The *In Vitro* Permeability Study of Valsartan through Excised Rat Skin

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Abstract

The in vitro permeability of valsartan through excised rat skin was investigated using Franz-type diffusion cells. The permeation was assessed by studying the effect of water, polysorbate-80 and eucalyptus oil on the permeability flux of valsartan through excised rat skin. The slope of linear portion of the graph obtained by plotting the cumulative amount of valsartan permeated per unit skin surface area as a function of time provided the permeability flux. Of the vehicles studied, only 6.0 % w/v polysorbate-80 solution provided most permeation of valsartan through the rat skin as evidenced by the highest permeability coefficient of valsartan.

Keywords: Valsartan, Anti-hypertensive, In-*vitro* permeability study

Introduction

The in *vitro study* of skin permeability plays an essential role in the selection of candidates for the development of transdermal dosage forms. Human skin either excised from surgical reduction or obtained from a cadaver is the membrane of choice. However, due to ethical considerations human skin is often being substituted with animal skin such as hairless rat, rabbit, pig and shed snake as a model membrane (Brisset and McBride, 1983; Priborsky *et al.,* 1987). Transdermal drug delivery avoids problems associated with gastrointestinal intolerance, reduces first-pass liver metabolism, increases drug efficacy and is applicable to drugs with short half-lives (Bhatia *et al.,* 1997) Due to the highly organized structure of the stratum corneum that forms an effective barrier to the permeation of drugs through the skin, a number of dermal permeation enhancing methods have been investigated in order to overcome the barrier property of the stratum corneum .Dermal permeation enhancement by chemical enhancers is one of those methods and chemical substances studied include water (Warster and Kramer, 1961), sulphoxides (Stoughton and Fritsch, 1964), propylene glycol (Toniton, 1986), azones (Watkinson, 1991), alcohols and acids (Doshi and Chien, 1989), esters (Catz and Heller, 1989), surfactants (Hwang and Danti, 1983) and essential oils (Monti *et al.,* 2002). Valsartan, N-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine is a potent long-acting non-peptide angiotensin II-receptor antagonist with high specificity for AT1 subtype (Latif *et al.,* 2001). It is used clinically to treat hypertension. Although valsartan is rapidly absorbed following oral administration, has moderate bioavailability and biological half-life (Flesch et al., 1997), it is considered a good candidate for transdermal drug delivery because of relative high inter and intra-patient variability associated with the only available dosage form of the drug. Also it will serve to provide an alternative dosage form of the drug that could benefit geriatric hypertensive patients. To explore the possibility of developing a transdermal therapeutic system for valsartan, we studied the effect of water, polysorbate-80 and eucalyptus oil on the permeability of valsartan through excised rat skin. Polysorbate-80 was chosen because the solubility of valsartan (a poorly water soluble drug) has been reported to be significantly increased by the aqueous solutions of the surfactant (Mbah, 2006). Furthermore, nonionic surfactants and essential oils have also been reported to be dermal absorption enhancers. To our knowledge, there is little or no study on percutaneous absorption of valsartan, and in this paper, the effect of these vehicles on the permeation of valsartan through excised rat skin was investigated.

Materials and Methods

Valsartan (Norvatis Pharmaceuticals, USA), eucalyptus oil (Sanoflore, France) and all other solvents were of analytical grade (BDH). Ultraviolet/Visible spectrophotometer (UV 2102 PC Unico).

Standard solution: Stock solution of valsartan (20 μg/ml) was prepared in methanol. Aliquots of the standard stock solution were pipetted into a 10 ml volumetric flask and diluted to volume with methanol to give the final concentration of 2-10 μg/ml of valsartan.

Preparation of rat abdominal skin: The male albino rats (140-200 g) used for the preparation of the skin was obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were allowed free access to food and water until used for the study. The rats were euthaniased using carbondioxide asphyxiation. The dorsal hair was removed with a clipper and full thickness skin was surgically removed from each rat. The entire abdominal skin was soaked in water at 60 º C for about 1 min, followed by careful removal of the epidermis (Shen et al., 1976). The epidermis was washed with water and used in the *in vitro* permeability studies. The skin samples were stored at –18 º C and placed at 4 º C the day before the study.

Table 1: Valsartan skin permeation parameters from polysorbate-80 solution using Franz cells

Polysorbate-80 solution (% w/v)	Permeation Parameters				ER
	Flux (µg/cm^2/h)	K_p (cm/h x 10^3)	D_{app} (cm^2/h)	K	
0.0	8.31 ± 0.164	1.66 ± 0.082	3.14 x 10^{-3}	29.6	1.00
0.5	3.91 ± 0.024	0.79 ± 0.054	3.14 x 10^{-3}	14.1	0.48
1.0	6.37 ± 0.073	1.27 ± 0.060	3.14 x 10^{-3}	22.7	0.72
2.0	8.68 ± 0.097	1.74 ± 0.031	3.14 x 10^{-3}	31.0	1.05
4.0	10.46 ± 0.150	2.09 ± 0.090	3.14 x 10^{-3}	37.3	1.26
6.0	11.07 ± 0.118	2.21 ± 0.046	3.14 x 10^{-3}	37.4	1.33

Table 2: Valsartan skin permeation parameters from emulsion formulation in 1.0 % (w/v) micellar solution using Franz cells

Emulsion (% v/v)	Permeation Parameters				ER
	Flux (µg/cm^2/h)	K_p (cm/h x 10^3)	D_{app} (cm^2/h)	K	
1.0	1.62 ± 0.017	0.32 ± 0.029	3.14 x 10^{-3}	5.7	0.19
5.0	3.52 ± 0.033	0.70 ± 0.015	3.14 x 10^{-3}	12.4	0.42
10.0	5.66 ± 0.049	1.13 ± 0.029	3.14 x 10^{-3}	20.15	0.68

Table 3: Valsartan skin permeation parameters from emulsion formulation in 2.0 % (w/v) polysorbate-80 solution using Franz cells

Emulsion (% v/v)	Permeation Parameters				ER
	Flux (µg/cm^2/h)	K_p (cm/h x 10^3)	D_{app} (cm^2/h)	K	
1.0	2.09 ± 0.086	0.420 ± 0.012	3.14 x 10^{-3}	7.49	0.25
5.0	5.36 ± 0.131	1.07 ± 0.037	3.14 x 10^{-3}	19.08	0.64
10.0	6.70 ± 0.094	1.34 ± 0.022	3.14 x 10^{-3}	23.90	0.81

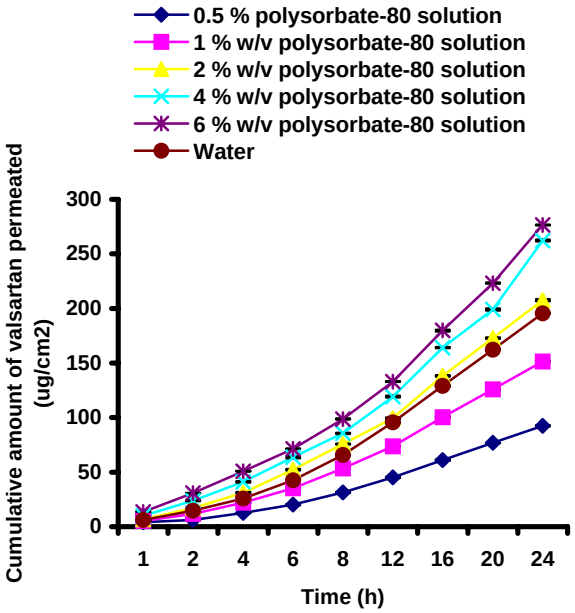

Fig.1: Plot of cumulative amount of valsartan permeated from polysorbate-80 solution versus time

Two hours before the beginning of the experiment, the skin was preequilibrated in phosphate buffer solution at 25 º C.

In vitro skin permeability studies: The Franz diffusion cells were used in the *in vitro* permeation studies. A circular specimen of the skin was sandwiched securely between the compartments of the diffusion cell with the stratum corneum facing the donor compartment. The effective diffusional area was 2.27cm^2 . The receiver compartment (25 ml) was filled with ethanol and water in the ratio of 70 : 30 % v/v in order to maintain sink conditions. The drug solution (5 mg/ml) in various vehicles was added to the donor compartment. The cells were maintained at 37 ± 1 º C by a magnetic stirrer with heater and the contents in the receiver cell were magnetically stirred. At appropriate intervals (1, 2, 4, 6, 8, 12, and 24 h) 2 ml samples were withdrawn from the receiver cell and replaced with an equivalent quantity of drug free solvent (70 : 30 % v/v ethanol-water) to maintain a constant volume. The samples were analyzed by spectrophotometry at a maximum wavelength of 260 nm and the valsartan content obtained from pre-constructed calibration graph.

Permeation data analysis and statistics: The steady-state flux (µg/cm^2/h) was calculated using linear regression analysis by plotting cumulative amount of valsartan permeated through the rat skin as a function of time and dividing a slope of the linear portion of the plot by the area of the skin surface through which diffusion occurred (Julreht *et al.,* 1998).

The lag time (t_l) was calculated from the x-intercept values of the regression lines The permeability coefficient (K_p) was calculated by dividing the flux value at steady-state by the donor cell initial concentration of valsartan.

The apparent diffusion coefficient (D_{app}) was estimated from the lag time values according to the following equation (Flynn et al., 1974) : $D_{app} = h^2 / t_l$, where h is the barrier (skin) thickness, t_l is the lag time.

The stratum corneum / vehicle partition coefficient (K) can be calculated from the following equation (Montenegro et al., 2007): $K = K_p\ h / D_{app,}$

The penetration enhancing effect of the vehicle was calculated in terms of enhancement ratio (ER) using the following equation (Williams

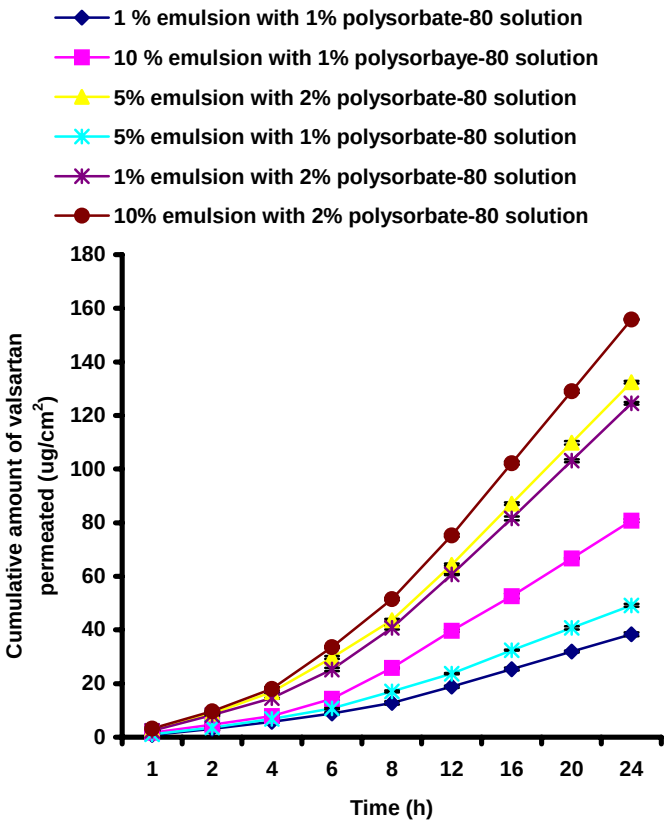

Fig. 2: Plot of cumulative amount of valsartan permeated from emulsion formulations versus time

and Barry, 1994): ER = K_p with vehicle / K_p with water.

Statistical comparisons were made using the student's t-test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

The *in vitro* skin permeation of valsartan was evaluated using the finite dose technique. The percutaneous absorption parameters obtained from water and polysorbate-80 solutions using Franz diffusion cells are listed in Table1. The skin permeation rates of valsartan were found to increase with increase in the concentration of the micellar solution. For example, the permeability flux (Table 1) of valsartan from 0.5 %, 1.0 %, 2.0 %, 4.0 % and 6.0 % w/v micellar solution was 3.91$\pm$ 0.02 µg/cm^2/h, 6.37 $\pm$ 0.07 µg/cm^2/h, 8.68 $\pm$ 0.10 µg/cm^2/h, 10.46 $\pm$ 0.15 µg/cm^2/h, 11.07 $\pm$ 0.12 µg/cm^2 respectively. The results also show the permeability flux of the drug from water to be 8.31 $\pm$ 0.16 µg/cm^2/h. The lag period for permeation of valsartan through the excised rat skin from micellar solutions and water respectively was found to be about 1h. The skin permeation profile of valsartan from water and polysorbate-80 solution is shown in Fig. 1.The effect of eucalyptus oil on the permeation rates of valsartan was studied by introducing varying volumes of oil into 1.0 % w/v and 2.0 % w/v polysorbate-80 solutions respectively. The results are given in Tables 2 and 3 respectively. It was found that the permeation rates of valsartan from the emulsions formed using 2.0 %w/v polysorbate-80 solution were higher than that obtained with 1.0 % w/v polysorbate-80 solution. For instance, the permeability flux (Table 2) of valsartan from 1.0 %, 5.0 % and 10.0 % v/v emulsion formed using eucalyptus oil and 1% w/v polysorbate-80 solution was 1.62$\pm$ 0.02 µg/cm^2/h, 3.52 $\pm$ 0.03 µg/cm^2/h and 5.66 $\pm$ 0.05 µg/cm^2/h respectively while the permeability flux (Table 3) of the drug at the same concentration of the emulsion with 2.0 % w/v polysorbate-80 solution was 2.09 $\pm$ 0.09 µg/cm^2/h, 5.36 $\pm$ 0.13 µg/cm^2/h and 6.70 $\pm$ 0.09 µg/cm^2/h respectively. The lag period for permeation of valsartan through the excised rat skin from the emulsions was noted to be about 1h. The skin permeation profile of valsartan from the emulsion formulations is shown in Fig. 2. The variability observed in the permeability flux values for the studied vehicles could be attributed to varying influences of the vehicles on the biophysical properties of the stratum corneum and differences in thermodynamic activity of the drug in the vehicles.. The biophysical properties of the treated stratum corneum with the vehicles were not studied to determine the probable mechanism of action. However, previous study has shown disruption of the lipid bilayers to be the probably mechanism of action for surfactants and emulsion formulations respectively (Shakeel *et al.,* 2008).

Conclusion: The effect of vehicles on the permeation of valsartan through excised rat skin using Franz-type diffusion cells in order to select a suitable vehicle in the development of a transdermal therapeutic system for valsartan was investigated. Of the vehicles studied, only 6 % w/v polysorbate-80 solution showed some degree of dermal permeation enhancing properties though insufficient to provide any potential use of it as vehicle in developing a transdermal therapeutic system for valsartan.

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