Research Paper

Novel Anti-Acne Drug Delivery System of Tretinoin

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Abstract

The objective of the present study was to formulate and evaluate Tretinoin niosomal gel and to carry out comparative skin irritation study with conventional Tretinoin solution and Tretinoin conventional gel. Topical Tretinoin (0.25%, 0.05%) has been a reliable treatment of acne vulgaris since 25 years but its major drawback is that it causes skin erythema on the applied area. The niosomol dispersion was prepared using different grades of non-ionic surfactants and cholesterol in different ratios along with Tretinoin. The transmission electron microscopy revealed that the niosome vesicles were of LUV type and polyhedral shape. The niosome vesicles prepared with SPAN™ 60 and cholesterol (20:1) showed maximum entrapment efficiency (53.77±1.57%) and minimum vesicle diameter (1.55±0.13 µm). The prepared niosome vesicles were incorporated into Carbopol® 971 gel base to prepare Tretinoin niosomal gel (0.05%). The stability study was carried out at different accelerated and non-accelerated conditions. The In-vitro diffusion study carried out using sigma dialysis membrane showed sustained release pattern of Tretinoin from niosomal gel. The comparative skin irritation study carried out on 18 healthy Wistar rats of either sex showed remarkable decrease in signs of skin erythema, lesions and scaling caused by Tretinoin.

Keywords: Tretinoin, Acne, Niosomes, Transmission Electron Microscopy, Diffusion Study, Skin Irritation Study

1. Introduction

The ideal drug delivery system delivers active moiety at the required rate as per the need of the body over the treatment time period to the site of action (Patel et al, 2007). At present there is no drug delivery system available for Tretinoin that achieves site specific delivery and control release kinetics of drug in predictable manner. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants of the alkyl or dialkyl polyglycerol ether class, with or without incorporation of cholesterol (Malhotra & Jain, 1994). It is established that topical and transdermal drug delivery system with niosomes appears promising for hydrophobic and amphiphilic drug molecules (Namdeo et al, 1999). The preparations containing niosomes give significantly higher epidermal and dermal concentration, protection from degradation and also act as sustained release system which decreases the irritation and sensitivity of certain drugs on the skin.

Topical treatment is widely useful in topical skin infections confined to the stratum corneum, squamous mucosa etc. Such diseases include acne, dermatophytosis, candidiasis, tinea nigra and fungal keratitis. The most commonly used anti-acne drugs for local action are Benzoyl peroxide, Clindamycin, Clarithromycin, Azithromycin, Erythromycin, Tretinoin and Isotretinoin (Webster, 1996). Topical Tretinoin works by both comedolysis and by normalizing the maturation of follicular epithelium so that comedo formation ceases (Webster, 1998). Griffiths et al (1993) studied the events in tretinoin-induced comedolysis in the rhino mouse model. Tretinoin produces irritation on applied skin so it is hypothesized that niosomal form of Tretinoin can overcome this drawback.

The aim of the present study was to encapsulate Tretinoin in niosome vesicles and further incorporate it into the Carbopol® 971 gel. The niosome vesicles were prepared...
using SPAN™ 60 as a non-ionic surfactant and cholesterol as a vesicle stabilizer. The Tretinoin niosomal gel, Tretinoin drug solution and Tretinoin gel were compared for In-vitro diffusion study and skin irritation study. The Tretinoin niosomal gel was also studied for stability study at different accelerated and non-accelerated conditions.

2. Materials and Methods

2.1. Materials

Tretinoin (TRT) was obtained as a gift sample from Solmag, Italy. SPAN™ 60 was obtained as gift sample from Croda chemicals Ltd, India. Cholesterol and dialysis membrane were purchased from Merck, USA and Sigma Aldrich, USA respectively. Carbopol® 971 gel base was purchased from Lubrizol advanced materials, USA. All other chemicals and solvents were of analytical reagent grade.

2.2. Formulation of Different Dosage Forms of Tretinoin

a) Formulation of Tretinoin Niosomes

The niosome vesicles were prepared by thin layer hydration technique using conventional rotary flask evaporation method (Table 1). (Azmin et al, 1985) SPAN™ 60, cholesterol and TRT at a ratio of 20:1:1 by weight (Sartorious digital weighing balance, India) were dissolved in 20 ml of methylene dichloride in 250 ml round-bottomed-flask. The flask was fitted onto a rotary flask evaporator (Buchi rotovapor R-210, India) and connected to a vacuum pump. The solvent system was evaporated under vacuum till the thin film was achieved. The flask was kept with desiccants over night to completely remove the traces of solvents. The dried film was hydrated with adequate amount of distilled water using bath sonicator (PCI Labs, India). The hydrated dispersion was kept in refrigerator (Kelvinator, India) for annealing of vesicles.

Table 1. Optimized Process Parameters For Thin Layer Hydration Technique

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ionic surfactant</td>
<td>SPAN™ 60</td>
</tr>
<tr>
<td>Surfactant: cholesterol ratio</td>
<td>20:1 by weight</td>
</tr>
<tr>
<td>Film formation time</td>
<td>10 min</td>
</tr>
<tr>
<td>Hydration volume</td>
<td>50 ml</td>
</tr>
<tr>
<td>Hydration temperature</td>
<td>50°C</td>
</tr>
<tr>
<td>Hydration time</td>
<td>45 min</td>
</tr>
<tr>
<td>Percentage drug entrapment</td>
<td>53.77±1.57%</td>
</tr>
<tr>
<td>Vesicle mean diameter</td>
<td>1.55±0.13 µm</td>
</tr>
</tbody>
</table>

b) Formulation of Tretinoin Niosomal Gel

Carbopol® 971 (1.0 g) was dispersed in 10 ml and added to 50 ml of niosomal dispersion to prepare the gel. The final weight of gel was adjusted to 100 g with addition of required amount of distilled water to get the 0.05% final strength of the gel. The pH of the gel was adjusted between 5.0-5.5 (Eutech pH 5, India). The gel was transferred to collapsible tubes and stored at 2-8°C in refrigerator.

c) Formulation of 0.05% Tretinoin Solution

TRT (50 mg) was added in 14.0 g of cremophor RH40 with continuous stirring. The Butylhydroxytoluene was accurately weighed 0.05 g and dissolved in 15.0 g of propylene glycol. The above both solutions were mixed together and added in 70.0 g of purified water. After stirring for some time clear yellow colored solution was obtained. The solution was stored in PE bottle at 2-8°C in refrigerator (Buhler, 1998).

d) Formulation of 0.05% Tretinoin Conventional Gel

TRT (50 mg) and cremophor RH40 (1.0 g) were added in ethanol containing appropriate amount of Butylhydroxytoluene. The Carbopol® 971 (1.0 g) was suspended in required amount of purified water and was allowed to swell for some time. Above both parts were mixed together and final volume make up was done using purified water to get 0.05% strength of the gel (Buhler, 1998).

2.3. Evaluation of Different Dosage Forms of Tretinoin

2.3.1 Microscopic Examination and Vesicle Size Analysis

The niosome vesicles were viewed under microscope to observe vesicle shape and lamellarity (Olympus B 201 Microscope). The niosome samples were evaluated for the Transmission electron microscope (Philips) by negative staining method using Uranyl acetate. Niosome vesicle diameter and zeta potential were determined by Malvern particle sizer.

2.4. Percentage Drug Entrapment

To determine percentage drug entrapment of TRT in niosome vesicles, 5 ml of niosomal dispersion was centrifuged at 15,000 rpm for 1 hour at 4°C in a centrifuge tube. The pellet settled at the bottom was collected and the supernant liquid were collected separately. The supernant liquid containing free unentrapped drug was measured using UV-visible spectroscopy method. The back calculation (Equation 1) was made to determine the entrapped drug remained
in the pellet. The percentage drug entrapment was calculated as per Equation 2.

2.5. In-vitro Diffusion Studies

Sigma dialysis membrane of 200 µm thickness was hydrated with pH 7.4 phosphate buffer saline for 24 hours before use. A modified Franz diffusion cell was used to carry out the diffusion study (Armengol & Estelrich, 1995 and Bhatia et al, 2004). The donor compartment contained TRT niosomal gel and recipient compartment consisted of phosphate buffer saline pH 7.4. Sampling was done from receptor compartment at regular time interval and equal volume of fresh phosphate buffer saline was replaced in the receptor compartment (Domecq et al, 2001; Bhalerao & Raje, 2003 and Garg et al, 2007). The amount of TRT present in the sample was determined by UV-Visible spectrophotometer (UV 1700, Shimadzu, Japan).

2.6. Stability Studies

The niosomal gel was packed in aluminum tubes and sealed properly. The required numbers of tubes were charged at different accelerated and non-accelerated conditions like 2-8°C, 25±2°C and 40±2°C for six months. The samples were withdrawn after each week and analyzed for percentage drug retained in niosome vesicles. (Franz,1975; Keshary & Chien, 1984; Spruance et al, 1984; New, 1990 and Jaafari et al, 2009).

2.7. Skin Irritation Study

The study was carried out with permission of animal ethical committee (reference number 197/99/CPCSEA). Total 18 healthy wistar rats of either sex having average weight of 3.5 kg were selected for study. The neck skin (4 cm² area) was shaved carefully (Dreher et al, 1996; Law & Shih, 2001; Brain et al, 2002 and Khan et al, 2005). The animals were divided into two equal groups. The adequate amount of TRT niosomal gel was applied to shaved skin area of one group. Same way, TRT conventional gel was applied to the shaved skin of other group which served as control. Both the formulations of same strength (0.05%) were applied on shaved rat skin for the determination of irritation characteristics. The applied area was covered by cotton and bandage. The visual observations were carried out at regular intervals of 12, 24, 48 hours for various symptoms such as scaling, lesions and erythema. The symptoms, lesions and erythema were graded as 3=severe, 2=moderate, 1=mild and 0=absent. The scaling was graded as 1=present, 0=absent.

3. Results and Discussion

3.1. Niosome Preparation

It is reported in some research works that niosome vesicle can be formed if the HLB value of non-ionic surfactant is between 4.0 to 8.0. Thus, in current experiment, SPAN™ 60 (sorbitan monostearate) was selected as non-ionic surfactant as its HLB value is 4.7. The phase transition temperature of SPAN™ is very high as compare to other non-ionic surfactants (around 50°C) and thus the stability of prepared vesicle is high as compare to others (Yoshioka et al, 1994). The SPAN™ has HLB value 1.8 whereas TWEEN® 80 has HLB value around 15.0 and thus both the non-ionic surfactants were rejected. The surfactant:cholesterol ratio is very important to be optimized because the cholesterol acts as stabilizer and itself is lipophilic in nature, so increased concentration of cholesterol causes reduction in percentage drug entrapment of lipophilic drug. Thus, the optimum concentration of cholesterol is needed.

The solvent system plays a major role in thin film formation because, it is necessary that the film must be thin enough otherwise sufficient hydration will not take place in hydration step. Mainly two solvents were tried ethanol and methylene dichloride in various proportions. The ethanol containing film showed stickiness and hence it remained unhydrated, whereas the film formed using methylene dichloride was easy to hydrate with warm water. The solubilization capacity of methylene dichloride was also found to be much higher than ethanol thus it is also cost effective.

Film formation time also plays major role in niosome vesicle formation because if the film is thin enough then the uniform vesicles will be formed whereas the thick film is not easy to hydrate and hence the vesicles formation will not occur properly. The film should be non-sticky easy to hydrate. Various factors affect the film quality like surfactant nature, solvent system, applied vacuum, flask size etc.
The hydration volume was also optimized in order to ensure the complete hydration of the thin film in rotary flask. The insufficient hydration leads to decreased percentage drug entrapment. But more hydration volume leads to dilution of the dispersion.

The hydration temperature was also optimized because it can affect the drug properties. The higher temperature may lead to degradation of drug whereas at the lower temperature, hydration of thin film will not occur properly. Ideally, the hydration temperature should be close to the phase transition temperature of the non-ionic surfactant and hence in current study, 50°C temperature was selected as hydration temperature.

The Figure 1 shows the photograph of negatively stained niosome vesicles by Uranyl acetate under Transmission electron microscope (TEM). The vesicle is of polyhedral shape and moreover; the vesicle wall is thick. Hence, we can state that vesicles are non-leaky and stealth.

3.2. Microscopic Examination and Vesicle Size Analysis

The Figure 2 shows the photograph of niosomes captured under conventional microscope at 40x magnification. The vesicles are of different size ranging from 1-3 µm. The shape and size of the vesicle also depends on the temperature applied during time of hydration. The high input of energy in form of temperature or sonication can further reduce the size of niosome vesicles. The thick vesicle indicates the more entrapment of lipophilic drug in the niosome vesicles as the lipophilic part gets entrapped in the vesicle wall. The Transmission electron microscopy (TEM) in Figure 2 reveals the actual shape (polyhedral shape) of the vesicles. The vesicle walls are found to be thick and hence the vesicles are predicted to be non-leaky and more stable.

3.3 Vesicle Size Analysis

The size of the vesicles was found to be 1.55±0.13 µm. The zeta potential of the niosomal dispersion was found to be -20 mV.

3.4. In-vitro Diffusion Study

The diffusion studies show the burst release effects of drug from TRT solution and TRT conventional gel (99.98% and 98.95% within 6 hours respectively), while the TRT niosomal gel shows the sustained release pattern over the treatment period. Figure 3 shows that the release pattern of TRT from the niosomal dosage form is of controlled and sustained manner. There were no signs of burst release of TRT from niosomal gel hence this characteristic of niosomal dosage form can lead to decrease sudden drug exposure to the naked skin. In niosomal gel formulation, the drug has to pass through two barriers to reach to the receptor component. Thus, the release rate is slow as compare to conventional gel formulation.
3.5. Skin Irritation Study

The Figure 4(a) reveals that the rats treated with TRT solution shows more irritation characteristics. The erythema signs are continuously increasing at regular time intervals. The lesions were absent initially but after 24 hours it appeared on applied skin portion. The scaling also increased as the therapy time increased. The Figure 4(b) shows data of the rats treated with TRT conventional gel which shows almost same irritation characteristics as the TRT solution. The TRT conventional gel shows the burst release pattern in in-vitro diffusion study. Thus, the sudden exposure to TRT takes place at the site of action which leads to erythema and other signs of irritations.

Figure 4(c) shows the graphical presentation of skin irritation studies of TRT niosomal gel on rat skin. The irritation characteristics were found to be almost absent in treatment with niosomal gel. This may be due to the sustained release pattern of TRT from the niosomal gel. The erythema signs were totally absent through out the treatment even after 48 hours of continuous exposure. The scaling was slightly observed after 48 hours of treatment.

3.6. Stability Studies

The stability studies of TRT niosomal gel was carried out at different accelerated and non-accelerated conditions. The Figure 5 shows continuous decrease in percentage drug retained in samples of TRT niosomal gel. It shows that niosome vesicles are maximum stable at 2-8°C as the percentage entrapment was found to be 94.81 % after 6 weeks. The preparation is less stable at 25±2°C and 40±2°C as the percentage entrapment were found to be below 80 % at both the conditions. Thus, it can be stated that the niosomal dosage forms should be stored in refrigerated conditions only.

4. Conclusion

The in-vitro diffusion study shows the sustained release pattern of TRT from niosomal gel and the skin irritation study reveals that the erythema, scaling and lesions due to
TRT treatment are reduced to the remarkable level. The stability study proves that the niosomes are more stable at 2-8°C hence proper attention should be paid in storage of niosomal dosage forms. From the present research work, it can be concluded that the niosomes are versatile dosage forms and hence must be explored further in development of topical drug delivery system.

Reference


