European Journal of Pharmaceutics and Biopharmaceutics 114 (2017) 154–163

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Anti-melasma codrug of retinoic acid assists cutaneous absorption with attenuated skin irritation

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ARTICLE INFO

Article history:
Received 7 November 2016
Revised 16 December 2016
Accepted in revised form 12 January 2017
Available online 1 February 2017

Keywords:
Codrug
Retinoic acid
Hydroquinone
Cutaneous absorption
Melasma

ABSTRACT

Melasma treatment with combined retinoic acid (RA) and hydroquinone (HQ) usually causes unsatisfactory outcomes and safety concerns. This study attempted to evaluate the cutaneous absorption and skin tolerance of the codrug conjugated with RA and HQ via ester linkage. The codrug's permeation of the pig skin was estimated using Franz diffusion cell. The codrug and parent drugs were comparatively examined for anti-inflammatory activity and tyrosinase inhibition. In vivo cutaneous irritation was assessed on nude mouse skin. Chemical conjugation of RA with HQ increased the lipophilicity and thus the skin absorption. The codrug absorption produced a 5.5- and a 60.8-fold increment compared to RA skin deposition at an equimolar (1.2 mM) and saturated solubility dose, respectively. The cumulative amount of HQ derived from the codrug in the receptor was comparable to or less than that of topically applied HQ. The RA-HQ codrug was partly hydrolyzed on penetrating the skin. The hydrolysis rate in intact skin was significantly lower than that in esterase medium and skin homogenates. The codrug showed an interleukin (IL)-6 inhibition activity comparable to RA. A therapeutic index 6-fold greater than RA was obtained with the topical codrug. The tyrosinase inhibition percentage of the codrug and HQ was 13% and 21%, respectively. The skin tolerance test determined by transepidermal water loss (TEWL), redness, and histopathology had exhibited minor skin irritation caused by the codrug compared to the physical mixture of RA and HQ at an equivalent dose. Topical codrug delivery not only promoted RA absorption, but also diminished the adverse effects of the parent agents.

1. Introduction

Melasma is a facial hypermelanosis characterized by irregular melanin accumulation in the skin. It can be caused by ultraviolet (UV) light exposure, pregnancy, oral contraceptive consumption, and endocrine dysfunction [1]. Melasma is found in 41% of females between the period of pregnancy and menopause [2]. The condition often develops slowly and continues for 9–12 years [3]. The use of topically applied hydroquinone (HQ) is the standard management of melasma due to the inhibitory ability of melanin synthesis. Topical retinoic acid (RA) therapy is also standard for melasma treatment owing to the bioactivity of cell-turnover regulation and its antioxidant property. The cutaneous photoaging and inflammation induced by UV can be reduced by RA [4]. Combined therapy using HQ plus RA is usually used in the treatment of melasma [5]. However, melasma is still recalcitrant to the dual drug treatment because it is prone to relapse with rebound hyperpigmentation. Subsequently, a prolonged treatment duration is necessary. This leads to a high incidence of adverse events (40%) in patients receiving combination therapy [6]. The undesired occurrence of skin itching, burning, and allergic dermatitis has led to the issue of patient compliance.

The simultaneous administration of two drugs in a physical mixture may not be an efficient method to achieve absorption to the target site and therefore may result in a poor response to the disease [7]. Structural manipulation of the drugs is a valid approach to enhance skin permeation. It is possible to covalently

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http://dx.doi.org/10.1016/j.ejpb.2017.01.016
0939-6411/

conjugate two drug molecules to form codrugs to ameliorate cutaneous delivery. Like the prodrug strategy, the codrugs can improve the biomembrane passage, extend the therapeutic period, increase the therapeutic outcome, and minimize the toxicity as compared to the parent drugs [8]. The production of unwanted promoieties by prodrug hydrolysis is absent in the case of codrugs [9]. RA possesses a carboxylic acid moiety, while HQ contains the hydroxyl groups, recognizing a possible formation of the esterified codrug for treating melasma. We aimed to synthesize the RA-HQ codrug to evaluate skin absorption and irritation via topical application. It was hypothesized that the codrug would increase the lipophilicity and thus enhance the cutaneous penetration relative to the parent drugs. Improved melasma therapy with attenuated irritation was anticipated.

The physicochemical properties of the codrug such as the melting point, lipophilicity, and solubility in aqueous solution and oil were characterized in the present study. The codrug hydrolysis was examined in esterases and skin homogenates to confirm the cleavage after entering the skin. The skin absorption was assessed by Franz diffusion cell with pig skin as the permeation barrier. The parent agent alone and the physical mixture of RA and HQ were included for comparison. The therapeutic effect of the codrug itself could be inactive, less active, or more active than the parent drugs [10]. The pharmacological activity of the RA-HQ conjugate could not be neglected. The anti-cytokine action and tyrosinase inhibition of the codrug were tested to elucidate this plausibility. Finally, the in vivo skin irritation elicited by the codrug and parent actives was analyzed based on the cutaneous physiology and histopathology.

2. Materials and methods

2.1. Synthesis of the codrug

Retinoic acid (1 mmol) and hydroquinone (1.2 mmol) dissolved in dry dichloromethane tetrahydrofuran with a ratio of 2:1, N,N-dicyclohexylcarbodiimide (DCC, 1.3 mmol) and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.8 mmol) were added to react under nitrogen. The reaction mixture was stirred for 48 h at room temperature. The solution was filtered and washed with an excess of dichloromethane. The filtrate was washed with water and dried over anhydrous magnesium sulfate. After removal of the solvent by evaporation under reduced pressure, the residue was purified by column chromatography on silica gel using ethyl acetate: n-hexane (44:5:6) as eluent. The scheme of synthesis is shown in Fig. 1. The structure of the codrug was verified by nuclear magnetic resonance (NMR, Avance 400, Bruker) and Fourier transform infrared spectroscopy (FTIR, FT/IR-4100, Jasco). IR (KBr) ν_{max} 3429, 2926, 2862, 1700, 1603, 1577, 1510, 1444, 1354, 1134, 955 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, dd, J = 15.8, 11.6 Hz), 6.96 (2H, d, J = 8.4 Hz), 6.77 (2H, d, J = 8.4 Hz), 6.37 (1H, d, J = 15.8), 6.31 (1H, d, J = 15.8 Hz), 6.18 (1H, d, J = 11.6 Hz), 6.16 (1H, d, J = 15.8 Hz), 5.98 (1H, s), 2.40 (3H, s), 2.02 (2H, m), 2.02 (3H, s), 1.76 (3H, m), 1.62 (2H, m), 1.47 (2H, m), 1.03 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6 (s), 155.6 (s), 153.2 (s), 144.1 (s), 140.4 (s), 137.6 (s), 137.2 (d), 134.7 (d), 132.0 (d), 130.2 (s),129.4 (d), 129.1 (d), 122.6 (2C, d), 117.1 (d), 115.9 (2C, d), 40.0 (t), 33.5 (t), 29.4 (2C, q), 22.2 (q), 19.6 (t), 14.5 (q), 13.4 (q). Figs. S1 and S2 show the spectra of RA, HQ, and RA-HQ codrug determined by NMR and FTIR, respectively.

2.2. Melting point

The melting points of the parent drugs and the codrug were measured by a differential scanning calorimeter (Q2000, TA Instruments).

2.3. N-octanol/water partitioning (log P)

A methanolic solution (1 ml) of RA, HQ, or codrug (0.5 mg) was prepared in a glass tube. Methanol was evaporated by a vacuum. The *n*-octanol and deionized water, 1 ml of each, were added to the tube. After being shaken for 24 h at 37 °C, the phases were separated by centrifugation at 10000*g* for 10 min. The compound concentration in organic solvent and water was detected by high-performance liquid chromatography (HPLC). The *n*-octanol/water partition coefficient was calculated as log *P*. The stationary phase of HPLC was a 25-cm-long, 4-mm inner diameter reverse-phase C18 column (Merck). The mobile phase for RA and the codrug consisted of methanol and pH 4.5 phosphate buffer (95:5) at a flow rate of 1 ml/min. The UV wavelength of the detector was set at 343 nm. The mobile phase for HQ consisted of methanol and pH 2 phosphate buffer (10:90). The flow rate and wavelength for HQ were 1 ml/min and 288 nm, respectively.

2.4. Saturated solubility

An excess amount of RA, HQ, or codrug was added to 1 ml of 50% propylene glycol (PG)/pH 7.4 buffer or mineral oil. The mixture in the test tube was shaken reciprocally at 37 °C for 24 h. After the

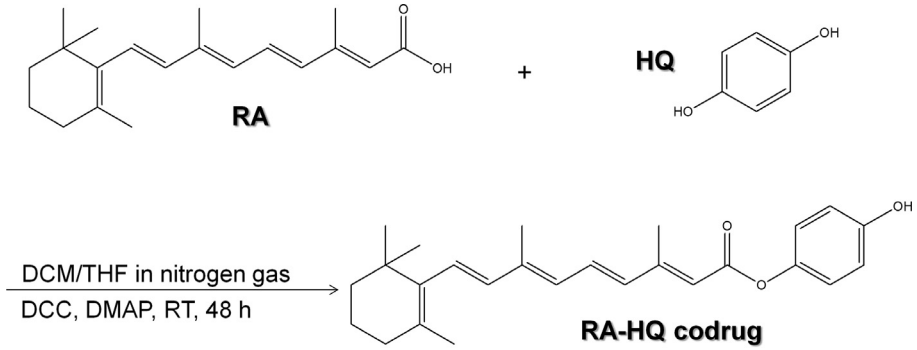

Fig. 1. Chemical reaction scheme for synthesizing retinoic acid (RA)-hydroquinone (HQ) codrug.