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Research paper

The co-drug of conjugated hydroquinone and azelaic acid to enhance topical skin targeting and decrease penetration through the skin

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ABSTRACT

A co-drug of hydroquinone (HQ) and azelaic acid (AA), bis(4-hydroxyphenyl)nonanedioate (BHN), was synthesized and investigated as a topical prodrug with the aim of improving the dermal delivery of the parent drugs. Physicochemical parameters were ascertained, and the enzymatic hydrolysis was examined. Skin permeation of HQ, AA, and BHN was studied by determining the skin deposition and flux across nude mouse skin under equivalent doses with the same thermodynamic activity. The partition coefficient (log *P*) of the co-drug increased by up to 5.0 with HQ and AA conjugation, which had respective log *P* values of 0.5 and 1.4. In the skin absorption experiment, BHN in ethanol/pH 7 buffer resulted in a 2-fold enhancement of skin deposition compared to both HQ and AA. With permeation using polyethylene glycol 400/pH 7 buffer as the vehicle, the co-drug, respectively, exhibited 8.1- and 1.4-fold enhancements of skin uptake compared to HQ and AA alone. The transdermal flux from BHN was negligible compared to those with HQ and AA treatments. The results of a preliminary safety evaluation showed no signs of stratum corneum disruption or erythema by BHN application within 24 h. The co-drug approach provides a viable option for the treatment of skin hyperpigmentation of HQ and AA.

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1. Introduction

Hyperpigmentation is a cosmetically important condition seen most often as a result of exposure to ultraviolet light, certain drugs and chemicals, and the existence of disease. Hyperpigmentation may also be a postinflammatory response to trauma, chemical peels, laser therapy, and acne [1]. The most commonly used treatment for all types of hyperpigmentary disorders is topical hydroquinone (HQ). HQ interacts with tyrosinase by binding histidines at the active site of the enzyme resulting in reduced skin pigmentation [2]. Azelaic acid (AA) was also shown to be effective in treating hyperpigmentation. It inhibits mitochondrial oxidoreductase activation, DNA synthesis, and tyrosinase [3]. In addition, it is used to treat rosacea, acne, melasma, malignant melanomas, and perioral dermatitis [4].

Many skin-lightening agents cause skin irritation and require months of use before results appear, and some agents are only partly effective [1]. For example, HQ induces the generation of reactive oxygen species, which can cause oxidative damage to membrane lipids and proteins. Exposure to HQ can cause skin irritation and sensitization, nail discoloration, and mutagenesis [5]. Moreover, it is known that this drug is rapidly transported from the skin to the vascular system and liver [6]. The most common side effects of topical AA are transient erythema, burning, and stinging, with an incidence of 38% [7]. Another disadvantage is that both HQ and AA may require several months to achieve significant improvement of the disorders, and some patients never achieve a completely satisfactory result with either treatment [8]. This has motivated clinicians and investigators to seek new treatments that have better efficacy for such skin disorders as hyperpigmentation and acne.

A rational way to improve the efficiency of drugs by the dermal route would be to manipulate the physicochemical properties to increase the rate of diffusion into the skin barrier. A promising approach in this respect is development of prodrugs [9,10]. Through the addition of a cleavable chemical group that typically increases a drug's lipophilicity, such prodrugs can facilitate the transfer of a drug into or across the skin. Because the prodrug approach is based on altering a drug's structure, prodrugs usually do not cause skin irritation [11]. A prodrug relies upon conversion within the body

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to release the parent active drug and pro-moiety to elicit its pharmacological effect. The main drawback of this approach is that the pro-moiety is essentially unwanted ballast which, when released, can lead to adverse effects. The term "co-drug" refers to the combination of two or more therapeutically active compounds via a readily cleavable covalent linkage. Advantages include synergistic modulation of the disease, enhancement of drug delivery, and the potential to enhance stability by masking labile functional groups [12].

To date, limited studies have been published on the delivery of co-drugs for dermatological purposes. HQ contains two hydroxyl functionalities, whereas AA contains two carboxylic acid groups, thus allowing the possibility of forming an ester co-drug. It is hypothesized that the co-drug would demonstrate enhanced dermal uptake properties relative to both parent drugs as it would be more lipophilic. In this study, we synthesized a novel co-drug that consisted of two HQ molecules and one AA bonded together by two carbonate ester linkages (bis(4-hydroxyphenyl)nonanedioate, BHN, Fig. 1). The physicochemical characteristics of the co-drug, including its melting point, partition coefficient, and solubility, were determined. The hydrolysis rates in esterases, skin homogenate, and plasma were investigated. Amounts of the compounds retained within skin reservoir and which penetrated across the skin were determined using in vitro Franz cells. Finally, in vivo skin uptake and skin irritation were examined. BHN can be used as a relatively more effective and safer agent to treat skin disorders, for example, in cases of combined hyperpigmentation and acne.

2. Materials and methods

2.1. Materials

HQ, AA, 4-dimethylamino-pyridine (DMAP), trifluoroacetic acid (TFA), and esterase from porcine liver were purchased from Sigma–Aldrich (St. Louis, MO, USA). *N*,*N*′-Dicyclohexylcarbodiimide (DCC) and triisopropylsilane (TIS) were obtained from Alfa Aesar (Ward Hill, MA, USA). 4-(Tert-butoxy)phenol was supplied by Matrix Scientific (Columbia, SC, USA). Polyethylene glycol (PEG)400 was from Kanto (Tokyo, Japan).

2.2. General procedure for the synthesis of Bis(4-tert-butoxyphenyl)nonanedioate (Ad4t)

Ad4t was synthesized by Steglich esterification as shown in Fig. 1. A solution of 4-(*tert*-butoxy)phenol (7.8 mmol), AA (4.8 mmol), and DCC (4.9 mmol) in anhydrous DCM (60 ml) was stirred at 0 °C for 1 h. DMAP (4.4 mmol) was added and stirred at room temperature for 24 h. The solvent was evaporated at reduced pressure. The residue was purified by silica gel column chromatography using a mixture of *n*-hexane: ethyl acetate (6: 1) to afford the products. A colorless powder and 52% yield was obtained. ^{1}H NMR ($CDCl_3$) δ 6.91 (8H, br.s), 2.48 (4H, t, J = 7.6 Hz), 1.70 (4H, br.t, J = 6.8 Hz), 1.36 (6H, m), 1.27 (18H, s). ^{13}C NMR ($CDCl_3$) δ 172.3 (s), 152.8 (s), 146.4 (s), 124.8 (d), 121.6 (d), 78.6 (s), 34.3 (t), 28.8 (t), 28.7 (t), 24.8 (t).

2.3. General procedure for the synthesis of BHN

Ad4t was de-protected by a TFA/TIS/DCM solution to afford BHN as shown in Fig. 1. A solution of Ad4t (3.6 mmol) in TFA:TIS:H_2O of 4:1:35 (40 ml) was stirred at room temperature for 2 h. The solvent was evaporated under a vacuum, and the reaction mixture was purified by silica gel column chromatography using a mixture of *n*-hexane:acetone (2:1) to afford the products. A colorless powder and 65% yield were obtained. ^{1}H NMR (acetone-d6) δ 8.31 (2H, br.s), 6.91 (4H, d, J = 8.8 Hz), 6.81 (4H, d, J = 8.8 Hz), 2.53 (4H, t, J = 7.2 Hz), 1.72 (4H, m), 1.43 (6H, m). ^{13}C NMR (acetone-d6) δ 172.4 (s), 155.3 (s), 144.1 (s), 122.9 (d), 115.9 (d), 34.1 (t), 29.2 (t), 25.1 (t). HRESI-MS m/z 395.1472 $[M + Na]^+$ (calcd. for $C_{21}H_{24}O_6Na$ 395.1471).

2.4. Fourier-transformed infrared (FTIR) absorption spectrometry

The chemical structure of BHN was verified by FTIR. Tablets of HQ, AA, and BHN were obtained using potassium bromide disc pellets. The measurement was taken on an FTIR spectrometer (FT/IR-4100, Jasco, Tokyo, Japan).

2.5. Melting points

The melting points of HQ, AA, and BHN were detected using a Fisher-Johns melting point apparatus (Fisher Scientific, Pittsburgh, PA, USA).

2.6. Capacity factor (log K')

Log K' values of HQ, AA, and BHN were isocratically determined using high-performance liquid chromatography (HPLC). The HPLC system included a Hitachi L-2130 pump (Tokyo, Japan), a Hitachi L-2200 sample processor, and a Hitachi L-2400 UV–visible detector. A 25-cm-long, 4-mm inner diameter C18 column (Merck, Darmstadt, Germany) was used. The mobile phase consisted of a methanol (pH 2.5) aqueous solution adjusted with 0.02 M potassium phosphate (15:85) at a flow rate of 1.0 ml/min. The UV wavelength was set to 289 nm. The retention time of each compound was measured, and K' values were calculated using the following equation: $\log K' = \log (t_r - t_0)/t_0$, where t_r is the retention time of each compound and t_0 is the retention time of the non-retained solvent peak, which was about 2.3 min from the sample injection time.

2.7. Partition coefficient (log P)

A predetermined amount of a methanolic solution of HQ, AA, or BHN was placed in a glass tube. After completely evaporating the methanol, 1 ml each of deionized water and *n*-octanol was added to the tubes. The mixture was shaken reciprocally in an incubator at 37 °C for 24 h. The phases were separated by centrifugation at 5500 rpm for 10 min. The aqueous phase was filtered through a polyvinylidene difluoride (PVDF) membrane with a pore size of 0.45 μm. The drug concentrations in both the organic solvent and water were determined by HPLC. Partitioning was calculated as the log P (compound concentration in the *n*-octanol phase/compound concentration in the water phase).

2.8. Solubility in different aqueous media

An excess amount of each compound was added to 1 ml of ethanol (EtOH)/pH 7 buffer or PEG/pH 7 buffer at a ratio of 3:7. The mixture was shaken reciprocally at 37 °C for 24 h. The suspension was then centrifuged at 10^4 rpm for 10 min and filtered through a 0.45-μm PVDF membrane. The compound concentration in the supernatant was determined by HPLC.

2.9. Animals

Female nude mice (8 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. The committee confirmed that the animal experiment followed

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