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Research Article

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# PREPARATION AND CHARACTERIZATION OF LIPOSOMES ENCAPSULATED WITH CLINDAMYCIN AND TRETINOIN

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## ABSTRACT

A formulation containing agents affecting the non-inflammatory like tretinoin as well as the inflammatory lesions of acne like clindamycin at the same time is preferable due to their high efficacy and shortening of the duration of treatment. Liposomes can improve the therapeutic effect of drugs and decrease the adverse effects. Therefore, liposomes containing clindamycin (Lip-CL), liposomes containing tretinoin (Lip-TRT) and liposomes loaded with both tretinoin and clindamycin (Lip-CL-TRT) were prepared and characterized. Lip-TRT were prepared by solvent evaporation method whereas Lip-CL and Lip-CL-TRT were prepared by dehydration-rehydration method. The morphologic, mean size and drug encapsulation efficiency were evaluated. Also, the amount of drug which was passed through or retained inside the skin was determined by Franz cell diffusion method and compared with the TRT cream. The particles of the liposomes were obtained in submicron size. The encapsulation efficiency (EE) of TRT and CL were high in the liposomal formulations. The retention of TRT and CL inside the skin from the Lip-CL-TRT were obtained more than 80%. Generally the results of the present study showed that it is possible to select liposomes as drug carrier for both CL and TRT.

**Keywords:** Acne, Liposomes, Clindamycin, Tretinoin, Skin.

## INTRODUCTION

Tretinoin (TRT) is a widely used drug in the topical treatment of acne, photo-aged skin, psoriasis and other skin disorders. The effect of TRT in the treatment of acne is to reduce the number and the size of comedones and therefore, TRT is commonly used at various dosage forms, such as lotions, hydrogels or creams.[1,2] As these formulations are easily removed by wetting, movement, and contacting, the efficacy is unpredictable.[3] Its use is limited by skin irritation, erythema, scaling, burning sensation and increased susceptibility to sunlight.[1,2]

Clindamycin (CL) is an antibiotic active against Grampositive aerobes and very active against both Gram-positive and Gram-negative anaerobic pathogens. Topical CL phosphate is effective in the treatment of acne.[4]

Some studies have shown that the combination of TRT and CL phosphate is clinically superior to either agent alone, when all lesion types are considered and increases patient compliance.[5]

The use of liposomes has provided for a higher concentration of drugs in deeper layers of the skin and a reduction in percutaneous absorption and unwanted side-effects.[6,7] Different investigators reported an increased skin accumulation of TRT *in-vitro* and a reduced irritancy *in-vivo* after treatment with these carriers. It was observed that the maximum comedolytic activity of TRT is reached at a concentration of five to ten times lower when TRT is incorporated into liposomes, compared to the conventional alcoholic gels.[8] On the other hand, it was found that the liposomal tretinoin (Lip-TRT) can protect the drug against photodegradation.[9]

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The superiority of liposome-encapsulated clindamycin (Lip-CL) solution vs CL solution in the treatment of acne was also demonstrated and no side-effects were reported [10]. Therefore, the efficacy and safety profiles of certain anti acne drugs may be improved by encapsulating both into liposomes. All of these advantages of liposomal formulations and the combination therapy of TRT and CL implied the importance of a study on the TRT-CL liposomal formulations.

In the current study we incorporated TRT, CL and TRT-CL into liposomes. Secondly, the characterization and penetration study of these formulations was evaluated and a comparison between the skin permeation of conventional TRT cream, CL gel, Lip- TRT and Lip-CL-TRT were also investigated.

## MATERIALS AND METHODS

TRT and CL, Cholesterol, α-tocopherol, $Na_2HPO_4$ and NaCl were obtained from Merck (Darmstadt, Germany). Soya Phosphatidylcholine (SPC) (PhospholiponR90, P90) was obtained from Avanti Polar Lipids (Alabaster, Alabama, USA). Chloroform, dichloromethane, glacial acetic acid,

acetonitrile, ethanol, methanol, triethanolamine (TEA) and Phosphate-buffered saline (PBS) ingredients were supplied from Sigma (USA). TRT cream and CL gel were purchased from the pharmacy. All solvents used in this study were high-performance liquid chromatography (HPLC) grade. All chemicals were of the purest grade available.

**Preparation of Lip-TRT**

Liposomes made of SPC and TRT were prepared in a similar way as described elsewhere. The composition of the lipid fraction of the liposomal formulation was 71.73% (w/w) SPC and 27.89 % (w/w) cholesterol known as the best composition to produce stable productions. The α-tocopherol 0.37% (w/w) was used as an anti-oxidant to protect the lipids and TRT against oxidation. TRT dissolved in chloroform-methanol (2: 1). Lipid fraction (SPC, Cholestrol and α- tocopherol) and the drug were then deposited as a thin film in a round bottom flask under nitrogen using a Rotary Evaporator (Heidolph, Laborota 4000, Germany). Residual solvent was removed under reduced pressure, and the resulting dried lipid-TRT film was then obtained. The films were then hydrated by the addition of a 2 ml of buffer pH=5 and homogenized in an overhead shaker (Heidolph, Swabach, Germany) resulting in turbid, white liposome dispersion. A buffer solution pH=5 was prepared by mixing 2.952 g $Na_2HPO_4.12H_2O$, 0.816 g citric acid $H_2O$, 0.105 g NaCl and water to 100 ml.[10]

**Preparation of Lip-CL**

Dehydration and Rehydration (DRV) Method was used for preparation of Lip-CL. In brief, liposomes consisted of phospholipid and cholesterol were prepared by solvent evaporation method and extruded 11 times through 100 nm polycarbonate filter by extruder (Lipex, Canada). CL solution in phosphate-buffered saline was added to this liposomes (phospholipid concentration= 32 µmole/ml) in the same volume. After freezing of this mixture, freezed liposome was vacuum dried in the freeze drier for 12 hours. The dried material was rehydrated by distilled water and vortexed to form liposome suspension. Non-entrapped CL was separated by centrifuge (Hettich, Germany) at 14000 rpm for 30 min and liposomes were washed twice with the same process.[11]

**Preparation of Lip-CL-TRT**

These liposomes were prepared with the method as mentioned above but instead of empty liposomes, Lip-TRT were used in DRV method.

**Determination of TRT by HPLC**

A Hitachi L-7100 liquid chromatography equipped with a 20 µl loop injector and Hitachi L-7420 UV-VIS detector (Hitachi, Tokyo, Japan) were used to carry out TRT determination. The column was a LiChrospher 100 RP-18 (12.5 cm× 4 mm, 5 µm particle size) (Merck, Darmstadt, Germany). TRT was determined at 350 nm. The mobile phase was water/ glacial acetic acid/acetonitrile, 15:0.5:84.5 (v/v/v), with a flow rate of 1.2 ml/min. The calibration curve was prepared with dichloromethane solutions of TRT at concentrations ranging from 0.1-0.001 mg/ml (n=6). The intra-and inter day variation for TRT was performed and there was no significant difference between day-to-day analysis. The quantitative determination of TRT in the tested samples was obtained from the calibration curve, which gave good linearity ($R2$ > 0.999, n=6) and reproducibility.[12]

**Determination of CL by HPLC**

The same HPLC instrument was used to obtain CL concentrations. CL was determined at 210 nm. The mobile phase was Buffer phase and acetonitril, 55: 45(v/v), with a flow rate of 1 ml/min. The calibration curve was prepared with distilled water solutions of CL at concentrations ranging from 0.001-4 mg/ml (n=6).

**Morphology, zeta potential and size analysis of liposomes**

An optical microscope (OLYMPUS, Germany) was used for studying the morphological features of the liposomes. The average particle size and charge of the liposomes were calculated with a particle size analyzer (PSA) (Malvern Instruments, Malvern, UK) at 25±1°C.

**Determination of encapsulation efficiency**

To determine the encapsulation efficiency, a known amount of liposomes was centrifuged at 14000 rpm (Beckman, USA) for 30 min. The supernatant and precipitate were analyzed by HPLC for both TRT and CL to determine the encapsulation percentage. The entrapment efficiency of liposomes was calculated by the following equation: EE (%) = (T-C)/T] × 100, where T is the total amount of drug that is detected both in the supernatant and sediment, and C is the amount of drug detected only in the supernatant.[6]

**Skin permeation studies**

*In-vitro* skin permeation studies were performed using diffusion Franz cells with an effective diffusion area of 3.37 cm2 and a receiver volume of 25 ml. The diffusion cells were maintained at 37 ±0.1°C using a re-circulating water bath and the fluid in the receptor chambers were stirred continuously. Phosphate buffer (pH 7.4) and ethanol (50:50 v/v) was used as the receiver medium for TRT formulations and Phosphate buffer for CL formulations to allow the establishment of the "sink condition". A suitable size of full-thickness skin of a BALB/c mouse was cut and mounted between the donor and receptor chamber of the diffusion cells, with the SC side facing upward. The mouse was properly shaved with electric clippers on the day before the experiment. The membranes were initially left in the Franz cells for 30 min in order to facilitate hydration. Subsequently, 1 g of the Lip-TRT, Lip-CL-TRT, TRT cream and CL gel were gently placed onto each membrane surface and the latter was covered with aluminum foil. A 5 ml was withdrawn from each receiver solution at 0.5, 2, 4, 8 and 24h intervals and replaced with the same volume of medium. Aliquots of the collected samples were analyzed for their contents, as described above. The derived concentration values were corrected by using the equation

$$Mt\,(n) = Vr \times Cn + Vs \times Cm$$

where $Mt(n)$ is the current cumulative mass of drug transport across the skin at time $t$, $Cn$ the current concentration in the receiver medium, $Cm$ the summed total of the previous measured concentrations [$m$=1 to ($n$−1)], $Vr$ the volume of the receiver medium and $Vs$ corresponds to the volume of the sample removed for analysis by HPLC.

For the determination of the amount of liposome retained in the skin, at the end of the experiment, the amount of the formulation remaining on the surface of the membrane was collected and assayed. The amount of the compound retained in the skin was then calculated by subtracting the sum of the amount of compound that remained on the surface and the amount of compound that was released (penetrated through the skin) from the whole amount applied.[13-15]

## RESULTS AND DISCUSSION

As it was previously proved, the topical effects of many drugs in the liposomal formulations were improved compared to the conventional formulations.[16-18] To increase the efficiency of topical therapy of acne vulgaris, Lip-CL-TRT were prepared and characterized in the present study. The prepared liposomes were heterogeneous, multilamelar vesicles with the range size of 399 to 879 nm (Table **1**). The encapsulation efficiency of TRT and CL in liposomal formulations was summarized in Table **2**.

**Table 1. Diameter of different liposomal formulations (Mean± SD, n = 3)**

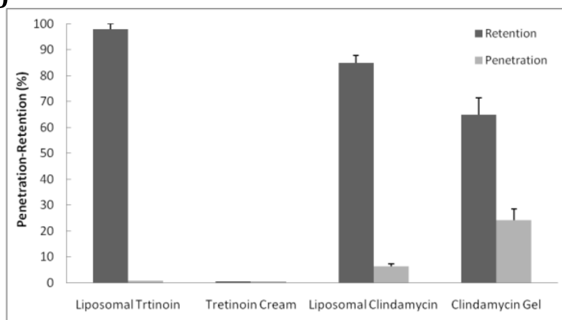
| Liposomal formulation | Z-average size (nm) |
|---|---|
| Liposomal tretinoin (Lip-TRT) | 879.4 ± 33.1 |
| Liposomal clindamycin (Lip-CL) | 719.0 ± 29.0 |
| Liposomes containing tretinoin and clindamycin (Lip-CL-TRT) | 398.8 ± 16.6 |

**Table 2. Encapsulation efficiency of CL and TRT in liposomal formulations (Mean ± SD, n=3)**

| Liposomal formulation | Encapsulation efficiency (%) |
|---|---|
| Tretinoin in Lip-TRT | 87.7 ± 5.3 |
| Tretinoin in Lip-CL-TRT | 58.3 ± 1.9 |
| Clindamycin in Lip-CL | 51.0 ± 1.5 |
| Clindamycin in Lip-CL-TRT | 40.0 ± 2.0 |

Figure **1** shows the permeation data obtained from liposomal and conventional formulations after 24 h. The penetration percentage of CL was higher for CL gel compared to Lip-CL. Also, almost all of the applied TRT was inside the skin after administration of liposomal TRT.

**Figure 1. Retention and penetration data of liposomal and conventional formulations after 24 h (Mean±SD, n=3)**




Topical application of TRT is limited by several disadvantages such as lipophilicity, instability and local irritation. In order to overcome these limitations, incorporation of TRT in liposomes has been studied by several researchers.[1,19,20] Reduced skin irritancy was reported by Sinico et al.[20] The concentration of TRT in epidermis and dermis increased after application of liposomal formulation compared to conventional creams or gels.

In the liposomal form, maximum comedolytic activity was achieved at a concentration of five to ten times lower and percutaneous penetration to systemic circulation was two times lower.[8] Also, protection of TRT against photodegradation was demonstrated by encapsulation in liposomes.[21]

In the other hand, liposomal CL composed of soya lecithin and cholesterol or hostaphat and cholesterol was prepared. Better efficiency of liposomal formulations was confirmed in 4 weeks clinical treatment.[22] In the study of Honzak and Sentjurc, *in vivo* experiment showed that liposomal CL was superior in treatment of closed comedons, papules and pustules compared to conventional solution.[10]

Because of the ability of liposomes to increase the accumulation of drug at the administration site and to incorporate both hydrophilic and lipophilic drugs, liposomes were chosen as carrier of CL and TRT in the present study. As previous studies have shown that the delivery of a lipophilic drug such as TRT can be modulated by varying the structure and/or membrane composition of liposomes. In the present study, the phospholipid, cholesterol amount and the buffer type were chosen according to the Brisaert study to achieve the highest TRT incorporation. Authors showed that these liposomes were chemically stable during 3 months.[1] Further *in vivo* work will show the efficiency of the prepared liposomal formulation.

## CONCLUSION

In the present study, liposomes containing both CL and TRT with suitable encapsulation efficiency were prepared. The skin deposition of drugs was changed in the liposomal forms.

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## DISCLOSURE

The authors report no conflicts of interest in this work.

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