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EFFECT OF PENETRATION ENHANCERS ON TRANSDERMAL ABSORPTION OF INSULIN ACROSS HUMAN CADAVER SKIN

V.U. Rao A.N. Misra*
Pharmacy Department,
Faculty of Technology & Engineering,
M.S. University, Kalabhavan, Baroda, India.

ABSTRACT

The transdermal diffusion of insulin, a model polypeptide drug, across the human cadaver skin (HCS) was evaluated in vitro, in presence of penetration enhancing solvents, anionic surfactants, biosurfactants, a natural moisturizing agent and combinations thereof. Also, an attempt was made to relate the enhanced penetration to physical parameters like distribution coefficient, surface tension and viscosity. The results of the permeation experiments indicate that the permeation enhancers used in the present investigation significantly enhance the amount of drug entering into the HCS and the amount reaching to the skin. A synergistic effect on permeation enhancement was observed in cases where combination of permeation enhancers were selectively used. Reasons for this synergism were critically examined and established.

INTRODUCTION

There have been several attempts at delivering therapeutic peptide and protein drugs by nonparenteral routes including oral, buccal, rectal and transdermal (1-4). Recent reports revealed transdermal permeation of macromolecules using penetration enhancers like dimethyl sulfoxide, azone and by physical means such as iontophoresis, streaming hot air flow, etc. (5). Considering the fact that the transdermal drug delivery provides the possibility of bypassing the hepato-gastrointestical first pass elimination and of achieving a better patient compliance, it is therefore logical to consider it as a potential route for delivery of peptide based pharmaceuticals.

The aim of the present study was to evaluate in vitro, the effect of penetration enhancing solvents, anionic surfactants, biosurfactants and a natural moisturizing agent on the transdermal diffusion of insulin, a model polypeptide drug across HCS. To establish whether physical parameters are responsible for enhancement in penetration, distribution coefficient, surface tension and viscosity were determined.

EXPERIMENTAL

A 24.8 IU/mg preparation of insulin (Novo, Denmark) was used. Dimethyl sulfoxide (DMSO), dymethylformamide (DMF), sodium deoxycholate (NDC) sodiumtaurocholate, sodiumtauroglyco-cholate (NTGC), sodiumphosphate, potasium di hydrogen orthophosphate and sodium chloride (National chemicals, India), Pluronic F68 (PF68), Pluronic F127 (PF127) (BASF, USA) were used.

Reagents:

1. pH 7.4 phosphate buffered saline (PBS):
   This buffer was prepared as per B.P. 1980.
2. Stock solution of insulin:
   It was prepared by dissolving 50mg of drug in 5ml PBS.
3. Stock solutions of the surfactants:These were prepared by dissolving 50mg of the powder in 10ml PBS.
4. Formulations:
   Each formulation contained 800ug/ml of insulin and specified concentration of penetration enhancer/s as shown in Table-1

Methods:

In vitro permeation tests:

The permeation study was conducted by applying 250µl of each formulation on the epidermal surface of a piece of HCS tied at one end of a glass permeation tube. The tube was dipped flush to the surface of 25ml PBS contained in a receptor compartment maintained at 37.5 ± 0.5°C and strirred at 50rpm with a magnetic stirrer. 2ml aliquots were withdrawn at each sampling interval from the receptor cell and insulin spectrophtometrically at 276mm on Hitachi 2000 UV-visible Spectrophtometer. Negative and positive blanks were also run simultaneously to ensure noninterference of skin leachings and the permeation enhancers. 2ml fresh PBS was added after each withdrawal. All permeation runs and sample analysis were carried out in triplicate over a period of 6 hours.

TABLE-1

Formulations Containing Insulin (40 IU/ml)) and Penetration Enhancers
(+ = present. - = absent.)

Formulation No.	30% DMF	30% DMSO	0.01% PF127	0.01% PF68	0.1% NDC	0.1% NTC	0.1% NTGC	10% Urea
Group A								
A1	+	-	-	-	-	-	-	-
A2	-	+	-	-	-	-	-	-
A3	-	-	+	-	-	-	-	-
A4	-	-	-	+	-	-	-	-
A5	-	-	-	-	+	-	-	-
A6	-	-	-	-	-	+	-	-
A7	-	-	-	-	-	-	+	-
A8	-	-	-	-	-	-	-	+
Group B								
B1	-	+	-	+	-	-	-	-
B2	-	+	-	-	-	+	-	-
B3	-	+	-	-	-	-	+	-
Group C								
C1	-	-	-	+	-	-	-	+
C2	-	-	-	-	-	+	-	+
C3	-	-	-	-	-	-	+	+

Determination of apparent distribution coefficient, viscosity and surface tension:

For the determination of apparent distribution coefficient, the HCS was cut in circular discs of 2 cm^2 area and put in glass vials each containing 10ml of solution. Each solution contained 1mg of insulin with composition of penetration enhancers as shown in Table-1. The vials were shaken thoroughly at 37 ± 0.5°C for 4 hours, centrifuged at 3000 rpm for 20mins after 24 hours and the supernatants were analysed.

Surface tension and single point viscosity of each formulation was determined using Stalagmometer and Ostwald Viscometer respectively. Negative and positive blanks were run for all measurements. Each measurement was carried out in triplicate.

DATA ANALYSES

The experimental model adopted here is a simple zero order flux case. The quantity of drug which permeates the HCS, $Q^1(t)$ and the quantity which passes through the HCS and reaches the sink $Q°(t)$ are the factors varying with time. These were calculated using the equations previously reported (6). The rate at which the macromolecule enters into the skin $(dQ°_{(t)}/dt$ and the rate which it enters into the sink $(dQ°_{(t)}/dt$ were calculated from the slopes of the regressed lines of $Q^1_{(t)}$ vst and $Q°_{(t)}$ vst values. The data of the apparent distribution coefficient, surface tension, viscosity, $dQ^1_{(t)}/dt$ and $dQ°_{(t)}/dt$ values for each formulation are given in Table-2.

Statistical analysis using ANOVA was applied to the data of $Q^1_{(t)}$, $Q°_{(t)}$, $dQ^1_{(t)}/dt$ and $d°_{(t)}/dt$ values. The data was considered significant for p 0.05.

RESULTS AND DISCUSSION

In case of 30% DMSO there is a significant enhancement in the $Q^1(t)$ and $Q°(t)$ values after 60 minutes with a 2 fold enhancement in the $dQ^1(t)/dt$ value and 2.3 fold enhancement in $dQ°(t)/dt$ value as compared to the control formulation. In case of DMF, statistically significant enhancement occures only after 90 minutes with 2 fold enhancement in $dQ°(t)/dt$ value and 2.22 fold enhancement in the $dQ°(t)/dt$ value. 30% DMSO and 30% DMF significantly enhance the viscosity of the formulations which may be contributory to the increased osmotic activity leading to distortion of the laminated structure of the stratum corneum (8). The effect of DMF is statistically less significant as compared to DMSO which may be because the transdermal water loss caused by DMF is reported to be less intense and of shorter duration as compared to DMSO (9).

Formualtions containig nonionic surfactants PF27 and PF68 show a significant enhancement in the $Q^1(t)$ and $Q°(t)$ values at all sampling time intervals. The significantly higher $dQ^1(t)/dt$ and $dQ°(t)/dt$ values for PF68 as compared to PF127 indicate that PF68 is a better penetration enhancer for insulin as compared to PF127, One reason may be that PF68 brings about significantly more reduction in the surface tension of the system as compared to the PF127 (Table-2).

All the three biosurfactants studied have demonstrated a significant increase in the $Q^1(t)$ and $Q°(t)$ values at all time intervals as compared to the control formulation. NTC and NTGC show a 2.27 and 2,42 folds enhancement in $dQ^1(t)/dt$ value respectively and 2.43 and 2.8 times enhancement in $dQ°(t)/dt$ value. On

TABLE-2

The Data for $dQ^{i}_{(t)}/dt$, $dQ°_{(t)}/dt$, Apparent Distribution Coefficient, Viscosity and Surface Tension for the Formulations containing Penetration Enhancers

Formulation No.	$\frac{dQ^{i}_{(t)}}{dt} \times 10^{-3}$	$\frac{dQ°_{(t)}}{dt} \times 10^{-3}$	Apparent Distribution Coefficient	Viscosity (Cps)	Surface Tension dynes/cm
0	4.01	3.50	0.073	0.787	68.360
A1	8.04	7.80	0.082	1.134	66.837
A2	8.50	7.10	0.086	1.255	65.081
A3	7.10	7.20	0.104	0.902	51.907
A4	8.80	8.20	0.122	0.868	44.024
A5	8.80	8.40	0.129	0.879	36.994
A6	9.10	8.50	0.132	0.837	34.480
A7	9.80	9.70	0.134	0.845	29.099
A8	8.30	7.50	0.096	0.845	67.168
B1	25.50	20.70	0.162	1.361	43.022
B2	39.90	36.50	0.194	1.673	32.172
B3	42.70	38.70	0.196	1.540	28.325
C1	18.60	17.20	0.164	0.873	51.654
C2	28.00	26.00	0.162	0.950	36.215
C3	31.50	30.60	0.168	0.904	30.567

Key 0 = Control Formulation
A1 = 30% DMF
A2 = 30% DMSO
A3 = 0.01% PF127
A4 = 0.01% PF68
A5 = 0.1% NDC
A6 = 0.1% NTC
A7 = 0.1% NTGC
A8 = 10% Urea
B1 = 30% DMSO + 0.1% NTC
B2 = 30% DMSO + 0.1% NTGC
B3 = 30% DMSO + 0.01% PF68
C1 = 10% Urea + 0.1% NTC
C2 = 10% Urea + 0.1% NTGC
C3 = 10% Urea + 0.1% PF68

the other hand, NDC shows only a 2.20 fold increase in $dQ^{i}(t)/dt$ and 2.34 fold increase in $dQ°(t)/dt$ values. Among the three biosurfactants, NDC reduces the surface tension least. This coupled with the fact that its aggregation number increases and hence solubility decrease at pH 7.4 must be responsible for the least activity.

Urea, a natural moisturing agent show significant enhancement in the $Q^1(t)$ and $Q°(t)$ values after 60 minutes as compared to the control formulation. There is a 2.07 fold values. From Table-2 it is apparent that the permeation enhancing property of urea cannot be attributed to its ability to modify the surface tension and viscosity of the formulation but may be due to its moisturising and keratolytic activity.

The penetration enhancing solvents, surfactants and urea all show a significant enhancement in the permeation of insulin. Since each of these agents act by different mechanisms, it was deemed worthwhile to study the penetration enhancing activity of formulations containing combinations of DMSO and surfactants and urea and surfactants (Table-1). As compared to the control formulation enhancement in $dQ^1(t)/dt$ value is 6.37 fold for formualtion B1, 9.97 fold for formulation B2 and 10.67 times for formulation B3. The $dQ^1(t)/dt$ value increase 2.89, 2.80 and 2.13 folds for formualtions B1, B2 and B3 respectively as compared to formualtions A4, A6 and A7.

In case of formulation C1, C2 and C3 there is a 2.11, 3.08 3.80 fold increase in the $dQ^1(t)/dt$ value respectively as compared to formualtions A4, A6 and A7.

Similar enhancement is observed in case of $dQ°(t)/dt$ values indicating that both, the rate at which the drug penetrates the skin and rate at which drug enters into the sink are enhanced in case of the combination.

in the case of combination of DMSO and srufactants the viscosity of the formualtions is significantly enhanced and the surface tension is also reduced to an extent which is greater than that for formualtions conataining single agents. Thus the increased osmotic activity coupled with the reduced thermodynamic activity may be the reason for synergistic effect. The ability of urea to hold water coupled with the reduction in the interfacial tension brought about by the surfactants may be the reason for the synergstic activity in case of formualtions C1 to C3.

CONCLUSIONS

All the penetration enhancers used in the present investigation significantly enhance the amount of drug entering into the HCS and the amount of drug reaching the sink. The extent of permeation enhancement vary from one enhancer to other and was found to follow the rank order:

B3 > B2 > C3 > C2 > B1 > C1 > A7 > A6 > A5 > A4 > A8 > A2 > A3 > A1 > 0

Physical parameters such as distribution coefficient, surface tension and viscosity were used for explaining the mechanism of permeation enhancement by different classes of enhancers and also can be helpful in predicating the degree of enhancement in that class. A synergistic effect was observed in cases where combination of enhancers were selectively used. It is assumed that to acheive same degree of enhancement the toxicities will be comapratively less in case of use of selective combinations as compared to single penetration enhancers. However, the role of combinations of enhancers can only be settled after biological investigation first on animal models followed by human subjects. Further efforts should concentrate on use of selective combinations of penetration enhancers in the development of transdermal delivery system for polyeptide drugs end evaluation of their toxicities.

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