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Formulation development of a transdermal drug delivery system for amlodipine base

D.M. McDaid[1], P.B. Deasy*

Department of Pharmaceutics, Trinity College, University of Dublin, Shrewsbury Road, Dublin 4, Ireland

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Abstract

Amlodipine base was prepared from its besylate salt and various physicochemical properties relevant to transdermal delivery determined. Permeation of the drug from a range of hydrophilic and hydrophobic bases through hairless mouse skin was studied and the influence of the penetration enhancers sodium lauryl sulphate 1% and propylene glycol 20% in a sodium carboxymethylcellulose 3% gel base was examined. The flux of drug could be further enhanced using variable percentage of ethanol in the donor phase. The influence of various rate controlling membranes and a contact adhesive on drug permeation was examined. In vivo studies using rabbits were performed to assess the suitability of a reservoir-type device. Employing data obtained from in vitro studies involving human abdominal skin, it was possible to predict the plasma profile resulting from the application of a similar device onto human skin over a period of 1 week and was found to be inadequate for clinical use. No adverse local effects in the animal model arising from the application of the transdermal device were observed.

Keywords: Transdermal drug delivery; Amlodipine base; Hairless mouse skin; Penetration enhancers; Ethanol; Rate-controlling membranes

1. Introduction

There has been much work done on the development of transdermal systems for calcium channel antagonists. Shah et al. (1992) investigated the passive transdermal delivery of verapamil through hairless mouse skin, while Wearley and Chien (1990) examined the iontophoresis-facilitated delivery of the compound. Diez et al. (1991) and Yu et al. (1988) examined a range of 1,4-dihydropyridines including nifedipine, but excluding amlodipine, using hairless rat skin and other animal models, and concluded that felodipine and nicardipine as the free base in particular would be the best candidates for transdermal delivery. The potential of transdermal patches to deliver effective levels of nitredipine in animal models and humans if containing penetration enhancer was demonstrated by Ruan et al. (1992). Ruan and Zheng (1991) also showed that by using a penetration enhancer (Azone) and a large contact area

* Corresponding author. Tel: + 353-1-2693333; Fax: + 353-1-2696457.

[1] Present address: Forest Laboratories Ltd., Clonshaugh Industrial Estate, Dublin 17, Ireland.

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(72 cm^2), it was possible to deliver effective levels of nifedipine to humans on once daily application using a matrix-dispersion type patch causing negligible local irritation.

Only limited work with hairless mouse skin suggesting the feasibility of developing an effective transdermal system for amlodipine besylate, involving use of ethanolic solutions with oleic acid or azone as penetration enhancers, has been published in the patent literature (Francoeur and Potts, 1988). Further work in publication by us (Deasy and McDaid, 1996) confirmed that amlodipine besylate was too hydrophilic for adequate transdermal delivery using a convenient area of device, despite the use of penetration enhancers and increase in the thermodynamic gradient across various rate-controlling membranes by ethanol. This project is concerned with the formulation development of a reservoir-type transdermal system using the more lipophilic amlodipine base.

2. Materials and methods

2.1. Materials

Acetic acid glacial, ammonium acetate, hydrochloric acid, magnesium sulphate Analar, polysorbate 80, propylene glycol, sodium carboxymethylcellulose low viscosity, sodium hydroxide Analar, sodium lauryl sulphate (BDH Chemicals), acetonitrile HPLC (Rathburn), acrylate Lewis acid based transfer adhesive between polyester release liners, type MSX 583, ethylene vinylacetate (EVA) membrane, type 987192, 19% VA content, 2 mil thickness, type 987292, 29% VA content, 2 mil, Scotchpack backing membrane, no. 1006 (3M), amlodipine besylate BP (Pfizer), ammonium carbonate, triethylamine (Aldrich), Celgard microporous polypropylene membrane, type 2400 (Celanese), Cremophor RH40, macrogol 300 and 4000, yellow soft paraffin (BASF), desipramine hydrochloride, 1-heptane sodium sulphonate, lidocaine (Sigma), diethylether, disodium hydrogen phosphate Analar/GPR, *n*-octanol, orthophosphoric acid, sodium dihydrogen orthophosphate Analar/GPR, tetrahydrofuran HPLC (Riedel de Haen), ethanol HPLC, methanol HPLC, methyl *tert*-butyl ether HPLC (Lab-Scan) and glass-distilled water. All reagents were GPR unless otherwise indicated.

2.2. Preparation of free base and its characterization

Amlodipine base was prepared from the besylate salt by dissolving it in methanol at 20°C, treated with 1.0 M aqueous sodium hydroxide solution, and extraction of the base formed into diethylether dried by filtering through heated dry magnesium sulphate, prior to evaporation of the solvent using a rotary evaporator. Alternatively the base was formed from an aqueous solution of the besylate at 50°C by treatment with the sodium hydroxide solution and allowed to crystallize out at 4°C, prior to collection on a 0.45 μm Millipore membrane filter and drying with dried magnesium sulphate in a desiccator. ^{1}H and ^{13}C nuclear magnetic resonance profiles for both amlodipine and amlodipine besylate were obtained by dissolving ~ 10 mg of compound in ~ 0.5 ml of deuterochloroform solution containing 1% deuterated tetramethyl silane as internal standard. Spectra were obtained using a Bruker MSL spectrometer, operating at 300 MHz, and equipped with an Oxford Magnet and an Aspect 3000 computer. The melting point of the besylate salt and base was determined using a Mettler DSC 20 with Mettler 10 A processor and output to a FX-800 printer.

2.3. Analysis of drug samples

Analysis of drug in solution was performed, where possible, using a double-beam Shimazdu UV-160 spectrophotometer set at 240 or 366 nm wavelengths of maximum absorbance. When interference or low drug concentration required, a HPLC method was employed using an LC-5A unit connected to a SPD-2A UV detector set at 240 nm with output to a C-R3A chromopack integrator (Shimadzu). An injection volume of 25 μl of unextracted solution was used. The column employed was an Ultratech ODS 5 μm (250 $\times$ 4.6 mm) guarded by a precolumn (10 mm) with similar packing, and the filtered and degassed

mobile phase, pumped at a rate of 1.5 ml/min, was composed of 6 mM 1-heptane sodium sulphonate, 42% (v/v) acetonitrile and 1% (v/v) tetrahydrofuran in a 0.1 M sodium dihydrogen orthophosphate buffer adjusted to pH 3.0 with 85% (v/v) orthophosphoric acid. The HPLC assay was validated for precision, linearity, recovery and specificity.

2.4. Determination of ionization constant

The ionization constant of amlodipine was determined by the method of Albert and Sergeant (1971), whereby the absorbance at 220 nm of aqueous solutions was examined over the pH range 8.5–9.2 (0.1 pH increments).

2.5. Determination of drug solubility

The solubility of amlodipine was determined in distilled water, phosphate buffer solution pH 7.0 and *n*-octanol. Analysis was carried out by placing an excess of the drug with 1 ml of the appropriate solvent in each of six 1.5-ml sealed polypropylene micro-vials, shaking in a water-bath at 37°C for 24–36 h until equilibrium was achieved and analysing the filtrate passing through a 0.22 μm Millipore filter by UV spectroscopy or HPLC with reference to a calibration curve.

2.6. Determination of partition coefficient

The partition coefficient of amlodipine was carried out in both *n*-octanol/distilled water and *n*-octanol/phosphate buffer, pH 7.0. The two phases were shaken together initially to ensure mutual saturation. An accurately weighed quantity of amlodipine was dissolved in 100 ml of the *n*-octanol phase and shaken at 37°C for 24 h against 50 ml aqueous phase in a sealed container. The separated *n*-octanol phase was assayed by UV spectroscopy to determine its residual concentration and hence the amount partitioned into the aqueous phase. The partition coefficient was expressed as the concentration of drug in the *n*-octanol phase (% w/v) divided by the concentration in the aqueous phase.

2.7. In vitro permeation studies

Abdominal skin from male hairless mice (6–8 weeks old), Sha-Sha strain, stripped if necessary using cellophane tape, pinna skin from the inner side of the ear or shaved full thickness abdominal skin from male New Zealand white rabbits (3 years old), and human abdominal skin obtained at autopsy from two elderly male subjects was used. Before use in permeation studies, each artificial membrane (Celgard or EVA types) was wetted to remove entrapped air and reduce surface tension by forcing phosphate buffer, pH 7.0, containing 0.05% polysorbate 80 through it, followed by rinsing and storage in buffer before use. When the donor phase included ethanol, the artificial membrane was wetted by sonication in the medium for 1 h before rinsing and storage in fresh medium. Donor systems, containing 1% (w/v) drug dispersions equilibrated overnight at 37°C, were prepared from sodium carboxymethylcellulose (NaCMC) 3% (w/v) in aqueous vehicles of variable pH, yellow soft paraffin, cremophor RH40 or macrogol ointment BP. Propylene glycol 20% or sodium lauryl sulphate 1% were substituted for water alone or in combination in NaCMC bases as penetration enhancers.

Permeation studies were carried out using diffusion cells, details of whose construction and use have been described previously (O'Neill and Deasy, 1988). At various time intervals, 1-ml samples were withdrawn from the receiver compartment and analysed by UV spectroscopy or HPLC for drug content. Experiments were replicated at least four times. When required, a transfer adhesive was used to secure the artificial membrane to the surface of the skin.

2.8. In vivo permeation studies

Each circular transdermal device or patch (diameter 3 cm) for amlodipine was fabricated by laminating at its circumference, using compression and heat at 160°C for 10 s, the drug loading in the intended weight/volume of donor system between the artificial membrane and an impervious backing membrane. The transfer adhesive was applied to the artificial membrane, its release liner being

removed just prior to use. Such patches were applied to the shaved abdominal area or pinna skin of male New Zealand rabbits (3.5–4.0 kg) each fitted with an Elizabethan-type collar to prevent interference with the patch. Blood samples withdrawn into heparinized tubes from the central ear artery at 12-h intervals for 3 days after sedation by intramuscular injection of Hypnorm 0.3 ml/kg (Janssen Animal Health). The separated plasma 1-ml samples, spiked with internal standard, desipramine hydrochloride or lidocaine, were shaken with 0.2 ml of ammonium carbonate 10% solution (~ pH 8.7), extracted with methyl *tert*-butyl ether, which after separation was evaporated to dryness at 55°C under vacuum. The residue was reconstituted in 0.1 ml hydrochloric acid, 0.01 M, washed with further methyl *tert*-butyl ether and 50 μl of the separated aqueous phase were injected into the HPLC system. The validated assay was based on that developed by Yeung et al. (1991) and was similar to that described above except that more sensitive Waters instrumentation was used and the mobile phase was composed of methanol/ammonium acetate (0.04 M)/acetonitrile (38:38:24) containing triethylamine 0.02%, final pH adjusted to 7.1 using acetic acid glacial.

Adverse skin reactions were assessed by examining and photographing both application sites on the rabbits immediately after termination of treatment.

3. Results and discussion

3.1. Physicochemical properties

Carbon and hydrogen NMR studies on amlodipine compared with amlodipine besylate confirmed that the free base was formed using either method of preparation, with no trace of the salt form remaining, and a yield of 90% conversion approx. Due to the suspected presence of methanol in the free base produced by the first method of manufacture, only the second method was subsequently used to produce free base for further experimental use. The melting point of the besylate salt was confirmed to be 208°C, while that of the base was 144°C. The pK_a of amlodipine was determined to be 8.91, indicating that the drug is highly ionized at all pH values over the physiologically relevant range 5–8. The surface of the skin is slightly acidic (pH 5.6), increasing at lower layers of the skin to that of plasma (pH 7.4), where the drug is slightly less ionized (99.95% compared with 97.00%). The aqueous solubility of amlodipine was determined to be 77.4 mg/l and the solubility in *n*-octanol was 56.08 mg/ml.

The observed partition coefficients of amlodipine were determined using *n*-octanol/water and *n*-octanol/phosphate buffer, pH 7.0, at 37°C, and corrected for fractional dissociation to give log *K* values of 2.96 and 3.09, respectively. The latter value is in good agreement with 3.18 derived from data presented by Mason et al. (1989) using partitioning between *n*-octanol and pH 7.2 buffer. There is an optimum log *K* value for most compounds for partitioning through lipophilic membranes. Compounds with values below these optimum log *K* values do not partition readily into the stratum corneum, while compounds with higher values of log *K* are so lipophilic that they remain dissolved in the stratum corneum. The log *K* values of amlodipine auger well for effective transdermal delivery because they are of the same order as those of glyceryl trinitrate (2.05), chlordiazepoxide (2.5) and timolol base (1.91), for which systemic transdermal delivery has been demonstrated.

3.2. Permeation across hairless mouse skin

The permeation profile of amlodipine across both intact and viable (stripped) hairless mouse skin is shown in Fig. 1. The calculated steady-state permeation rate was 5.92 $\mu g/cm^2$ per h for intact skin and 28.6 $\mu g/cm^2$ per h for viable skin with corresponding lag times of 2.17 h and 0.76 h. As the stratum corneum is lipophilic in nature and represents a major barrier to permeation of most drugs, its removal by stripping has resulted in significant increase in amlodipine permeation rate and reduction in lag time. The lag time is often considered as the first limiting factor for a transdermal delivery system (TDS) and 10% of

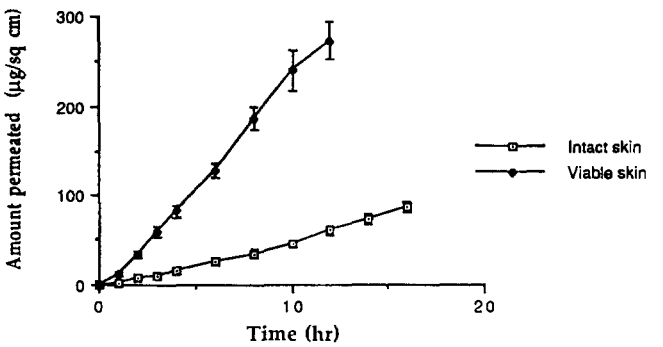

Fig. 1. Permeation of amlodipine across intact and viable hairless mouse skin (mean ± S.D., n = 4).

the exposure time of the TDS is considered a reasonable upper value for the lag time (Diez et al., 1991). Since an exposure time of 72–120 h is envisaged for the TDS being developed, the lag time obtained from these preliminary experiments does not preclude the possibility of developing a successful TDS.

3.3. Formulation effects

The choice of reservoir system, its pH and rate-controlling membrane will influence the transfer of drug from a TDS into skin layers. Fig. 2 shows the effect of yellow soft paraffin, Cremophor RH40, macrogol ointment and sodium carboxymethylcellulose gel on the transdermal penetration of amlodipine through hairless mouse skin, the plots confirming achievement of steady-state permeation rates after elapse of an initial lag period. The steady-state fluxes were 2.78 ± 0.43, 3.62 ± 0.57, 4.14 ± 0.69 and 5.92 ± 0.72 $\mu g/cm^2$ per h for yellow soft paraffin, Cremophor, macrogol and NaCMC bases, respectively (mean ± 1 S.D.). These results indicate greater delivery from hydrophilic compared with hydrophobic bases, which may be due to the greater solubility in the base and ease of partitioning through the skin. Also components of the various bases may affect properties of the skin such as hydration or act as penetration enhancers.

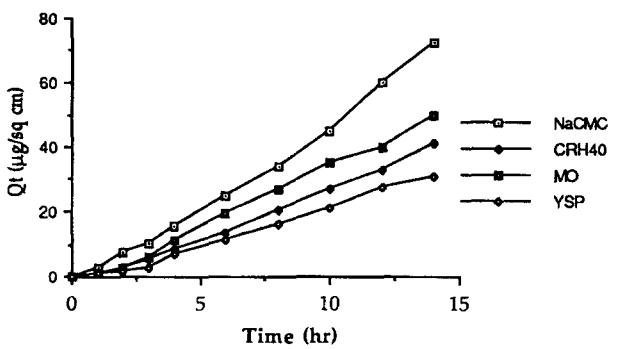

Fig. 2. Effect of various donor systems on the permeation of amlodipine through hairless mouse skin, where Q_t is the cumulative amount of base transferred (NaCMC is sodium carboxymethylcellulose gel, CRH40 is Cremophor RH40, MO is macrogol ointment and YSP is yellow soft paraffin).

As maximum flux was observed with the NaCMC base, the effect of altering the pH of this vehicle was studied as shown in Fig. 3. Because amlodipine is a weak base, increase in pH would be expected to give rise to greater thermodynamic activity of the increasing amount of undissociated form (Higuchi, 1960). It can be seen from the permeation profiles illustrated that although no significant differences ($P > 0.05$) were observed over the pH range studied, the permeation rates for amlodipine were higher at higher pH (4.18 ± 0.65, 5.76 ± 0.77, 5.92 ± 0.72 and 5.79 ± 0.69 $\mu g/cm^2$ per h at pH 5, 6, 7 and 8, respectively).

The previous results indicated that whereas changes in formulation and vehicle pH could affect the flux of drug through hairless mouse skin, clinically useful transdermal delivery was unlikely to be achieved without further enhancement of the effect. When sodium lauryl sulphate (SLS) 1% and propylene glycol (PG) 20% as penetration enhancers were included into the NaCMC 3% gel base (pH 7), either alone or in

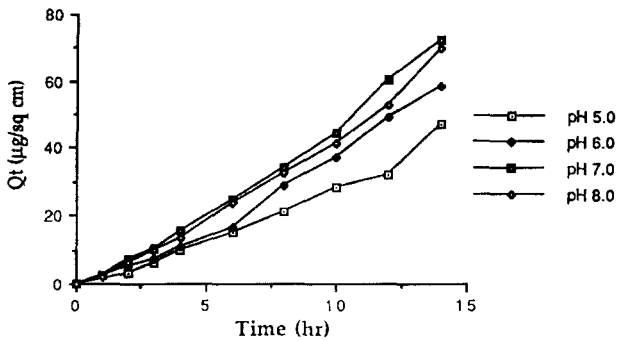

Fig. 3. Effect of NaCMC gel pH on the steady-state permeation rate of amlodipine through hairless mouse skin.

combination, the effect on permeation was as shown in Fig. 4. When SLS was included alone, the flux increased slightly ($P > 0.05$) over the control (6.19 ± 0.83 compared with 5.92 ± 0.72 $\mu g/cm^2$ per h). When PG was employed alone in the donor phase, the resultant flux was increased to 7.43 ± 0.88 $\mu g/cm^2$ per h ($P > 0.5$). Significant ($P < 0.05$) increase in flux to 9.87 ± 1.14 $\mu g/cm^2$ per h compared with the control was observed only when the penetration enhancers were used in combination. The mode of action of SLS is related to its ability to disrupt keratin resulting in increased hydration of the stratum corneum, apart from possible emulgent, solubilization and competitive binding effects (Barry, 1987). In addition to increasing skin hydration because of its hygroscopic property, propylene glycol probably acts mainly as a cosolvent, increasing the drug gradient in solution for percutaneous absorption (Barry, 1983). It is probable that the combination of the two enhancers produced a synergistic effect as their major modes of promoting enhancement are different.

The effect of ethanol on the permeation of the free base form of amlodipine through hairless mouse skin at both pH 5 and 7 was examined. Fig. 5 shows the permeation profiles obtained at pH 7 with variable percentage of ethanol incorporated into both the donor and receiver phases. As can be seen from Table 1, the resultant increase in flux of amlodipine can be attributed in part to the increased solubility of amlodipine in the donor phase, in addition to greater membrane fluidity and pore formation associated directly with increasing ethanol content. At 0% and 10%, the permeability coefficient values ($K_p \times 10^5$) are very similar (5.00 cm/s and 5.19 cm/s, respectively), indicating that the incorporation of ethanol up to a level of 10% into the formulation produces no significant effects on the permeability of hairless mouse skin with respect to amlodipine base, even though there are large differences in the fluxes observed. At 20% ethanol, however, the permeability coefficient increased significantly ($P < 0.05$) and thus the resistance to the passage of amlodipine through the skin has been decreased. The difference between the lag times was not significant ($P > 0.05$), leading to the conclusion that any enhancing effect of ethanol occurs very soon after exposure of the skin to the donor phase. When 20% ethanol is incorporated into both the donor and receiver phases, the flux of amlodipine is 85.1 $\mu g/cm^2$ per h, which could deliver a theoretical daily dose of 40.8 mg using a 20 cm^2 device. When the receiving phase was changed to 100% pH 7 as a more realistic simulation of in vivo conditions, the steady-state flux dropped to 9.50 ± 1.73 $\mu g/cm^2$ per h when using a donor phase of the same pH with 20% added ethanol. This flux could deliver a theoretical daily dose of 4.56 mg of base from a device of 20 cm^2. The theoretical transdermal dose of amlodipine is ~ 3.2 mg and thus a device employing the formulation described could produce therapeutic levels in vivo. The effect of inclusion of a rate-controlling artificial membrane and adhesive layer in parallel would be to further reduce the transdermal steady-state flux and provide some degree of control over the permeation of the drug from the composite device into the systemic circulation.

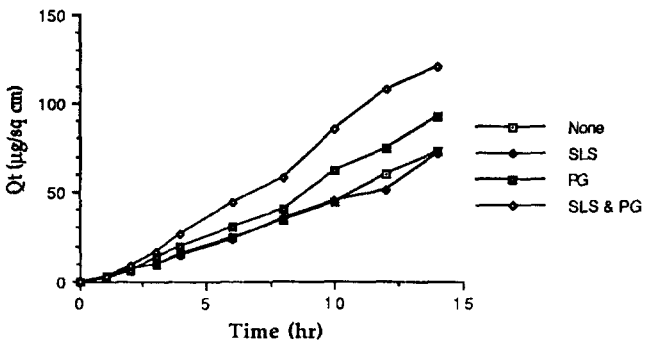

Fig. 4. Effect of various penetration enhancers on the steady-state permeation rate of amlodipine through hairless mouse skin (SLS is sodium lauryl sulphate and PG is propylene glycol).

3.4. Effect of artificial membranes and other skin types

The solubility of the ionized form of amlodipine base can be increased by a decrease in pH of the dissolution medium, although the intrinsic solubility of the undissociated molecule is unaffected by changes in pH. It follows that if an artificial membrane employed is permeable towards both the ionized and unionized species, the flux from a saturated solution will be affected by changes in the pH of the donor system. Fig. 6 shows the effect of pH on the permeability of amlodipine base through the microporous polypropylene membrane, Celgard 2400. It is obvious from the permeation profiles that the steady-state fluxes from these saturated solutions increase with a decrease in pH; thus both the ionized and unionized forms of amlodipine are capable of crossing the membrane.

The permeation profile of amlodipine base through the EVA copolymer membranes 987192 and 987292 compared with Celgard 2400 at pH 6 using a saturated solution of drug in the donor phase is shown in Fig. 7. The fluxes of amlodipine base recorded at steady-state are 0.21 $\pm$ 0.01 and 0.47 $\pm$ 0.03 μg/cm^2 per h using EVA 987192 and EVA 987292, respectively. Employing an exposure area of 20 cm^2, corresponding inadequate delivery of 0.103 and 0.227 mg of amlodipine base would result over a 24 h period. Fig. 8 shows the permeation profile of amlodipine base through Celgard 2400 using a donor phase composed of 20% ethanol and 80% pH 7 into a receiver phase composed of 100% pH 7. The resultant flux associated with the increased thermodynamic gradient of the drug across the membrane was 16.8 μg/cm^2 per h, corresponding to an acceptable daily delivery of 8.06 mg of amlodipine base for an exposure area of 20 cm^2 when a saturated solution of amlodipine in equilibrium with excess drug was used as the donor phase. This amount of free base permeated compares favourably with the estimated transdermal daily delivery dose of 3.2 mg required and suggests that a system employing this vehicle could be useful for the design of a transdermal delivery system for amlodipine base.

In an attempt to develop a final effective TDS system, artificial membranes and adhesive were tested in parallel with various natural epidermal barriers. Celgard 2400 was chosen as the rate-controlling membrane as the previous studies had shown that both EVA membranes tested were too impermeable. Using a donor vehicle of 20% ethanol and 80% pH 7 containing excess drug, the steady-state flux of amlodipine was 7.1 $\pm$ 1.63 μg/cm^2 per h through Celgard 2400 and hairless mouse skin in parallel into a receiver phase of pH 7, corresponding to adequate daily dose for a 20 cm^2 device. Although the flux is lower than that acquired using hairless mouse skin alone, the difference is not significant and so it was concluded that the microporous polypropylene membrane cannot be regarded as an adequate rate controlling membrane. However, it does provide a substrate for the application of the optimum contact adhesive determined experimentally, acrylate based MSX 583, the combination in parallel hav-

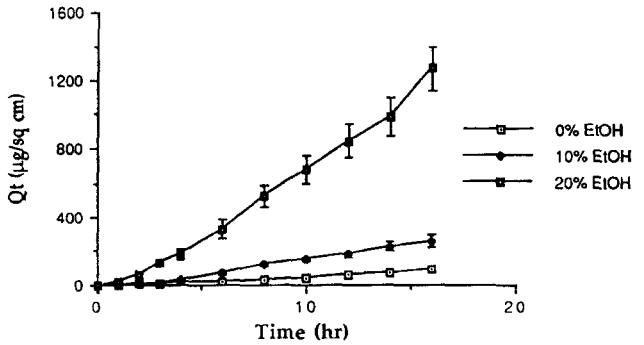

Fig. 5. Permeation profiles obtained with amlodipine base through hairless mouse skin using identical donor and receiver phases consisting of 0, 10 and 20% ethanol (EtOH) incorporated into aqueous buffer pH 7.

ing the potential for composite membrane control. The resultant steady-state flux, when the adhesive layer was used to bind the Cegard 2400 and hairless mouse skin membranes together, was 3.21 ± 0.78 $\mu g/cm^2$ per h, with a lag time of 3.43 ± 0.51 h. Thus the adhesive has become a rate-controlling layer, offering some resistance to the transfer of drug from the donor phase to the receiver phase. As the lag time showed little change with the addition of the adhesive layer, it was concluded that amlodipine partitions readily into this layer.

It is possible to quantify the amount of control the artificial membrane and adhesive have on the overall permeation process using the method of Guy and Hadgraft, 1992. The fractional control by the device (F_d) was determined to be 0.662, greater than that exerted by the skin (F_s = 0.338). This adequate degree of control by the device is desirable, as it has the advantage that inter-subject variabilities in skin permeability result in less variation in the overall steady-state permeation of the drug, as well as improving the safety of the device by reducing the risk of overdosage. Unfortunately the effect of using this adhesive to decrease the steady-state flux, was to reduce the anticipated daily dose from the device to 1.54 mg for a device of 20 cm^2. A backing membrane was examined, but no difference in drug permeation was observed.

Table 1
Physiochemical data obtained for the permeation of amlodipine base through hairless mouse skin employing ethanol in donor and receiver phases at concentrations of 0, 10 or 20%, pH 7

Parameter	Ethanol (% v/v)		
	0	10	20
Flux ($\mu g/cm^2$ per h)	5.92 ± 0.72	20.1 ± 2.65	85.1 ± 6.33
Lag time (h)	2.30	2.64	2.29
Solubility (mg/l)	328	1076	3628
$K_p \times 10^5$ (cm/s)	5.00	5.19	6.52

Theoretically, the steady-state flux from the donor vehicle through the skin and into systemic circulation should be constant over the chosen period (3 days minimum), as the drug is in suspension with a saturated solution providing a constant thermodynamic gradient. Apart from the reported increased permeability of hairless mouse skin after ~20 h (Lambert et al., 1989), there is

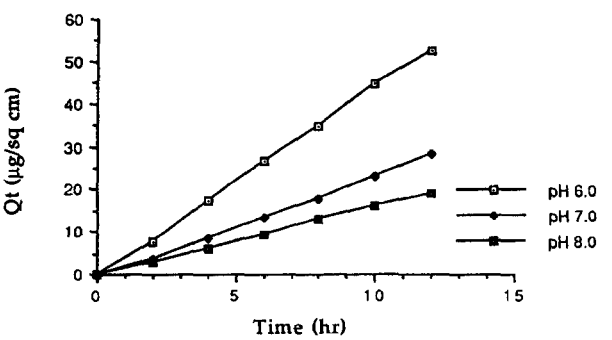

Fig. 6. Effect of pH on the permeation profile of amlodipine base through Celgard 2400.

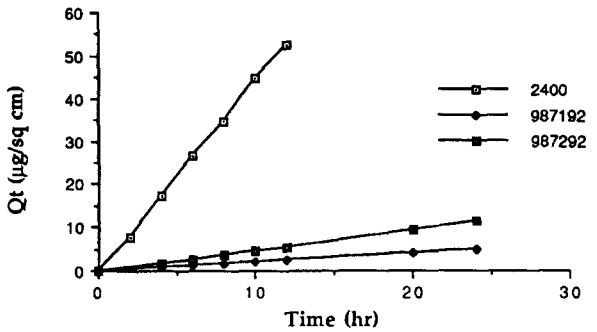

Fig. 7. Permeation of amlodipine at pH 6 through Celgard 2400 and EVA 987192, 987292 membranes.

reason to believe that over an extended period the concentration of ethanol in the donor phase will decrease from 20% due to the permeation of the ethanol from the donor vehicle into the systemic circulation and also due to the migration of water into the donor vehicle. By diluting the ethanol concentration in the donor phase, the saturated concentration of amlodipine will decrease, thus lowering the steady-state flux. Coupled with this, the possibility exists also that as the percentage of ethanol decreases, any penetration enhancing properties associated with ethanol could be concentration dependent and thus dilution of the donor phase with water could further lower the steady-state flux of amlodipine.

For subsequent studies, it was decided to use rabbits and two application sites were chosen, pinna and abdominal skin. Table 2 lists some physicochemical data derived from the permeation profiles of amlodipine from 20% ethanol and 80% pH 7 containing excess drug into pH 7 receiver phase through hairless mouse skin, rabbit pinna skin, rabbit abdominal skin and human abdominal skin, obtained during preliminary experiments to select an experimental model. The lowest flux was observed through human skin when studied over a 24 h period. Many literature references have indicated that hairless mouse skin is more permeable than human cadaver skin. For example Bond and Barry (1988) compared the permeability of the two skin types using 5-fluorouracil and found that the mean permeability through the animal skin was ~ 2.2 times that through human skin, suggesting that hairless mouse skin may act as a reasonable substitute for human skin in permeation experiments, at least for simple aqueous solutions employing short contact times. When the effect of acetone on the permeability of both skin types was examined, the permeability of human skin was not significantly altered, whereas that of mouse skin increased 16-fold. It was therefore suggested that data using acetone-exposed hairless mouse skin and possibly other solvents as examined in this project may not relate well to data for human skin. Similar differential enhancement of the permeability of both skin types to verapamil hydrochloride by azone treatment was reported by Agrawala and Ritschel (1988).

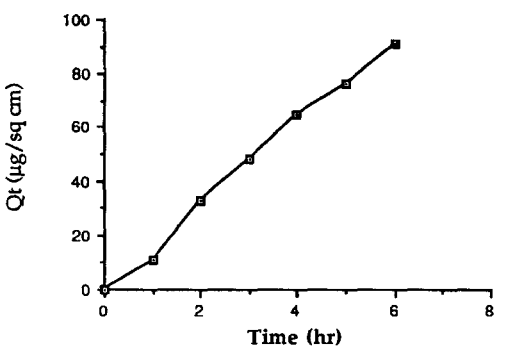

Fig. 8. Permeation profile of amlodipine base through Celgard 2400 using a donor phase of 20% ethanol and 80% pH 7 into a receiver phase of 100% pH 7.

In order to assess the longer-term effect of the ethanolic donor system on natural membranes, the permeation profiles of amlodipine through Celgard 2400/MSX 583/rabbit pinna or abdominal skin into pH 7 medium was studied. The steady-state flux through rabbit pinna skin over 3 days was 3.19 ± 0.53 $\mu g/cm^2$ per h with an initial lag time of 3.91 ± 0.39 h. When this result was compared with the flux of amlodipine obtained through rabbit pinna skin alone (7.61 ± 1.92 $\mu g/cm^2$ per h), it can be seen that the flux is decreased significantly ($P < 0.05$) and that the degree of control exerted by the device, F_d, was 0.58. Similarly, the flux of amlodipine at steady-state through Celgard 2400/MSX 583/rabbit abdominal skin was 2.49 ± 0.43 $\mu g/cm^2$ per h, with an extrapolated lag time of 4.31 ± 0.52 h. Rabbit abdominal skin alone, using comparable donor

Table 2
Physicochemical data obtained from permeation experiments for amlodipine through various skin types

Skin	Flux (μg/cm^2 per h)	Lag time (h)	K_p (cm/s $\times$ 10^7)
Hairless mouse	9.5 $\pm$ 1.73	3.02 $\pm$ 0.42	7.27
Rabbit pinna	7.61 $\pm$ 1.92	3.8 $\pm$ 0.61	5.82
Rabbit abdominal	3.62 $\pm$ 0.87	4.1 $\pm$ 0.52	2.77
Human abdominal	0.96 $\pm$ 0.24	5.2 $\pm$ 0.63	0.74

and receiver conditions, produces a higher flux of amlodipine (3.62 $\pm$ 0.87 μg/cm^2 per h), but the difference was not statistically significant ($P > 0.05$), and the fraction of control the device had on the overall permeation process was lower at 0.31. Although this fraction of control is lower than that exerted with rabbit pinna skin, the composite device has acceptable rate-limiting capacity at both skin sites of use in controlling possible in vivo fluxes of the drug.

3.5. In vivo evaluation

Figs. 9 and 10 show the mean plasma profiles for rabbit pinna and abdominal skin, respectively, obtained on application of a device containing a saturated amlodipine solution with excess drug in 20% ethanol, 80% pH 7 as reservoir, and with Celgard 2400 and MSX 583 adhesive as composite rate-controlling membrane.

Analysis of the plasma concentration-time data for amlodipine following transdermal administration was carried out assuming that the drug was delivered at a constant rate. The predicted profiles calculated therefrom are shown also in Figs. 9 and 10. It is possible to convert values of the input absorption rate, K_o (66.6 and 81.0 μg/h for rabbit pinna and abdominal skin, respectively) to μg/cm^2 per h by adjusting for the relevant exposure area. For rabbit pinna skin an exposure area of 16 cm^2 was used and this yields an absorption rate of 5.06 μg/cm^2 per h, while for the thicker rabbit abdominal skin an exposure area of 20 cm^2 was used, leading to a reduced absorption rate of 3.33 μg/cm^2 per h. A comparison of in vitro and in vivo absorption results shows that through rabbit pinna skin the flux of amlodipine is slightly higher in vivo than in vitro (5.06 as compared with 3.19 μg/cm^2 per h). Using rabbit abdominal skin, the in vivo flux of amlodipine is also slightly higher (3.33 as compared with 2.49 μg/cm^2 per h). However, both results obtained are in good agreement with those obtained in vivo and illustrate the validity of the proposed model. It is possible that the higher absorption rates of amlodipine obtained in vivo arise from an adhesive loading dose as proposed by Guy and Hadgraft (1985), which produce elevated plasma levels in the initial absorption phase. The in vivo data obtained was acquired over a duration of 72 h, which may not be sufficient time for the effect of the adhesive loading dose to be eliminated and for the zero-order membrane-controlled contribution to establish. Thus, it is possible that all data points are artificially high, and the fitted absorption rates are also too high and may be closer to those obtained in vitro.

The apparent conversion factor for absorption rates acquired from in vitro experiments to those obtained in vivo is 1.59 for pinna skin and 1.33 for abdominal skin. Although there is a difference between the two conversion factors, it is proposed to assume that the true conversion factor lies between these values. Human abdominal skin was examined in vitro, together with rabbit pinna skin and rabbit abdominal skin, with absorption rates of 0.96 $\pm$ 0.24, 7.61 $\pm$ 1.92 and 3.62 $\pm$ 0.87 μg/cm^2 per h being obtained, respectively. These absorption rates for both rabbit pinna and rabbit abdominal skin are higher than those reported in the previous section, where the effect of the rate-controlling membrane and adhesive is to decrease the steady-state fluxes through both rabbit pinna skin and rabbit abdominal skin. It is possible to calculate the contribution of the rate-controlling membrane and adhesive to the overall flux using the following equation:

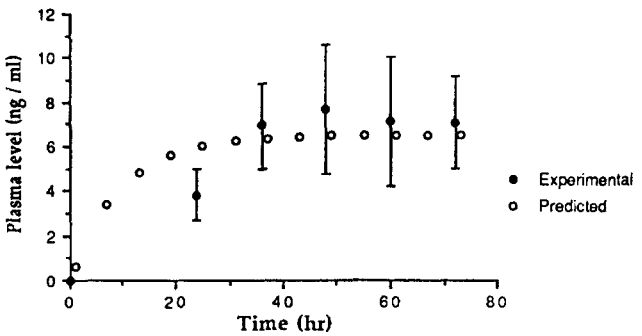

Fig. 9. In vivo plasma concentration-time profile of amlodipine obtained after application of a device onto rabbit pinna skin, using an exposure area of 16 cm^2, together with the predicted profile (results expressed as the mean ± S.D., $n = 6$).

$$1/J_t = 1/J_m + 1/J_a + 1/Js$$

where J_t, J_m, J_a and J_s are the rates of permeation across the total combination, membrane, adhesive and skin, respectively (Guy and Hadgraft, 1992).

Thus the flux of amlodipine through the rate-controlling membrane and adhesive, as calculated from rabbit pinna skin experiments conducted in vitro, is 5.49 $\mu g/cm^2$ per h, while the corresponding calculated value from rabbit abdominal skin data is 10.47 $\mu g/cm^2$ per h. It is then possible to calculate a theoretical value for the predicted in vitro flux of amlodipine through the membrane/adhesive/human skin combination, $J_{\text{human/in vitro}}$, using the above equation. Using rabbit pinna skin, a value of 0.88 $\mu g/cm^2$ per h is obtained for $J_{\text{human/in vitro}}$, while using rabbit abdominal skin a value of 0.82 $\mu g/cm^2$ per h is obtained. These values corresponding to the situation in vitro, can be converted to predicted in vivo fluxes by means of the conversion factors reported earlier. To create a range in which the true in vivo flux is likely to occur, each of the in vitro flux rates, $J_{\text{human/in vitro}}$, is converted using both conversion factors, thus yielding four in vivo flux rates of 1.17, 1.40, 1.09 and 1.30 $\mu g/cm^2$ per h. These permeation rates have an average of 1.24 ± 0.14 $\mu g/cm^2$ per h. It is possible then to predict the plasma concentration-time profile in humans using these permeation rates (mean ± S.D.) for an exposure area of 20 cm^2 and body weight of 70 kg, assuming from the data published by Faulkner et al. (1986) that the elimination rate constant is 0.0204/h and the volume of distribution is 1498 l. Fig. 11 illustrates the predicted profile of amlodipine in a human subject over 1 week. As can be seen, steady-state plasma levels of ~0.8 ng/ml are achieved only after 5 days, indicating that a loading dose may be necessary. The therapeutic plasma level of amlodipine is in the range 3–10 ng/ml. The average absorption rate of 1.24 $\mu g/cm^2$ per h would result in a daily dose of 0.59 mg amlodipine being delivered using an exposure area of 20 cm^2. This dosage falls short of the desired level of ~3.2 mg, but the approach illustrates that with further refinement of formulation and/or increase in area it may be possible to develop a successful transdermal system for human use.

Both application sites in rabbits were examined visually for signs of local irritation after wearing the device for 3 days. No local irritation was observed at either application site, confirming that the device was well tolerated on dermal application even after several days of wear. These results are not surprising as several researchers (Srivastara et al., 1988; Sharpe et al., 1989; Chen et al., 1990) have shown that various Ca^{2+} antagonists produce inhibition in acute and chronic models of inflammation in a dose-dependent manner, presumably by rendering cells devoid of calcium.

4. General discussion

It appears from the work presented that the formulation of an effective TDS for the delivery

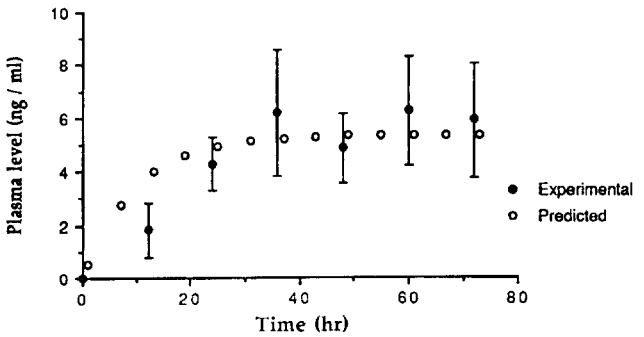

Fig. 10. In vivo plasma concentration-time profile of amlodipine obtained after application of a device onto rabbit abdominal skin, using an exposure area of 20 cm^2, together with the predicted profile (results expressed as the mean ± S.D., $n = 6$).

of amlodipine base may be possible. Further work is needed to refine the device in order to attain clinical levels in humans. It is certainly possible to increase the flux of amlodipine from the type of TDS formulated in this work by a number of methods. The available surface area could be increased or else the rate-controlling membrane/adhesive combination could be varied so that the delivery rate could be tailored to that necessary for human use. Alternatively the formulation of the donor system could be changed so as to increase the steady-state flux from the reservoir. There are a number of options open for exploration here including the inclusion of penetration enhancers or an increase in the % ethanol in the donor system. Other possible methods of increasing the flux include the use of ultrasound and iontophoresis. As amlodipine exists predominantly in the ionized state at pH 7, the use of iontophoresis to 'push' the ions across the skin seems an attractive avenue to explore.

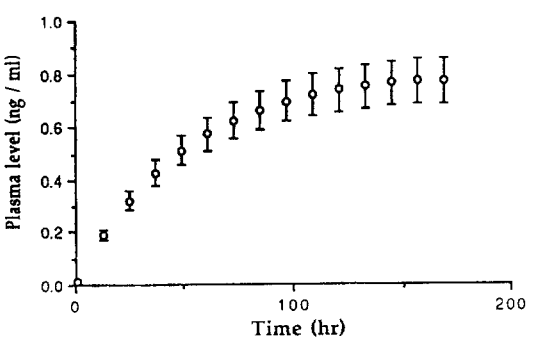

Fig. 11. Predicted in vivo plasma concentration-time profile of amlodipine after application of a TDS onto human abdominal skin, using an exposure area of 20 cm^2.

As yet the only treatment with Ca^{2+} antagonists involves oral drug delivery systems that require frequent dosing and the development of successful TDSs for their delivery would produce many advantages over traditional therapy. This research has attempted to illustrate that the formulation of an effective TDS for the delivery of amlodipine may be possible. This avenue of drug delivery should be further investigated so that the successful treatment of angina, hypertension and other clinical conditions for which Ca^{2+} antagonists are indicated may become possible with increased benefit to patients.

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