SHORT COMMUNICATIONS

Evaluation of Liposomal Clobetasol Propionate Topical Formulation for Intra-Dermal Delivery

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Hydroxy propyl methyl cellulose (HPMC) K4M gels containing free (HCF), liposome encapsulated clobetasol propionate (HCL) and physical mixture of the drug and lipids (HCP) were prepared and subjected to *in vitro* drug diffusion studies using rat skin to determine diffusion parameters. Data obtained were analyzed to calculate the amount of the drug in the skin (Qm) and the quantity of the drug in the recipient fluid (Csf) and its ratio (Qm/Csf). *In vivo* skin blanching assay of these formulations in human volunteers showed low blanching scores for liposomal gel formulations indicating low absorption of the liposomal drug in to the blood stream resulting in it's accumulation in the skin. Out of all the formulations tested, liposomal gel prepared with drug, phosphatidyl choline and cholesterol in the ratio of 2:4:1 showed highest Qm/Csf ratio and minimum blanching score.

Rapid absorption of topically applied corticosteroids in to the circulation results in a short duration of local effect and undesirable systemic toxicity on chronic usage in conditions such as eczema, psoriasis and dermatitis[1]. The approach of achieving selective drug delivery to the skin by incorporating liposome-encapsulated drugs in topical formulations has been reviewed[2]. Mezei and Gulasekaram[3] reported alteration in the disposition in rabbits with lower triamcinolone acetonide levels in the blood stream when administered topically as liposomal drug creams in comparison to conventional creams. Misini *et al.*[4] reported the retention of tretinoin in epidermis and dermis associated with the reduced systemic absorption following the topical administration of liposomal tretinoin to hairless rat skin. In the present communication, a comparative study of *in vitro* diffusion and *in vivo* disposition of plain and liposome encapsulated clobetasol propionate (CLP) from HPMC K4M gel based topical formulation is reported. *In vivo* disposition of CLP has been studied by skin blanching assay in human volunteers.

Clobetasol propionate was obtained as a gift sample from M/s Gufic Labs, Mumbai. Phosphatidyl choline (PC), type X-E, content 99% (TLC) of Sigma Chemical Company, USA was obtained from CSIR Centre for Biochemicals, New Delhi. Cholesterol (Chol) was obtained from S.D. Fine Chemicals, Baroda. Hydroxy propyl methyl cellulose K4M (Methocel K4M) was obtained from CDH (P) Ltd., New Delhi.

Clobetasol propionate was estimated by the method reported by Sethi[5]. The sample to be analyzed was taken in a 25 ml volumetric flask, and enough methanol was added to make up to 10 ml. Ten millilitres of isoniazid reagent (1mg/ml solution in acidified methanol) was added and the mixture was warmed at 50±2° for 45 min, cooled and the volume was made up with methanol. The absorbance was read at 410 nm against a suitable reagent blank using UV/Vis spectrophotometer (Hitachi 2000). The concentration of the drug was measured using the calibration curve in the range of 2 to 20 µg/ml. PC was estimated by the assay method of Stewart[6], which involves the reaction of phospholipids with ammonium ferrothiocyanate reagent (0.1 M solution in distilled water) to form a blood red colored complex. Absorbance was measured at 485 nm against a reagent blank. Concentration of PC was estimated using the calibration curve in the range of 50-350 µg/ml. Chol. was estimated by a

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reported method[7] involving the reaction of cholesterol with ferric chloride reagent (0.05% w/v solution in glacial acetic acid) to form a complex. Absorbance was measured at 550 nm against a suitable reagent blank. Concentration of cholesterol was estimated using the calibration curve in the range of 10 to 100 µg/ml.

Liposomes were prepared by lipid film hydration method as described by Mezei and Gulasekaram[8]. The Drug, PC and Chol dissolved in chloroform:methanol mixture (2:1) in a 50 ml round bottom flask was added with alpha-tocopherol (1% of PC by weight), mixed and evaporated under vacuum in a rotary flash evaporator. The film formed was hydrated with 10 ml of calcium chloride solution (25 mM solution in distilled water), gently shaken and then vortexed for 10 min to get a uniform dispersion. The suspension was centrifuged at 2750 rpm for 30 min to recover liposomes. The supernatant was assayed for the quantity of un-entrapped CLP and the percent drug entrapment in liposome was calculated. All batches of liposomes were also analyzed for phosphatidyl choline and cholesterol content by the methods described above.

Liposome samples were subjected to microscopic observation using Carl Zeiss Jena microscope for morphological examination at 40 X. Surface characteristics and lamellar pattern were observed by negative stain transmission electron microscopy. A drop of liposomal suspension was put on a wax bed and a drop of 2% ammonium molybdate solution was added and was mounted on the surface of a formver grid of Jeol Jem 100 SX transmission electron microscope and observed at various magnifications[9]. Particle size analysis[10] was carried out using a BH_2 Olympus microscope at a magnification of 100X and frequency- distribution curve was drawn.

HPMC Gel base was prepared by dispersing 50 g of the HPMC powder in distilled water containing 0.001% phenyl mercuric nitrate and stirred under a triple blade stirrer. The mixture was allowed to stand overnight to free the gel from entrapped air. Gel formulation containing free drug was prepared by triturating the base with weighed quantity of the drug (120 #) and finally passed through triple roller mill. The same procedure was adopted in preparing gel formulations containing liposome by taking calculated quantities of the liposome pellets obtained by centrifugation. Gel formulation containing the physical mixture of the drug, PC and Chol was prepared by triturating the drug (120 #), PC and Chol with the gel. All formulations were prepared in two drug concentrations, 1.0 % w/w for *in vitro* studies and 0.05 % w/w for *in vivo* studies.

In vitro permeation study of the drug from formulations was conducted using Franz diffusion cell[11] using rat skin model. Skin of Wistar female rats was removed from abdominal portion (Approx. 5cm^2), shaved off and the subcutaneous fat was removed by established method[12]. The skin (thickness approximately 0.04 cm) was then tied to the mouth of the sample cylinder (diameter-4.8 cm.) so as to expose the epidermal surface to the donor compartment. Two hundred milligrams of the formulation was applied uniformly on the surface of the skin in the donor compartment. The cylinder was then placed immersed in 50 ml of the recipient liquid (Phosphate buffer pH 7.4) maintained at 37°, and stirred at 50 rpm. Samples were withdrawn at suitable time intervals and were analyzed for the drug content by the method described above.

The skin blanching assay[13] was used to compare the efficacy of the liposomal formulation with conventional formulation containing the free drug. The assay was conducted on healthy volunteers of either sex (age 22 to 50 y). Volunteers, selected based on their ability to elicit a skin blanching response, were applied with adhesive tapes of 7 mm x 7 mm cut squares on the flexor aspect of both forearms. The squares were filled with formulations (10 mg of formulation per cm^2) and the site applied was occluded using another adhesive tape. After 6 h, the tapes were removed gently and the forearm washed with water. One hour after washing, the blanching response was evaluated by a panel of three unbiased observers using a 0 to 4 scale rating as per Barry and Woodford's method[14]. The blanching scores were subjected to 't' test to study the level of significance.

The mean cumulative amount of drug diffused (n =6) was plotted against time (fig. 1). Slopes of the linear curves ('r' value is between 0.97 and 0.99) obtained represent 'R', the diffusion rate[15]. Permeability coefficient of the drug through the membrane (P), Diffusion coefficient of the drug in the skin (D) and Partition coefficient of drug between skin and vehicle (K) were calculated using the equations suggested by Chien[16], Higuchi[17] and Roberts[18] respectively, as given below;

$P = R/Cd$; % Diffusion $= 200\ (Dt/\pi h^2)^{1/2}$; $K = P\ h / D$

Where, Cd = drug conc. on the surface of the skin;

h= thickness of the skin. Percentage diffusion was calculated from the value of cumulative amount of drug diffused at time 't'. Since the sink condition is prevailing in the flux of the drug from donor to recipient compartment across the membrane, the theoretical quantity of the drug remaining in the membrane (Qm) is given by the formula[18]; Qm = KhCo' /2 , Where , Co' = drug concentration in the donor compartment. Quantity of drug in the blood stream at steady state(Css) following topical application may be calculated using the equation[19] ,

Css = A. Co'. P / Vd. Ke; Csf = Co'. P

Where, A is the area of the skin, Vd is the volume of distribution and Ke is the elimination rate constant of the drug. Since A, Vd and Ke are constants, for the purpose of comparison of formulations, a factor (Csf) is calculated to indicate the quantity of the drug in the blood following topical application by using the above simplified formula.

In the present context, concentration of the drug in the dermal compartment (Qm) is a beneficial parameter and the concentration in the blood (Csf) is a non-beneficial parameter. Hence overall assessment of the formulation for effectiveness may be calculated as the ratio of Qm to Csf (Qm/Qsf).

Liposomes prepared were spherical and multilamellar as confirmed by negative stain electron microscopy. Particle size distribution of all batches of liposomes was found normal and symmetric around the mean (4.38 to 5.13 µm). Analysis of particle size data of various batches by 'ANOVA' showed calculated F value (0.128) less than the table F value (4.26) at $p< 0.05$ indicating no significant difference among their particle size distribution. The percentage drug entrapment in liposomal batches varied between 72 to 84 % with highest entrapment efficiency (84.28%) in the batch with 2:4:1 ratio of Drug:PC:Chol. Table 1 gives the details regarding different batches of gel formulations along with values of various diffusion parameters. Liposomal gels exhibited low diffusion rate(R), diffusion coefficient (D) and permeability coefficient(P) across the skin but high values of (Qm/Csf) particularly with HCL2 and HCL3 batches in comparison to gels containing free drug or the physical mixture. This result indicates the possibilities of drug accumulation in the skin coupled with low diffusion rate in to the recipient fluid.

Skin blanching assay has been reported conventionally with Caucasian volunteers. Indian volunteers have

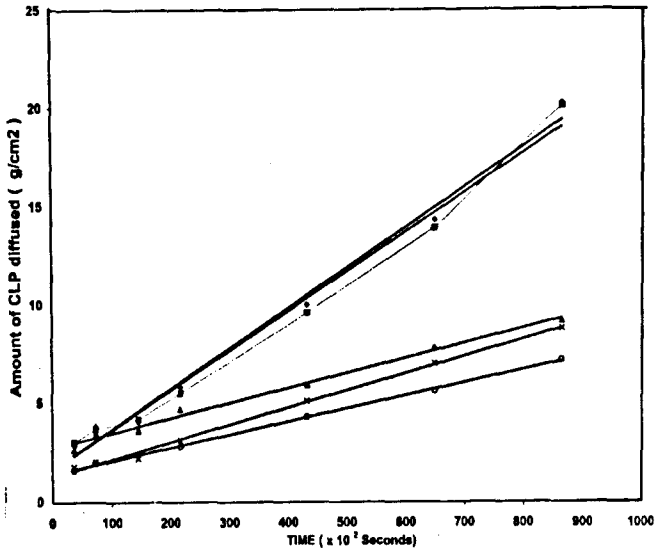

Fig. 1. Clobetasol release from formulations

Amount of Clobetasol (CLP) released with time from plain HCF (—◆—) and liposomal gel batches, HCP (—□—), HCL1 (—▲—); HCL2 (—X—) and HCL3 (—O—)

also shown promising response in our studies. Out of 30 volunteers, 27 have exhibited detectable blanching after 6 hours of occlusion. However 13 volunteers among them were found to be very good discriminators when tested with gels of different drug concentration and so the assay was restricted to these volunteers only. The blanching scores for all the volunteers, for each formulation, were summed and expressed as the Mean Blanching score (MBS) in Table 1. Liposomal gel formulations particularly batch HCL2 exhibited low degree of blanching in comparison to those containing free drug and physical mixture. Since blanching occurs as a result of vasoconstriction of superficial vasculature caused by the penetration of the topically applied corticosteroid in to blood vessels of the skin, low blanching score indicate low concentration of the drug in the superficial blood vessels and obviously high concentration in the skin[13]. These results are in good correlation with the results obtained with the *in vitro* model described above indicating the possibilities of using the model for rapid screening of formulations and also to predict the efficacy of topical preparations. The results also highlight the potential of topical preparations containing liposome encapsulated drugs for selective accumulation in skin.

TABLE 1 : *IN VITRO* DIFFUSION RATE AND DIFFUSION PARAMETERS

Formulation code →	HCF	HCL_1*	HCL_2*	HCL_3*	HCP@
Content	CLP	L-CLP	L-CLP	L-CLP	PM-CLP
Ratio (CLP: PC: Chol)	-	(4:8:1)	(2:4:1)	(1:2:1)	(4:8:1)
Cd (mg/cc)	52.00	52.80	59.40	38.90	52.80
Co'(x 10^3µg/g)	13.00	13.20	14.85	9.73	13.20
% Diffusion at t= 6 h	4.04	3.22	1.89	2.61	3.77
R (x10^{-2}(µg/cm^2)/sec)	2.05	0.76	0.87	0.66	2.02
P (x 10^{-4} cm/sec)	3.94	1.44	1.47	1.70	3.83
D (x 10^{-10} cm^2/sec)	0.95	0.60	0.21	0.40	0.83
K (x 10^5)	1.66	0.96	2.82	1.71	1.85
Qm (x10^6µg)	43.15	25.20	83.72	33.30	48.89
Csf (µg)	5.12	1.90	2.18	1.65	5.05
Qm/Csf (x 10^4)	842.00	1327.00	3847.00	2018.00	968.00
MBS	7.16	6.54	6.06	6.46	7.06

Assessment of HPMCK4M gels containing clobetasol propionate across rat skin model.
*Gel containing liposome encapsulated drug (L-CLP). @ Gel containing physical mixture of drug:PC:Chol. (PM-CLP)

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