Research Article

DEVELOPMENT AND CHARACTERIZATION OF PREDNISOLONE LIPOSOMAL GEL FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT

The present objective for the study was to prepare prednisolone liposomal gel intended for topical application. Various liposome formulations were prepared using Vortexing Sonication technique using vacuum rotator evaporator by varying the lipid phase composition (lecithin/cholesterol). Liposome formulations were characterized for drug content, entrapment efficiency, surface morphology, surface charge, and stability studies. Topical liposomal gels were prepared by incorporation of liposome dispersion into structured vehicle carbopol (2 %). Alternatively, hydrogels containing prednisolone were prepared and their drug release properties were investigated. The percentage entrapment of drug was increased with increase in phospholipid composition in the range of 85-98 %. Liposomal gel showed prolonged release of prednisolone than the hydrogels. Results of all the studies suggested that Prednisolone liposomal gel formulation was therapeutically effective drug delivery system for treatment of Rheumatoid Arthritis.

Keywords: Prednisolone, liposome, Anti-inflammatory, Rheumatoid arthritis, Phospholipids.

INTRODUCTION

Prednisolone is a steroidal anti-inflammatory drug with predominant glucocorticoid and low mineral corticoid activity. It is used in the treatment of rheumatoid arthritis hence also known as 'disease modifying anti arthritic drugs' because of its anti-inflammatory action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase¹⁻². When steroidal anti-inflammatory drugs such as prednisolone are given orally results in systemic side effects like bone loss, increased susceptibility to infection, osteoporosis, peptic ulcers and buffalo hump. Parental route of administration results in rapid clearance rate of drug which ultimately compels invasive and frequent administration of drug. Despite noteworthy advances have been made over recent years for the management of rheumatoid arthritis, the currently available methods, have a dose limiting therapeutic index with compromised safety implications. Attempts will be made in developing and characterizing a specific drug delivery system targeting drugs to synovium or specific tissues which in turn increase drug efficacy with minimum extra synovial toxicity. The term Rheumatoid Arthritis (RA) is a chronic auto-immune disease that causes pain, stiffness and swelling and limit function of many joints that can cause disability and have a negative socio-economic impact. The term RA was first given by "Sir Alfred Baring Garrod" in 1859. The synovial fibroblast activates macrophages and transcription factor NF- κ B which aids in progression of disease as well as mediating inflammation. As a result of inflammation, the synovial thickens, the cartilage begins to disintegrate gradually leads to destruction³⁻⁵. Recent research suggests that calcifying Nanoparticles (also known as nanobacteria) are present in synovial fluid and are responsible for provocation of inflammation leading to bone and joint destruction⁶. Drugs useful in treatment of rheumatoid arthritis are classified as first line agents having Non-steroidal anti-inflammatory drugs and steroidal anti-inflammatory drugs like prednisolone. Liposomes are a tiny bubble (vesicle), made out of the same material as a cell membrane. It is a novel generation of carrier mediated drug delivery system having several advantages over other conventional methods. It has shown better stability and ease of

sterilization on large scale by preventing drug over loading. Liposome can be composed of naturally derived phospholipids with mixed lipid chain, increases efficacy and therapeutic index. The main objective of the study is to develop and evaluate prednisolone liposomal gel formulation for the treatment of rheumatoid arthritis. Detailed literature survey revealed that there are only ophthalmic formulations of prednisolone 1 % for treatment of eye infections but no attempts or formulations are developed till date to evaluate the topical delivery of prednisolone.

MATERIALS AND METHODS

Prednisolone was gift sample from AKUMSLaboratories Ltd., Haridwar, Uttarakhand, India. Saturated Soya lecithin was purchased from Hi media, India. Carbopol- 940 purchased from Lubrizol USA. Methanol, chloroform, n-Octanol and all other reagents Thomas Baker USA and solvents were of analytical grade.

Preparation of Liposome Formulation

Liposome formulation containing prednisolone was prepared by using Vortexing Sonication method using vacuum rotatory evaporator. Optimization of liposome formulation was done by preparing varying concentration of lecithin and cholesterol. Prednisolone 20 mg and lecithin, cholesterol was dissolving in chloroform: methanol mixture in the ratio 2:1 (v/v) for various formulations as shown in Table 1 and kept for vortexing for 30 min and then to bath sonicator for 5-10 min above the T_c of the lipid. Initially 5 ml aliquot of organic solvent was introduced into round bottomed flask at 37°C and rotated. After complete drying remaining 5 ml aliquots of solution was used. The flask containing liposome formulation was kept in vacuum desiccator overnight and then sieved with 100 mesh.

Preparation of liposomal gels

The appropriate amount of carbopol 940 P was weighed and added slowly in a citrate buffer solution (pH 5), under constant vortexing.

After addition of full amount of solid material, the gel was allowed to swell under moderate vortexing for at least 24 h or until transparent and swollen. Other ingredient such as 15 % w/v polyethylene glycol-400 (PEG 400) and triethanolamine (0.5 % w/v) were added to obtain homogeneous dispersion of gel and sodium benzoate (0.5 % w/v) was added in the buffer used for gel preparation. Liposomal gel formulation was prepared by mixing the liposomal dispersion with the gels in the ratio of 1:5 (w/w) (liposomal dispersion/gel).

Table 1: Composition of optimized prednisolone liposome formulation

Formulation code	Prednisolone (mg)	Soya Lecithin (mg)	Cholesterol (mg)
F1	20	150	10
F2	20	210	10
F3	20	230	10
F4	20	290	10

Characterization of Liposomes

Prepared liposomal formulation was characterized for the following parameters⁶.

Vesicle Size and Count

A drop of distilled water was added to few liposome granules on glass slide without cover slip to observe formation of liposome from liposome formulation. Vesicle size and count was recorded under Digital optical microscope [Metzer, India] with magnification 4X.

Surface charge

Liposome formulation of the optimized batches was dissolved in phosphate buffer pH 7.5 and made a higher serial dilution 1000 X until a clear solution is obtained. Sample was analyzed using Zeta Analyzer [Haroba, Japan] for determination of surface charge.

Drug content

1 g of liposome formulation was weighed and vesicles were lysed with 25 ml of methanol by sonication [citizen, India] for 15 min. The clear solution was diluted to 100 ml with methanol. Then 10 ml of solution was diluted to 100 ml with saline phosphate buffer pH 7.4. Aliquots were withdrawn and drug content was calculated for prednisolone using RP HPLC developed method⁷.

Entrapment efficiency

1 g of Liposome formulation was weighed equivalent to 10 mg of drug and was transferred to a 100 ml volumetric flask containing 25 ml of mobile phase, then sonicated and filtered through 0.45 µm membrane filter. The filtrate was finally diluted to 10X with mobile phase and appropriate dilutions were made to obtain concentration in the range of 1-5 µg/ml. The steady base line was recorded by using the optimized chromatographic conditions. The assay was subjected for calculating regression equation. The procedure was repeated for 6 times and the percentage of drug in the formulation was calculated for optimized batches of liposomal formulations.

$$\% \text{ Drug Entrapped (PDE)} = [(C_t - C_f)/C_t] \times 100$$

*C_t is the concentration of total drug content, and
C_f is the concentration of free drug

Yield of Liposomes

After complete drying, the drugloaded liposomes was collected and weighed accurately. The yield of liposomes was calculated by

$$\% \text{ yield} = \text{Total weight of liposomes (g)} \times 100 / \text{Total weight of drug + Total weight of excipients}$$

Characterization of Gels

Optimized gel base was evaluated for following parameters for both plain gel and gel loaded with liposomes⁸.

Physical examination

Macroscopic examination for visual (aspect, consistency, homogeneity, color), olfactory (smell), tactile (touch and thermal sensation); features was done for liposomal gel formulation.

pH optimization

Triethylamine was used to neutralize the gel and to prepare gels with different pH values, 5.5; 6.5 and 7.5 using pH meter [Lab India, India].

Viscosity and Rheological properties

The rheological analysis of the experimental gels was performed using a Brook field viscometer pro D II + apparatus, equipped with standard spindle LV1 with 61 marking. Viscosity of 1, 1.5 and 2 % carbopol gel was determined and optimized concentration was selected.

Drug content and content uniformity

The gel sample (1 g) was withdrawn and drug (prednisolone) content was determined using RP HPLC developed method. Similarly, the content uniformity was determined by analyzing drug concentration in gel taken from 3 to 4 different points from the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using RP-HPLC method⁷.

Stability studies as per ICH guidelines

For stability studies, formulations were stored in tight sealed, ambered colored glass containers at various temperatures 8°C, room temperature and at 40°C for a period of three months using stability chambers [Cintex, India]. Regular tested for changes in surface morphology, color, residual drug content.

In-vitro studies

Percentage cumulative amount of Drug Release from Dialysis Membrane

An *in vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, molecular weight 5000 Daltons) was placed between receptor and donor compartments. Liposomal gel equivalent to 1 g was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37 ± 0.5°C with stirring at 500 rpm [Remi, India] throughout the experiment. At fixed time interval, 5 ml of aliquots was withdrawn for every 1, 2, 3, 4, 6, 8, 12, 14 hours from receiver compartment through side tube and analyzed by RP-HPLC method. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism and order of drug release from liposomal gel¹⁰.

Percentage cumulative amount of Drug Release from Rat skin

Preparation of skin

The abdominal hair of Wistar male rats, weighing 150 ± 25 g, was trimmed using trimmer [rexino, India] 24 h before treatment. After anesthetizing the rat with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable

enzymes, the dermal side of the skin was in contact with a saline solution for 1 h before starting the diffusion experiment. A system having modified Franz's diffusion cells with a diffusional area of 2.15 cm was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment. Drug encapsulated liposomal gel equivalent to 1 g was applied to the skin surface in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). During the experiments, the diffusion cell was maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 500 rpm [Remi, India]. After application of the test formulation on the donor side, at fixed time intervals, 5 ml of aliquots were withdrawn from receiver compartment through side tube for every 1, 2, 3, 4, 6, 8, 12, 14 h and analyzed by RP-HPLC method for determining the cumulative amount of drug permeated through skin⁹.

Drug Retention Study

The skin was removed from the diffusion cells after completion of experiments. The surface of skin specimens was washed 10 times with 1 ml distilled water and dried on filter paper. The effective surface area of the skin was separated and minced with a surgical sterile scalpel then finally homogenized in a vial filled with methanol by using Homogenizer (REMI RQT-124A) at 16,000 rpm for 5 min (REMI Cooling Centrifuge TR 01). The tissue suspension was centrifuged for 15 min at 9000 rpm and then the supernatant was filtered. Then filtered supernatant tissue suspension was further extracted with methanol and filtered. The filtrate was assayed for cumulative amount of drug retained on the skin by using RPHPLC method.

RESULTS AND DISCUSSION

In a pre formulation study the optimum concentrations of phospholipid and cholesterol was determined to obtain stable liposomes devoid of aggregation, fusion and sedimentation. Prednisolone liposome was prepared using vortex sonication technique and method was found to be well suited for the production of liposomes without aggregation. Amount of phospholipid and cholesterol was found to be very critical in the preparation and stabilization of liposomes.

Effect of variables on vesicle size

In the present study, this technique was effective to produce poly dispersity index within the range which indicates obtained liposome population have narrow size distribution when compared to other method. Results showed that with increase in the concentration of phospholipid vesicle size was found to be increased as shown in Table 2.

Effect of variables on entrapment efficiency

Results show that with increase in the concentration of phospholipid and entrapment efficiency found to be increased. From the factorial design experiment F1, F2, F3, F4 M which had maximum vesicle size and percentage entrapment efficiency, selected for the further study of gel formulation (Table 2).

Determination of vesicle count and size

Results of average vesicular size and distribution were calculated for count and distribution as shown in Table 2 and Figure 1.

Determination of Zeta ζ potential

Surface charge was determined and the liposomal formulations showed potentials ranging from -31.6 to -63.2 mv which was sufficient to avoid aggregation of vesicles (Table 3).

Percentage yield of liposomes

The percentage yield for optimized batches of prednisolone liposome formulations was found to be increased with increase in the phospholipid concentration shown in Table 3.

Table 2: Characterization results for prednisolone liposome Formulations

Prednisolone Formulation	X1 % Entrapment efficiency	X2 Vesicle size (μm)	Poly dispersity index
F1	84.32	5.23	0.522
F2	89.75	5.45	0.361
F3	92.45	5.83	0.231
F4	96.46	6.71	0.248

Table 3: Zeta potential and Percentage yield for optimized liposome formulations

Formulation	Prednisolone Liposomes [mv]	Prednisolone liposome
F1	-63.2	92.82
F2	-61.3	91.65
F3	-49.2	90.79
F4	-31.6	86.32

Characterization of liposomal gels

Prepared liposomal gel formulation was evaluated for the following parameters:

Viscosity measurements

Viscosity measured for optimized plain gel and liposomal gel showed 10560 and 11007 cps respectively.

Content uniformity and pH measurement

There was no significant difference observed in the percent drug at various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory. The pH values of the prepared liposome gels were within the limits of 5.79-6.06.

In-vitro Studies

The release rate of prednisolone liposomal formulation over dialysis membrane was significantly higher than its flux across skin, indicating the barrier properties of skin for drugs. *In vitro* permeation rate studies such as steady state transdermal flux (SSTF or Jss) for transport of prednisolone across skin was estimated for different formulations. Calculations for the *in-vitro* rate permeation are as follows:

$$\text{Jss/ SSTF} = Q/TA = \text{Amount of drug permeated/ time} \times \text{area of skin}$$

*Jss is steady state flux measured as the slope of the profile after regression analysis

The cumulative amount of drug release of various liposomal gel formulations are shown in Table 4 and 5.

Skin permeation and drug deposition studies

Results obtained from *in-vitro* drug permeation studies for prednisolone liposomal gel formulations are shown in Figure 3 and 4. Results clearly indicate that the amount of drug retained in the skin was considerably higher in case of liposomal gels when compared to non-liposomal formulation (free drug gel). This shows that liposomes not only enhance the penetration of drug molecules but also helps to localize the drug within the skin Figure 2.

Table 4: Cumulative drug release of prednisolone formulations for 14 h over dialysis membrane

Formulations	% Cumulative Release
F1	55.61
F2	66.71
F3	76.63
F4	82.93
Free Drug Gel	98.01

Table 5: Cumulative amount drug released of liposomal gel over rat skin for 14 hours

Formulations	% Cumulative permeation
F1	52.53
F2	62.56
F3	82.33
F4	82.46
Free Drug Gel	94.19

Figure 1: TEM of liposomal formulation

Figure 2: Percent of prednisolone liposome formulation retained over skin

*F2 has more percent retain on skin when compare to other F1, F3, F4 and free drug gel

Figure 3: Percent cumulative amount prednisolone liposomal gel release over membrane

Figure 4: Percent cumulative amount prednisolone liposomal gel release over rat skin

CONCLUSION

The liposomal product of Prednisolone was prepared with the view to improve therapeutic response and reduce the possible adverse symptoms. Here liposomes of prednisolone were prepared using vortex sonication technique. Percentage entrapment efficiency was optimized after studying the effect of various process and formulation variables. The percentage cumulative drug release was also studied. Stability studies showed maximum percent drug retention at refrigerated temperature (2-8°C). The drug entrapment efficiency can be attributed to phospholipids' ability to vesiculate independently because they carry two bulky nonpolar lipid chains and a polar head group, which helps them spontaneously form into closed bilayer systems.

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