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Percutaneous permeation of betamethasone 17-valerate from different vehicles

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Summary

In vitro percutaneous permeation of betamethasone 17-valerate through excised human skin was examined using four different vehicles. The mean drug flux from the pressure sensitive silicone adhesive which contained the drug as a suspension was 2.4-times greater than that from a 1.2 mg/g commercially available cream which also contained the drug as a suspension. The latter was comparable to the mean flux from aqueous saturation (5.5 μg/ml) and 4.1-times larger than that from 0.12 mg/g cream. The aqueous vehicle did not show an enhancing effect on the percutaneous permeation of betamethasone 17-valerate because the flux from the aqueous saturation was within a factor of 2.5 when compared to the value from the suspension in the silicone adhesive and commercially available cream. Aqueous saturation concentration may provide the representative unit activity flux of corticosteroid through the skin.

Introduction

According to the concept of thermodynamic activity (Higuchi, 1978; Barry, 1983; Flynn and Stewart, 1988), any vehicle maintaining the saturation concentration of a drug in the vehicle at the vehicle-skin interface should produce the same rate of drug penetration under the condition that the vehicle does not alter the properties of the skin as a diffusion membrane (Poulsen et al., 1978). However, to date no vehicle has been established as providing unit activity flux. If a representative or standard unit activity flux can be determined from some vehicle, specific topical formulations (Broggini et al., 1991; Walker et al., 1991) may be classified in terms of this standard value. When a formulation provides steady state flux near the standard unit activity flux, it may contain the drug as a finely ground suspension, while if the flux does not reach steady state and the maximum flux is below the unit activity flux, the drug may be dissolved in the vehicle and its concentration is below the maximum solubility. On the other hand, when the flux exceeds the standard unit activity flux, the vehicle may provide the supersaturation of drug in the stratum corneum and/or have an enhancing effect on percutaneous drug absorption (Barry, 1983).

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In this article, the steady state flux of betamethasone 17-valerate from aqueous saturation concentration is compared to that from (1) a commercially available cream containing the drug in suspension, (2) a pressure sensitive silicone adhesive which also contains the drug in suspension and (3) a commercially available 'reduced strength' cream with a decreased concentration. The drug was assayed using high-performance liquid chromatography (HPLC).

Materials and Methods

Aqueous saturation solution of betamethasone 17-valerate

Betamethasone 17-valerate was a generous gift from Schering Plough (Bloomfield, NJ). To prepare saturated aqueous solutions of betamethasone 17-valerate, an excessive amount of corticosteroid was added into 0.1 M acetate buffer (pH 4.5) for 48 h at 33 °C while stirring with a magnetic stirrer. The suspension was filtered by a syringe filter with pore size 0.45 μm (Micron Separations Inc., MA). The first 20 ml of filtrate was discarded because some betamethasone 17-valerate may be adsorbed to the filter. The resultant saturation solution was diluted when necessary. The acetate buffer (pH 4.5) was employed because unless the pH is maintained between 4 and 5, betamethasone 17-valerate is rapidly converted to betamethasone 21-valerate (Yip and Li Wan Po, 1979; Cheung et al., 1985; Smith et al., 1985).

A silicone adhesive patch containing betamethasone 17-valerate

Betamethasone 17-valerate was dissolved in a mixture (64:36 (w/w)) of dichloromethane-BIOPSA® (Dow Corning, Midland, MI). 3 ml of the solution was placed on a backing membrane, Scotchpak® (Type 1009, 3M, St. Paul, MN). The solution was then developed using a casting device (2.5 inch in width and 0.010 inch in thickness, designed and made by SRI international, Menlo Park, CA) into the approx. 6×20 cm area. The low adhesion polyester film (a release liner), Scotchpak® (Type 1022, 3M, St. Paul, MN) was then placed on BIOPSA® and the patch was stored at room temperature. The drug concentration in the dichloromethane-BIOPSA® mixture was 0.15% (w/v). Thus, the drug amount per unit area was 38 $\mu g/cm^2$ since the mixture was applied as a 0.010 inch thick layer. The silicone adhesive, BIOPSA®, was translucent after dichloromethane evaporated when the drug concentration was 0.02% (w/v) or less but turned cloudy when the concentration was 0.03% (w/v) or more in this mixture. This suggests that the patch made from 0.15% (w/v) betamethasone 17-valerate in the mixture may be a suspension in which the concentration is more than 5-times larger than the drug solubility in BIOPSA®. The thickness of adhesive in the patch was approx. 50 μm when measured using a Light Wave Micrometer (Van Keuren Co., Watertown, MA).

Commercially available creams

A commercially available Valisone® cream 0.1% (Schering Plough, Bloomfield, NJ) which contains 1.2 mg/g betamethasone 17-valerate (0.1% as betamethasone) in an aqueous hydrophilic emollient cream and Valisone® reduced strength cream 0.01% (Schering Plough, Bloomfield, NJ) which contains 0.12 mg/g betamethasone 17-valerate were used in the experiment without modification. According to the manufacturer, Valisone® cream 0.1% is a suspension. On the other hand, no structure suggesting the presence of suspended particles of betamethasone 17-valerate was observed under the microscope in Valisone® reduced strength cream 0.01%. However, no attempt has been made to determine the solubility of betamethasone 17-valerate in that cream.

Penetration of betamethasone 17-valerate through the split thickness skin

Human cadaver split thickness skin was obtained from four subjects. The skin samples were mounted in glass diffusion cell with 1 cm^2 area (LG-1084-LPC, Laboratory Glass Apparatus Inc., Berkeley, CA). The outside of the cell was maintained at 37°C with running water. The following four vehicles were employed at the donor site: (1) 1 ml of the aqueous saturation solution of be-

tamethasone 17-valerate in 0.1 M acetate buffer (pH 4.5); (2) 10 mg of 0.12 mg (w/w) betamethasone 17-valerate 'reduced strength' Valisone® cream; (3) 10 mg of 1.2 mg/g (w/w) betamethasone 17-valerate Valisone® cream and (4) a round 1 cm^2 area piece excised from a 38 $\mu g/cm^2$ (w/v) betamethasone 17-valerate patch in BIO-PSA®. The experiment was conducted in duplicate for each vehicle. The aqueous solution was exchanged at 5, 10, 24, 34, 48 and 58 h; the donor cell solution was carefully removed with a polystyrene transfer pipet to minimize skin damage and then the remaining solution was removed with absorbent paper (Kimwipe®, Kimberly-Clark, Roswell, GA). The donor cell was refilled with a new aqueous drug solution. The cream was weighed on the round area (1 cm^2) of the aluminum foil and placed, together with this foil, on the skin. The donor cells were covered by Parafilm® (American National Can, Greenwich, CT). 3 ml of 0.1 M acetate buffer (pH 4.5) was used as a receptor fluid. The receptor fluid was collected and replaced at 10, 24, 34, 48, 58 and 72 h. The flow-through cell technique was not used because the concentration of betamethasone 17-valerate in the receptor fluid attained during the 10–14 h sampling period was usually less than 10% of the aqueous saturation (5.5 $\mu g/ml$). In addition, in the preliminary study, the flux vs time profiles with or without the flow-through cell technique did not significantly differ from each other.

When the study was finished, the 1 cm^2 area of the skin sample which had been exposed to the receptor fluid was excised after the vehicle was removed. The cream was removed gently by the spatula. The epidermis and dermis were physically separated. They were then serially placed in the following five solutions (1 ml each) for a total of 5 days (24 h for each solution): (1) methanol-0.4 M acetate buffer (pH 4.5) solution (1:1 (v/v)); (2) methanol-0.4 M acetate buffer (pH 4.5) solution (9:1 (v/v)); (3) 100% methanol; (4) chloroform-methanol (2:1 (v/v)); and (5) another portion of chloroform-methanol (2:1 (v/v)). All the extracts were combined and the volume was reduced by a gentle stream of air until the total volume became less than 0.5 ml. Betamethasone 17-valerate in this volume was then extracted as described in the following section. The mean ($\pm$SD, $n = 12$ each) recoveries of betamethasone 17-valerate from epidermis and dermis were 84.8 $\pm$ 39.2 and 67.2 $\pm$ 13.3%, respectively. To establish these values, other skin pieces had been dipped in the aqueous betamethasone 17-valerate solution for 4 days and then the drug was extracted; the amounts absorbed into the skin were calculated from the differences between the aqueous concentrations before and after equilibrium was attained and compared with the amount actually extracted.

Measurement

To the aqueous samples, 2 μg of prednisone in methanol, as an internal standard, was added. To a small vial, 0.5–2 ml of the sample was then transferred and 2 ml of 2% methanol in dichloromethane was added and vortexed for 1 min. The samples were centrifuged at 3000 rpm for 5 min. The upper aqueous phase was discarded. The organic phase was filtered using a column packed with Celite 545® (Manville, Denver, CO) and evaporated to dryness. When betamethasone 17-valerate in the cream was measured, the vehicle was placed in 1–5 ml of dichloromethane and vortexed for 30 s. When adding 2–80 μg of an internal standard, prednisone, the organic solution was filtered and evaporated as above. The sample was reconstituted in 2% methanol in dichloromethane and injected into the instrument. Betamethasone 17-valerate was measured by a normal phase HPLC method using a Rabbit-HP constant flow pump (Rainin Instrument Co., Berkeley, CA), a Knauer variable wavelength UV detector (Spektralphotometer, No. 731.87, Bad Homburg, Germany) set at 240 nm and Shimadzu Chromatopac (CR 601, Kyoto, Japan). A silica gel column, LiChrosorb Si-100, 10 μm, 250 $\times$ 4 mm i.d. (Merck, Darmstadt, Germany) was used for compound separation. The column was washed with 0.5% sulfuric acid and then with distilled water until the eluent became neutral, and then finally with methanol before the analysis (Kubota et al., 1989). The chromatographic solvent system consisted of 0.1% water, 3.5% methanol and 30% dichloromethane in *n*-hexane. The flow rate was 2 ml/min. The

retention times for betamethasone 17-valerate and prednisone (internal standard) were 3.9 and 11.4 min, respectively. The detection limit was 4 ng per sample (signal-to-noise ratio of 4:1). The recoveries of betamethasone 17-valerate and prednisone from the aqueous solutions, cream and BIOPSA® were > 96%.

Results

Fig. 1 shows the mean (±SD, $n = 4$) cumulative amount vs time profiles of betamethasone 17-valerate from the four different vehicles through the skin with the 1 cm^2 area. At 72 h, the cumulative amount from 1.2 mg/g Valisone® cream (3.49 ± 1.51 μg) was similar to that from the aqueous saturation (3.96 ± 1.25 μg) and 4.1-times larger than that from 0.12 mg/g reduced strength Valisone® cream (0.86 ± 0.12 μg). The mean (±SD, $n = 4$) cumulative amount at 72 h released from the 38 $\mu g/cm^2$ patch (9.61 ± 0.87 μg) was 2.4-times larger than that from the aqueous saturation. The mean drug amounts excreted during the 48–72 h period were 98, 76, 70 and 102% of those during the 24–48 h period from the aqueous saturation, 0.1% Valisone® cream, 0.01% Valisone® reduced strength cream and silicone patch, respectively. The maximum flux was observed during the 24–34 h period for both 0.1 and 0.01% Valisone® creams.

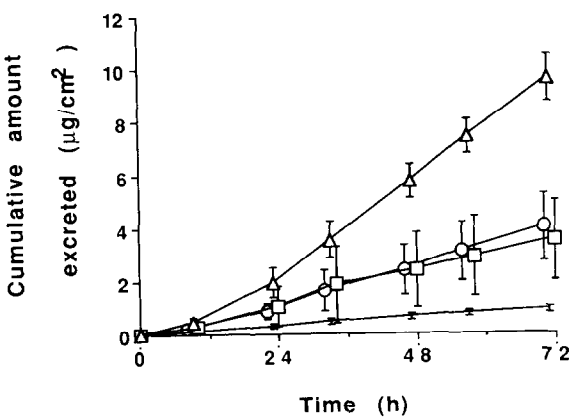

Fig. 1. The mean (±SD) cumulative amount vs time plots of betamethasone 17-valerate excreted into the receptor fluid through the skin until 72 h after the application of the 38 $\mu g/cm^2$ suspension in the silicone adhesive, BIOPSA® (△), aqueous saturation (○), 10 mg of Valisone® cream 0.1% (□) and 10 mg of Valisone® cream 0.01% (no symbol).

TABLE 1

The mean (± SD, $n = 4$) amounts of betamethasone 17-valerate in the skin after 72 h absorption from various vehicles

Formulation	Epidermis ($\mu g/cm^2$)	Dermis ($\mu g/cm^2$)
Aqueous saturation	0.31 ± 0.08	0.20 ± 0.13
Valisone® cream 0.1%	0.57 ± 0.17	0.17 ± 0.14
Valisone® cream 0.01%	0.10 ± 0.05	0.04 ± 0.04
Suspension in BIOPSA® silicone patch	0.55 ± 0.14	0.22 ± 0.09

The amounts of the drug extracted from the epidermis and dermis excised at 72 h (area = 1 cm^2) are presented in Table 1. The overall mean (±SD, $n = 32$) weight of the dermis sample excised at 72 h was 29 ± 20 mg.

The mean amount (±SD, $n = 4$) of betamethasone 17-valerate in the 0.12 mg/g cream removed by the spatula at 72 h was 0.40 ± 0.15 μg. The sum of the amount in the cream, that in the skin and cumulative amount appeared in the receptor fluid was calculated as 115 ± 5% of the applied dose. The mean (±SD, $n = 4$) value in the 1.2 mg/g cream removed at 72 h was 5.49 ± 2.54 μg. The sum of the drug in the vehicle, skin and receptor fluid was 87 ± 8% of the applied dose.

Discussion

Theoretically, the unit activity flux of a drug is the same from any vehicle. In spite of the simplicity of this principle, it is practically difficult to determine the standard value of the unit activity flux; an ideal standard vehicle which does not interfere and change the drug solubility and/or diffusivity of the skin (stratum corneum) has not been established for most compounds.

It has been argued that an aqueous vehicle acts as a potent enhancer for corticosteroids. For instance, according to Barry (1985), "without exception, the prevention of water loss from the

stratum corneum and subsequent increased water content in the skin apparently enhance the penetration; the penetration of corticosteroids may increase 100-fold under occlusion". Nevertheless, the quantitative data clearly indicating that the drug flux through a hydrated stratum corneum is larger than that through a less hydrated stratum corneum are scarce. For instance, the early report that occlusion enhanced the percutaneous absorption of hydrocortisone (Feldmann and Maibach, 1965) has been challenged by the recent 'mass balance' technique, when different time of occlusions and volume of the vehicle were applied (Bucks et al., 1989). In this regard, the fact that flux of betamethasone 17-valerate from aqueous saturation was within a factor of 2.5 when compared with that from a commercially available cream (Valisone®) and pressure sensitive silicone adhesive (Fig. 1) may pose an additional question on the role of water as a potent enhancer. Particularly, the mean value of the cumulative drug amount from the commercially available cream (Valisone® cream 0.1%) which contains the drug as a suspension was similar to that from aqueous saturation. This may also suggest that the aqueous saturation of corticosteroid could be a good candidate to assess the standard unit activity flux.

Rather, the possible mechanisms which accounted for this 2.5-times difference observed among the flux values from the three different vehicles are worth discussing supposing that they have minimal effects on the diffusivity/solubility in the skin. In this study, we tried to keep the constant saturation concentration in the donor cell for the aqueous solution which was replaced six times by a new portion of the solution during the 72 h study period. However, the observed flux could be smaller than the possible unit flux attainable by the saturated aqueous solution. First, on average, the amount absorbed into skin (amount appeared in the receptor fluid plus that in skin) was 4.47 $\mu g/cm^2$ which was more than 10% of the total amount applied to the donor cell ($5.5 \times 7 = 38.5$ $\mu g/cm^2$). In addition, in the receptor cell the concentration reached around 5% of the aqueous saturation after 24 h. It is also possible that the thin aqueous layer formed in the skin-receptor fluid boundary, known as the unstirred layer (Pedley, 1983), may have decreased the flux. This aqueous boundary layer effect may also not be negligible at the aqueous donor solution-skin boundary; the concentration gradient can be generated within the aqueous donor phase. Similarly, the concentration gradient may be formed within the Valisone® 0.1% cream when 12 μg was applied as a suspension in 10 mg cream. It is known that once the vehicle is applied and drug molecules are released to the skin, the concentration gradient in the continuous phase can be formed in the vehicle even while suspended phase is not yet completely depleted if the dissolution rate is not rapid enough (Ayres and Lindstrom, 1977). Indeed, the decrease in the driving force was observed in both 0.1 and 0.01% Valisone® cream (particularly, in the latter formulation); the flux became smaller after it reached the maximum value during the 24–34 h period. On the other hand, the relatively large amount (38 $\mu g/cm^2$) was applied in the thin silicone adhesive (with a thickness ≈ 50 μm). If the decrease in the concentration or driving force in silicone adhesive is minimal, the flux obtained from this vehicle may be near the unit flux.

However, another mechanism could account for the 2.5-times difference of the flux from the different vehicles; e.g., supersaturation of betamethasone 17-valerate in the stratum corneum was reached when the silicone adhesive patch was applied. It is not possible to determine the exact mechanism for the 2.5-times difference of the flux values from the different vehicles in this study. The effects of the vehicles on diffusivity/solubility of betamethasone 17-valerate in the skin as well as the saturation concentration of betamethasone 17-valerate in the vehicles (particularly in Valisone® cream) and the dissolution rate of solid phase and diffusivity of drug in the vehicles should be determined to give an unambiguous explanation for the difference.

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