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Astragaloside IV-loaded nanoparticle-enriched hydrogel induces wound healing and anti-scar activity through topical delivery

Xi Chen[a,1], Li-Hua Peng[a,1], Ying-Hui Shan[a], Ni Li[a], Wei Wei[b], Lian Yu[b], Qi-Mei Li[c,d], Wen-Quan Liang[a], Jian-Qing Gao[a,d,*]

[a] Institute of Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, PR China
[b] Department of Pharmaceutics, College of Pharmaceutical Sciences, Jiamusi University, Jiamusi, Heilongjiang, PR China
[c] Changzhou Nanfang Sanitation Materials Company, Changzhou, Jiangsu, PR China
[d] Technology Center for Transdermal Drug Delivery System of Jiangsu Province, Jiangsu, PR China

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ABSTRACT

This study aims to investigate the novel preparation of solid lipid nanoparticle-enriched hydrogel (SLN-gel) for the topical delivery of astragaloside IV and to determine the effects of astragaloside IV-based SLN-gel on wound healing and anti-scar formation. Solid lipid nanoparticles (SLNs) were prepared through the solvent evaporation method. The particle size, polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE), drug release, and morphological properties of the SLNs were characterized. The optimized SLNs were incorporated in carbomer hydrogel to form an SLN-enriched gel (SLN-gel) carrier. The effects of astragaloside IV-enriched SLNs on wound healing were determined using the wound scratch test, and their uptake by skin cells was tested *in vitro*. With the rat full-skin excision model, the *in vivo* regulation of astragaloside IV-based SLN-gel in the wound stages of re-epithelization, angiogenesis, and extracellular matrix remodeling was investigated. The best formulation of astragaloside IV-based SLNs had high EE (93% ± 5%) and ZP (−23.6 mV ± 1.5 mV), with a PDI of 0.18 ± 0.03 and a drug loading percentage of 9%. Astragaloside IV-based SLNs and SLN-gel could release drug sustainably. Astragaloside IV-based SLNs enhanced the migration and proliferation of keratinocytes and increased drug uptake on fibroblasts *in vitro* ($P < 0.01$) through the caveolae endocytosis pathway, which was inhibited by methyl-β-cyclodextrin. Astragaloside IV-based SLN-gel strengthened wound healing and inhibited scar formation *in vivo* by increasing wound closure rate ($P < 0.05$) and by contributing to angiogenesis and collagen regular organization. SLN-enriched gel is a promising topical drug delivery system. Astragaloside IV-loaded SLN-enriched gel was proven as an excellent topical preparation with wound healing and anti-scar effects.

1. Introduction

The wound healing process involves a complex and dynamic series of events leading to the repair of injured tissues. These events, triggered by tissue injury, involve three overlapping but well-defined phases: inflammatory, proliferative, and remodeling (Rhett et al., 2008). Re-epithelialization and angiogenesis are two important activities determining the wound lesion. The former starts a few hours after the lesion and includes the movement of cells, especially the keratinocytes from the margins and the epidermal appendices. In the latter, new blood vessels are formed from preexisting vessels. The remodeling phase is marked by the maturation of elements and affections to the extracellular matrix, leading to proteoglycan and collagen deposits, which are closely related to scar formation (Gurtner et al., 2008).

Wound treatments ideally accelerate healing and reduce scar complications. However, scarless healing is difficult in adult tissues. Accordingly, increasing attention has been directed to screening and developing products that are effective in both accelerating wound healing and preventing scars. Nowadays, Traditional Chinese Medicine (TCM) is widely practiced and is viewed as an alternative to conventional medicine for wound healing in the Chinese population. In TCM, Astragali Radix (AR) (the root of *Astragalus membranaceus*) is an herb widely used in enhancing the repair and recovery of tissues and organs such as lung (Dong et al., 2010), heart (Liu et al., 2010), and neurons (Fang et al., 2009), because of its tonic property. In our previous research, we reported that astragaloside IV, the chief component of AR, could accelerate wound

* Corresponding author at: Institute of Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, Zhejiang, PR China. Tel.: +86 571 88208437; fax: +86 571 88208437.
E-mail addresses: gaojianqing@zju.edu.cn, gaojianqing1029@yahoo.com.cn (J.-Q. Gao).
[1] These authors contributed equally to this work.

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healing and reduce scars (Chen et al., 2012). The chemical structure of astragaloside IV is shown in Fig. 2A. Atragaloside IV-incorporated sodium alginate-gelatin hydrogel was reported effective in skin wound repair, leading to a significant improvement in wound closure, collagen synthesis, and skin tensile strength recovery (Peng et al., 2012).

However, the daily dosing frequency of astragaloside IV-incorporated sodium alginate-gelatin hydrogel rendered it not a good choice for wound treatment. To reduce the dosing frequency of astragaloside IV and to enhance patients' compliance, a suitable pharmaceutical carrier for the topical application of astragaloside IV must be discovered. Among the many nanovesicles, solid lipid nanoparticles (SLNs) have attracted increasing attention as a more alternative dosage form to liposomes and polymeric nanoparticles. SLNs possess good tolerability and stability, scaling-up feasibility, and the ability to incorporate hydrophobic/hydrophilic drugs (Uner and Yener, 2007). SLNs are well suited for use on damaged or inflamed skin because they are composed of non-irritant and non-toxic lipids. Moreover, researchers reported that SLNs exhibit good epidermal-targeting effects (Chen et al., 2006). Many drugs such as retinol (Jenning et al., 2000), ascorbyl palmitate (Uner et al., 2005), clotrimazole (Souto et al., 2004), triptolide (Mei et al., 2003), and podophyllotoxin (Chen et al., 2006) were incorporated by SLNs for topical delivery. However, SLN dispersions have low viscosity for topical application on skin. To address this concern, SLNs can be incorporated into semi-solid systems (*e.g.*, hydrogels) to increase the consistency of final formulations and promote the long-term stability of incorporated nanoparticles. Triamcinolone acetonide acetate-loaded SLN gels possess good stability, rheological properties, and high electric conductance (Liu et al., 2008). Moreover, SLNs and nanostructured lipid carrier (NLC)-enriched hydrogels for the transdermal delivery of nitrendipine (NDP) are advantageous, with the suitable viscosity, transparency, and high chemical stability of drugs in the formulation (Bhaskar et al., 2009). Controlled release was achieved by incorporating NDP into the solid matrix of Dynasan114-based lipid nanoparticles. An additional advantage was the possibility of combining drug-loaded SLN/NLC with gel hydrophilic matrices, resulting in excellent adhesion and constant release formulation.

In this study, astragaloside IV-based SLNs and SLN-gel were prepared and optimized. The effects of astragaloside IV-based SLN-gel on wound healing and scar formation, including re-epithelization, angiogenesis, and collagen deposition, were demonstrated *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Astragaloside IV was purchased from the Zhejiang Institute of Food and Drug Control (purity >99.3%, HPLC). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Gaithersburg, MD, U.S.A.). Purified mouse anti-rat CD31 monoclonal antibody was purchased from BD (New Jersey, U.S.A.). Horseradish peroxidase (HRP)-labeled goat anti-mouse IgG1 antibody was purchased from Boster, Inc. (Wuhan, China). Masson's trichrome staining kit was purchased from Nanjing Keygen, Inc. (Nanjing, China). CytoSelect™ 24-well wound healing assay was purchased from Cell Biolabs, Inc. (San Diego, CA, U.S.A.). The immortalized human fibroblast line was obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The immortalized keratinocyte line was obtained from Jiangsu Biomics, Inc. (Jiangsu, China). Chloral hydrate, rhodamine b, glycerol tristearate, and carbomer 934 were purchased from the China National Medicines, Inc. (Beijing, China). Buffered paraformaldehyde (4%) was purchased from Boster, Inc. (Wuhan, China). Finally, Poloxamer 188 was purchased from BASF (Rhineland-Palatinate, Germany).

2.2. Animals

Six-week-old Sprague-Dawley rats (170–200 g) and New Zealand rabbits (2.5–3 kg) were supplied by Zhejiang University Experimental Animal Center, China. All animals were maintained under constant conditions (temperature 25 °C ± 1 °C), with free access to standard diet and drinking water. All experimental procedures were in accordance with the Zhejiang University guidelines for the welfare of experimental animals (Animal Experimentation Ethics Approval No.: Zju2010-1-02-015).

2.3. Preparation and formulation screening of SLNs and SLN-gel

SLNs were prepared through the solvent evaporation method with modifications (Shah and Agrawal, 2012). Glycerol tristearate and astragaloside IV were dissolved in 5 mL ethanol. The aqueous phase containing poloxamer 188 was transferred with a magnetic stirrer, and the lipid phase was dispersed dropwise into the aqueous phase at a stirring speed of 1000 rpm at 80 °C. After stirring for 1 h, the resultant emulsion was cooled by adding ice-cold water to solidify the nanoparticles, centrifuged at 3000 rpm to remove unentrapped or undissolved drug crystals, and then centrifuged again at 18,000 rpm for 30 min at 4 °C to remove the free drug in the aqueous water. The resultant solid mass was dispersed in distilled water to obtain SLN dispersion.

SLNs were prepared by varying the contents of lipid, drug, and surfactant in water (Table 1). The size, ZP, and encapsulation efficiency (EE) of the SLNs were evaluated.

In the preparation of SLN-gel, Carpomer 934 was dispersed in an appropriate amount of double-distilled water, stored overnight, and then neutralized with NaOH. After adding the SLN dispersion (using the best formulation obtained from the formulation optimization), the sample was mixed using a high-speed stirrer at approximately 500 rpm for 5 min. Finally, the pH of the sample was adjusted to 7. The concentration of Carpomer 934 in the SLN-gel was 0.5%.

In Section 2.6, rhodamine b SLNs were used to investigate the effect of the SLNs on drug uptake. The preparation method for rhodamine b SLNs was the same as that for astragaloside IV SLNs.

2.4. Evaluation of SLNs and SLN-gel

2.4.1. Particle size analysis and ZP measurement

To determine the size of nanoparticles and ZP, the SLN dispersion and SLN-gel were diluted with double-distilled water and then adjusted to a conductivity of 50 mS/cm with sodium chloride. The measured electrophoretic mobility was converted to ZP using the Helmholtz–Smoluchowski equation. This process was performed using the software included in the system.

2.4.2. EE and drug loading

The freshly prepared SLN dispersion was centrifuged at 3000 rpm to remove unentrapped or undissolved drug crystals (Das et al., 2011). The obtained formulation was dissolved in methanol and then vortexed to extract the drug from the lipid. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant was appropriately diluted with methanol, and drug concentration (denoted as M_1) was measured by HPLC assay. The amount of drug entrapped in the SLNs was determined by centrifugation. Briefly, the nanoparticle dispersion was centrifuged at 18,000 rpm for 30 min at 4 °C to remove the free drug in the aqueous water. After centrifugation, the solid mass was dissolved in methanol and