Development of Topical Diclofenac Sodium Liposomal Gel for Better Antiinflammatory Activity

A.V. Jithan and M. Swathi
Vaagdevi College of Pharmacy, Kakatiya University, Warangal, India-506001.

ABSTRACT: The objective of this study was to develop a diclofenac liposomal gel intended for better anti-inflammatory activity compared to a normal gel after topical administration. Spherical multilamellar vesicular liposomes (MLVs) consisting of soya lecithin, cholesterol and diclofenac were prepared via thin film hydration methodology. Small unilamellar vesicles (SUVs) were then prepared out of MLVs. Entrapment efficiency, the drug associated with SUVs was subsequently determined. Liposomal gel formulations were then prepared by incorporation of SUVs into carbopol 934 gel previously prepared. The formulations were then characterized for in vitro drug release, ex vivo permeation studies, drug-lipid compatibility and rheological behaviour. Vesicle size/Zeta potential of the liposomes containing in one selected formulation were determined. Pharmacodynamics was also determined for selected diclofenac liposomal gel. Diclofenac liposomal gel formulation prepared showed more sustained and prolonged anti-inflammatory effect compared with diclofenac gel. Taken together, the results indicate that the diclofenac liposomal gel is better than the regular gel without liposomes. Drug-lipid compatibility studies indicated no interaction with drug and the polymer.

KEY WORDS: Liposomes; Liposomal gel; Diclofenac; Transdermal delivery; Anti-inflammatory activity

Introduction
Skin has been considered as a promising route for the administration of drugs because of its accessibility and large surface area. Transdermal drug delivery system, designed to deliver a variety of drugs to the body through diffusion across the skin layers, is appealing for several reasons including avoidance of the variable absorption and metabolic breakdown associated with oral treatments, drug administration can be continuous, and minimal intestinal irritation can be avoided. Liposome is has been used in transdermal drug delivery system because of its much higher diffusivity in skin compared to most bare drugs (Barry et al., 2001; Cevec et al., 2004; El Maghraby et al., 2008). Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used to administer drugs by several routes such as the oral, parenteral, and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have to act topically, such as local anaesthetics, but also for enhancing transdermal delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration. Due to the forementioned advantages, in this study liquid-state liposomes were chosen to serve as the drug delivery system (Grace J et al., 2008; Esposito et al., 1998). Although liposomes demonstrated promise for Transdermal drug delivery, the practical application of these formulations onto the skin is less. However, these can be incorporated into the gels than can applied onto the skin. It has been found that liposomes incorporate into the gels are stable (Shan-shan et al., 2007; Zeljka Pavelic et al., 2001). In this study such as application for Diclofenac has been investigated.

Hydrogels are clinically acceptable systems that offer many advantages, such as suitable rheological properties, good tissue compatibility and convenience in handling and ease of application. Carbopol gels are approved for pharmaceutical use in several different administration routes. Cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration and they provide higher and sustained skin concentrations of drugs compared to conventional gels and creams. Moreover, carbopol gels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH (Zeljka Pavele et al., 2004; Beukelman et al., 2008).
Sodium diclofenac (DFNa, C14H10Cl2NO2Na) is a widely used nonsteroidal anti-inflammatory drug (NSAID) that exhibits antirheumatic, analgesic, osteoarthritis and antipyretic activities. It has a short half-life in plasma (1-2 hours) and only 50% of the drug reaches the circulation. The most common adverse effects of the drug are gastritis, peptic ulceration, local mucosal irritations, and depression of renal functions. Because of the short biological half-life and associated adverse effects, alternate routes other than oral as well as topical route for systemic delivery are preferable (Chuaasuwan B et al., 2009). Topical dosage forms are desirable for the chronic use of this drug, especially in the case of rheumatic symptoms. The efficacy of topical diclofenac sodium depends greatly on the capacity of the preparation to allow the drug penetrates through the skin. Since the permeability of intact skin for diclofenac sodium is low, in order to increase skin permeability, the promoting effect of ethanol on percutaneous absorption of diclofenac and the combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac have been performed. However, these approaches do not only have limited skin permeability, but also cause skin irritation as well. Stratum corneum (SC) is a main barrier of many compounds passing through the skin. Several approaches have been developed to weaken this skin barrier. One possibility for increasing the penetration of drugs and many cosmetic chemicals is the use of vesicular systems, such as liposomes and niosomes (Amnon Sintov C et al., 2006; Manosroi et al., 2008).

The aims of this study were to develop liposome enriched diclofenac sodium liposomal hydrogels for transdermal delivery, perform in vitro release studies and in vivo permeation studies through rat skin, and evaluate the efficacy of liposomal gels against inflammation induced rats. The purpose was to provide the delivery of the transdermal drug at a controlled rate across intact skin to improve bioavailability and inflammation control for longer period from liposomal gels.

Materials and Methods

Materials

Diclofenac Sodium was obtained from Alkha pharmaceuticals, Hyderabad. Carbopol 934 gifted by Genuine Chemical Co., Mumbai. Chloroform and methanol were purchased from Finar chemicals, Ahmedabad. Propylene Glycol was purchased from Qualikems Fine chemicals, Mumbai, Soya lecithin from Hi Media Laboratories Pvt. Ltd. and Carrageenan from SD Fine chemicals. Mumbai, India, The other chemicals were of analytical reagent grade.

Preparation of Liposomes

Multi lamellar liposomes (MLV) consisting of Diclofenac Sodium were prepared using the thin film hydration method. Diclofenac, Soya lecithin, Cholesterol were dissolved in mixture of chloroform and methanol (9:1) and a thin film lipid layer was obtained by evaporating the organic solvent (for 15min, at 60°C, 90rpm) using a Rotavapor (Laborota 4000, Heidolph, Germany) and water jet vacuum. The obtained thin film layer was dried overnight in a vaccum oven to ensure complete removal of organic solvent. Then the lipid film was suspended in Phosphate buffered saline (PBS) (pH 7.4) by vortexing for 10 min, and was allowed to hydrate for1 h at 70°C, 90rpm. The liposomal suspension was then centrifuged 3000rpm for 30min. The settled liposomes were resuspended in PBS. Then this suspension was subjected for sonication for 15min (3min each cycle, 5cycles, 150V/T probe) using a ultrahomogenizer to get SUVs. Temperature was maintained at around 65°C. For the current study, three batches of liposomes were prepared and the composition is shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>FORMULATIONS</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diclofenac</td>
</tr>
<tr>
<td>I</td>
<td>25mg</td>
</tr>
<tr>
<td>II</td>
<td>50mg</td>
</tr>
<tr>
<td>III</td>
<td>75mg</td>
</tr>
</tbody>
</table>

Organic solvents: Chloroform-9ml, methanol-1ml

Gel Preparation

As a vehicle for incorporation of Diclofenac Sodium liposomes for transdermal delivery a gel was made. Carbopol 934 (1g) was dispersed in demineralised water (88ml) by stirring at 800 rpm (Remi, Mumbai, India) for 60 minutes. Then, propylene glycol (10ml) was added and the mixture was neutralised by dropwise addition of 10% NaOH. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 6.5 (Zeljka Pavelic et al., 2005).

Incorporation of Diclofenac liposomes into the gel

Liposomes containing Diclofenac Sodium were mixed into the 1% (w/w) Carbopol hydrogel by an electrical mixer 25rpm for 5 min to get Diclofenac sodium liposomal gels (DSLG).

Characterization of diclofenac sodium liposomal gel (DSLG)

Physicochemical Properties of DSLG

The liposomes enriched hydrogels were characterized for their physicochemical properties such as colour, odour and pH.
Microscopy
Prepared liposomes (MLVs and SUVs) were observed under Binoculer microscope (PZR-700, Quasmo, India) at suitable magnification.

Particle size and zeta potential of liposome enriched hydrgels
The mean size, polydispersity index of the size distribution and zeta potential of liosomes was determined by photon correlation spectroscopy (PCS) using Zetasizer 3000 HAS (Malvern Instruments, Malvern, UK). The DSLGs were diluted 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was performed at 25°C with an angle of detection of 90°. Each value reported is the average of three measurements. The polydispersity index measures the size distribution of the nanoparticle population (Gande Suresh et al., 2007)

Entrapment Efficiency
Separation of unentrapped drug from the prepared liposomes was carried out by mini column centrifugation method. Liposomal suspension (0.2ml) was placed in Sephadex G-50 column (pre-saturated with empty liposomes) and centrifuged at 2000rpm for 3min. Elutes containing drug loaded liposomes were collected and observed under optical microscope to ensure the absence of unentrapped drug particles. Appropriate amount of elute was digested with chloroform-methanol (2:1,v/v) and the clear solution thus obtained was analyzed using UV Visible spectrophotometer for the drug content estimation at a λmax of 276nm. Liposomes prepared without drug were treated in similar manner and served as blank for the above study. Studies were conducted in triplicate.

%Entrapment efficiency = \[
\frac{\text{Entrained drug (mg)}}{\text{Total Drug Added (mg)}} \times 100
\]

In vitro Release Studies
In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane (Hi Media molecular weight 5000) was placed between receptor and donor compartments. Diclofenac Sodium liposomal gel (0.5ml of liposomal suspension in 0.5 gm) was applied to the skin surface in the donor side, at fixed time intervals, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible spectrophotometer at 276nm.

Preparation of skin
The abdominal hair of Wister male rats, weighing 150±25 g, was trimmed using trimmer 24 hr 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 1hr before starting the diffusion experiment. All surgical and experimental procedures were reviewed and approved by the animal and ethics review committee, Vaagdevi College of Pharmacy, Warangal, Andhra Pradesh, India.

Ex Vivo Permeation studies
A system employing improved Franz diffusion cells with a diffusional area of 4.15cm² 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. Diclofenac Sodium liposomal gel (0.5ml of liposomal suspension in 0.5 gm) 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±0.5°C and stirred at 500rpm. 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 and analyzed by UV-Visible spectrophotometer at 276nm.

Rheological Behaviour
The rheological measurements were performed on a rheometer Brookfield Programmable Rheometer LVDV-III + CP 230 equipped with a cone and plate test geometry (plate diameter 20 mm, cone angle 4°). All measurements were carried out at a temperature of 20±0.1°C. The rheological properties of the developed liposomal gel were studied by continuous shear investigations, which were performed in order to evaluate the shear rate [1/s] as a function of shear stress [Pa]. This study started applying 0Pa up to a maximum shear stress of 50Pa and the resulting shear rate was measured(Kesavan Bhaskar et al., 2009 ; Nina Dragicevic-Curic et al .,2009).

Pharmacodynamic Studies
The animals used for in vivo experiments were adult male Wister rats (150±170gm) purchased from the Mahaveer Enterprises, Hyderabad. The animals were kept under standard laboratory conditions, at 25±1°C and 55±5% relative humidity with a 12hr light/dark cycle. The animals were housed in polypropylene cages, with free access to a standard laboratory diet and water. The study was
approved by the Institutional Animal and Ethics Committee (Vaagdevi College of Pharmacy, Warangal, Andhra Pradesh, India). Guidelines of the institutional animal ethics committee were followed for in vivo experiments.

Carrageenan induced paw edema method was used to study the in vivo performance of the prepared drug delivery system. Anti-inflammatory activity was determined by measuring change in the volume of inflamed paw, produced by injection of Carrageenan (0.1ml of 1% w/v) using plethysmometer. Male wister rats selected for the study were weighed and marks were made on the right hind paw just behind tibia-tarsal junction on each animal. Thus, every time the paw was dipped in the plethysmograph (mercury displacement method) up to the fixed mark to ensure constant paw volume. Wister rats were divided into four groups including one controlled group with each group comprising of 3 animals. The paw volume was noted at 0, 1, 2, 4, 6, 8, 12 and 24hr (Fairaz Shakeel et al., 2007; Perez Grm et al., 1996).

The formulations were applied transdermally to albino rats of respective groups, excluding the animals of controlled group. The controlled group animals were injected with saline (0.9% NaCl) containing no drug. After 30min of transdermal application of formulations, 0.1ml of 1%w/v Carrageenan (in 0.9% normal saline) was injected in the sub planter region of the right hind paw of rats. The initial reading just after injection and subsequent paw volumes was measured up to 24hr. The percent inhibition of edema induced by carrageenan was calculated for each group using the following equation:

$$\% \text{ Inhibition of edema} = 100 \left( 1 - \frac{a - x}{b - y} \right)$$

Where

- $a$ = mean paw volume of treated animals after carrageenan injection
- $x$ = mean paw volume of treated animals before carrageenan injection
- $b$ = mean paw volume of control animals after carrageenan injection
- $y$ = mean paw volume of control animals before carrageenan injection

Fourier Transform Infrared (FTIR)

Diclofenac Sodium, Soya lecithin and diclofenac liposomes were subjected to FTIR analysis so as to predict if there is any interaction is possible between the drug and the polymer.

Results and Discussion

Characterization of diclofenac sodium liposomes and DSLG

Physicochemical properties

The liposome suspensions were white in colour, odourless and fluid in nature. Gels loaded with liposome suspensions were colourless, odourless with smooth appearance.

Microscopy

All the batches of the liposomes prepared were viewed under binocular compound microscope. Figure (1) shows the microscopic view of diclofenac multilamellar vesicular liposomes. The SUVs incorporated into the gels were of nanorange size and could not be seen ever under 100X of the microscope.
**Particle size and Zeta potential**

Particle size, Polydispersity index (PDI) and Zeta potential values of the in DSLGIII was found to be 230.1±4.2nm, 0.247±0.032 and -41±2.16 (mean±S.D(n=3)).

**Entrapment Efficiency**

The Entrapment Efficiency values were determined for three three batches of liposomes were observed as 58±0.545%, 60±0.62% and 62±0.75% for I, II and III respectively.

**In vitro Release Studies**

The cumulative amount release of DS from DSLGI, DSLGII, DSLGIII was investigated for a period of 24hr; each sample was analyzed in triplicate. Figure (2) shows the in vitro release profile of DSLGs. DSLGI, DSLGII, DSLGIII could prolong the drug release by the fact that the drug molecules are entrapped in the lipid matrix. The amount of drug release at the end of 24 hr in DSLGI, DSLGII and DSLGIII formulations was found to 1.42, 3.52 and 5.83mg respectively. All the Liposomes enriched gel formulations showed controlled drug release and also an increase in release rate was observed after 24hr. The log percent cumulative drug released was plotted as a function of log time and the slope of the curves was determined as the values of diffusional release exponent (\( \eta \)). The values of diffusional release exponent (\( \eta \)) from the straight lines were noted to be 1.062, 0.636 and 0.495 in formulations of DSLGI, DSLGII and DSLGIII respectively, which showed that the release of drug from formulations followed a non-Fickian pattern. From the cumulative amount drug released versus time plot, the slope values were determined as release rate constants. The release rate constants were 0.065, 0.145 and 0.221 mg/cm²/hr for DSLGI, DSLGII and DSLGIII respectively. Thus the formulations with higher drug content shows higher release rate constants. The slower release of drug from Liposomes enriched carbopol gel maintained the drug concentration for longer period of time. Burst releases as well as sustained release both are of interest for dermal application. Burst release can be useful to improve the penetration of drug. Sustained release supplied the drug over a prolonged period of time.

**Ex Vivo Permeation studies**

The cumulative amount permeated of DS from DSLGI, DSLGII and DSLGIII were investigated for a period of 24hr; each sample was analyzed in triplicate. Figure (3) shows the ex vivo permeation profile of DSLGs of DSLGI, DSLGII and DSLGIII. Cumulative amount of drug permeated in 24 hr were 327.51, 720.04 and 1176.7µg/cm² for DSLGI, DSLGII and DSLGIII formulations respectively. The release kinetics was established by determining the diffusional release exponent from the plot of log of cumulative drug permeated versus log time. This plot yielded a straight line from which diffusional release exponent (\( \eta \)) were calculated and found to be 0.868, 0.663 and 0.555 in Liposomal gel formulations of DSLGI, DSLGII and DSLGIII respectively, which showed that the release of drug from these formulations followed a non-Fickian pattern.

**Rheological Behaviour**

The rheological status of a semisolid drug carrier system is a very important physical parameter. Rheology measurements provide essential information about different aspects concerning semisolid preparations. Concerning application and performance on skin they provide essential information. Furthermore, drug release from semisolid vehicles is influenced by the rheological behaviour. The rheological behaviour of hydrogels loaded with DSLG was evaluated and the flow curve of the gel was shown in Figure (4).

**Pharmacodynamic Studies**

The in vivo performance of selected DSLGIII hydrogel were carried out using carrageenan-induced rat paw edema method. Formulations DSLGIII under study not only decreased the inflammation to the larger magnitude, but also sustained this magnitude. In DSLGIII formulation the maximum inhibition was observed at 6th hr with higher value 83.65%, and even after 24hr, 36.6% inhibition was observed. However, in case oral administration, inhibition was displayed at 1hr with magnitude of 82.5% and just after 4hr it scored below 50% and in case DS gel administration, inhibition was displayed at 2hr with magnitude of 78% and just after 6hr it scored below 46% (Figure 5). The possible reason could be the drug concentration in the blood, which was maintained for longer duration in case formulation DSLGIII in comparison to the drug administered orally and gel form. In comparison to orally administered DS and DS gel, the formulations DSLGIII which was applied transdermally gave good results. The maximum inhibition for DSLGIII was observed at 6hr and the inhibition was maintained up to 12hr and also even after 24hr inhibition was observed. The anti-inflammatory activity of the formulation (DSLGIII) was maintained for longer period of time due to slow release of the drug. This was attributed to gel structure and the surface active properties of the gel.

**Fourier Transform Infrared (FTIR)**

To see if there is any probable interaction between the drug and the lipids, the samples of pure drug, Soya lecithin and diclofenac loaded liposomes were analyzed by the FTIR. The reports revealed by the FTIR study confers that there is no interaction between the lipids and drug.
**Fig. 2** In Vitro Drug Release Profiles from Various Formulations.

**Fig. 3** Ex Vivo Drug Permeation Profiles from Various Gel Formulations.
Conclusion
This study reveals that Liposomal gels were not rapid and fast, but the effect was maintained for prolonged periods. We conclude that liposomal gels can be successfully utilized for the sustained delivery of Diclofenac via the Transdermal route.

Acknowledgements
The authors would like to thank principal and management of Vaagdevi College of Pharmacy for providing necessary facility useful in conduction of this work.
References


