Nanoparticulate Systems of Acyclovir for Topical use in Herpes treatment – Formulation, Characterization and Evaluation

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ABSTRACT

Of the numerous drugs that are available for systemic treatment of HSV infection, a cream containing an antiviral agent is still the most convenient method of treating herpes simplex labialis/facialis in the general population. Acyclovir (ACV) is a synthetic purine nucleoside analog derived from guanine indicated for the treatment of infections caused by the herpes virus and is considered the safest and most efficacious for this purpose. Topical application of ACV in polyethylene glycol (PEG) is a common practice, but has been shown to be less effective compared to oral and intravenous routes. Considering these aspects, development of ACV-loaded Solid Lipid Nanoparticulate (SLN) drug delivery system by transdermal route was attempted for possible improvement in the permeation efficiency for effective treatment in herpes infections. The characterization and evaluation of the developed formulation was also carried out.

Keywords: : Acyclovir , Herpes ,Transdermal ,Flux

1. Preparation of ACV-SLN

1.1 Preparation of ACV-SLN

50mg of Acyclovir was dissolved in 1ml of DMSO .To this, 450 mg of Soya Lecithin and 50mg of Eudragit –E previously dissolved in 9ml of methanol-toluene (i.e.2ml methanol & 7ml toluene), were added. 165mg of Polysorbate-80 was dissolved in 10ml water. This aqueous phase was then added to the organic phase under stirring at room temperature. Emulsification was achieved by homogenizing (DIAX 900, Heidolph Homogenizer, Germany) the primary emulsion for 3mins .The resultant emulsion was cooled and was sonicated using ultrasonic solid probe (Sonics, Vibracell, USA) for two minutes. The solvent was removed from the emulsion using Flash Evaporator (Heidolph,Germany). Trace solvent removal was ensured by applying only vacuum for about 30-60 minutes at end of preparation. The volume of the aqueous SLN dispersion obtained was made up to 10ml with water and sterilized by autoclaving at 121°C for 15 minutes.

2.Characterization of ACV-SLN

The mean size and polydispersibility index of the size distribution of the particles in the ACV-SLN dispersion were determined by photon correlation spectroscopy using zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). Each sample was diluted to a suitable concentration (1:50) with filtered distilled water. Analysis was performed at 25ºC with the angle of detection of 90°.The zeta potential (ZP) of the systems was determined by using Zetasizer 3000 HAS (Malvern Instruments, Malvern, UK) .The sample was diluted suitably with filtered double distilled water. Charge on emulsion droplets and their mean ZP values (±SD) were obtained from the instrument.Prepared nanoparticles systems were assayed for the drug content using High Pressure Liquid Chromatography (HPLC). 0.05 ml of ACV-SLN dispersion was dissolved in methanol/DMSO (9:1) mixture. 20µl of the solution was injected in the HPLC column.The percentage of incorporated Acyclovir (entrapment efficiency) was determined by HPLC analysis at 254nm ,after centrifugation of the aqueous dispersion.The amount of free drug was detected in the supernatant and the amount of incorporated drug was determined as a result of the initial drug minus the free drug .The entrapment efficiency could be calculated by the following equation:

$$EE\% = \left[\frac{W_{intial\ drug} - W_{free\ drug}}{W_{intial\ drug}}\right] x\ 100 \qquad (1)$$

Where $W_{initial\ drug}$ is the mass of the initial drug used for the assay and the $W_{free\ drug}$ is the mass of the free drug detected

in the supernatant after centrifugation of the aqueous dispersion.

3.Transdermal permeation studies

Permeation of Acyclovir across excised rat skin on its application in the form of solid lipid nanoparticles when compared to the control solution and the marketed cream was investigated by using Franz (vertical) diffusion cell. The available diffusion area was 3.8sq.cm.The skin was mounted between the receptor and the donor compartments with the side of the stratum corneum facing upwards. The receptor fluid was pH 2.8-phosphate buffer. The contents of the receptor chamber were continuously agitated by a magnetic stirrer throughout the experiment. 0.2%w/v of sodium azide was added to the receptor compartment to prevent microbial growth. The study was conducted at 37±1°C. Finite doses of the formulations approximately equal quantities measured by weight equivalent to 5mg of drug were charged in the donor compartment .A sample of 0.2 ml was collected from the receptor compartment at each predetermined time intervals up to 48h and replaced with equal volume of fresh receptor solution.Samples were analyzed for Acyclovir content by injecting 20µl of sample onto HPLC column .The amount of drug diffused at different time points was obtained from a standard .The concentration-peak height ratio plots were found to be linear (r^2 0.999) over a concentration range of interest 250ng-10µg/ml and the limit of detection was 0.1µg/ml.

50mg of Acyclovir dissolved in 1ml DMSO was made up to 10 ml with water. The permeation profiles of SLN were also compared with the marketed ACIVIR cream containing 5%w/w Acyclovir from Cipla, India. 100 mg of the cream, which corresponds to 5mg of drug, was charged in the donor compartment.

4. High Pressure Liquid Chromatography

A HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with LC-10 AT solvent delivery unit, SPD-10 AVP UV-spectrophotometric detector, Sphinchrom software and Rheodyne injector fitted with 20µl capacity loop was used for analysis. An octadecylsilane (C-18) reverse phase stainless steel analytical column (250×4.6mm) with a 5µ-particle size was employed for chromatographic separation.Mobile phase consisted of pH 2.58 phosphate buffer and Methanol in the ratio of 90:10. The flow rate was maintained at 1 ml/min. Samples were detected using UV-Spectrophotometric detector at 254 nm and 0.05 a.u.f.s at ambient temperature. The retention time for Acyclovir was 6.1 min.

5. Permeation parameters and data analysis

The cumulative amount of Acyclovir permeated per unit area of skin was plotted against time [1]. The steady state flux (J ss)(mg/cm^2/h) was calculated as slope of the linear portion of the plot. Linear regression analysis was applied to draw the best-fit line for the linear portion. The permeability coefficient was calculated from the steady state flux (Jss) according to the equation

$$K_P = Jss / Co \qquad (2)$$

Where Kp is permeability coefficient (cm/h)

Co is Initial concentration of Acyclovir in donor compartment.

Lag times were obtained by extrapolating the cumulative amount of ACV permeated Vs time curves to the time axis. Data expressed as mean ± SD (N= 3)

Cumulative amount of acyclovir permeated per unit area of skin was plotted against time, linear regression analysis was approached to draw the best-fit line for the linear portion from this data.

Statistical analysis

Cumulative amount of acyclovir permeated and the study state flux values of SLN versus Control Systems were statistically compared using t-test. Results were considered significantly different at $p<0.05$.

The mean size and zeta potential of the polysorbate 80 stabilized ACV-SLN system using Soya lecithin as lipid was found to be 108.35 ± 3.5 nm and -29.5±2.8mV respectively. The drug content was found to be 4.56mg.

The plots of mean cumulative amount of Acyclovir permeated versus time across rat skin from different formulations evaluated as function of time are shown in Fig. 4.1 and data given in Table 4.1. Efficiency of various systems in promoting the permeation of Acyclovir can be ranked in the following order:

ACV-SLN > Control Suspension > ACIVIR Cream

The plots of mean flux (µg/cm2.h) from different Acyclovir formulations through in vitro rat skin evaluated as a function of time are shown in Fig.4.2 and the data is given in Table 4.2. As seen from the plots, the ACV-SLN Systems show significantly higher mean flux compared to the other formulations.

Table 4.1: Mean cumulative amount permeated(CAP) from the different ACV systems through in vitro rat skin

Time (h)	Control (µg)	ACIVIR Cream (µg)	ACV-SLN (µg)
0.25	5.8±1.7	3.2±0.7	8.85±1.5
0.5	11.8±3.7	6.6±0.7	16.9±2.0
0.75	23.6±4.7	15.4±0.7	26.6±2.8
1	32.0±6.0	23.5±3.9	36.7±2.9
1.5	59.5±10.0	40.7±4.6	46.7±3.5
2	76.0±8.7	70.2±5.2	86.2±9.5
2.5	87.2±7.7	77.5±7.3	103.4±5.8
3	133.8±8.2	97.3±6.3	123.5±11.5
4	185.9±6.3	154.5±10.2	159.2±13.2
6	325.6±9.7	278.9±12.5	228.3±15.6
8	516.9±15.3	497.9±14.2	400.9±17.8
12	1004.0±22.1	805.8±17.3	546.3±263
14	1104.3±14.2	862.0±23.3	781.2±19.6
24	1123.5±13.5	901.3±15.6	1300.2±25.6

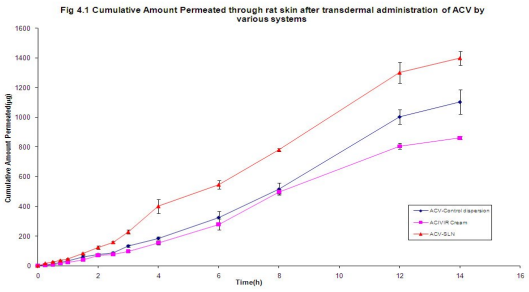

Table 4.2 Mean flux µg/cm^2.h from the different ACV systems through *in vitro* rat skin

Time (h)	ACV-Control Mean Flux(µg/cm^2.h) ±S.D	ACIVIR CREAM Mean Flux(µg/cm^2.h)± S.D	ACV-SLN Mean Flux(µg/cm^2.h)±S .D
0.25	21.36±6.13	11.07±2.54	61.72±6.73
0.5	43.62±13.54	24.22±2.73	98.36±9.46
0.75	87.02±17.17	56.84±2.84	131.65±12.67
1	117.83±22.13	86.59±14.48	170.35±13.38
1.5	219.17±37.21	149.89±32.26	307.58±16.07
2	280.05±32.09	258.53±18.35	410.37±42.91
2.5	321.09±38.42	285.45±26.98	583.25±26.54
3	493.07±32.8	358.33±23.48	784.77±52.18
4	684.81±39.16	569.21±45.12	1366.03±174.34
6	1199.73±145.02	1027.86±138.29	2136.51±108.11
8	1904.55±147.27	1834.63±55.48	2918.99±37.37
12	3699.01±177.89	2969.04±72.49	4530.14±261.87
14	4068.67±301.82	3176.05±36.98	4993.6±172.07

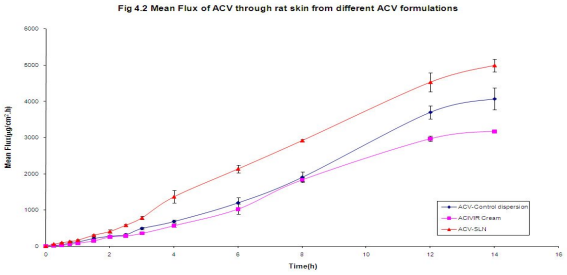

Table 4.3 Permeation Parameters of Acyclovir from different Acyclovir Systems across rat skin

Formulation	Steady state Flux Jss (mg/cm^{2}h)	Lag time (avg±sd) (h)	Permeability Coefficient (avg±sd) Kp (cm/h)
ACV-Control Dispersion	*0.137±0.16	0.26±0.03	0.0275±0.012
ACIVIR CREAM	*0.149±0.32	0.36±0.15	0.0299±0.013
ACV-SLN	0.250±0.84	0.18±0.02	0.0501±0.025

*Statistically different at $p<0.05$

As given in Table 4.3,the values of The maximum flux exhibited by the formulations at 14 hrs was also highest in

case of ACV-SLN systems when compared to the control and cream systems. The Lag time was seen to be highest in case of ACIVIR cream (0.36±0.15h), which is twice the time taken by the SLN systems. This is followed by the ACV-Control system showing a lag time of 0.26±0.03h. The lowest lag time was seen with SLN systems with a mere 0.18±0.02h. The permeability coefficient Kp was calculated and found to be highest in case of SLN systems when compared with that of the control and the cream preparations.Enhancement ratio was calculated as the ratio of Acyclovir flux in SLN to that of the control and cream systems. When compared with the control the ratio is 1.58±0.63 and when compared with the cream the value is found to be 2.19±1.22.

Conclusions

1.From our results, Acyclovir SLN formulations have clearly shown higher permeation efficiency when compared to both the marketed ACIVIR cream and the control suspension in water. This might be due to the fact that Soyalecithins contain unsaturated fatty acids, oleic acid and linoleic acids which have penetration enhancing properties of their own as compared to egg lecithin, which contains saturated fatty acids [2] .

2.ACIVIR cream was found to show least permeation. The preparation contains drug dissolved in Poly ethylene glycol (PEG) where PEG is known to improve the penetration enhancement effect via skin. Even in the presence of this skin penetration enhancer, i.e. PEG, the permeation of ACV across skin seems to be limited. The flux of the formulations through the rat skin was also evaluated. The increase in the flux of the SLN systems with time indicate that these systems escape into the target sites much faster when compared to the control systems. Several reports in the literature have pointed out that the *in vivo* efficacy of a formulation is basically proportional to the drug flux [3]. The higher flux values of the SLN system can be of high value as the ACV released from the transdermal delivery system can act locally to prevent the evolution of skin lesions.The SLN systems exhibit lowest lag times when compared to both the cream and the control systems. This lower lag time combined with the highest permeation amounts observed in case of SLN indicate that these systems can show the highest permeation efficiency .

In accordance with the above-cited mechanisms, we presume that ACV-SLN might have significantly improved the skin permeation of ACV. Besides, the use of DMSO in the SLN and Control systems might also be the factor in promoting permeation through skin. Absorption of drugs or chemicals through skin can be enhanced using chemical enhancers such as DMSO. The exact mechanism is unknown. However, it has been shown that the mode of action involves a combination of elution of DMSO soluble components (in this case, drug) from stratum corneum, delamination of the horny layer and denaturation of its proteins(Scott et al 1987)[4].This indirectly supports the conclusion that the stratum corneum is the barrier layer to the absorption of drugs such as Acyclovir.

References

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