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Advantages of cross-linked versus linear hyaluronic acid for semisolid skin delivery systems

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ABSTRACT

Cross-linking of the hyaluronic acid (hyaluronan, HA) molecule may be a possible chemical modification via which to improve the problem of the penetration of HA. The successful preparation and characterization of stable nanoparticulate systems based on HA were described and discussed previously. The purpose of the present study is to compare hydrogels based on linear or cross-linked HA as potential semisolid drug delivery forms from the aspects of deep HA penetration through the skin. The rheological properties, hydration, irritation effect, *in vitro* and *in vivo* skin penetration were studied. The hydration effect was kept and the rheological parameters were slightly changed after the cross-linking procedure. Diffusion and penetration studies demonstrated that the formation of smaller particles of HA by means of cross-linking resulted in better diffusion through a synthetic membrane and better penetration through the human epidermis and living animal skin as compared with linear HA, which did not penetrate.

1. Introduction

Hyaluronic acid (HA) also known as hyaluronan and sometimes presented as a hyaluronate (poly)anion from a chemical/structural aspects, is a non-sulfated glycosaminoglycan. In aqueous solution at physiological pH, HA exists as negatively charged hyaluronate macromolecules with an extended conformation. In a polyanionic form, the functional groups of HA make the biopolymer so hydrophilic that its binds 1000 times more water than might be predicted from its molar mass. HA is present in almost all biological fluids and tissues of the vertebrates, the highest amount being found in the extracellular matrix of the soft connective tissue. The skin contains slightly more than 50% of the total HA content present within the human body [1]. After the age of 20 years, the amount of HA continuously decreases.

Thanks to its versatile properties, such as its biocompatibility, non-immunogenicity, biodegradability and viscoelasticity, HA is an ideal biomaterial for cosmetic, medical and pharmaceutical applications. It is widely utilized for the treatment of osteoarthritis, vesico-urethral reflux and urinary incontinence. HA is incorporated in many moisturizing creams and wound-healing dressings, and it is also applied in ophthalmology [2–6]. However, with the exception of the vitreous body, where its half-life is 20–70 days, the turnover of HA in most tissues in the body is surprisingly rapid. The typical half-life in the human skin is 2–5 days, in the joints and pleura it is 0.5–1 day, and in the anterior chamber of the eye it is merely 1–2 h [1]. A novel approach is the use of HA-based nanoparticles as an effective carrier in transdermal drug delivery [7–11]. In view of the problem of the enzymatic degradation of HA, a number of research studies

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have been made with the aim of elongating the presence of HA in the body [12]. Another problem in the application of HA is the administration route. In most cases, HA is injected, which is a painful application procedure and sometimes causes inflammatory complications and bacterial infections. HA exhibits good gelling properties and leads to a hydration effect in the uppermost layer of the stratum corneum following dermal application, which in most cases promotes penetration of the drug. The water may cause the compact structure of the horny layer to swell and open up, leading to an increase in the extent of penetration. However, the penetration of HA into the deeper layers of the skin is very slow or may be inhibited, depending on the molecular weight of the HA [13].

Cross-linking of the HA molecule may be a possible mode of chemical modification with the aims of preventing the degradation and improving the penetration of HA [12]. Various methods have been developed for the production of cross-linked HA systems for potential use in hydrogels [9,14,15], films [16,17] or particulate systems [10,18,19]. Several attempts have also been made to produce cross-linked HA particles through a carbodiimide technique in aqueous media [20–22]. This procedure has the advantage that stable colloid systems may be obtained in water without the use of any surfactant or other solvent.

We earlier [23,24] described and discussed the successful preparation and characterization of stable nanoparticulate systems based on HA. Covalent cross-linking through the carboxyl functional groups of HA was carried out with a diamine via a carbodiimide technique in aqueous media at room temperature. The effects of the molecular weight of HA, the cross-linking ratio and the environmental conditions were investigated. It was established that cross-linked HA particulate systems could be prepared which can form stable colloid systems in aqueous media.

In the present work, a stable cross-linked HA-based nanoparticulate semisolid preparation was investigated in comparison with a hydrogel containing linear HA. The rheological properties, hydration, irritation effect, *in vitro* and *in vivo* skin penetration abilities were studied.

2. Experimental part

2.1. Materials

The sodium salt of HA (M_W = 4350 kDa) was obtained from Gedeon Richter Ltd., Hungary. Its quality met the European Pharmacopoeia (Ph. Eur. 6) requirements. For the cross-linking reaction, 2,2-(ethylenedioxy)bis(ethylamine) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (CDI) were purchased from Sigma–Aldrich, Co. Transcutol® and Labrasol® were from S & D Chemicals Ltd., Hungary, and glycerol 85% was from Molar Chemicals Kft, Hungary. All other chemicals were of analytical grade.

2.2. Preparation of cross-linked HA nanoparticles

Cross-linked HA nanoparticles were prepared according to the procedure described earlier [23,24]. Briefly, the sodium salt of HA was dissolved in water to produce a clear solution with a concentration of 1 mg/ml. The pH of the solution was adjusted to 5.5. The diamine solution (1.0 v/v% in water, pH = 5.5) was added to the HA solution and mixed for 30 min at room temperature. A CDI solution was next added dropwise, and the reaction mixture was stirred at room temperature for 24 h. The aqueous system containing cross-linked HA nanoparticles was subsequently purified by dialysis for 7 days against distilled water and freeze-dried. The cross-linking ratio was 25%.

2.3. Semisolid gel formulation

Glycerol 85%, and purified water as solvents, with Transcutol® and Labrasol® as penetration enhancers comprised the hydrophilic base of the gel. 1% of either the cross-linked or the linear HA was dispersed into this base. The gels were stirred at intervals until complete dissolution was attained.

2.4. Rheological measurements

Rheological measurements were carried out with a Physica MCR101 rheometer (Anton Paar, Austria). A cone-plate measuring device was used in which the cone angle was 1°, and the thickness of the sample in the middle of the cone was 0.046 mm. Flow curves of the different samples were also determined. The shear rate was increased from 0.1 to 100 1/s (up-curve), and then decreased from 100 to 0.1 1/s (down-curve) in the CR mode. The shearing time in both segments was 300 s. Oscillation measurements were used to analyze both storage modulus (G') and loss modulus (G'') for frequencies between 0.01 and 100 Hz in the linear viscoelastic region. The measurements were performed at 32 °C.

2.5. Hydration and irritation tests

The Corneometer® CM 825 (Courage and Khazaka Electronic GmbH, Cologne, Germany) is the instrument commonly used worldwide to determine the level of hydration of the skin surface, mainly the stratum corneum [25,26]. The investigation is based on measurement of the capacitance of a dielectric medium. The Tewameter® TM 300 (Courage and Khazaka Electronic GmbH, Cologne, Germany) is the most generally accepted measuring device for the assessment of transepidermal water loss (TEWL). This is the most important parameter for evaluation of the barrier function of the stratum corneum. High TEWL values indicate a greater of water loss and are consistent with increased damage to the barrier function of the stratum corneum, such as may occur during irritant exposure. The probe indirectly measures the density gradient of water evaporation from the skin via the two pairs of sensors inside the hollow cylinder [27,28].

Six hairless mice strain (SKH-1) without any dermatological disease or allergy were used in the experiment. All interventions were in full accordance with the NIH guidelines relating to experimentation with animals. The procedures and protocols of all animal experiments in the present study were approved in advance by the Ethical Committee for the Protection of Animals in Scientific Re-