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Temperature-Induced Release of All-*trans*-Retinoic Acid Loaded in Solid Lipid Nanoparticles for Topical Delivery

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Abstract: The aim of this work was to develop and evaluate solid lipid nanoparticles (SLN) containing all-*trans*-retinoic acid (ATRA) for topical delivery. SLN composed of coconut oil and curdlan improved the suspension instability of ATRA in aqueous solution. The photodegradation of ATRA by light was reduced by incorporation in SLN. The loading efficiency of ATRA in SLN was higher than 95% (w/w). The amounts of ATRA released from SLN at 4 °C and at 37 °C were less than 15% and more than 60% (w/w) for 96 h, respectively. The ATRA-loaded SLN can be used as a potential carrier for topical delivery.

Keywords: all-*trans*-retinoic acid, coconut oil, curdlan, solid lipid nanoparticle, topical delivery.

Introduction

All-*trans*-retinoic acid (ATRA) is an active metabolite of retinol with the acidified form. ATRA attracts the spotlight of attention due to its physiological effects such as regulation of epithelial cell growth and differentiation, collagen synthesis, as well as anti-cancer activities in various types of cancer cells.[1] In particular, ATRA is effective in the treatment of wrinkled and pigmented skin.[2] However, the use of ATRA is limited in topical delivery because it has the poor aqueous solubility and is chemically unstable. ATRA is dissolved in organic solvents such as methanol and ethanol, but it is insoluble in water. When ATRA is exposed on light, heat, and oxidants, it is readily and rapidly transferred to an isomerized form. Moreover, ATRA topically treated in skin can lead to local irritation such as erythema, peeling and burning at the application site.[3] *In vivo* the therapeutic effects of ATRA are limited due to its rapid metabolism and catabolism by cytochrome P450 enzymes.[4] To overcome these problems many attempts have been carried out using drug incorporation techniques such as liposomes, solid lipid nanoparticles (SLN), and polymeric micelles.[5-7] Park *et al.* suggested that incorporation of ATRA using the nanoparticles of methoxy poly(ethylene glycol) (MPEG)-grafted chitosan progressed its therapeutic effect in apoptosis of CT-26 tumor cells.[8] Na *et al.* proposed the self-assembled hydrogel nanoparticles based on carboxymethylated-curdlan with a hydrophobic moiety as a controlled delivery system for ATRA.[9] In the study, they showed the usefulness of the hydrogel nanoparticles in the treatment of cancer and the controlled release of ATRA. Brisaert *et al.* have reported that photodegradation of ATRA in lotions was rapidly generated under light and could be delayed by incorporation of ATRA into liposomes.[10] In spite of these advantages, the utility of liposomes may suffer difficulty due to their physical instability and cost factor.[11]

SLN have received attention as a promising drug delivery carrier, particularly for lipophilic drugs due to their low toxicity, high drug incorporation efficiency, and easy scale up

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for industrial production.[12] SLN may allow the improvement of the poor solubility of lipophilic drugs in aqueous and the protection of the incorporated drugs against light, heat and oxidants.

In this study, we have used coconut oil and curdlan for the preparation of the SLN incorporating ATRA. Coconut oil was chosen as the solid core and curdlan was applied as the surfactant. Coconut oil leads to the formation of stable nano-dispersions in water. Coconut oil is solid state at lower temperature than 24 °C and is liquid state at higher temperature.[13] Curdlan, one of the microbial polysaccharides, can surround the nano-dispersions and be solidified around the oil.[14] The main aim was to develop and evaluate the SLN based curdlan and coconut oil for topical delivery of ATRA.

Experimental

Materials. All-*trans*-retinoic acid (ATRA), coconut oil, phosphotungstic acid solution and ammonium water were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Curdlan was obtained from Takeda Chemical Industry Ltd. (TCI, Osaka, Japan). Eudragit L100-55 was gifted from Deggusa (Degussa-Hüls, Germany). Tween 80 was obtained from Junsei Chemical Co. (Tokyo, Japan). All other chemicals were of analytic grade and used directly without further purification.

Preparation of ATRA-Loaded SLN. The preparation of SLN consisted of coconut oil and curdlan was achieved as the previous method.[14] ATRA was firstly dissolved in 1 mL of ethanol solution containing 20 mg of Eudragit L100-55 under dark condition. The ATRA solution was added to melted coconut oil and then the mixture was sonicated for 0.2 min at room temperature. The emulsion was added to curdlan solution (0.05% (w/v)) dissolved in 2.5% (v/v) ammonium solution containing Tween 80. After homogenizing, the mixture was vigorously stirred and the pH of the solution was slowly adjusted to 7.0 with 0.01 N HCl solution.

Characterization of ATRA-Loaded SLN. The size distribution of ATRA-loaded SLN was determined by dynamic light scattering (DLS-7000, Otsuka Electronics Co. Ltd., Japan). The ATRA-loaded SLN was filtered with a syringe filter of 0.8 μm pore-size and diluted to an appropriate scattering intensity. Transmittance electron microscopy (TEM) for the ATRA-loaded SLN was performed using a JEOL JEM-2000 FX II at 80 kV. The nanoparticle suspension was dropped onto a carbon film-coated copper gird. After negative staining using phosphotungstic acid, the observation was carried out.

Suspension Stability of ATRA-Loaded SLN. The solubility improvement of ATRA by encapsulation using the SLN was investigated with transmittance determination at 500 nm in aqueous media. The ATRA-loaded SLN was diluted with distilled water and the transmittance of the solution was determined with an UV spectrophotometer (UV-1200, Shimadzu Co. Ltd., Kyoto, Japan) at 4 and 37 °C, respectively (n=3).

Chemical Stability of ATRA Encapsulated in SLN. To evaluate effect of encapsulation on photodegradation by light, free ATRA in methanol or ATRA-loaded SLN suspension was poured into a glass vial and a light source with 50-W was placed at 30 cm distance from the vials. The amount of the remaining ATRA was directly measured with an UV spectrophotometer (UV-1200, Shimadzu Co. Ltd., Kyoto, Japan) at 340 nm.

ATRA Loading Efficiencies and Release Properties. Free ATRA from the ATRA-loaded SLN suspension was isolated using a centrifugal filter device (Microcon, MW 5 KDa cut off; Millipore, MA). 0.5 mL of the suspension was placed into the filter tube and then centrifuged at 10,000 rpm for 5 min. After dissolving the supernatant in DMSO, the amount of ATRA was determined using the UV spectrophotometer at 340 nm. The measurement of ATRA release from the ATRA-loaded SLN was carried out in phosphate buffer (0.1 M, pH 7.4) at 4 and 37 °C. One milliliter of the ATRA-loaded SLN suspension was place into dialysis tube (MW 12 KDa cut off; Sigma) and the released ATRA was determined with the UV spectrophotometer at 340 nm.

Results and Discussion

Characteristics of ATRA-Loaded SLN. The ATRA-loaded SLN is briefly composed of curdlan and coconut oil. Curdlan was used as a shell material and coconut oil was selected as a core material. Curdlan is dissolved in alkaline solution but is insoluble in neutral solution. Curdlan solution in alkaline pH forms a gel as the pH is reduced to neutral or acidic pH. Curdlan used as a surfactant can surround coconut oil in alkaline pH and be solidified around the oil as pH reached to neutral. Therefore, we hypothesized that ATRA can be incorporated in coconut oil by an emulsion formation and curdlan can surround the ATRA-incorporated coconut oil.

The ATRA-loaded SLN was prepared with various concentrations of ATRA and coconut oil (Table I). As shown in Figure 1(a), the SLN-2 observed by TEM was spherical and relatively uniform. The spherical morphologies of all samples with uniformity were also confirmed by TEM. Figure 1(b) shows the size distribution of the SLN-2 in aqueous

Table I. Characterization of ATRA-Loaded SLN

Sample	ATRA (mg)	Coconut Oil (mL)	Mean Size (nm)[a]	ATRA Loading Efficiency (%, w/w)[b]
SLN-1	0.05	0.04	89.2 ± 15.1	95.8 ± 0.2
SLN-2	0.01	0.08	99.8 ± 23.1	97.8 ± 0.1
SLN-3	0.20	0.16	50.5 ± 8.60	98.7 ± 0.2

[a]Mean size of the ATRA-loaded SLN was measured by dynamic light scattering (n=3). [b]ATRA loading efficiency was calculated using a following formulation (n=3): Loading efficiency (%)=(Residual amount of ATRA in the SLN/Feeding amount of ATRA)×100.

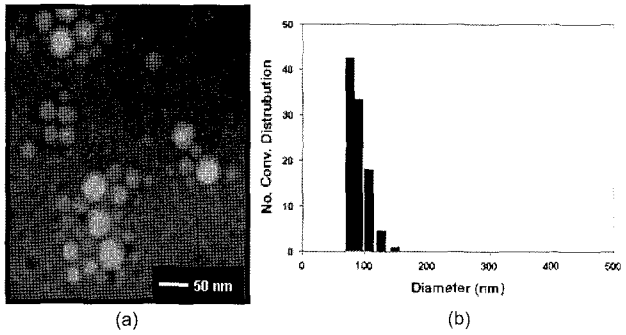

Figure 1. TEM image (a) and size distribution (b) of the ATRA-loaded SLN.

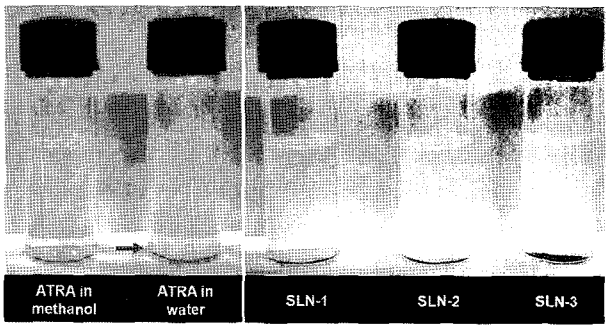

Figure 2. Photographs of suspension of ATRA-loaded SLN in water. ATRA is dissolved in methanol but it is precipitated in water. Arrow indicates ATRA precipitated in water. When ATRA was incorporated in SLN, it was well dispersion without sedimentation.

solution measured by DLS. The mean diameter of the SLN-2 was 99.8±23.1 nm. There was no significant change in the particle size of the SLN-1 and SLN-2. However, the particle size decreased significantly in SLN-3 (Table I).

Suspension Stability of ATRA-Loaded SLN. To be able to suspense ATRA in aqueous solution, formulations such as polymeric particles, micelles, and liposomes are required for various applications. As shown in Figure 2, ATRA was insoluble in water but ATRA incorporated in SLN was well suspended without sedimentation. Suspension stability of ATRA-loaded SLN in distilled water was measured by monitoring the change in transmittance at 4 and 37 °C, respectively. There were no the changes in transmittance at 4 °C for 6 h in all samples. In contrast, the changes in transmittance at 37 °C were observed. As shown in Figure 3(b), the SLN-3 exhibited more the change in transmittance. Coconut oil is solid state at lower temperature than 24 °C and is liquid state at higher temperature.[13] Hence, the ATRA-loaded SLN showed the temperature dependent property as phase transition of coconut oil by temperature. This result means that release of ATRA loaded in the SLN can be effectively induced at higher temperature than 24 °C such as human skin temperature.

Chemical Stability of ATRA-Loaded SLN. ATRA is

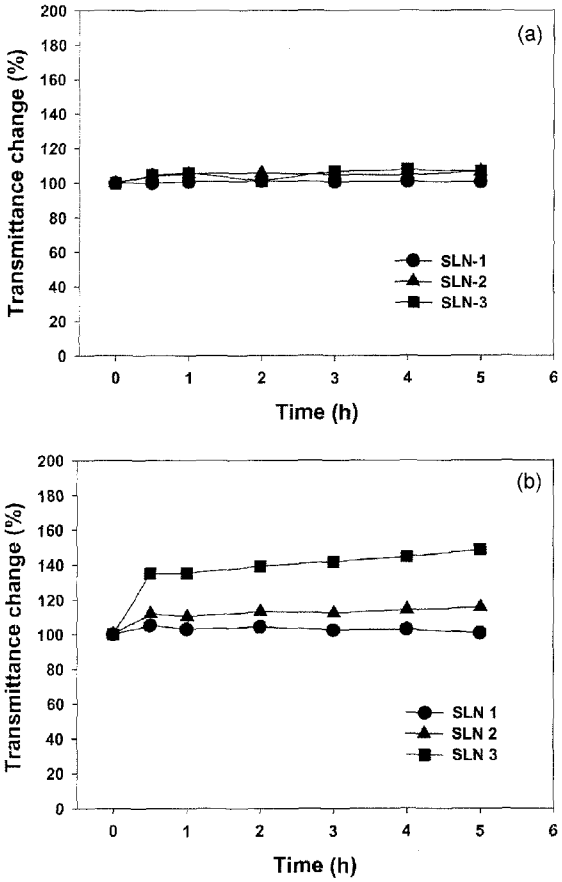

Figure 3. Stability of ATRA-loaded SLN suspended in water at 4 °C (a) and 37 °C (b). Each experiment was performed in triplicate. Values are expressed as mean±S.D.

known to be chemically unstable. Above all, it shows the light-sensitive degradation. To determine the stability of the SLN against light, we investigated the chemical stability of ATRA loaded in the SLN under light at room temperature. The intact ATRA in methanol was rapidly degraded under light exposure (Figure 4). On the other hand, ATRA in the SLN showed more slow degradation than bare ATRA. After 5 h of incubation, intact ATRA in methanol remained less than 55% and the content of intact ATRA incorporated in the SLN remained more than 70% in all samples until 5 h of light exposure. The content of intact ATRA remained in the SLN-3 was 82.9%, resulting in the good protection for ATRA from light exposure. This result indicates that ATRA incorporated in the SLN can be kept without the rapid degradation by light exposure.

ATRA Loading Efficiencies and Release Profiles. ATRA loading efficiencies of the ATRA-loaded SLNs were shown in Table I. The loading efficiencies of ATRA were higher than 95% (w/w) of feeding amounts used at the beginning for all formulations. To investigate temperature-induced release, the release studies of ATRA from the SLNs were performed at 4 and 37 °C. The release profiles of ATRA from

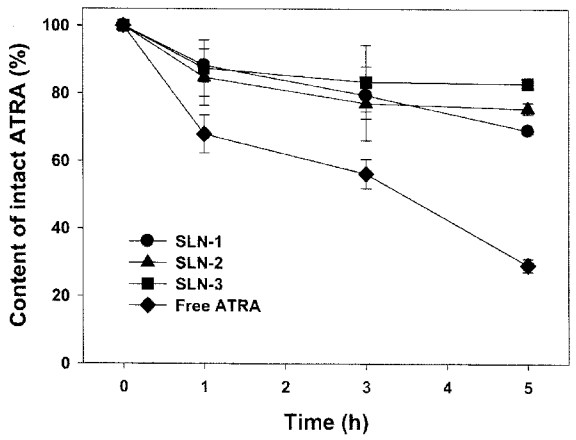

Figure 4. Stability of bare ATRA and ATRA loaded in SLN against light. Each experiment was performed in triplicate. Values are expressed as mean±S.D.

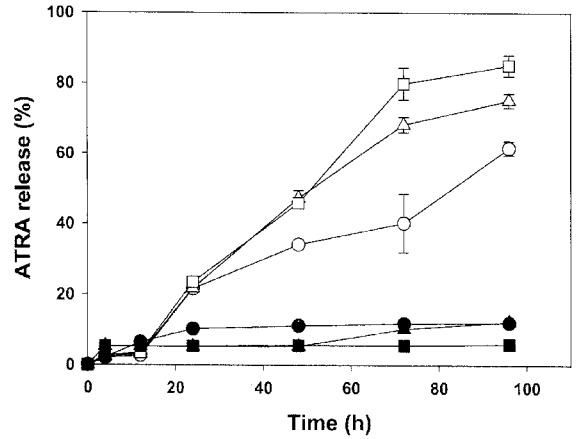

Figure 5. Release profiles of ATRA from SLN 1 (circle), 2 (triangle), and 3 (square) at 4 °C (close) and 37 °C (open). Each experiment was performed in triplicate. Values are expressed as mean±S.D.

the SLNs are shown in Figure 5. The cumulative amounts of ATRA released from the SLNs at 4 °C were less than 15% (w/w) of the drug loading for all formulations within 96 h. In contrast, ATRA release at 37 °C was more than 60% (w/w) for all the SLNs within the same time. As the results in the suspension stability study, the rate of ATRA release was induced at body temperature. In previous many studies, it has been reported that SLN have improved the dermal localization of several topical therapeutic agents.[15,16] Recently, Shah *et al.* suggested that the SLN encapsulating tretinoin, a metabolite of Vitamin A, might be transported across the skin through permeation study with rabbit skin *in vitro*.[17] Thus, the ATRA-loaded SLN presented in this study can be employed as a topical delivery system for enhancing the treatment of skin diseases.

Conclusions

In this study, we prepared the ATRA-loaded SLN made of coconut oil and curdlan. Coconut oil and curdlan were used as the lipid core and the shell material respectively. The formulation for ATRA improved the poor solubility in aqueous solution and chemical instability against light. The release rate of ATRA from the SLNs increased at 37 °C. Therefore, we expect that the SLN for ATRA can be used as a potential carrier in topical delivery.

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