Tyrosine-derived Nanospheres (TyroSphere™): A New Carrier System for Topical Delivery of Adapalene

Tannaz Ramezanli1,2, Z. Zhang3, J. Kohn3, B.B. Michniak-Kohn1,2,3
1Ernest Mario School of Pharmacy, 2Center for Dermal Research, 3The New Jersey Center for Biomaterials
1,2,3Rutgers-The State University of New Jersey, Piscataway, NJ 08854, USA, tr243@eden.rutgers.edu

ABSTRACT SUMMARY

In this study we investigated the applicability of tyrosine-derived nanospheres (TyroSphere™) as a nano-sized carrier for topical delivery of adapalene. Adapalene was successfully encapsulated in TyroSphere™ and generated spherical vesicles with diameter ranging from 65 to 80 nm. TyroSphere™ significantly increased adapalene solubility. Binding efficiency and drug loading were found to be up to 70% and 1.3% (w/w), respectively. Partition coefficient of adapalene-TyroSphere™ into human artificial sebum was recorded as 39.5. Human skin distribution studies were also conducted. Higher delivery of the drug to epidermis was obtained using the adapalene-TyroSphere™ formulation when compared to Differin®. Hydroxypropyl methylcellulose was used as a thickening agent to prepare a gel formulation. Delivery of adapalene into hair follicles using TyroSphere™ was confirmed by fluorescent imaging.

INTRODUCTION

Adapalene is a synthetic naphthoic acid derivative with retinoid activity and is used topically for the treatment of acne. This compound is highly lipophilic (logP = 8) and practically insoluble in water; therefore, formulating this drug is very challenging1,2. To date, there is no publication on using polymeric nano carriers for the delivery of adapalene.

Tyrosine-derived nanosphere (TyroSphere™), is based on ABA triblock copolymers where the hydrophilic A blocks are poly(ethylene glycol) and the hydrophobic B block is composed of desaminotyrosyl-tyrosine octyl ester and subaric acid. TyroSphere™ has been shown to encapsulate lipophilic compounds such as Nile Red3, paclitaxel4 and curcumin. TyroSphere™ was found to be biodegradable and compatible with skin5. This study reports on the characteristics of adapalene-loaded in TyroSphere™ (Ada-Tyr): morphology, particle size, binding and loading efficiencies, sebum partition coefficient and diffusion through artificial sebum, and delivery of adapalene into epidermis and hair follicles using TyroSphere™.

EXPERIMENTAL METHODS

Adapalene (purity>99%) was purchased from BOC Sciences (Shirley, NY, USA). Maximum solubility of adapalene was examined in phosphate buffer saline (PBS) pH=7.4 and in presence of different amounts of Tween 80. PEG38-b-oligo(desaminotyrosyl-tyrosine octyl ester suberate)-b-PEG5k (Mw=22.9kDa) is an amphiphilic ABA block copolymer used for preparation of TyroSphere™. The Ada-Tyr formulations were formed by methods described elsewhere1.

For characterization of Ada-Tyr, dynamic light scattering (DLS) was used to measure particle size and polydispersity index. Transmission electron microscopy (TEM) analysis was performed to confirm the size and spherical shape of the particles. To determine the adapalene binding efficiency (BE%= mass of drug in the TyroSphere TM/mass of drug in the feed) and loading efficiency (LE%= mass of drug in the TyroSphere TM/mass of TyroSphere TM), aliquots of nanoparticle suspension were lyophilized; subsequently, adapalene was extracted with dimethylformamide (DMF), and drug content was determined by HPLC equipped with a UV/vis detector and a reverse phase C18 column. A mixture of water (0.1% TFA): acetonitrile 87:13 (v/v) was applied as the mobile phase. The UV/vis detector was set at 319 nm.

Artificial human sebum was prepared by mixing squalene, fatty acids, wax and oils found in human sebum. 10 mg of artificial sebum was added into 10 times diluted Ada-Tyr suspension and placed inside a bath shaker (37°C and 100 rpm). Sebum partition coefficient (PQ) of adapalene at the 15 h time point was determined. PQ = drug content in 1 g of sebum/drug content in 1 g of aqueous solution. In addition, Ada-Tyr diffusion through artificial sebum was studied using 24-well plate (Transwell®) with 0.4 µm supporting membrane at 37°C. The receptor media (1% (w/v) Tween 80 in phosphate buffer) was collected at every time point and analyzed using HPLC.

Skin distribution of Differin® and Ada-Tyr was studied using vertical Franz diffusion cells. Following 12 h of application on dermatomed human skin, adapalene delivered into the epidermis layer was extracted with the aid of homogenizer and quantified by HPLC.

In the next study, a viscous formulation of Ada-Tyr was prepared using high viscosity hydroxypropyl methyl cellulose (HV HPMC) (Sigma Aldrich). 2% HV HPMC gel in PBS was mixed with Ada-Tyr liquid dispersion (1:1 v/v). TEM was used to study size change and possibility of agglomeration of nanoparticles. The capability of TyroSphere™ to deliver adapalene to hair follicles was examined using pig ear skin in vitro. 400 µL of Ada-Tyr viscous formulation was added on 0.64 cm² of skin and massaged for 3 minutes. Fluorescent images of vertical cross-sections and surface of the porcine skin were taken to visualize distribution of adapalene in the epidermis and follicular orifices.

RESULTS AND DISCUSSION

Table 1 shows various formulations of Ada-Tyr that were prepared. The size of the TyroSphere™ was approximately 65nm (PDI=0.22) regardless of the adapalene incorporation. Increase in polymer input resulted in higher particle diameter, to about 80 nm. Final filtration of nanospheres did not have a significant effect on particle size. The adapalene BE with 2 different initial drug and polymer inputs were determined (Figure 1). LE in those formulations ranged from 0.5 to 1.3%. Further increase in adapalene initial input resulted in significant lower BE and LE.

Table 1: Ada-Tyr formulations prepared in this study.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Adapalene input (µg)</th>
<th>copolymer input (µg)</th>
<th>PBS (ml)</th>
<th>pH=7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ada-Np-1</td>
<td>600 µg</td>
<td>60 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Ada-Np-2</td>
<td>800 µg</td>
<td>60 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Ada-Np-3</td>
<td>1 mg</td>
<td>60 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Ada-Np-4</td>
<td>800 µg</td>
<td>80 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Ada-Np-5</td>
<td>1 mg</td>
<td>80 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Empty-Np-1</td>
<td>-</td>
<td>60 µg</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Empty-Np-2</td>
<td>-</td>
<td>80 µg</td>
<td>14.4</td>
<td></td>
</tr>
</tbody>
</table>
agglomeration narrow particle size distribution and lack of particle dispersion on dermatomed human cadaver skin, adapalene extracted from epidermis was measured as 3.43±1.14 µg/ml. Maximum solubility of adapalene in PBS was below our HPLC detection limit and estimated to be < 5 ng/ml. Maximum solubility of the drug in presence of 0.1, 0.5, and 1% Tween 80 in PBS (w/v) was determined as 3.7±0.8, 15.4±3.9, and 37.6±7.7 µg/ml respectively. While highest concentration of adapalene in TyroSphere™ formulations was obtained as 265±35.3 µg/ml. TyroSphere™ enhanced the solubility of adapalene in PBS more than 10,000 times. This significant increase in the solubility by nanospheres is critical in the delivery of therapeutics for topical applications.

Following 12 h application of Ada-Tyr aqueous dispersion on dermamotad human cadaver skin, adapalene extracted from epidermis was measured as 3.43±1.14 µg/cm², while Differin® lotion delivered 1.25±1.28 µg/cm² of drug into the epidermis (n=8). Nanosphere formulation showed significantly higher delivery of drug to the epidermis compared to commercial formulation (p value < 0.05).

TEM images of final Ada-Tyr formulations as liquid dispersions and viscous formulation (Figure 3) show a narrow particle size distribution and lack of particle agglomeration in the presence of HPMC.

Adapalene is a fluorescent molecule, and is used to visualize follicular delivery of the drug via the TyroSphere™ formulation. Images taken from surface of the skin clearly showed accumulation of adapalene in the opening of follicular ducts (Figure 4). Fluorescent images from cross sections of pig skin samples treated with Ada-Tyr gel confirmed the presence of adapalene in the hair follicles, as well as upper epidermal layers 24 h after skin application (see Figure 4).

**CONCLUSION**

TyroSphere™ successfully incorporated adapalene and provided substantial enhancement of its solubility in PBS. TyroSphere™ formulations improved delivery of adapalene to the epidermis compared to the marketed formulation Differin®. A viscous formulation of Ada-Tyr was successfully prepared for ease of application on skin. Small particle size in addition to good partitioning of adapalene in human sebum contributed to targeted delivery of Ada-Tyr to the hair follicles, where acne originates.

**REFERENCES**