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EVALUATION OF VERAPAMIL HYDROCHLORIDE PERMEATION THROUGH HUMAN CADAVER SKIN

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ABSTRACT

Preformulation studies were conducted to determine the feasibility of a transdermal dosage form of verapamil hydrochloride (VPHCl). The apparent partition coefficient (octanol/water or buffer) of VPHCl in buffers of different Ph values was determined. The saturation solubility of VPHCl in different buffers and propylene glycol was determined. The target drug flux through the human skin to attain therapeutic concentrations in blood was determined. The maximum flux attainable through the human skin was determined. An attempt was made to increase the flux of the drug using azone as a penetration enhancer. The rate limiting barrier for the permeation of VPHCl through the skin was determined.

INTRODUCTION

Verapamil hydrochloride (VPHCl) is a calcium ion influx inhibitor (slow channel blocker or calcium ion

TABLE I: Relevant Physico Chemical and Pharmacokinetic Properties of Verapamil HCl

Molecular weight	491.08
Melting point	143.5 °C
Approximate required therapeutic concentration	100 ng/ml
Clearance	15 ml/min.kg
Volume of distribution	5 L/kg
elimination half life	3-4 hr
Oral Bioavailability	22 %

antagonist) used in the management of essential hypertension. The physicochemical and pharmacokinetic properties of VPHCl are shown in Table I (1).

The low bioavailability due to extensive first pass hepatic metabolism (associated with the oral route) can be avoided by the transdermal route of drug administration. Also the drug has a short half life and hence requires more frequent dosing by the oral route. A prolonged duration of action is possible with a single application of a transdermal patch. This will lead to better patient compliance by eliminating frequent dosing.

The advantages and limitations of transdermal route of drug administration are well documented (2). Based on the physicochemical and pharmacokinetic properties VPHCl appears to be a good candidate for transdermal delivery. Ritschel et. al reported the permeation of VPHCl through rat skin (3). The transport behavior of VPHCl across artificial membranes as a function of pH or the ionic strength of the reservoir was reported in the literature (4). Iontophoretic delivery of verapamil was reported also reported by Wearley and Chien. (5). The goal of this study was to determine if the target VPHCl flux through the human skin can be attained to get therapeutic levels of the drug in the blood. The effect of azone as a drug flux enhancer was also determined.

MATERIALS AND METHODS

Materials:

Human cadaver skin (57 year old white male, leg) was obtained from the local hospital. All chemicals and reagents were purchased from Sigma Chemicals.

Analytical:

A sensitive HPLC assay for the analysis of VPHCl was developed. The liquid chromatograph used was from Laboratories Date Control Analytical, Riviera Beach, FL, USA, which is equipped with a ConstaMetric 1 pump and a variable UV detector (Spectromonitor 3). The integrator used was from Hewlitt Packard (HP 3394 A). A phenyl

column was used and the mobile phase was 40 % acetonitrile in water. The effluent was monitored at 230 nm. Terbutaline was used as the internal standard at a concentration of 5 μg/ml. The samples from the solubility and partition coefficient studies were analyzed by a spectrophotometric assay at 230 nm.

Determination of target flux:

The flux was determined by the following mass balance equation at steady state:

I.R. = Css X Cl

where I.R. is the input rate into the body through the skin, Css is the steady state concentration (100 ng/ml) and Cl is clearance of the drug from the body (15 ml/min/kg or 1050 ml/min for a 70 kg person).

Solubility Studies:

An excess of drug was mixed in 5 ml of propylene glycol, or buffer solution (pH 2.2, 5, 8 or 10). The solution was agitated at room temperature for 12 hours. The solution was then filtered through a Whattman 1 filter paper, suitably diluted and analyzed for the drug content.

Determination of the apparent partition coefficient (octanol/water):

The appropriate buffer was mixed with octanol and agitated overnight at room temperature. The two phases were then separated and used for the partition coefficient study. To 15 ml of the buffer, VPHCl was

added and the concentration was determined exactly. Ten ml of this solution was mixed with 10 ml of the octanol (equilibrated with buffer phase) in a sealed test tube. The mixture was agitated at room temperature for six hours. The two phases were then separated and the concentration in the buffer phase was again determined. A separate standard curve was constructed with each of the buffer solution to analyze the sample at that particular pH.

Skin Preparation (6):

The human cadaver skin was defatted and used within 48 hours. The skin was soaked in 5 % ethylene diamine tetra acetic acid solution for 12 hours. The epidermis was then separated from the dermis with a forceps. The epidermis was washed with distilled deionized water blotted with a Kim Wipe and dried in a desiccator till use. To obtain delipidized epidermis the epidermis was gently shaken in a mixture of chloroform:methanol (2:1) for 2 hours. The epidermis was then removed and washed with deionized water.

Test Solutions:

Saturated solution of VPHCl in propylene glycol was used as the control solution. Sixty mg of azone was weighed and the volume was made up to 2 ml with saturated solution of VPHCl in propylene glycol (3% w/v azone). The presence of azone did not increase the solubility of

the drug significantly. Hence no significant change in the thermodynamic activity of the drug was expected.

Permeation Study:

Modified Franz diffusion cells were used for the permeation study. The human skin barrier (epidermis or delipidized epidermis) was mounted between the donor and receptor chambers. The receptor phase was saline phosphate (pH 7.4) buffer maintained at 37 °C by circulating water from a water bath. A magnetic stirrer ensured uniform mixing of the diffusate. One hundred microliter of the test solution was added onto the stratum corneum in the donor phase. Samples were taken at predetermined time intervals and analyzed for drug content by the developed HPLC assay.

Skin Retention:

At the end of the experiment the exposed skin was blotted dry and cut into small pieces. This was then homogenized with 5 ml of methanol thrice. The homogenate were mixed, filtered and evaporated to dryness. The residue was reconstituted with the mobile phase and after suitable dilution analyzed for the drug content.

Permeation Data Analysis: (7)

The flux of VPHCl is calculated from the amount permeated vs time plot. The slope of the linear portion of the plot is equal to the flux. The x-intercept of the linear portion of the curve give the lag time (T). The

TABLE II: pH Solubility Profile Of Verapamil Hydrochloride

Vehicle	Solubility (microgram/mL)
Propylene glycol	61500 ± 621
pH 2.2 buffer	82800 ± 750
pH 5.0 buffer	55000 ± 312
pH 8.0 buffer	17550 ± 217
pH 10.0 buffer	934 ± 19

Each value is the mean of five reading

permeability coefficient of the drug was calculated from the following equation:

$Kp = J/C$

where J is the flux and C is the concentration of the drug added to the skin. The partition coefficient of the drug is expressed by the equation:

$Km*d = Kp/(1/6T)$.

Statistical Analysis:

The control and enhancer treated groups were compared by using a Students t test ($p < 0.05$).

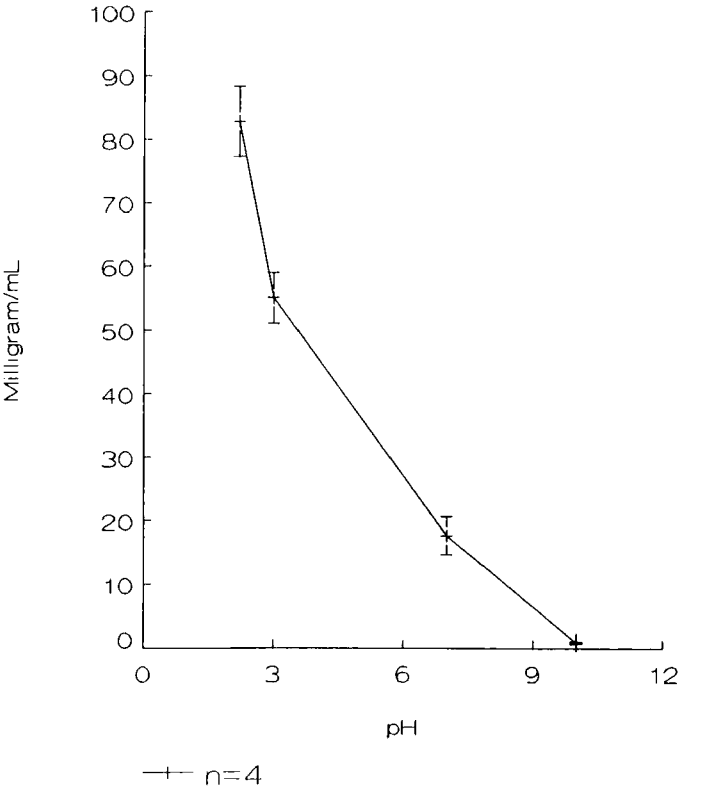

FIGURE 1: pH-Solubility Profile of Verapamil Hydrochloride (n = 5)

RESULTS AND DISCUSSION

Target flux:

The input rate required based on the steady state equation was 6300 μg/hr. This translates into a target flux of 315 $\mu g/cm^2/hr$ for a 70 kg person with a 20 cm^2 dosage form applied to the skin.

Solubility:

The solubility of VPHCl in various solvents is shown in Table II. The pH solubility profile is shown in Figure 1.

TABLE III: pH-apparent partition coefficient of Verapamil HCl (octanol/buffer)

Octanol/water	---------	0.22 ± 0.02
Octanol/pH 2.2 buffer	---------	0.74 ± 0.1
Octanol/pH 5 buffer	---------	5.75 ± 0.7
Octanol/ pH 8 buffer	---------	24.0 ± 1.7
Octanol/pH 10 buffer	---------	45.0 ± 5.5

Each value is the mean of 5 readings.

Propylene glycol was chosen as the solvent since it is a commonly used solvent in many pharmaceutical preparations and its safety and efficacy was well established.

Partition coefficient:

The partition coefficient values are shown in Table III. The pH - partition coefficient profile is shown in Figure 2. The apparent partition coefficient in water (octanol/aqueous phase) was 0.22 ± 0.02. The VPHCl concentration in the water and octanol phase was essentially constant after 12 hours.

Permeation Studies:

The permeation parameters obtained with the various barrier membranes were shown in Table IV. The permeation profiles of VPHCl and without 3 % w/v azone was shown in

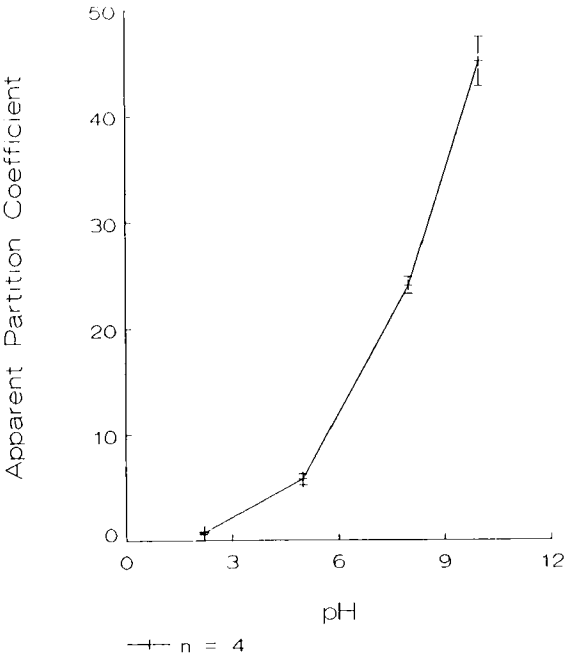

FIGURE 2: pH-Apparent Partition Coefficient Profile of Verapamil Hydrochloride (n =5)

Figure 3. The drug flux through the epidermis of the human skin was small. The presence of a 3 % w/v azone increased the flux dramatically to 53.9 $\mu g/cm^2$.hr. There was a 22 fold increase in the flux of the drug with azone. There was no statistically significant difference in the lag time. It appears that azone acted as a penetration enhancer by increasing the partitioning of the drug into the skin. Delipidizing the epidermis completely eliminated the barrier properties of the epidermis. The permeation profiles with and without azone

TABLE IV: VPHCl Human Skin Permeation Parameters

Barrier membrane	Flux ($\mu g/cm^2.hr$)	Lag-Time (hr)	Permeation Co-Efficient 10^{-5} (cm/hr)	Partition Coefficient 10^{-3} (Km*d)	Diffusion Co-efficient 10^{-3} (D/d^2)
Epidermis No azone	2.4	31.8	3.9	6.2	5.2
Epidermis with azone	53.9	31.2	87.6	165.3	5.3
Delipidized epidermis No enhancer	600.0	0.08	975.6	4.7	2083.3
Delipidized epidermis with azone	573.9	0.08	933.2	4.5	2083.3

Each value represents the mean of 4 or 5 readings

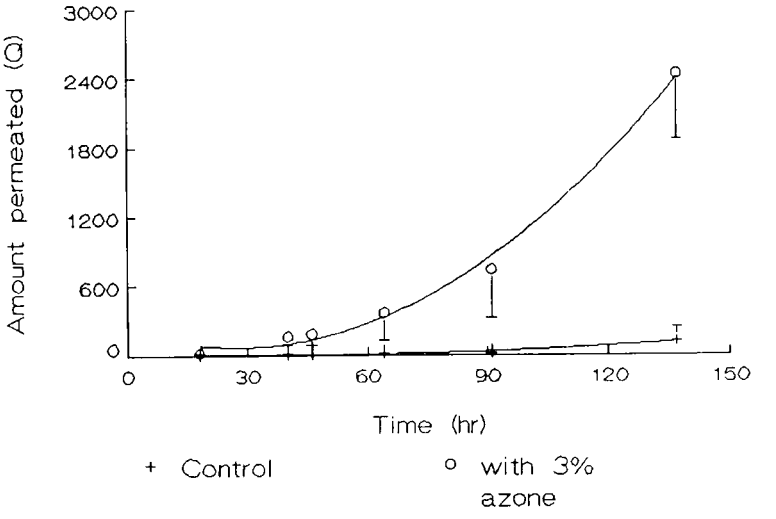

FIGURE 3: Verapamil Hydrochloride Permeation Profile through Human Epidermis with and without 3% w/v azone (n= 4 or 5 for control and azone treated respectively)

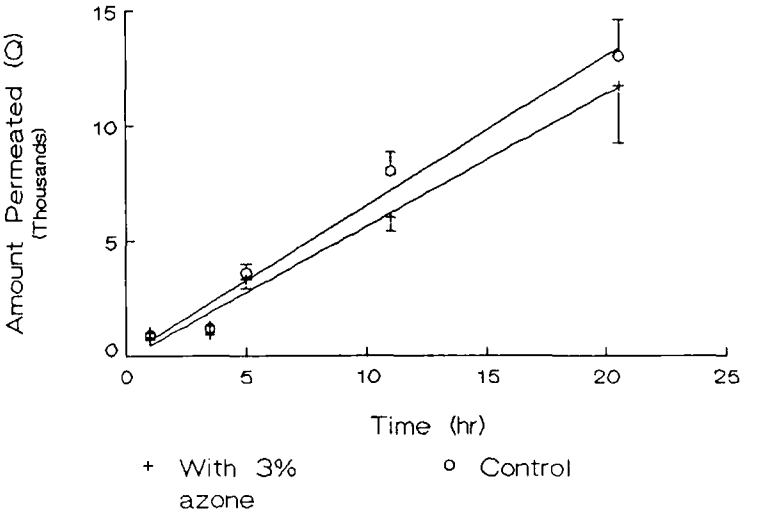

FIGURE 4: Verapamil Hydrochloride Permeation Profile through Delipidized Human Epidermis with and without 3% w/v Azone (n = 4 or 5 for control and 3% w/v azone treated respectively)

through delipidized epidermis were shown in Figure 4. The maximum flux attained was 600 $\mu g/cm^2.hr$. The lag time was extremely short. There was no significant increase in the flux of the drug with azone for the delipidized epidermis suggesting that the effect of azone is on the lipids of the skin. As can be seen from the diffusion coefficient values, delipidizing the epidermis completely eliminated the resistance to the diffusion of the drug.

Skin Retention:

The presence of azone increased the amount of drug retained in the skin. The amount of VPHCl retained in the

skin increased from 3.4 ± 1.4 to 6.7 ± 2.2 % of the applied dose. This suggests that the presence of azone increased the binding of VPHCl to the skin. For delipidized epidermis there was no significant increase in the binding of the drug to the skin with azone. (1.1 ± 0.4 vs 1.3 ± 0.3 % of the applied dose for control and azone treated skin respectively).

CONCLUSIONS

A stability indicating HPLC assay was developed for the quantitation of VPHCl in diffusate and skin extract samples. The apparent partition coefficient of VPHCl suggests that it was a fairly lipophilic compound. Hence significant permeation of the drug through the skin was expected. The target flux to attain therapeutic concentrations of VPHCl was set at 315 $\mu g/cm^2/hr$ from a 20 cm^2 patch. The passive diffusion of VPHCl through the human cadaver skin was small. The presence of 3% w/v azone increased the flux of VPHCl significantly (22 fold). The maximum flux attained with 3 % azone was 53.9 ± 15 $\mu g/cm2/hr$. Since 6300 μg need to be delivered through the skin, a transdermal patch of 116 cm^2 would be required with azone as a penetration enhancer. Such a size is cosmetically not feasible and hence transdermal delivery by passive diffusion is not feasible with azone as a penetration enhancer. There was significant binding of VPHCl to the skin. The amount of drug retained in the

skin increased significantly with azone suggesting that azone increased the partitioning of the drug into the skin. Delipidizing the epidermis completely destroyed the barrier property of the skin. This is in agreement with other reports that the major barrier for drug permeation through the skin lies in the epidermis. Thus transdermal delivery of VPHCl by passive diffusion is feasible only with a chemical enhancer which is capable of removing the lipids from the epidermis of the skin.

ACKNOWLEDGEMENT

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