J. Pharm. Pharmacol. 1996, 48: 1138–1142
Received November 7, 1995
Accepted March 21, 1996

Terbutaline Transdermal Delivery: Preformulation Studies and Limitations of In-vitro Predictive Parameters

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Abstract

A transdermal dosage form of terbutaline may be useful to prevent nocturnal wheezing by providing prolonged duration of action. It will also improve patient compliance and bioavailability. Controlled input of the drug would be an additional advantage as this will reduce the intersubject variablity. Preformulation studies were conducted to determine the feasibility of a transdermal dosage form of terbutaline.

The drug solubility in propylene glycol was 6·3 mg mL^{-1}. The apparent partition coefficient (*n*-octanol/deionized-water, pH 6·5) of terbutaline was 0·03. A pH-partition coefficient (octanol/buffer) profile indicated that the partition coefficient values were 0·02, 0·05 and 0·4 in buffers of pH 3, 7·4 and 9, respectively. The required drug flux through the human skin to attain therapeutic concentrations in the blood was calculated to be 3·3 $\mu g\ cm^{-2}\ h^{-1}$ for a 10-cm^2 transdermal delivery system. Rabbit, guinea-pig and human skin was tested as the penetration barrier using modified Franz diffusion cells. Terbutaline flux values through the rabbit and guinea-pig skin were 8·3 and 7·7 $\mu g\ cm^{-2}\ h^{-1}$, respectively. The flux through human full-thickness skin and human epidermis were 0·6 and 3·6 $\mu g\ cm^{-2}\ h^{-1}$. Azone (3% w/v), a skin penetration enhancer, significantly increased the drug flux through all the membranes tested.

Based on these studies, transdermal delivery of terbutaline appears to be promising.

Terbutaline (1-(3,5-dihydroxyphenyl)-2-tertiarybutyl amino ethanol) is a β_2 selective bronchodilator. It is used for the long term treatment of obstructive airway diseases and in the treatment of bronchospasm (Davies et al 1974; Reynolds, 1993). Currently, terbutaline is given by the oral, inhalational, subcutaneous and parenteral routes. A 5-mg oral dose provides bronchodilation for about 6 h which may not be sufficient to prevent nocturnal wheezing. A sustained release terbutaline tablet formulation was also reported (Taudorf et al 1981; Erikson et al 1982). Drug delivery by the transdermal route has many advantages over the oral route. To overcome the inter-subject variability after oral dosing and to obtained prolonged action, the feasibility of transdermal delivery of terbutaline was investigated.

Materials and Methods

Materials

All chemicals were from Sigma Chemical Co., St Louis, MO, USA. Human cadaver skin (46-year-old white male and 65-year-old white male from the abdomen area) was obtained from a local hospital. Male rabbit (Myrtle Rabbitry, TN, 12-week-old) skin from the abdominal area was used. The guinea-pigs (8-week-old) were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN, USA).

Analysis

A sensitive HPLC assay was used for the quantitation of terbutaline in both the diffusate samples and in the skin extract samples (Tenjarla et al 1995). A cyano column (Alltech, 150 × 4·6 mm, E fittings) was used to achieve separation between the drug and the internal standard. The flow rate was 1·4 mL min^{-1}. The isocratic mobile phase was 30% acetonitrile in pH 5·6 buffer. The effluent was monitored at 225 nm. The internal standard was propranolol at a concentration of 2·5 $\mu g\ mL^{-1}$.

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Target input rate

The target input rate was determined by the pharmacokinetic steady-state equation:

$$\text{Input rate} = \text{steady-state concentration} \times \text{clearance} \quad (1)$$

A pharmacokinetic model (Guy & Hadgraft 1985) was also used to determine the target flux. The relevant parameters and the equation used are shown in Table 1.

Solubility study

An excess of terbutaline was added to 2 mL propylene glycol and the mixture was agitated at room temperature for 12 h. The sample was filtered through Whatman No 1 filter paper. The filtrate was suitably diluted and analysed by HPLC.

Partition coefficient study

n-Octanol and water (or buffer) were mixed and equilibrated with each other for 6 h and separated. Drug (2 mg) was added to the mixture, which was agitated for 12 h. The phases were separated and analysed by HPLC. The partition coefficient was determined by calculating the ratio of the drug concentrations in the octanol and water phases.

Skin preparation

Freshly excised full-thickness rabbit or guinea-pig skin from the abdominal area was used. The hair was lightly clipped and any adhering muscle or fat were removed from the dermal side. For human cadaver skin, the fat and muscle from the dermal side was removed and stored at −70°C until use. The mem-

Table 1. Parameters used in the pharmacokinetic model (Guy & Hadgraft 1985) to simulate plasma concentration assuming terbutaline flux of 3 $\mu g\ cm^{-2}\ h^{-1}$.

Molecular weight (MW)	= 260
Clearance (CL)	= 103·9 mL min^{-1}
Volume of distribution (V)	= 126000 mL (70-kg person)
Partition coefficient (PC)	= 0·05
K_2	= 2·9 × $(122/MW)^{0.33}$
K_3	= (K2 × PC)/5
Elimination rate constant (K_4)	= 0·0495 h^{-1}
Patch surface area (A)	= 10 cm^2
K_0, input rate	= 3 $\mu g\ cm^{-2}\ h^{-1}$
C_3	= (A × K_0/V) × [1/K_4 + (be^{-at} − ae^{-bt})/ K4(a − b)]

Where $a = [(K_2 + K_3 + K_4) + \sqrt{\{(K_2 + K_3 + K_4)^2 - 4K_2K_4\}}]/2$
$b = [(K_2 + K_3 + K_4) - \sqrt{\{(K_2 + K_3 + K_4)^2 - 4K_2K_4\}}]/2$

brane was used within 48 h after death. The human epidermis was obtained by treating the full-thickness skin with 0·5% ethylenediaminetetraacetic acid solution for 12 h (Raykar et al 1988).

Skin permeation study

Modified Franz diffusion cells were used for the skin permeation study (Tenjarla et al 1994). Rabbit or guinea-pig full-thickness skin, human cadaver full-thickness skin or the human epidermis were used as the penetration barriers. The membrane was loaded between the donor and receptor chambers of the cell with the stratum corneum facing upwards. The receptor chamber was filled with phosphate-buffered saline (pH 7·4) and maintained at 37°C by circulating water from a water bath. A magnetic stirrer was placed in each cell to ensure uniform mixing. The test solution (200 μL saturated terbutaline solution in propylene glycol with or without 3% w/v azone) was added to the skin in the donor chamber and sealed with parafilm. Samples were taken at predetermined time intervals and analysed by HPLC.

A plot of the amount of drug permeated per square centimeter vs time was constructed. The slope of the linear profile was equal to the drug flux (J) through the skin at steady state. The lag-time (T) was determined by extrapolation of the linear phase to the x-axis. It should be noted that the diffusional pathlength is probably longer than the thickness of the membrane. This is because the diffusional path length is believed to be tortuous around the cells of the stratum corneum. This may have an effect on the calculated partition coefficient (K_m × d) value. The other parameters, diffusion coefficient (D) in the membrane, the permeability constant (K_p) and the partition coefficient (K_m) were calculated as follows:

$$D/d^2 = 1/6T \quad (2)$$

$$K_p = J/C \quad (3)$$

$$K_m \times d = K_p/(D/d^2) \quad (4)$$

where d is the thickness of the membrane.

Drug retention in the skin

At the end of the permeation study, the excess drug on the skin was removed by gently washing three times with 200 μL of water. The drug-exposed skin was then cut into small pieces and homogenized with 5 mL of methanol. The homogenate was filtered. An additional 10 mL of methanol was added in two installments to the residue and homogenized and filtered again. The filtrate was combined, evaporated to dryness, reconstituted with the mobile phase, suitably diluted and analysed by the developed HPLC assay.

Results

The target input rate at steady state was calculated to be 32·8 $\mu g\ h^{-1}$ using the steady-state equation. If a 10- or 20-cm^2 transdermal patch were to be formulated, this would translate into a required flux through human skin of 3·28 and 1·6 $\mu g\ cm^{-2}\ h^{-1}$, respectively. The pharmacokinetic model suggests that with an input rate of 3 $\mu g\ cm^{-2}\ h^{-1}$ (10 cm^2 patch) a steady-state concentration of 4·6 ng mL^{-1} will be obtained (Fig. 1).

The partition coefficient (*n*-octanol/water, pH 6·5) of terbutaline was 0·03 ± 0·01 indicating that it is a very hydrophilic compound. The apparent partition coefficient (octanol/buffer) values at pH 3, 7·4 and 9 were 0·02, 0·05 and 0·43, respectively (n = 4).

Terbutaline was freely soluble in water. A significant discoloration was observed in an aqueous medium after 72 h at

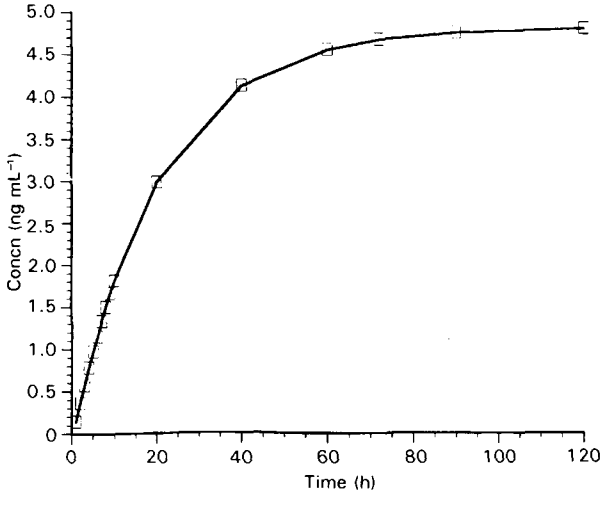

FIG. 1. Simulated terbutaline plasma concentration using a pharmacokinetic model at an input rate of 3 $\mu g\ cm^{-2}\ h^{-1}$ from a 10-cm^2 transdermal patch.

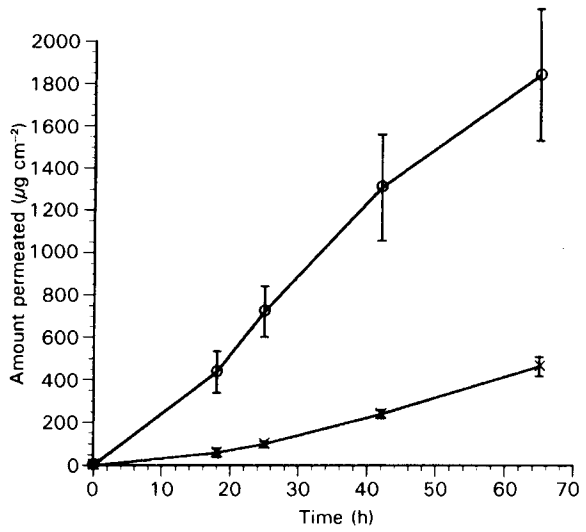

FIG. 2. Permeation of terbutaline through rabbit skin without and with 3% w/v azone. Control (X), 3% azone (O), (n = 4 or 5).

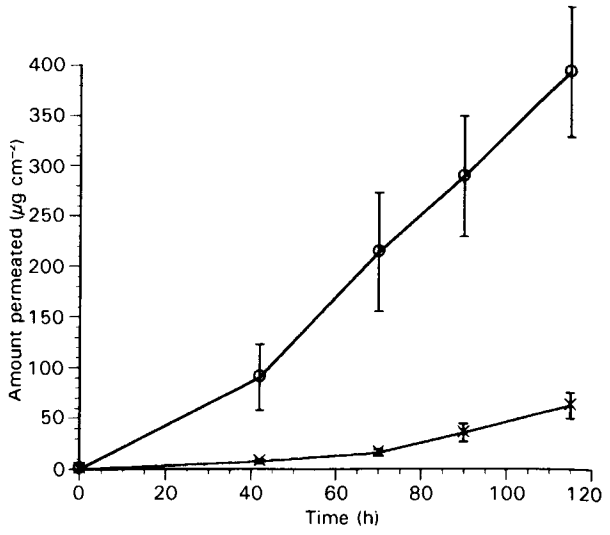

FIG. 4. Permeation of terbutaline through the human full-thickness skin without and with 3% w/v azone. Control (X), 3% w/v azone (O) (n = 4 or 5).

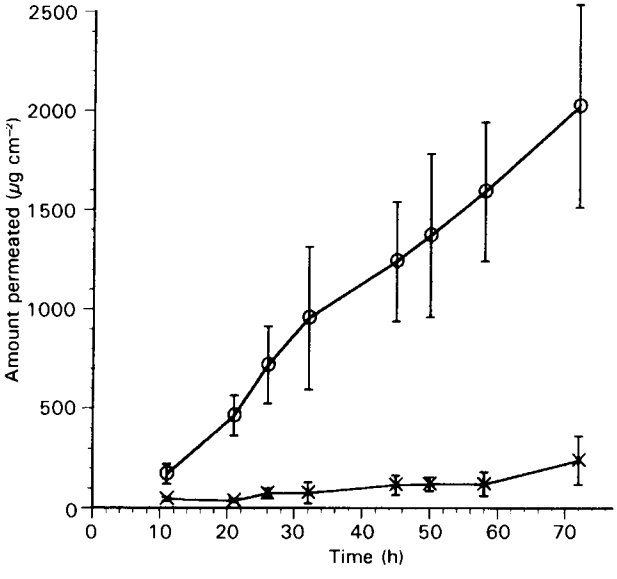

FIG. 3. Permeation of terbutaline through guinea-pig skin without and with 3% w/v azone. Control (X), 3% azone (O) (n = 4 or 5).

room temperature. The solubility of terbutaline in propylene glycol was $6{\cdot}3 \pm 1{\cdot}9$ mg mL^{-1}. Propylene glycol was used as a solvent because it is a commonly used solvent in dermatological formulations and its safety and efficacy had been established.

The maximum terbutaline flux attained through the rabbit full-thickness skin without and with 3% w/v azone was 8·3 and 28·5 $\mu g\ cm^{-2}\ h^{-1}$ (Fig. 2). Terbutaline flux through the guinea-pig skin was 7·7 and 56·1 $\mu g\ cm^{-2}\ h^{-1}$ without and with azone, respectively (Fig. 3). The corresponding values for human full-thickness skin (46-year-old white male, abdomen) were 0·6 and 3·6 $\mu g\ cm^{-2}\ h^{-1}$ respectively (Fig. 4). Due to the known variability in drug permeation through the human skin, the experiment was repeated with a second sample of human cadaver full-thickness skin (56-year-old white male, abdomen) and the flux obtained was 1·3 $\mu g\ cm^{-2}\ h^{-1}$ without an enhancer. Azone increased the flux of terbutaline 3·6-, 7·3- and 6-fold for the rabbit, guinea-pig and the human full-thickness skin respectively. The rabbit and guinea-pig skin membranes were about 13 times more permeable than the human full-thickness skin. Terbutaline flux through the human epidermis (46-year-old white male human skin without the dermis) without and with 3% azone was 1·6 and 6·5 $\mu g\ cm^{-2}\ h^{-1}$respectively (Fig. 5). Terbutaline skin permeation parameters for the various penetration barriers tested are reported in Table 2. The order of terbutaline permeation through the various membranes tested were: rabbit skin = guinea-pig skin > human epidermis > human full-thickness skin. The enhancement factor (flux with azone treatment/flux of control) for the various penetration barriers was of the order: guinea-pig skin > human full-thickness skin > human epidermis skin > rabbit skin.

The amount of drug retained in the rabbit skin at the end of the permeation study was 3 and 6·5% of the applied amount without and with azone respectively. The corresponding values for human full-thickness skin were 1·1 and 2·8% respectively. No statistically significant difference was found in the amount of drug retained in the skin without and with azone for the human epidermis or the guinea-pig skin.

Discussion

Partition coefficient

The octanol/water partition coefficient is generally believed to be a good representation of the partitioning of a drug between the stratum corneum and the underlying hydrophilic viable epidermis. The partition coefficient (octanol/water) of terbutaline was 0·03. Even with a pH 9 buffer as the aqueous phase, the partition coefficient was only 0·4, indicating the hydrophilic nature of the compound. The outer layer of the skin

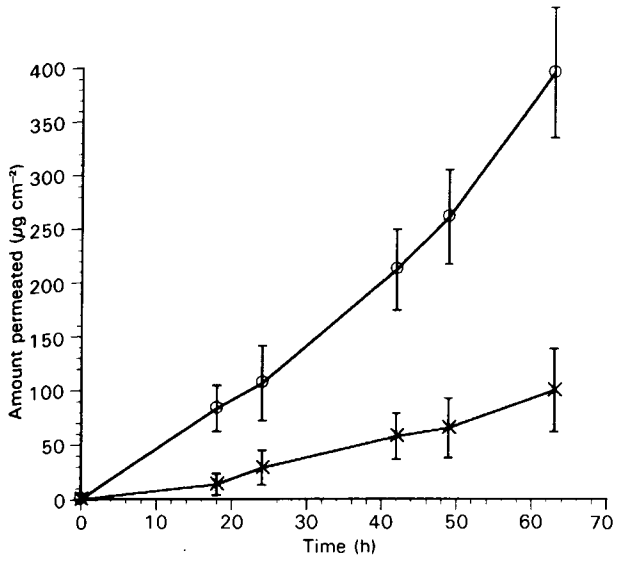

FIG. 5. Permeation of terbutaline through human epidermis without and with 3% w/v azone. Control (X), 3% w/v azone (O) (n = 4 or 5).

(stratum corneum) is known to be very lipophilic because of the presence of lipids. Based on the partition coefficient experiment alone, one would predict that the partitioning of terbutaline from a solution in water into the stratum corneum would be small. In this report propylene glycol was used as the solvent for the drug because of its wide use and reported action as a skin-penetration enhancer. Also, with propylene glycol, the maximum thermodynamic activity can be attained with a smaller amount (the solubility of terbutaline in propylene glycol was $6{\cdot}3 \pm 1{\cdot}9$ mg mL^{-1}, whereas it is freely soluble in water). However, once terbutaline enters the stratum corneum, its partitioning into the viable epidermis was expected to be swift. Ideally a drug should have affinity for both the vehicle (used to introduce the drug to the skin) and the stratum corneum and also a balanced octanol/water partition coefficient to facilitate drug transport from the stratum corneum into the viable epidermis. A single experiment using water as the vehicle may lead to an erroneous prediction of transdermal feasbility. A polymeric vehicle similar to the one used in transdermal delivery may provide a more realistic prediction.

Choice of the penetration barrier

Terbutaline flux of 3·3 $\mu g\ cm^{-2}\ h^{-1}$ through human skin was required to attain steady-state therapeutic concentrations in blood (from a 10-cm^2 patch dosage form). The maximum flux attained (without enhancer) through the rabbit and guinea-pig full-thickness skin, human full-thickness skin and the human epidermis were 8·3, 7·7, 0·6 and 1·6 $\mu g\ cm^{-2}\ h^{-1}$ respectively. This further confirms the existing pool of literature that the rabbit and guinea-pig membranes are significantly more permeable to drugs than human skin. Extrapolation of data from animal to human skin should take this factor into consideration. Given the hydrophilic nature of the permeant, one would expect the flux from the full-thickness skin and the epidermis to be the same. The observed higher flux through the epidermis may be due to slight disruption of the integrity of the membrane during the separation of the epidermis from the full-thickness skin. Azone significantly increased terbutaline flux in all cases. The enhancement factors, due to presence of the azone, for the rabbit, guinea-pig, human full-thickness and human epidermal skins were 3·4, 7·3, 6 and 4·1, respectively. Since the drug is taken up by the blood capillaries at the intersection of epidermis and dermis, the flux through the human epidermis in-vitro is more representative of the in-vivo situation. Without a penetration enhancer, the human skin area required for diffusion to attain therapeutic concentration was 29·8 cm^2 (or a transdermal patch dosage form of about 30 cm^2). Such an area may not be feasible cosmetically. With azone as a penetration enhancer a 10-cm^2 patch is sufficient to attain therapeutic levels of terbutaline.

Site of application and choice of vehicle on terbutaline flux attained

Jain et al (1992) reported a terbutaline flux of 4·5 $\mu g\ cm^{-2}\ h^{-1}$, without an enhancer, across full-thickness human cadaver skin from the underarm area. The source of the drug was a transdermal patch with a rate-controlling membrane made of Eudragit and polyethylene glycol. The stated steady-state flux was attained with a very short lag-time of about 2 h. In our study, where the drug source was a saturated terbutaline solution in propylene glycol, the maximum flux across human cadaver skin from the abdomen area was only 0·6 $\mu g\ cm^{-2}\ h^{-1}$ and the lag-time was significantly higher. The higher terbutaline flux of 4·5 $\mu g\ cm^{-2}\ h^{-1}$ suggests that

Table 2. Terbutaline skin permeation parameters through rabbit, guinea-pig and human skin.

	J ($\mu g\ cm^{-2}\ h^{-1}$)	T (h)	D/d^2 (h^{-1} ($\times 10^{-2}$))	K_p ($cm\ h^{-1}$ ($\times 10^{-4}$)	$K_m \times d$ (cm ($\times 10^{-2}$))
Rabbit skin					
control	8·3 ± 2·3	10·0 ± 2·1	1·7 ± 0·4	13·1 ± 3·6	7·7 ± 1·8
+ 3% azone	28·5 ± 6·2	5·5 ± 1·5	3·3 ± 1·0	45·2 ± 9·8	14·1 ± 2·6
Guinea-pig skin					
control	7·7 ± 1·9	26·7 ± 6·2	0·7 ± 0·2	12·3 ± 3·0	18·4 ± 8·7
+ 3% azone	56·1 ± 6·6	9·3 ± 0·5	1·6 ± 0·1	89·0 ± 10·4	49·7 ± 3·6
Full-thickness human skin					
control	0·6 ± 0·1	30·0 ± 4·9	0·6 ± 0·1	5·7 ± 1·3	9·6 ± 2·7
+ 3% azone	3·6 ± 0·8	28·5 ± 5·6	0·6 ± 0·1	5·7 ± 1·3	9·6 ± 2·7
Human epidermis					
control	1·6 ± 0·4	10·5 ± 2·2	1·7 ± 0·4	1·9 ± 0·6	1·2 ± 0·3
+ 3% azone	6·5 ± 1·8	7·5 ± 1·1	2·3 ± 0·3	10·3 ± 2·9	4·8 ± 1·9

Mean ± standard deviation, n = 4 or 5.

variation in the site of application can lead to a significant difference in the input rate (unless the release rate from the dosage form itself is the rate-limiting step in drug permeation). The higher flux reported by Jain et al (1992) may also be attributed to the different vehicles used (Eudragit transdermal patch in their study vs a saturated drug solution in propylene glycol in our study). Terbutaline obviously has a greater affinity for propylene glycol and this led to decreased partitioning into the stratum corneum. There has been a number of reports where a single vehicle and human cadaver skin was used in an in-vitro experimental setting and the results were used to predict the feasibility (or the lack of it) of transdermal delivery. These predictions based on a single set of conditions (vehicle, membrane barrier, etc.) may have led to the discarding of potentially good drug candidates for transdermal delivery (especially in the pharmaceutical industry in the preliminary preformulation and development stages). This is well illustrated by the above example where different conditions lead to a totally different prediction, in the absence of a skin penetration enhancer.

Time required to attain therapeutic concentration in plasma after transdermal application

Based on first principles of pharmacokinetics, steady-state plasma concentration will be attained in four to five half lives of any drug. When applied to transdermal delivery, this translates into at least 56 h before steady-state plasma levels are obtained for terbutaline ($t_{\frac{1}{2}} = 14$ h). For transdermal delivery, additional time (usually referred to as lag-time) is required, to saturate the skin with drug, before steady-state input is attained. The pharmacokinetic model predicted an interval of about 60 h before therapeutic concentrations in plasma were attained. This obviously is unacceptable unless an oral or parenteral loading dose is given prior to transdermal patch application. Initially one would have predicted that terbutaline is not a good candidate for transdermal delivery based on its half-life of 14 h. However, in the clinical report by Jain et al (1992) after a single patch application, a therapeutic plasma concentration of about 2·7 ng mL^{-1} was attained within 6 h, maintained for 24 h and declined only after the transdermal patch was removed. The pharmacodynamic effects (% increase in forced expiration volumes) correlated well with the plasma concentration. Why was such a large difference between predicted and actual time required to obtain therapeutic concentration in the plasma? Some contributing factors may be greater terbutaline skin permeation from a polymeric patch than from a saturated aqueous solution, site of skin source, duration of storage of the cadaver skin and reduced drug clearance in the actual clinical population. This suggests that a drug candidate should not be rejected for transdermal delivery based on its long half-life alone. It may not be necessary to obtain pharmacokinetic steady-state concentration which takes about 4–5 half-lives. Therapeutic plasma concentrations and desired pharmacodynamic effects for terbutaline were attained with one single patch application and were sustained for at least 24 h (which was significantly better than the oral tablet).

Lag-time

It is possible to obtain a wide range for the lag-time for a given drug candidate. The lag-time depends on the choice of the vehicle, the drug concentration in the vehicle, the effect of vehicle on the barrier property of the skin, and the individual subjectivity of what is considered to be the linear phase of the permeation profile. All these factors should be considered before rejecting a drug candidate based on a high lag-time. Use of lag-time in the absence of steady state will result in inaccurate estimates of diffusion coefficient (D) and partition coefficient (K_m) (Shah et al 1994). The present study illustrates the importance of taking all factors into consideration before predicting transdermal delivery, namely the in-vitro methodology, the barrier membrane used, use of full-thickness human skin or only the epidermis, site of application, the lag-time, the vehicle used to introduce the drug, effect of the vehicle on the integrity of the skin, the affinity of the drug for the vehicle, desired plasma concentration and the drug clearance values in the actual clinical populations. Hydrophilic drugs such as terbutaline may also be good candidates for transdermal delivery depending on their physicochemical and pharmacokinetic parameters. From these studies transdermal delivery of terbutaline appears to be promising.

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