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Skin permeation and *ex vivo* skin metabolism of *O*-acyl haloperidol ester prodrugs

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ABSTRACT

Ethyl (HE), propyl (HP), butyl (HB), octyl (HO) and decyl (HD) *O*-acyl esters of haloperidol (HA) were evaluated for permeation across full-thickness human and guinea pig skin. The inclusion of 0.5 mg mL^{-1} cetrimide as a receptor phase solubilising agent did not significantly alter the barrier properties of the membranes. The permeation of the parent drug, HA, across guinea pig skin was found to be greater than that of its derivatives. Prodrug hydrolysis by cutaneous esterases was minimal. The permeation of HE, HP and HB across freshly excised guinea pig skin was subsequently investigated, however, prodrug hydrolysis remained low. Hydrolysis studies using a skin extract revealed only limited prodrug metabolism. However, in the presence of a liver extract, hydrolysis of all prodrugs was rapid. It was proposed that GGGX esterases, required for the hydrolysis of tertiary esters, were not present at a sufficiently high concentration within the skin for substantial prodrug hydrolysis to occur. This does not necessarily detract from the system as post-transdermal delivery liberation of HA *in vivo* is an equally useful mode for delivering this drug to the systemic circulation.

1. Introduction

Haloperidol, 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone (HA), continues to be a useful agent in the treatment of schizophrenia (NICE, 2002). A recent analysis of antipsychotic prescribing has shown that 'typical' antipsychotic drugs, such as haloperidol, remain popular and that their use is even on the increase (Poon et al., 2007). HA is commonly given orally, in tablet, capsule or liquid form, but it may also be given intra-muscularly or intra-venously. In addition to its neuroleptic indications, parenteral HA is also used for anti-emesis in palliative care (British National Formulary, 2008). Although HA is well absorbed from the gastro-intestinal tract it undergoes extensive metabolism in the liver. Its bioavailability after dosing with an oral solution was found to vary between 53% and 97% (Holley et al., 1983). HA is well tolerated by many, although dosage-related extrapyramidal symptoms are often reported (Martindale, 2005).

Non-compliance can be a major issue in the mentally ill (Corrigan et al., 1990), and since oral HA is administered up to three times daily there is the potential for problems of this nature. Monthly intra-muscular administration of haloperidol decanoate (HD), a *O*-acyl ester of HA, reduces the problems of confused or agitated patients forgetting (or refusing) to take their medication. This allows scope for patients who would have required in-patient care to be treated in the community, thus reducing the overall cost of care. However, there are drawbacks to the administration of HD by this route. Local reactions and tenderness at the site of injection have been reported (Hamann et al., 1990). Furthermore, parenteral depot preparations require a healthcare professional to administer them, and short of removing the depot site by surgery, it is not possible to terminate treatment once it has commenced. Like the depot formulation, a transdermal HA device would benefit the patient by having a reduced dosage interval. Transdermal devices may potentially be left in place for up to 1 week without interfering with the patient's lifestyle. A transdermal HA formulation would have the additional advantage of not requiring a health professional to administer it. Furthermore, treatment by this route can be terminated simply by removing the transdermal device. HA delivered transdermally, and therefore avoiding the GI route, could also be used to combat nausea and vomiting associated with opiate therapy in palliative care.

The transdermal delivery of HA has been investigated by a number of research groups in recent years (Almirall et al., 1996; Vaddi et al., 2001, 2002; Lim et al., 2006; Elgorashi et al., 2008). These studies have all demonstrated that therapeutic plasma levels of HA from transdermal systems are unattainable from simple donor phase solutions without penetration enhancement, where terpenoids have typically been proposed. The risks associated with long-term skin exposure to terpenes are not well established, although it has

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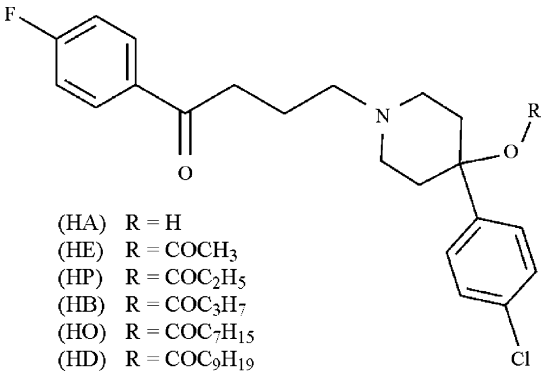

Fig. 1. Chemical structures of HA and *O*-acyl ester prodrugs.

been reported that 1,8-cineole is readily absorbed through the skin (Heard et al., 2006).

The current study concerns the transdermal delivery of HA via the prodrug approach, using ethyl (HE), propyl (HP), butyl (HB), octyl (HO) and decyl (HD) *O*-acyl derivatives. For any prodrug to be efficacious it must be metabolised to an active molecule post-delivery and it is established that HD is readily converted to HA *in vivo* (Martindale, 2005). Previous studies showed that HA was liberated from a range of acyl prodrugs when incubated with porcine liver esterase (PLE), and that the rate of hydrolysis of the octanoate derivative (HO) was significantly greater than that of the other prodrugs evaluated ($p < 0.0001$) (Morris et al., 2008). The aim of the current study was to probe the transdermal delivery and *ex vivo* skin metabolism of the same group of acyl ester HA prodrugs (Fig. 1).

2. Materials and methods

2.1. Materials

Haloperidol, gentamicin sulfate, lactate diagnostic kit (procedure no. 826-UV), Hanks' balanced salt solution (HBSS), and 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) were all obtained from Sigma (Poole, UK). The buffer salts used to prepare the phosphate-buffered saline (PBS) [Sørensen, modified; pH 7.4, 142 mM], in addition to all other chemicals and reagents were obtained from Fisher Scientific (Loughborough, UK). The synthesis and characterisation of haloperidol ethanoate (HE), propanoate (HP), butanoate (HB), octanoate (HO) and decanoate (HD) esters was reported previously (Morris et al., 2008).

2.2. Solubility, stability and C log P determination

The solubility of HA and its prodrugs was determined at 37 °C in PBS and in HEPES modified Hanks' balanced salt solution (HHBSS), and in the presence and absence of the solubilising agent cetrimide (0.5 mg mL^{-1}). Excess solute was added to 1 mL of the dissolution media in Eppendorf centrifuge vials which were agitated using a blood tube rotator that was held at 37 °C. After 72 h the vials were centrifuged at 10,500 × *g* for 10 min in a Centurion 8000 centrifuge that had been pre-warmed to 37 °C. A 200 μL aliquot of the supernatant was removed and diluted with an equal volume of methanol to avoid solute precipitation on cooling. Samples were analysed for HA and the respective prodrug by HPLC. KowWin software (Syracuse Research Corporation) was used to calculate *C* log *P* values for all permeants.

2.3. Skin preparation

Throughout this study two types of skin membrane were employed: full-thickness human breast skin obtained post-surgery from one female donor and full-thickness guinea pig skin from the dorsal region of male animals (weight 250–350 g), obtained *post mortem*. The subcutaneous fat was removed by blunt dissection and the hair was trimmed from the guinea pig skin using electric clippers. Skin samples were cut into sections of approximately 2 cm^2 and stored at −20 °C prior to use. When the prolongation of skin viability was required the skin samples were used immediately without prior freezing.

2.4. Skin permeation

2.4.1. Effect of cetrimide in receptor phase

Due to the low aqueous solubilities of the HA prodrugs it was necessary to add a solubilising agent to the receptor phase in order to maintain sink conditions. The effect of the presence of cetrimide (0.5 mg mL^{-1}) in the PBS receptor phase on the flux of HA was evaluated. The aqueous solubility of HA was sufficiently high (without cetrimide) to enable its permeation in the presence and absence of cetrimide to be evaluated. Skin samples from one human and one guinea pig donor were mounted in upright static Franz-type diffusion cells, which were maintained at 37 °C. Throughout the experiment the degassed isotonic PBS receptor phases were agitated with magnetic stirring bars. Skin hydration effects in animal models can be much more pronounced than with human skin (Bond and Barry, 1986). Consequently, HA was applied to the skin surface dissolved in 50 μL of acetone. The vehicle evaporated within 5–10 min leaving a thin film of the permeant (1.330 μmol) on the skin surface. Receptor phase samples (200 μL) were removed at predefined intervals over a 48 h period and stored at −20 °C prior to analysis by HPLC. The receptor phases were immediately replenished with an equal volume of pre-warmed buffer. A control cell, where only 50 μL of acetone was applied to the skin surface, showed that there were no co-eluting peaks originating from the skin.

2.4.2. HA and HA prodrug permeation

The method was the same as that outlined in Section 2.4.1, except that full-thickness guinea pig skin was used throughout. Only this source of skin provided sufficient replicates to enable robust comparisons to be made between the different permeants. Skin samples from nine animal donors were distributed evenly throughout the six permeation experiments. HA and its prodrugs were again applied to the skin surface dissolved in 50 μL of acetone, which quickly evaporated leaving the permeant (1.330 μmol) deposited on the skin surface.

2.5. Skin permeation—freshly excised skin

2.5.1. HA prodrug permeation

The method followed was analogous to that described in Section 2.4.1 except that the guinea pig skin was used immediately post-mortem. HHBSS was used as the receptor phase instead of PBS since it has been previously shown to be capable of prolonging skin viability in the hairless guinea pig (Ng et al., 1992). Cetrimide was not included in the receptor phase because of its detrimental effect on skin viability (Thomas and Heard, 2007). In the absence of the antimicrobial cetrimide, gentamycin sulfate was added to reduce microbial growth. Percutaneous permeation determinations were restricted to HE, HP and HB using the fresh guinea pig skin; a control diffusion cell, where only acetone was applied to the skin surface confirmed that there were no additional peaks originating from the skin. Three further diffusion cells, to which acetone was also

applied, were assembled for the determination of metabolic activity of the skin using the lactate formation method. Since the effect of HA prodrugs on the lactate assay was unknown, no permeant was applied to the skin mounted in these cells.

2.5.2. Skin metabolic activity

There is no wholly accepted definition of viable skin. Enzyme activity, anaerobic carbohydrate metabolism, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) metabolism, gene expression and products of gene expression (e.g., cyclooxygenase and lipoxygenase) have all been used as indicators of skin viability (Sartorelli et al., 2000; Wester et al., 1998; Haberland et al., 2006; Birchall et al., 2000; Thomas et al., 2007). Authors also frequently refer to the viable epidermis as 'viable skin' even when performing *ex vivo* studies. In the current study, a lactate dehydrogenase kit was used to assess skin viability through anaerobic carbohydrate metabolism. The lactate dehydrogenase kit was used to assess skin viability through anaerobic carbohydrate metabolism. The main by-product of anaerobic glucose metabolism is lactate, therefore, the concentration of lactate in the receptor phase can be used as an indicator of skin viability. Lactate UV absorption is low, but quantification can be improved by converting lactate to pyruvate, which has a higher UV absorption. To assess carbohydrate metabolism within the skin, receptor phase samples were removed from three control diffusion cells at various timepoints up to 48 h. De-proteinisation was achieved by vortex mixing the samples with 400 μL of trichloroacetic acid, followed by chilling at approximately 2 °C for 10 min. The mixture was then centrifuged for 10 min at 10,500 × *g* in a Centurion 8000 centrifuge to remove the suspended protein. From the lactate diagnostic kit, NAD vials were reconstituted with water (4 mL), glycine buffer (2 mL), and lactate dehydrogenase suspension (0.1 mL). Receptor phase samples (0.1 mL) were combined with 2.9 mL portions of the resultant lactate dehydrogenase mixture in a UV cuvette, which was held at 37 °C in a thermostatted PerkinElmer Lambda 5 UV/vis double-beam spectrophotometer. The UV absorption at 340 nm was monitored until the conversion of lactate to pyruvate was complete (typically 5–10 min).

2.6. HA prodrug hydrolysis by skin and liver extracts

2.6.1. Preparation of extracts

Skin from the dorsal region of one male guinea pig (250–350 g) was removed as described in Section 2.3. Following removal of the subcutaneous fat, a piece of full-thickness skin (5.76 g) was fully immersed in 31 mL of PBS and left stirring for 24 h at room temperature. The PBS was subsequently filtered under gravity through Whatman No. 1 filter paper. The filtrate was then divided between 1.5 mL Eppendorf vials and centrifuged for 20 min at 10,500 × *g* in a Centurion 8000 centrifuge. The clear extract was stored at −20 °C prior to use. The use of skin homogenates was avoided in order to prevent the prodrugs being exposed to intracellular enzymes which they may not ordinarily encounter on permeation through the skin. To prepare the guinea pig liver extract, the liver was removed from the same animal and chopped into pieces of approximately 4–5 mm^3. The tissue (5.49 g) was added to 29.5 mL of PBS in a 50 mL centrifuge tube. The resultant mixture had the same tissue weight/PBS volume ratio as the skin extract. Whilst being held in an ice-bath, the mixture was homogenised (Silverson Machines) and the homogenate centrifuged at 2500 × *g* for 15 min in a Beckman-Coulter Optima LE-80K ultracentrifuge. The supernatant, which remained turbid, was divided and transferred to 1.5 mL Eppendorf vials and centrifuged for a further 20 min at 10,500 × *g* in the Centurion 8000 centrifuge. The final clear supernatant was stored at −20 °C prior to use.

2.6.2. Prodrug hydrolysis

The hydrolysis of HA prodrugs was determined in the presence of the *ex vivo* skin and liver extracts in a similar manner to that described previously (Morris et al., 2008). Briefly, the skin and liver extracts were allowed to equilibrate for 1 h at 37 °C prior to the addition of 20 μL of the methanolic prodrug solution. The final substrate concentration in the reaction vessel was 53.3 nmol mL^{-1}. The prodrugs were therefore present at levels in excess of their solubility. This was unavoidable since the saturation solubilities of HO and HD in the reaction medium were below the limits of detection of the HPLC-UV analytical method employed. As a control, the prodrugs were also incubated in PBS alone. The parent drug was also incubated with the same tissue preparations. At various timepoints up to 50 h, 100 μL samples were removed to 1.5 mL Eppendorf vials containing an equal volume of acetonitrile. Acetonitrile has been previously used to quench enzyme-catalysed reactions (Seville et al., 2000; Morris et al., 2008) and this was subsequently confirmed again here. The samples were centrifuged at 10,500 × *g* for 10 min to remove any suspended protein, upon which the supernatant was removed to 200 μL glass autosampler vials and stored at −20 °C prior to analysis by HPLC.

2.7. HPLC analysis

Quantitative analysis was by reverse-phase HPLC using an automated Agilent 1100 series instrument in combination with Chemstation software (Version A.09.01). A Kingsorb 5 μm ODS 250 mm × 5 mm column (Phenomenex, Macclesfield, UK) was used

Table 1
HPLC analysis of HA and prodrugs: retention times, capacity factors (k'), alpha values (α) relative to HA and the gradient timetable[a].

Analyte	HA	HE	HP	HB	HO	HD
Retention time (min)	7.5	11.2	14.4	14.7	15.0	16.6
k'	3.16	5.22	7.00	7.16	7.33	8.22
α	–	1.65	2.22	2.27	2.32	2.60
Time (min)	Gradient timetable (mobile phase A:mobile phase B)[b]					
	(A:B)	(A:B)	(A:B)	(A:B)	(A:B)	(A:B)
0	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5
6	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5
8	23.5:76.5	23.5:76.5	21:79	16:84	6:94	4:96
15	23.5:76.5	23.5:76.5	21:79	16:84	6:94	4:96
18	–	–	21:79	16:84	6:94	4:96
20	–	–	23.5:76.5	23.5:76.5	23.5:76.5	23.5:76.5

[a] Mobile phase A: 0.5% aqueous triethylamine; mobile phase B: methanol containing 0.5% triethylamine.
[b] Morris et al. (2008).

Table 2
Haloperidol and prodrugs: molecular weights, C log *P* values, solubilities, predicted k_p and predicted flux.

Compound	MW	*C* log *P*[a]	Solubility in PBS (nmol mL^{-1})	Solubility in PBS containing cetrimide 0.5 mg mL^{-1} (nmol mL^{-1})	Solubility in HHBSS (nmol mL^{-1})	Predicted k_p (cm h^{-1})	Predicted flux (nmol cm^{-2} h^{-1})
HA	375.86	4.2	60.2 ± 1.1	51.6 ± 4.3	38.5 ± 0.3	0.0089	0.536
HE	417.90	5.2	10.8 ± 0.8	25.6 ± 0.5	8.2 ± 0.1	0.0253	0.273
HP	431.93	5.7	6.9 ± 0.1	40.6 ± 0.5	3.5 ± 1.3	0.0470	0.324
HB	445.95	6.19	4.1 ± 0.1	60.1 ± 1.7	2.6 ± 0.4	0.0860	0.353
HO	502.06	8.15	0.9 ± 0.4	51.1 ± 1.8	nd	0.9637	0.867
HD	530.11	9.13	nd	41.9 ± 4.9	nd	3.2257	–

[a] KowWin software (Syracuse Research Corporation); solubility study performed in triplicate ± SEM; nd = not detectable.

throughout. The flow rate was 1 mL min^{-1} and detection was by UV at 242 nm. A gradient elution system had been developed previously (Table 1) which enabled the resolution of haloperidol and prodrug within the same chromatogram (Morris et al., 2008). Mobile phase A consisted of 0.5% aqueous triethylamine; mobile phase B was methanol containing 0.5% triethylamine. Retention times, capacity factors (k') and alpha values (α) relative to HA are listed in Table 1.

2.8. Statistical analysis

Statistical analyses were carried out using Instat 3 for Macintosh GraphPad Software, Inc. (Hercules, CA, USA). Unpaired *t*-tests with Welch correction were used to investigate differences between two data sets. A *p*-value of <0.05 was considered significant.

3. Results

3.1. Solubility determination and predictions of flux

The solubilities of HA and its prodrugs in PBS in the presence and absence of cetrimide and in HHBSS are listed in Table 2. No HA was detected in any of the prodrug samples indicating that the prodrugs were stable in aqueous media over the time course of the solubility experiment. The addition of cetrimide to PBS increased the solubility of all the HA prodrugs.

The Potts and Guy equation (1992; Eq. (1)) uses the permeant molecular weight (MW) and log *P* to predict its permeability coefficient (k_p):

$$\log k_p(\mathrm{cm\,h^{-1}}) = -2.74 + 0.71(\log P) - 0.0061(\mathrm{MW}) \quad (1)$$

Using the MW and *C* log *P* values listed in Table 2, predicted k_p values were calculated for HA and its prodrugs. Permeant fluxes were then estimated by multiplying the estimated k_p by the aqueous solubility of the permeants in PBS. It was not possible to estimate the HD flux because its aqueous solubility could not be determined.

3.2. Transdermal delivery studies

3.2.1. Effect of cetrimide on HA flux

The addition of cetrimide (0.5 mg mL^{-1}) to the PBS receptor phase had no significant effect on the flux of HA across either human or guinea pig skin (Table 3), with a Student's *t*-tests giving *p*-values of 0.68 and 0.49 for human skin and guinea pig skin, respectively. The assumption was made that this hypothesis would hold true for the HA prodrugs under investigation in this study. Consequently, cetrimide was added to the PBS receptor phase as a solubilising agent to provide sink conditions in subsequent transdermal diffusion studies. Cetrimide also possesses intrinsic antibacterial and antifungal activity (Hugo and Russell, 1992) conferring the additional advantage of reducing microbial growth within the receptor phase.

Table 3
HA flux across human and guinea skin using a PBS receptor phase in the presence and absence of cetrimide 0.5 mg mL^{-1} (n = 6, ±SEM).

Receptor phase	$\mathrm{Flux_{human}}$ (nmol cm^{-2} h^{-1})	$\mathrm{Flux_{GP}}$ (nmol cm^{-2} h^{-1})
PBS	0.172 ± 0.034	0.911 ± 0.370
PBS (cetrimide)	0.182 ± 0.014	0.687 ± 0.080

3.2.2. HA and HA prodrug permeation

The cumulative permeation profiles of HA and its prodrugs are shown in Fig. 2. The flux of HA was considerably greater than that of its derivatives (Table 4) and only the lowest molecular weight prodrug, HE, permeated the skin to any appreciable extent (Table 4). Minimal levels of HP and HB were detected in the receptor phase samples, whilst HO and HD permeation was below the analytical limit of detection. The permeation profile of HA flattened towards the end of the permeation experiment as the drug concentration in the receptor phase approached 40% of the saturation solubility. The flux of HA was therefore calculated using data from the first 24 h only.

When HE and HP were dosed, a peak corresponding to HA was detected in sample chromatograms. This indicated that these two prodrugs underwent limited hydrolysis on their passage through the skin. However, any hydrolysis of the other prodrugs was below the limit of detection.

3.2.3. Fresh skin

The cumulative permeation profiles of HE, HP and HB using fresh guinea pig skin are shown in Fig. 3. As the prodrug ester chain length increased, the flux of the prodrugs decreased (Table 4). A peak corresponding to HA was detected in the HE and HP (but not

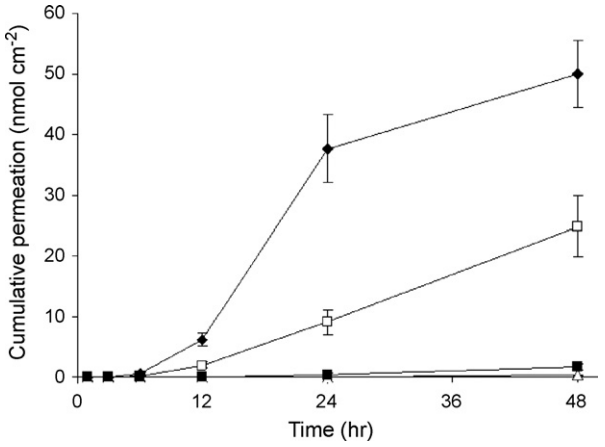

Fig. 2. Mean cumulative amount of HA and five prodrugs permeated through full-thickness guinea pig skin. Key: (♦) HA; (□) HE; (△) HP; (■) HB (HO and HD permeation not detected; n = 10; ±SEM).

Table 4
Haloperidol and prodrugs: fluxes, hydrolysis in skin and liver extracts.

Compound	Permeant flux (nmol cm^{-2} h^{-1})[a]	HA flux ($\times 10^3$ nmol cm^{-2} h^{-1})[a]	Flux–fresh skin (nmol cm^{-2} h^{-1})[b]	HA flux $\times 10^3$–fresh skin (nmol cm^{-2} h^{-1})[b]	Rate of HA liberation $\times 10^2$ by skin extract (nmol mL^{-1} h^{-1})[c]	HA liberated ($t_{50\,h}$) by skin extract (%)[c]	HA liberated ($t_{3\,h}$) by liver extract (%)[c]
HA	2.136 ± 0.316	–	–	–	–	–	–
HE	0.664 ± 0.123	1.426 ± 0.485	1.367 ± 0.231	3.185 ± 0.705	10.837 ± 3.973	9.56	74.54
HP	0.014 ± 0.004	8.160 ± 1.315	0.301 ± 0.064	1.050 ± 0.628	6.943 ± 2.395	6.58	96.85
HB	0.049 ± 0.014	nd	0.041 ± 0.022	nd	10.077 ± 0.852	9.17	95.21
HO	nd	nd	–	–	6.900 ± 0.238	6.07	106.15
HD	nd	nd	–	–	4.063 ± 0.566	3.56	22.92

Replicates: [a]($n = 10$); [b]($n = 4$); [c]($n = 3$); ±SEM; nd = not detectable.

the HB) sample chromatograms. The levels of HA present were comparatively low indicating that only limited prodrug hydrolysis had occurred even with the use of fresh skin. The omission of cetrimide from the receptor phase ruled out any possibility that it may have inhibited cutaneous enzymes thus preventing prodrug hydrolysis.

3.2.4. Skin metabolic activity

It was demonstrated that the HHBSS receptor phase was capable of maintaining the viability of full-thickness guinea pig skin for at least 48 h. However, it can be seen from Fig. 3 that the rate of lactate formation declined after approximately 16 h. This is comparable to a similar study using full-thickness human skin, which demonstrated a fall-off in lactate production between 12 h and 24 h (Stoddart, 2002).

3.3. HA prodrug hydrolysis by skin and liver extracts

In the presence of the skin extract the HA prodrugs only underwent limited hydrolysis to the parent drug. The plots of HA liberation were approximately linear, as such, it was possible to determine rates of prodrug hydrolysis, which were taken to be the gradients of the straight-line portions of the respective plots (Table 4). Whilst the hydrolysis rates of HE and HB were greatest, it was notable that HO and HD, the two prodrugs with the longest ester chains, were hydrolysed at the slowest rates. Because the prodrugs were present as saturated solutions, it was presumed that the drug in solution would be constantly replenished over the course of the experiment. Consequently, the concentration of the dissolved drug would remain approximately constant throughout. When HA was incubated with the skin extract as a control its concentration remained constant throughout the course of the experiment.

The prodrugs incubated with the liver extract underwent almost complete hydrolysis in just 3 h (Table 4). Clearly, the esterase activity of the liver extract far exceeded that of the skin extract. Following the initial sharp increase in HA concentration, the levels of the parent drug then declined over the remainder of the experiment. Furthermore, when HA was incubated with the liver extract as a control its concentration fell by approximately 50% over the 50 h. It has been previously reported that in human liver homogenates HA is extensively metabolised to an alcohol metabolite usually referred to as 'reduced haloperidol' (Eyles et al., 1998). It therefore seems likely that HA in the control samples (and also that liberated from the prodrugs) would have undergone further metabolism by the guinea pig liver extract to reduced haloperidol. This explains the fall in HA concentration over time. No HA was detected in any of the PBS prodrug control samples confirming that prodrug hydrolysis was via an enzymatic mechanism.

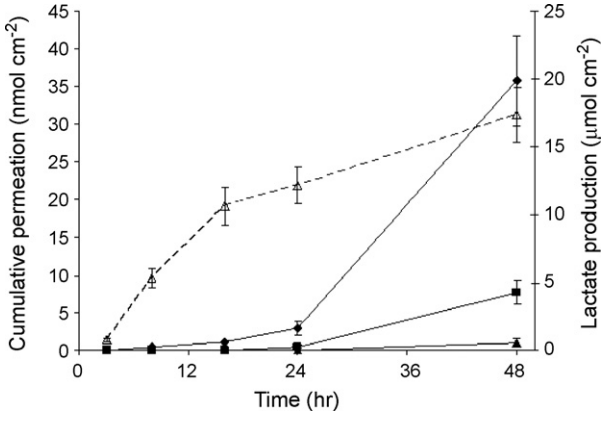

Fig. 3. Mean cumulative amount of HE, HP and HB permeated through full-thickness fresh guinea pig skin. Key: (♦) HE; (■) HP; (▲) HB; (–△–) lactate formation (prodrugs: $n = 4$, ±SEM; lactate formation: $n = 3$, ±SEM).

4. Discussion

Due to the low aqueous solubility of the HA prodrugs it was necessary to add a solubilising agent to the receptor phase in order to improve their solubilities and thus prolong sink conditions. Cetrimide was selected as it has been used as a receptor phase additive to improve the solubility of highly lipophilic transdermal permeants (Thomas et al., 2007). Cetrimide was able to increase the solubilities of the HA prodrugs substantially, providing more effective sink conditions. Furthermore, a cetrimide concentration of 0.5 mg mL^{-1} was found to have no deleterious effect on the barrier properties of either full-thickness human or guinea pig skin.

The flux of HA was generally lower than that which has been reported previously. However, earlier studies examining the transdermal delivery of HA have employed either lactic acid 0.03% (v/v) (Vaddi et al., 2001, 2002) or ethanol 50% (v/v) (Almirall et al., 1996) as receptor phase additives. It is known that ethanol can act as a penetration enhancer (Oh et al., 2002), whilst pH can also affect permeant flux (Roy and Flynn, 1990). Of greater importance is probably the use of epidermal membranes in the studies by Almirall et al. (1996) and Vaddi et al. (2001, 2002). Heat separated epidermal membranes are sometimes used in skin permeation studies. However, this can lead to erroneously high data when lipophilic compounds are under investigation, as the largely aqueous dermis is a significant barrier to the permeation of these permeants (Williams, 2003; Heard and Screen, 2008). Furthermore, the preparation of epidermal membranes is likely to eliminate the activity of cutaneous esterases (Bonina et al., 2001), therefore, full-thickness skin was used throughout this study.

All HA derivatives exhibited lower fluxes than the parent drug. It is speculated that this may have been due to the lower aqueous solubilities of the prodrugs, their increased molecular weight and size or preferential binding to skin components. There was a strong correlation between the solubilities of the permeants in PBS and their experimental fluxes. The fall in aqueous solubility, as the ester chain length increased, was generally mirrored by a decline in the fluxes of the permeants. Similar relationships have been reported

previously with Mannich base derivatives of theophylline and 5-fluorouracil, and also with 6-mercaptopurine prodrugs (Sloan et al., 1984; Waranis and Sloan, 1987, 1988). Furthermore, mathematical models, incorporating permeant aqueous solubility, have been used to predict the transdermal fluxes of 41 compounds from an isopropyl myristate vehicle. In addition to accurate flux predictions, these models appear to suggest the existence of a lipid-aqueous series diffusional path across the skin (Roberts and Sloan, 2000). This hypothesis is supported by the solubility and permeation data presented in this study.

The increased MW of the bulkier prodrugs may have contributed to the reduced flux. The MW of transdermal permeants has long been known to be a major factor determining the degree of percutaneous absorption (Scheuplein et al., 1969). However, it seems doubtful that a modest 19% increase in MW (HA vs. HB) would have been the sole cause of a 44-fold reduction in flux. Similarly, it appears unlikely that the increased molecular size or volume of the prodrugs, which would have been proportional to MW, would have been the primary reason for the reduced flux.

Alternatively, the reduced permeation of the prodrugs may have been due to increased binding of the permeants to components in the stratum corneum. Esterification of HA would have caused considerable changes in the physicochemical properties of the drug. These changes, in turn, may have altered the drug's affinity for the stratum corneum. Whilst the flux predictions were largely inaccurate, it should be noted that the *C* log *P* values of HO and HD were outside the range of the dataset from which Eq. (1) was derived. Interestingly, the rank order predictions made using the Potts and Guy equation were also incorrect. The flux predictions for HA and HE (which have the lowest *C* log *P* values of all the permeants) were more accurate. Flux predictions also take no account of permeant hydrolysis in the skin—largely immaterial in this study, but possibly relevant if cutaneous metabolism is rapid. It is often quoted that transdermal permeants should possess a log *P* of between 2 and 3 for optimal flux (Williams, 2003). This rule of thumb, rather than the Potts and Guy equation, appears to be more applicable here.

All permeants were deposited onto the surface of the skin using volatile acetone solutions to avoid potential artifacts from skin hydration effects. When employing this method one must make two key assumptions. Firstly, that the volatile solvent applied to the skin does not alter the integrity of the barrier. There is some evidence suggesting that this should not be presupposed. Bond and Barry (1988) noted that the pre-treatment of hairless mouse skin did result in a significant increase in the flux of 5-fluorouracil. However, no significant difference in the flux of the same permeant was revealed when human skin was used. The second supposition is that the solid deposited on the skin surface will quickly dissolve (and saturate) the minute volumes of water that result from transepidermal water loss. Variations in the dissolution rates of these dry surface films could result in non-uniform thermodynamic activities in the skin surface water. Whilst this cannot be ruled out, the positive relationship between the aqueous solubilities of the HA prodrugs and their fluxes does match other studies using saturated aqueous donor phases (Sloan et al., 2003). These similarities suggests that drug dissolution was probably not a major contributory factor in determining rank order of diffusion.

Non-specific cutaneous esterase activity has been known about for many years (Montagna, 1955). The extensive hydrolysis of ester prodrugs during their passage through the skin has been reported, even when the skin has been previously frozen (Hewitt et al., 2000; Valiveti et al., 2005). However, the current study of tertiary ester prodrugs only revealed very minimal hydrolysis of just two of the five HA prodrugs. The hydrolysis studies using skin and liver extracts largely backed up the transdermal data from this study and our previous work using PLE (Morris et al., 2008). It is also in agreement with Cheung et al. (1985) who noted that the tertiary ester betamethasone-17-valerate was stable to hydrolysis by cutaneous enzymes.

Esterase enzymes are typically divided into type-GX and type-GGGX, based upon the presence of a GX or GGGX structural motif within the active site of the enzyme. Typically only GGGX-type esterases, for example PLE, are capable of metabolising bulky substrates, such as tertiary esters (Henke et al., 2002). From the transdermal and prodrug hydrolysis data presented, it seems likely that GX-type esterases are the predominant form found in guinea pig skin. As such, significant cutaneous hydrolysis of tertiary esters within guinea pig skin is unlikely. This suggests that if the researcher wishes to accurately model the cutaneous hydrolysis of tertiary esters, some form of skin extract should be used.

The use of purified esterases as a model for cutaneous esterases should not be ruled out if the prodrugs in question are primary, secondary or phenolic ester prodrugs. Previous studies have demonstrated that ester substrates of this nature are readily hydrolysed within the skin (Cheung et al., 1985; Hewitt et al., 2000; Ostacolo et al., 2004). It should also be noted that tertiary ester prodrugs can be rapidly hydrolysed within the bloodstream (Martindale, 2005). In view of this, transdermal tertiary ester prodrugs can still be efficacious if the target is the systemic circulation.

5. Conclusions

The transdermal permeation of HA and its ester prodrugs was evaluated. The predicted fluxes of HA and the HA prodrugs were shown to be inaccurate underlining the importance of obtaining empirical data. The HA prodrugs were found to be relatively stable to cutaneous esterases encountered during transdermal permeation. Incubation of the prodrugs with a skin extract yielded similar findings. Whilst HA prodrugs are unlikely to be delivered at clinically significant levels via passive transdermal diffusion they should not be discounted as candidates for controlled release formulations via other delivery routes.

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