Development of Celecoxib Transfersomal gel for the Treatment of Rheumatoid Arthritis

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ABSTRACT

The aim of the present study was to investigate the potential of Transfersomal gel formulations for transdermal delivery of Celecoxib and to evaluate the effect of concentration of Soya PC and Sodium deoxycholate. Transfersomal vesicles containing Soya PC mixed with Sodium deoxycholate and Celecoxib were prepared by conventional rotatory evaporation (Film hydration method) and characterized for various parameters including vesicle shape, size and size distribution, surface morphology, entrapment efficiency, in-vitro and ex-vivo drug release and in-vivo anti-inflammatory activity. Vesicles were also evaluated for skin irritation study and permeation studies. Results of all the studies suggested that Celecoxib Transfersomal gel formulation was therapeutically effective drug delivery system for treatment of Rheumatoid Arthritis.

Introduction

The term Rheumatoid Arthritis (RA) was first given by “Sir Alfred Baring Garrod” in 1859. RA is a chronic auto-immune disease that causes pain, stiffness, swelling and limit function of many joints that can cause disability and have a negative socio-economic impact. Many DMARDs (Disease modifying anti-rheumatic drugs)[1]. Cytotoxic drugs and NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) are commonly used for the treatment of RA. NSAIDs are commonly used to reduce pain and inflammation caused in RA. Celecoxib is a selective Cyclo-oxygenase-2 (COX-2) inhibitor that has been recommended orally for the treatment of RA. But, long-term oral administration of Celecoxib may result in serious gastrointestinal side effects [2-3]. To overcome the gastrointestinal side effects it is needed to develop a safer formulation of Celecoxib that increase both skin permeation and amount of drug delivered to skin for better therapeutic effect for longer duration. But, presumably due to its poor solubility in hydrophilic media Celecoxib is unable to penetrate the skin sufficiently to permit clinically useful transdermal application. Hence, an improved Celecoxib formulation with higher skin permeation efficiency could be useful to relieve the pain of local skin and joint inflammation. Drug permeation across the stratum corneum barrier can be enhanced by augmentation of the skin permeability and secondly by activation of concentration independent transport–driving forces. Some topical drug delivery systems use chemical penetration enhancers and solvents which may cause skin irritation especially in chronic applications. It is therefore desirable to develop a vesicular carrier system with higher penetration efficiency to overcome all these problems. Recently the drug carrier systems such as lipid vesicles (liposomes and Proliposomes) and nonionic surfactant vesicles (Niosomes and Proniosomes) are gaining importance owing to their ability to act as a means of sustained release of drugs[4-7]. Celecoxib is one of the most selective specific Cox-2 inhibitor drug. It is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of rheumatoid arthritis. Due to specific...
COX-2 inhibition inflamed tissues could be targeted without disturbing the homeostatic functions of prostaglandins in non-inflamed organs and it also preserves the anti-inflammatory efficacy without causing the associated toxicities of NSAIDs. The rate of absorption of Celecoxib is moderate when given orally and peak plasma drug concentration occurs after 2 to 4 hours [8]. To overcome this problem, Transfersomal gel approach is applied. These self-optimized aggregates, with the ultra flexible membrane, are highly adaptable, stress responsive, complex aggregate which are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. Transfersomes are several orders of magnitude more elastic than the standard liposome’s and thus well suited for the skin penetration. These are usually composed of phospholipids and an edge activator. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. This carrier system is preferred over other carrier systems as it is a biodegradable, biocompatible and non-toxic carrier with better potential for skin permeation [9-12]. This paper describes the Transfersomal gel carrier system which is efficient for the delivery of Celecoxib for Rheumatoid Arthritis treatment. Transfersomes are incorporated into hydrogel system using Carbopol-940 gel because it acts as a dermal vehicle and this gel is used to have a proper semisolid consistency to facilitate convenient transdermal application.

Materials and methods

Celecoxib was provided as a gift sample from Synmedic Laboratories (Faridabad, India). Soya phosphatidylcholine was purchased from Himedia, India. Sodium deoxycholate and Sephadex G-50 were purchased from Otto, India. Carbopol-940 purchased from Burgoyne, India. Methanol, chloroform, n-Octanol and all other reagents and solvents were of analytical grade.

Preparation of Transfersomes

Transfersomes containing Celecoxib were prepared with slight modification [13-15]. First, Phospholipid (85mg), Sodium deoxycholate (15mg) and the drug Celecoxib (1mg) were taken in a clean, dry, round bottom flask, and then lipid mixture was dissolved in 3:1 v/v Chloroform : Methanol. The organic solvent was evaporated until complete dryness by hand shaking method. The deposited lipid film was hydrated with PBS (pH 7.4) by rotation in reverse direction for 1 hr. at room temperature and the resulting vesicles were swollen for 2 hr. at room temperature to get large multi-lamellar vesicles (LMLV). LMLV were then sonicated using (Bath Sonicator) for 15 min. to obtain Transfersomes of nanometric size range. These were further centrifuged through Sephadex G-50 minicolumn at 2000 rpm for 3 min. for separation of free unentrapped drug.

Incorporation of Transfersomes in Carbopol-940 gel

The Carbopol-940 gel was prepared as reported. Carbopol resin 940 (1 g) was dispersed in distilled water (60gm). The mixture was stirred until thickening occurred. After complete dispersion, the solution was kept in dark for 24 hrs. for complete swelling of Carbopol-940. Then PEG-400 (10g) was added slowly into the aqueous dispersion of Carbopol-940. Then, other ingredients such as isopropyl alcohol (IPA) 10g, polyethylene glycol (PG) 10g and triethanolamine (TEA) 0.5g were added. Distilled water (q.s.) for 100g of gel was also added to get homogeneous dispersion of gel. Transfersomes dispersion was incorporated into carbopol gel [16]. Finally, transfersomal dispersion (free from unentrapped drug) was mixed into dermal vehicle (Carbopol-940 gel) by using a mechanical stirrer (Remi Instruments, Mumbai, India) for 5 min.

Vesicle Characterization and Morphology

Transfersomal formulation was characterized for various parameters like size and size distribution, visualization by transmission electron microscopy (TEM).

Morphological characterization by TEM: Negatively stained samples were prepared by placing a drop of Transfersomal dispersion on a carbon-coated 300-mesh copper grid. The suspension was left to adhere on the carbon substrate for about 2 min., to allow its absorption in carbon film and the excess liquid was drawn off with the filter paper. Subsequently a drop of 2% (w/v) aqueous solution of uranyl acetate was applied for 35 seconds for contrast enhancement and again the solution in excess was removed by the tip of filter paper. The sample was air-dried and observed under the Transmission electron microscope (PU Chandigarh) at 90kV.

Vesicle Size and polydispersity index

Vesicle size, size distribution and zeta potential were determined by (Zeta sizer 2000, Malvern UK). For size measurement Transfersomal gel was dispersed into distilled water with the help of cyclo-mixer to get a homogeneous dispersion. Dispersion was put into cuvettes of Zeta sizer and measured the size of Transfersomes and polydispersity index in triplicate.

Measurement of entrapment efficiency

For determination of entrapment efficiency of Transfersomes separation of unentrapped drug was done using mini-centrifuge column of Sephadex G-50 and then evaluation of entrapment efficiency was done by rupturing the vesicles in n-Propanol and then analyzed spectrophotometrically by measuring absorbance at 252 nm using UV spectrophotometer (UV-Labindia, USA) for drug content. % entrapment efficiency was calculated according to the equation [17-19].

\[
\text{Drug entrapment efficiency (\%) } = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug used}} \times 100
\]
Measurement of elasticity of Transfersomes
Degree of deformability is an important and unique parameter of Transfersomal formulations because it differentiates Transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum. The deformability of Transfersomes was determined by extrusion method [20]. Transfersomal formulations were passed through a pore of known size (50 nm) at a constant pressure for 5 minutes. Vesicle size and size distributions were noted after each pass by digital microscopy. The degree of deformability was calculated by using the following formula:

\[ E = J \times \left( \frac{v}{r_p} \right)^2 \]

where, 
- \( E \) = elasticity of vesicles membrane
- \( J \) = amount of suspension extruded in 5 min.
- \( r_v \) = vesicle size
- \( r_p \) = pore diameter.

Counting of number of vesicles per cubic mm
Transfersomal formulation was diluted five times with 0.9% of NaCl solution, and the number of vesicles per cubic mm was counted by optical microscopy by using a haemocytometer. The vesicles in 80 small squares were counted and calculated by using the following formula:

\[ \text{Total no. of vesicles per cubic mm} = \frac{\text{Total no. of vesicles counted} \times \text{dilution factor} \times 4000}{\text{Total no. of squares counted}} \]

In Vitro drug release studies
For drug release studies, 2 ml of drug loaded Transfersomal gel/ simple gel were put into donor compartment. Dialysis membrane was mounted between donor and receptor compartment of the Franz diffusion cell with an effective diffusion area of 2.26 cm² and a cell volume of 25 ml. Initially the donor compartment was empty and the receptor compartment was filled with methanolic phosphate buffer saline (methanolic PBS), pH 7.4 (30:70%, v/v). The diffusion cell was maintained at 37°C and receiver fluid was stirred continuously using a magnetic stirrer at a speed of 100 rpm. The formulation was gently placed in the donor compartment. The samples were withdrawn periodically for 24 hours from the receptor compartment while following each sampling the withdrawn volume was replaced with equal volume of PBS (pH 7.4) in order to maintain the volume and sink condition. The samples were then analyzed spectrophotometrically at 255 nm for presence of the drug [16]. Each preparation was studied in triplicate and the result of each preparation is the average value of three experiments.

In Vivo studies
Anti-inflammatory activity
Approval to carry out in vivo studies was obtained from the animal ethical committee of the Rajendra institute of technology and sciences, Sirsa, India. All animal studies were performed according to the guidelines compiled by the ethical committee CPCSEA (Committee for the Purpose of Control and Supervision of experiments on animal, Ministry of Culture, Government of India).

Young male wistar rats were used for the study and the rats were maintained under hygienic condition and supplied with a standard laboratory diet and water ad libitum and monitored on a regular basis. The experimental rats were then divided into three groups, each carrying six animals. The anti-inflammatory and sustaining actions of the optimized formulation were evaluated by the Carrageenan-induced hind paw edema method developed by winter et al. in wistar rats. The paw volume was measured at 0.5, 1, 2, 3, 4, 6, 12, 24 hr after Carrageenan injection using Plethysmometer and expressed as edema hind paw volume (ml) relative to the initial hind paw volume. Decrease in the amount of paw swelling was determined from time to time and effect produced by each formulation treated group was calculated against the respective control group [22].

Confocal Laser Scanning Microscopy
Confocal laser scanning microscopy (CLSM) is an imaging technique which offers the possibility of visualizing the distribution of fluorescent probe (Coumarin-6) in sample by optical sectioning at high resolution and without cryo-fixation or embedding the tissue. For CLSM studies, Coumarin-6 loaded Transfersomes were prepared using the same method as discussed previously. Unentrapped dye was removed by mini column centrifugation method. The abdominal hair of albino rats (Wister strain), weighing 150 - 200 g, was shaved.
using a hand razor without damaging the skin surface. For each formulation one animal was used. First, the Transfersomal gel loaded with the Coumarin-6 and Carbopol 940 gel containing dye were formulated. Animals were then treated topically with fluorescent lable formulations for 6 hrs and after 6 hrs rats were sacrificed, treated skin was dissected and fixed and evaluated for Coumarin-6 penetration for above prepared formulations under the CLSM (Olympus FV-1000) [23]. The excitation wavelength was 430 nm and a 485 nm long pass filter was used for emission.

Hence, using this technique penetration of molecules into skin can be reported, and it is a valuable method for identifying transport pathways as evidenced by hotspots of fluorescence intensity. In other words localization and the permeation pathways of a fluorescent model compound can be studied using CLSM technique.

Skin irritation study

The skin irritation was done as a test of product safety. The animal albino wistar rats were used for this study. The back of animals were clipped free of fur with a hand razor exposing a bare patch of skin at least 24 hr before application of the sample. The rats were divided into three groups (n=3) as follows: Group 1: Standard irritant (0.8% v/v Formalin), Group 2: Carbopol-940 Gel entrapped drug (Standard), Group 3: Transfersomal Gel (Celecoxib) and formulations were topically applied and spread uniformly with a glass rod [24-25].

The treated area of skin was observed for any visible changes such as erythema/edema at 24, 48, 72 hrs post-treatment and the primary irritation index was calculated. The scores for each animal was added and divided by the total no of animals to give the primary irritation index. Primary irritation index was characterized by score and response category.

Figure 1: TEM image of Transfersomes

In vitro drug release study

The In vitro drug release studies gives an idea about the release pattern of the drug from the vesicles. Release studies were carried out in methanolic PBS (30:70%, v/v). 87.91% entrapped drug was released from the transfersomal gel within 24 hrs (Figure 2).

Result

Preparation and characterization

The present work was aimed at preparing transfersomes, which were incorporated into Carbopol-940 gel for transdermal delivery of Celecoxib for the treatment of Rheumatoid arthritis. Transfersomes were prepared by the conventional film hydration method. The composition of transfersomes was Soya PC: Sodium deoxycholate (85: 15) and drug amount 1 mg. The size and polydispersity index of transfersomes were 100.2 ± 0.7 and 0.219 ±0.001 respectively. Polydispersity index showed the uniformity of preparations. The % entrapment efficiency (EE) of Celecoxib in transfersomes was found to be 66.29 ± 0.64. Entrapment efficiency decreased with decrease in surfactant amount this is due to the possible decrease in solubilization of drug due to the limiting amount of surfactant and hence decreased entrapment efficiency. Sonication time also affected the vesicle size when it was increased from 10 to 15 min. vesicle size decreased but when it was further increased to 20 min. vesicle size decreased and resulted into possible aggregate formation and rupture of vesicles which resulted in decreased entrapment efficiency. The TEM photomicrographs of Blank and drug loaded transersomal gel formulation confirms its shape. For ease of dermal application transfersomal dispersion was incorporated into a gel base. Pavelic et al. (2001) reported that transfersomal vesicles are compatible with polymers derived from poly (acrylic acid) polymer, such as Carbopol. Hence in the present study, transfersomes were incorporated into Carbopol-940 gel and Ex vivo skin permeation studies and In vitro release kinetics of the drug entrapped formulation was studied. Carbopol-940 was chosen for gel preparation because of its biodhesive property and it also retains for longer time at the site of administration.
**Figure 2: Comparison of *in vitro* drug release profile of transfersomal gel and simple Celecoxib gel formulation**

*In vitro skin permeation study*

The goal of permeation study is to compile a kinetic profile that reflects the concentration of active ingredient changes in time as it diffuses through the skin. The studies were performed on hairless abdominal skin of rat using the Franz diffusion cell. A significant increase in the skin permeation of Celecoxib was recorded from Transfersomal gel in comparison to simple Celecoxib gel. The permeation parameters were calculated by plotting a curve between cumulative amounts of drug permeated per unit area ($\mu$g/cm$^2$) vs time. The flux was obtained from the slope of the linear portion of graph. Transdermal flux was more for transfersomes than simple celecoxib gel. Permeation coefficient was found to be 0.0131 ± 0.00026 for transfersomal gel formulation and 0.0160 ± 0.00123 for simple CXB Gel.

Skin permeation efficiency of the Transfersomal formulation was determined by CLSM study. Both the formulations permeated through the skin but the penetration efficiency was greater in case of transfersomal gel loaded with Coumarin-6 marker. It was confirmed by bright fluorescent intensity of Transfersomal gel formulation. Hence, Transfersomal gel formulation may be more effective in the treatment of Rheumatoid arthritis.

**Figure 3: CLSM image of skin optically scanned at different increments through the Z-axis of CLS microscope showing fluorescence up to a depth of 39.89 µm on application of Carbopol-940 gel (a) containing Coumarin-6 dye and TG containing Coumarin-6 dye(b) at an excitation/emission of 430-485 nm.**

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Table 1: Permeation parameters of Transfersomal Gel & Simple Celecoxib Gel formulation

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Transdermal flux (Jss) (µg/cm²/hr)</th>
<th>Permeation coefficient (cm²/hr)</th>
<th>Enhancement ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfersomal Gel</td>
<td>32.19 ± 2.38</td>
<td>0.0160 ± 0.00123</td>
<td>1.22 ± 0.11</td>
</tr>
<tr>
<td>Simple Celecoxib Gel</td>
<td>26.27 ± 0.62</td>
<td>0.0131 ± 0.00026</td>
<td></td>
</tr>
</tbody>
</table>

All the values are given as mean ± SD, n = 3

Skin irritation study

The skin irritation was done as a test of product safety. Transfersomal gel of Celecoxib should be non-irritant, so it was tested for skin irritation on wistar albino rats. The effect of formulation, simple CXB gel and standard irritant (0.8% v/v Formalin) on rat skin was observed after 24, 48 and 72 hrs. It was concluded from the skin irritation studies that Transfersomal gel formulation can be considered safe for topical application. When standard irritant was applied moderate skin irritation was produced, as shown by the primary irritation scores. Hence, prepared transfersomal formulation is non-irritant to skin.

In vivo anti-inflammatory activity

In vitro anti-inflammatory activity of the transfersomal gel and Simple celecoxib gel formulation was tested by using Carrageenan induced hind paw edema method developed by winter et al. in wistar rats. Dose of Celecoxib was calculated according to weight of rats. Rats were observed for 24 hrs for any change in paw volume. Transfersomal gel formulation showed maximum anti-inflammatory effects. It may be due to better skin penetration and drug release rate than simple Celecoxib gel formulation.

Stability studies of transfersomes

The storage stability studies were evaluated by measuring the changes in mean vesicle size and % entrapment efficiency of three batches of same formulation after three months of storage in refrigerator (4 ±1 °C) and room temperature (25 ± 2 °C) in sealed glass ampoules. % entrapment efficiency of the drug in the transfersomal gel formulation was determined after 10, 20, 30, 60 and 90 days to know the amount of drug leaked out. % entrapment efficiency and increase in vesicle size was more in the formulations those were stored at 25 ± 2 °C than at 4 ± 1 °C. At 4 ±1 °C there were no significant changes (p>0.05) in vesicle size of transfersomal gel as compared to initial vesicle size. While at 25 ± 2 °C increase in vesicle size was significant (p<0.05). Thus transfersomal gel formulation is more stable at 4 ±1 °C. Leakage of drug was minimum in gel formulation at all the temperature. It may be due to high viscosity of the Carbopol-940 gel base as it retarded movement and fusion of vesicles which were responsible for drug leakage.

Conclusion

The above overall results of present investigation showed that Celecoxib transfersomal gel formulation is therapeutically effective transdermal drug delivery system for the treatment of Rheumatoid arthritis. Transfersomal gel formulation differ from Simple Celecoxib gel as it possess better skin permeation efficiency and stability due to simultaneous presence of different stabilizing (phospholipids) and destabilizing (surfactant) molecules having tendency to redistribute in bilayers. Hence, it can be concluded from the results that transfersomal gel of Celecoxib possessed better skin permeation potential, better stability and higher entrapment efficiency, easy to scale up and ability as a self penetration enhancer.

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Conflict of interest statement

We declare that we have no conflict of interest.

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