Department of Pharmaceutics[1], Faculty of Pharmacology[2], Dermatology[3], P.S.G College of Pharmacy and Hospitals, Peelamedu, Coimbatore, Department of Pharmaceutical Engineering and Technology[4], Trichy, Tamilnadu, India

Formulation and evaluation of a proniosome hydrocortisone gel in comparison with a commercial cream

V. Sankar[1], C. Praveen[1], K.G. Prasanth[2], C.R. Srinivas[3], K. Ruckmann[4]

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V. Sankar, M. Pharm, Asst. Professor, P.S.G. College of Pharmacy, P. O. Box, No: 1674, Peelamedu, Coimbatore-641004 Tamilnadu
sansunv@yahoo.co.in

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Proniosomes, a novel drug delivery approach for increasing permeation of hydrocortisone through the skin, were investigated. Proniosome hydrocortisone gel was prepared by a coacervation-phase separation method using different combinations of non-ionic surfactants with cholesterol and lecithin. Proniosome formulations were characterized for vesicle size, entrapment efficiency, and drug content uniformity. Span 20:Span 40, Span 20:Span 60 and Span 20:Span 80 combinations showed good entrapment compared with Span: Tween combinations (Span 20:Tween 40, Span 20:Tween 60, Span 20:Tween 80). *In vitro* release in 8 h from a Span 20:Span 80 proniosome 1 % hydrocortisone formulation was high (58.29 %) compared to the other proniosome formulations. Proniosome hydrocortisone gel shows diffusion type release which was confirmed by Higuchi and Peppas plot. *In vivo* studies in mice confirmed that the proniosome 1% hydrocortisone formulation was more active than a commercially marketed 1 % hydrocortisone cream. Topical application of hydrocortisone in the form of proniosomes leads to prolonged action.

1. Introduction

A novel approach to minimizing the physical instability of niosomes is proniosomes - derived niosomes for the delivery of sparingly soluble drugs. This is based on the liposome production method. Proniosomes consist of maltodextrin powder coated with surfactant or a surfactant/drug mixture to yield a dry powder. Upon addition of hot water and brief agitation, the maltodextrin dissolves and the surfactant forms a suspension of multilamellar vesicles (niosomes) containing the poorly soluble drug. The niosomes slowly release the drug into solution (Blazek-Welsh and Rhodes 2001).

Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via the transdermal route. This would be possible if proniosomes formed niosomes on hydration with water from the skin following topical application under occlusive conditions (Fang et al. 2001). Proniosomes minimize problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, storage and dosing.

Proniosomes are mostly used in and are of interest for topical formulations. In this study hydrocortisone was formulated in the form of proniosomes for the treatment of dermatitis.

2. Investigations, results and discussion

2.1. Formulation preparation and vesicle characterisation

Preformulation studies indicated that a non-ionic surfactant ratio of 1:9 shows better vesicle formation than 1:1 or 9:1. Hence proniosome hydrocortisone gel was formulated with different non-ionic surfactant combinations in a 1:9 ratio containing 1% and 2.5% hydrocortisone with soya lecithin (Ankur Gupta et al. 2007).

The vesicle size of the proniosome hydrocortisone gel formulations was determined using an optical microscope (Gupta et al. 2007). Vesicle formation was good in S20:80 1 % (PHG 3) and poor in S20: T80 2.5 % (PHG 12).

Vesicles obtained from proniosome formulations with Span combinations were found to be in the size range 3 – 6 μm and in Span, Tween combinations the size range was 4 – 7 μm. Average vesicle size is shown in Table 1. Encapsulation of proniosome hydrocortisone gels prepared using different surfactant combinations was determined by ultra centrifugation at 13000 rpm for 30 min. The percentage entrapment of proniosome hydrocortisone gel varied from 27 – 89 %.

Entrapment was high in S20: 40 (89.67 %) and poor in S20:T80 (27.2 %) based 1 % proniosome hydrocortisone gel, and for 2.5 % proniosome hydrocortisone gel entrapment was found to be high in S20:40 (83.55 %) and poor in S20: T80 (57.34 %). This shows hydrocortisone entrapment was high in S20:40 and poor in S20: T80 for both 1 % and 2.5 % formulations. Percentage entrapment efficiency of proniosomes is shown in Table 1. (Gupta et al. 2007).

Among the 12 proniosome formulations, entrapment efficiency was highest in S20:40 1 % (89.67 %) and lowest in S20:T80 1 % (27.20 %).

The drug content analysis of proniosome hydrocortisone gels showed that 1 % proniosome hydrocortisone gel formulations had a more uniform distribution than 2.5 % PHG formulations, but drug concentration was higher in 2.5 % proniosome hydrocortisone gel formulations. Drug content uniformity in

Table 1: Vesicle size, entrapment efficiency and drug content uniformity of hydrocortisone proniosome gels

S.No	Formulation Type	Surfactant Type	Drug conc.	Vesicle size Range(μm)	Average size(μm)	% Entrapment	Amt. of Drug (mg)	% of drug content
1	PHG 1	S20:S40	1%	2 – 6	3	89.67%	1.399594	55.9837
2	PHG 2	S20:S60	1%	2 – 8	4	79.66%	1.399594	55.9837
3	PHG 3	S20:S80	1%	2 – 12	4.5	70.66%	1.395287	55.8114
4	PHG 4	S20:T40	1%	2 – 12	4	58.88%	1.399594	55.9837
5	PHG 5	S20:T60	1%	2 – 10	3.5	65.15%	1.394507	55.7802
6	PHG 6	S20:T80	1%	2 – 8	3	27.20%	1.399594	55.9837
7	PHG 7	S20:S40	2.5%	2 – 8	4	83.55%	1.507501	24.3145
8	PHG 8	S20:S60	2.5%	2 – 14	5	80.00%	1.433335	23.1183
9	PHG 9	S20:S80	2.5%	4 – 12	6	76.42%	1.430687	23.0755
10	PHG 10	S20:T40	2.5%	2 – 16	6.5	76.12%	1.428865	23.0462
11	PHG 11	S20:T60	2.5%	2 – 20	7	77.02%	1.430687	23.0755
12	PHG12	S20:T80	2.5%	4 – 10	6	57.34%	1.399594	22.5741

proniosome hydrocortisone gel 1 % and 2.5 % formulations is shown in Table 1. (Pundit and Bharathi 2007)

2.2. In vitro and in vivo evaluation of proniosome hydrocortisone gel formulations

2.2.1. In vitro release

Drug release from proniosome hydrocortisone gel was investigated using a dialysis membrane with UV detection at 248 nm. Proniosome hydrocortisone gel formulations were compared with a marketed 1 % hydrocortisone cream. The initial 1-hour release from the marketed formulation was found to be higher compared with PHG 1 %. But the cumulative percentage release from 1 % hydrocortisone cream was not regular and linear with respect to time when compared with the 1 % PHG formulation. A maximum of 34.7 % of the drug was found to be released from the marketed 1 % cream through the dialysis membrane in 2 h whereas 58.29 % of drug was found to be released from the S20: 80 1 % PHG formulation and the release extended up to 8 h. Cumulative release is compared for 1 % PHG formulations and the marketed 1 % hydrocortisone cream in Fig. 1.

The cumulative release from the 2.5 % PHG formulation shows sustained release in a similar way to 1 % PHG. But here good release was observed from Span 20: Span 40 (53.81 %) and poor from Span 20: Span 80 (29.05 %) as shown in Fig. 2.

The kinetic parameters of release from proniosome 1 % hydrocortisone gel (Span 20:Span 80) were studied out using zero order, first order, Higuchi and Peppas kinetics.

The regression coefficient for PHG 1 % (Span 20:Span 80) was 0.99833 for zero order and 0.9631 for first order kinetics. The results show that the formulation obeys zero order kinetics.

The Higuchi plot value for proniosome 1 % hydrocortisone gel (Span 20:Span 80) was more than 0.998. Hence it follows a diffusion release mechanism. The slope value of the Peppas plot was 0.8930which confirms non-fickian diffusion type.

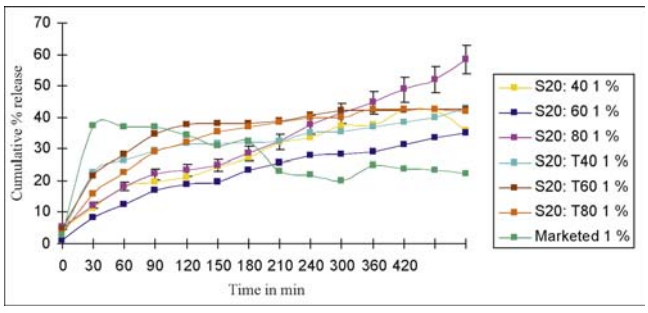

Fig. 1: Comparative *In-vitro* release of 1 % hydrocortisone proniosome gel formulation with marketed hydrocortisone cream 1%

Drug release from rat skin for all the proniosome formulations was studied using a Franz diffusion cell (Fang et al. 2001; Pundit and Bharathi 2007). Release from rat skin using the Franz diffusion cell was found to be low. However, it showed linearity in release with respect to time. The cumulative percent release of 1 % PHG formulations through rat skin is shown in Fig. 3. The cumulative percent release of 2.5 % PHG formulations through rat skin is shown in Fig. 4.

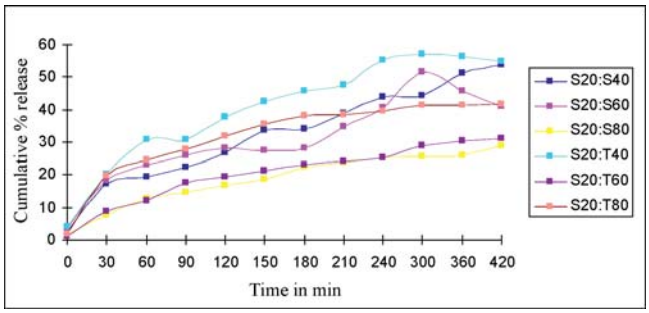

Fig. 2: Comparative *In-vitro* release of 2.5 % hydrocortisone proniosome gel formulations

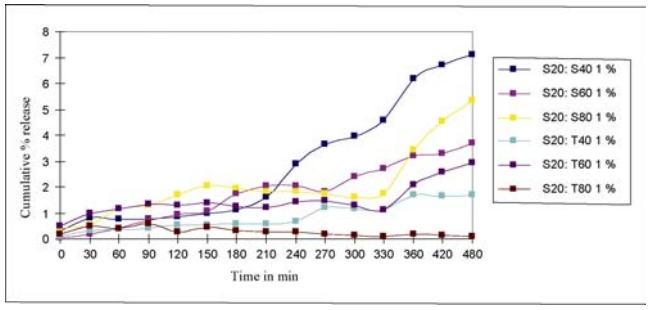

Fig. 3: Comparative release of 1 % hydrocortisone proniosome gel formulations through rat skin

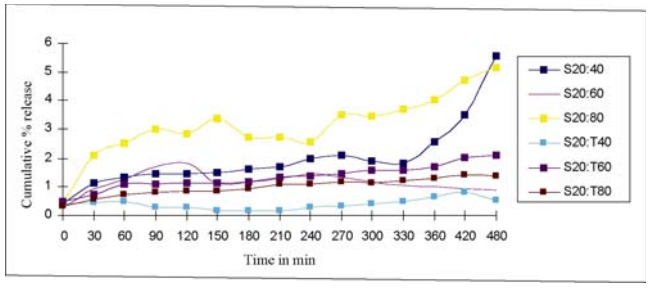

Fig. 4: Comparative release of 2.5 % hydrocortisone proniosome gel formulations through rat skin

Table 2: Comparative anti-inflammatory activity of hydrocortisone proniosome gels in mice

S.No.	Group Type	Animals in group	Paw size before inflammation(mm)	0 hr (mm)	1hr (mm)	2hr (mm)	3hr (mm)	4hr (mm)
1	I	1	2.65	3.5	4.7	5.0	5.1	4.0
2		2	2.87	3.82	4.5	5.1	5.3	4.4
3		3	2.6	3.4	4.6	5.0	5.2	4.2
4	II	1	3.05	3.90	3.64	3.6	3.45	3.4
5		2	2.75	3.55	3.67	3.46	3.3	3.25
6		3	2.80	3.60	3.38	3.2	3.1	3.1
7	III	1	3.25	4	3.72	3.29	3.1	3.05
8		2	2.90	3.75	3.84	3.6	3.05	2.8
9		3	2.64	3.49	3.55	3.28	3.09	2.75

The release from Span 20:Span 40 1 % (7.09 %) was highest and Span 20:Tween 80 2.5 % lowest. The lower extent of drug release through rat skin may be due to lower adsorption and fusion of proniosome on to the surface of the skin and the lipid bilayers of the proniosomes might have formed a more rate-limiting membrane barrier.

2.2.2. In vivo studies

In vivo studies were carried out on 9 mice using the carrageenan induced mouse paw edema method. (Shahiwala and Misra 2002). The results of the studies calculated as percentage edema inhibition are shown in Table 2. This study shows that the marketed formulation was good in the initial hours but later the activity was reduced. But with the proniosome hydrocortisone gel formulation the activity was less in the initial hours but later it showed promising anti-inflammatory action, exceeding the marketed formulation 4 h. (Table 3)

Statistical analysis was done for the *in-vivo* studies, the results indicating that, compared with controls, the marketed 1 % hydrocortisone cream and proniosome 1 % hydrocortisone gel (S 20: S 80) showed significant anti-inflammatory activity ($P < 0.001$). When comparing the marketed 1 % hydrocortisone cream and proniosome 1 % hydrocortisone gel (S 20: S 80) the significance was $P < 0.05$.

Proniosomes prepared by the coacervation-phase separation method (Gupta et al. 2007) using an optimized ratio of non-ionic surfactants showed enhanced anti-inflammatory action. Drug entrapment was found to be higher in hydrophobic surfactant combinations than in hydrophobic and hydrophilic surfactant combinations. Vesicle formation from hydrophobic surfactant combinations was found to be stable. Proniosome hydrocortisone gel showed good *in vitro* drug release compared with the marketed hydrocortisone cream. Proniosome hydrocortisone gel did not show encouraging drug release through rat skin.

In vivo studies for proniosome 1 % hydrocortisone gel (Span 20:Span 80) showed better anti-inflammatory action than the marketed 1 % hydrocortisone cream in mice. It also showed a more prolonged action than the marketed cream. (Shahiwala and Misra 2002)

Proniosome hydrocortisone gel showed diffusion type release as confirmed by Higuchi and Peppas plot. Comparing the proniosome formulation with the marketed 1 % hydrocortisone cream, the proniosome formulation showed better release and anti inflammatory activity than the marketed formulation. Phospholipids and non-ionic surfactants in an optimum ratio in the proniosomes may act as penetration enhancers, which are useful for increasing the permeation of hydrocortisone through skin.

3. Experimental

3.1. Materials

Hydrocortisone USP (Samarth Labs, Mumbai), soya lecithin (phosphatidyl choline) (Hi-Media Laboratories) cholesterol (Loba Chemie), Span 20,40,60,80 (Loba Chemie), Tween 40, 60, 80 (Loba Chemie, Mumbai), dialysis membrane was purchased (Hi-Media Laboratories, Mumbai, India), ethyl alcohol 99.9%

3.2. Formulation procedure

Proniosomal gel was prepared by a coacervation-phase separation method. Precisely weighed amounts of drug (hydrocortisone), surfactant, lecithin, and cholesterol were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and ethyl alcohol and water added. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent and it was warmed over a water bath at 60-70 °C for about 5 min until the surfactant mixture was completely dissolved. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath until a clear solution was formed, which was converted into proniosomal gel on cooling. The pronio-

Table 3: Composition of hydrocortisone gel formulations

S.No.	Formulation Type	Drug conc.	Surfactant Type	Ratio	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Water (ml)
1	PHG 1	1%	S20:S40	1:9	100	100	2	0.5
2	PHG 2	1%	S20:S60	1:9	100	100	2	0.5
3	PHG 3	1%	S20:S80	1:9	100	100	2	0.5
4	PHG 4	1%	S20:T40	1:9	100	100	2	0.5
5	PHG 5	1%	S20:T60	1:9	100	100	2	0.5
6	PHG 6	1%	S20:T80	1:9	100	100	2	0.5
7	PHG 7	2.5%	S20:S40	1:9	100	100	2	0.5
8	PHG 8	2.5%	S20:S60	1:9	100	100	2	0.5
9	PHG 9	2.5%	S20:S80	1:9	100	100	2	0.5
10	PHG10	2.5%	S20:T40	1:9	100	100	2	0.5
11	PHG 11	2.5%	S20:T60	1:9	100	100	2	0.5
12	PHG 12	2.5%	S20:T80	1:9	100	100	2	0.5

some formulations were prepared with 1 % and 2.5 % drug concentrations. The proniosome hydrocortisone gel formulation compositions are given in Table 1.

3.3. Vesicle size analysis

Proniosomal hydrocortisone gel (100 mg) was hydrated in saline solution (0.9 % solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under an optical microscope.
The size of 50 vesicles was measured using a calibrated ocular and stage micrometer fitted to the optical microscope. Vesicle size was calculated using Eq. (1).

$$\text{Size of each division} = \frac{\text{Number of divisions of stage micrometer}}{\text{Number of divisions of eye piece micrometer}} \times 10 \quad (1)$$

3.4. Encapsulation efficiency

Encapsulation of hydrocortisone drug in the proniosomal gel was evaluated by dispersing the proniosomal hydrocortisone gel (100 mg) in distilled water and warming the dispersion gently to form niosomes. Then the dispersion was centrifuged at 13,000 rpm for 1 h at 5 °C. The supernatant was taken for the spectrophotometric determination of free drug at 248 nm. Percentage encapsulation efficiency was calculated from Eq. (2).

$$\% \text{ Encapsulation efficiency} = [(C_t - C_r)/C_t] \times 100 \quad (2)$$

Where,

C_t – Concentration of total hydrocortisone.
C_r – Concentration of free drug in supernatant solution.

3.5. Drug content uniformity

The formulated proniosomal gel was mixed well. 100 mg of gel was weighed and transferred into a vial. The gel was dissolved in 25 ml of phosphate buffer saline (pH 7.4) with vigorous shaking, and the solutions were assayed for hydrocortisone content at 248 nm. The amount of drug in 100 mg gel was calculated by Eq. (3).

$$\text{Amount of drug} = [(\text{concentration}) \times (1) \times (100)/1000] \quad (3)$$

3.6. *In vitro* release studies

Drug release from proniosome hydrocortisone gel was carried out using Himedia dialysis membranes 50 with a molecular weight cut-off range from 12000 - 14000. A weighed amount (100 mg) of proniosomal gel formulation was applied to the dialysis membrane and the open ends of the membrane were closed with membrane closure clips. The membrane containing gel formulation was allowed to dip in 50 ml of pH 7.4 phosphate buffer saline receptor medium. The receptor medium was stirred using a magnetic bead with a magnetic stirrer at 60 rpm. Samples were withdrawn and replaced by equal volumes of fresh receptor medium at each sampling interval. Samples withdrawn were analyzed spectrophotometrically at 248 nm.

3.7. Ex-vivo studies

The institutional animal ethics committee of the (PSG Institute of Medical Sciences and Research) granted approval for animal usage (Reg No: 158 / 1999 / CPCSEA) on 5th January, 2009.
The permeation of proniosomal hydrocortisone gel was determined by Franz (vertical) diffusion cell using excised rat skin as membrane mounted on the receptor compartment with the stratum cornea side facing upwards into the donor compartment. A weighed amount of proniosome hydrocortisone gel was applied to the stratum corneum facing upwards into the donor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer saline. Sampling was done regularly at predetermined time intervals. The available diffusion area of the cell was 1.5 cm^2. The receptor medium was stirred by a magnetic bead. Samples withdrawn were analyzed spectrophotometrically at 248 nm.

3.8. In vivo studies

Acute and chronic inflammation models were used to evaluate the anti-inflammatory activity. The study was carried out in mice with weights ranging from 35 -49 g. In the acute model carrageenan was used to induce inflammation in hind paws of mice. 0.1 ml of 1% carrageenan in normal saline was injected into the paw. Three groups of animals were used.
Group I was the control (0.1 ml 1% carrageenan in normal saline). Group II received the marketed 1% hydrocortisone cream (100 mg). Group III received the proniosome 1% hydrocortisone gel (100 mg).
The anti-inflammatory effects of the marketed and proniosomal formulations were determined by plethysmometer. The paw volume was measured initially and then at 1, 2, 3 and 4 hr after carrageenan injection.

3.9. Statistical analysis

The *in vivo* results from the S20:80 proniosome 1 % hydrocortisone gel formulation were subjected to analysis of variance (one way ANOVA with Turkey's multiple comparison post test) with Graph Pad Prism (version 3.0) software.

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