Software, USA.

Skin permeation theory tells us that entropy is the major diffusional driving force, and that this can be considered in two ways. Firstly, concentration differentials where drug in high concentration should spontaneously migrate to an area of low concentration—the greater the differential, the greater the flux. Secondly, thermodynamic activity considerations tell us that two different solutions of the same drug at the same level of saturation should provide equal fluxes, as the molecules in both scenarios are equally proximal to the point of being excluded from the formulation or precipitated. Major flaws include the inherent assumptions that the vehicle remains entirely above the skin/vehicle interface and the solute does not bond to the vehicle.

Across full-thickness skin, permeation from saturated solution in PEG was below the limit of detection (Table 2), as observed previously, although the whole-receptor sampling protocol employed may have 'masked' the *cumulative* appearance of drug in the receptor over the 48 h period. Incorporation of 10% ethanol provided significant enhancement, from <limit of detection to 0.83 ± 0.21 $\mu g\,cm^{-2}\,h^{-1}$, which compares very well to the value of 0.77 $\mu g\,cm^{-2}\,h^{-1}$ observed previously. The majority of relevant published articles tell us that such a phenomenon is due to some kind of reversible fluidisation of the intercellular lipids of the stratum corneum, even though the fate of the enhancer was not established in such papers (Williams and Barry, 2004; Trommer and Neubert, 2006). In our earlier paper this behaviour was demonstrated to be due to the transit of the ethanol permeating through skin which gave rise to co-transportation of the mefenamic acid—the so-called pull or drag effect. PEG400 is poorly absorbed into the skin and so the pull effect is low or negligible.

The results for the heat-separated epidermal membrane tell a similar story (Table 2), with 2.42× the flux when ethanol was present ($p = 0.0095$). Examination of the results for heat-separated dermal membrane reveals a similar enhancement of 2.34× ($p = 0.0027$). The significance of this may not be immediately apparent, but consider the above discussion about the mechanism of penetration enhancers being based upon the modulation of lipid domains of the stratum corneum. The dermis is

Table 2
Permeation data (J_{ss}, steady state flux; ER, enhancement ratio; k_p, permeability coefficient) for the enhanced permeation of mefenamic acid across porcine ear skin membranes by ethanol ($n \geq 5 \pm$ S.D.)

Skin Type	Donor Phase	J_{ss} ($\mu g\,cm^{-2}\,h^{-1}$)	p	ER	Donor concentration ($\mu g\,cm^{-3}$)	k_p ($\times 10^{-6}$ cm h^{-1})	ER
Full-thickness	PEG 400 + 10% ethanol	0.83 ± 0.21			26363	31.4 ± 8.0	
	PEG 400	0			21461	0	
Heat-separated dermis	PEG 400 + 10% ethanol	1.45 ± 0.31	0.0027	2.34	26363	55.0 ± 11.7	1.90
	PEG 400	0.62 ± 0.13			21461	28.9 ± 6.1	
Heat-separated epidermis	PEG 400 + 10% ethanol	28.8 ± 7.72	0.0095	2.42	26363	1090 ± 293	1.97
	PEG 400	11.9 ± 2.4			21461	554 ± 111	

largely comprised of packed collagen bundles, almost devoid of lipoidal domains. Therefore, the mechanism that gave rise to the observed 2.34-fold enhancement across such a membrane must have been entirely independent of lipid bilayer modulation. Again, the only rational explanation is one concerning solvation of the mefenamic acid by ethanol molecules, followed by a pulling transport process. This is further supported by the close agreement of the enhancement factors, which indicates a stoichiometric relationship between mefenamic acid and ethanol association. Thermodynamic activity and Fickian considerations (k_p data) were in agreement.

Another fundamental assumption of skin permeation theory many have become conditioned to is that "the stratum corneum is the main barrier to skin permeation". This of course seems entirely plausible, as the stratum corneum is the end product of keratinocyte differentiation—however, it is a paradigm that is rarely put to the test.

The permeation of mefenamic acid across heat-separated epidermal membrane from PEG the flux was not ~0, as expected on the basis of the full-thickness skin data. The flux was a relatively large 11.9 $\mu g\,cm^{-2}\,h^{-1}$. This can possibly be accounted for if the membrane had sustained damage during the heat separation process. However, inspection of the permeation data for the permeation of mefenamic acid across heat-separated dermis is LOWER than across epidermis by a factor of 20.

Several works have made the distinction between the apolar (lipodal domains of the) stratum corneum and polar (aqueous) viable epidermis in terms of location of the major barrier to the ingress of exogenous chemicals. Lipophilic compounds can readily partition into the stratum corneum but are retarded by the viable epidermis; polar compounds are poorly absorbed into skin unless via a shunt route (Williams, 2004).

The fact that the dermis presented a 20-fold increased barrier compared to the epidermis indicates that the prevalent use of heat-separated membranes for modelling transdermal and transcutaneous delivery of lipophilic compounds may be highly inappropriate by being the source of a significant gross error. The dermis is host to the microvasculature of the skin and permeant molecules must diffuse through at least some of this domain prior to uptake by the blood vessels (transdermal) or progress into deeper tissues (transcutaneous). Furthermore, as flux across the dermal tissue was the lower of the two isolated membranes from PEG400, one would expect this value to be reflected in the overall value for the full-thickness skin. This is clearly not the case as permeation across the full-thickness was below the limit of detection and indicates either that the true rate-limiting step resides within the dermal/epidermal junction or that the process of heat-separation causes catastrophic modulation within the skin barrier function. This could include structural changes within the collagen bundles of the dermis or the hemidesmosomes that degrade in order to liberate the dermis to the epidermis and the thermal leaching of lipids from the stratum corneum.

To conclude, complexation and co-permeation with ethanol via a pull effect is confirmed as the mechanism of enhanced skin permeation of mefenamic acid. Native, full-thickness skin provides a more effective barrier than either isolated dermis or epidermis, casting doubt over the widespread practice of using heat-separated epidermal membranes to model skin permeation and penetration. Clearly more work needs to be carried out to determine if these conclusions apply to other drugs.

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