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Hydroquinone-salicylic acid conjugates as novel anti-melasma actives show superior skin targeting compared to the parent drugs

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ABSTRACT

Background: Hydroquinone (HQ) and salicylic acid (SA) are drugs for treating melasma through the mechanisms of tyrosinase inhibition and chemical peeling, respectively. Their high frequency of causing skin irritation has led to limited use of both drugs.
Objectives: We designed the new conjugates obtained by joining HQ and SA by the co-drug concept for evaluating cutaneous absorption capability.
Methods: Monoester (4-hydroxyphenyl 2-hydroxybenzoate, HPH) and diester (1,4-phenylene *bis*(2-hydroxybenzoate), PBH) forms of the conjugates were synthesized and physicochemically characterized. The enzymatic hydrolysis to the parent drugs was examined. Both an equimolar dose and a saturated solubility were utilized as the applied dose for testing cutaneous absorption via pig and nude mouse skins.
Results: The conjugates had higher lipophilicity, less aqueous solubility, and a lower melting point/crystallinity than the parent drugs. Both conjugates showed a quick conversion into the parent drugs in esterases and skin homogenates, with PBH showing the greater hydrolysis. The hydrolysis level in skin after topical application was less as compared to that in esterases and homogenates. The tyrosinase inhibition (%) and molecular docking demonstrated that the conjugates possessed skin-lightening capability (3% for HPH and 7% for PBH) although this activity was lower than that of HQ (23%). The conjugates showed an increased skin deposition compared to the respective parent drugs. Total absorption of HPH and PBH led to a 13- and 19-fold enhancement in cutaneous retention compared to HQ alone. A similar increment of skin deposition was shown for the conjugates when compared to SA. Contrary to skin reservoir retention, transdermal transport across the skin was decreased by the conjugates, especially for PBH. This indicates the maximization of cutaneous targeting by the conjugates.
Conclusions: Topically applied HPH and PBH can be the new candidates for treating melasma due to efficient skin absorption and acceptable skin tolerance.

1. Introduction

Melasma is a dermatological disease of hyperpigmentation commonly triggered by solar irradiation and hormones [1]. It affects millions of people worldwide, appearing most often on the forehead, cheek, and lip [2]. Although not life-threatening, melasma is usually difficult to treat and has a negative influence on patients' emotions and quality of life [3]. Hydroquinone (HQ) as a skin-lightening drug is a first-line medication for treating melasma. It is also a gold standard when treating postinflammatory hyperpigmentation and lentigo [4]. Superficial peeling agents used to exfoliate the epidermis are also employed to treat melasma due to their ability to remove keratinocytes containing melanin

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[5]. Salicylic acid (SA) is commonly used in clinics as a drug for peeling. It is also known as an ultraviolet B sunscreen for the possible prevention of melasma [6]. Because of the different action mechanisms, HQ in combination with peeling agents offers a synergistic therapy for treating melasma [7]. HQ is categorized as a hydrophilic molecule with a limited skin permeability. Moreover, HQ poses some problems with storage instability and skin toxicity such as contact dermatitis and allergic dermatitis. Up to 70% of patients experience cutaneous irritation after HQ application [8]. Chemical peels also cause skin irritation and inflammation in patients with melasma [9].

To improve the efficacy and safety of melasma therapy, intense efforts should be devoted to enhance drug absorption and reduce toxic risk. One of the strategies is the design of prodrugs. The prodrug approach represents chemical modification of an active into a bioreversible form for overcoming permeation barriers and local irritation [10]. The prodrug concept can be extended to the conjugates of two or more actives. Such a derivative is called a co-drug. A co-drug aims to deliver different drugs simultaneously with a favorable permeation and greater stability compared to the parent compounds. Combinative therapy can be administered in the form of a co-drug to ameliorate skin transport and targeting [11]. It may be beneficial for melasma patients since they should apply two or more drug products during the course of treatment [12]. An all-in-one co-drug can improve patients' compliance and therapeutic outcome.

In order to promote cutaneous delivery of HQ and SA for treating melasma, and to take advantage of the synergistic activity of skin lightening and chemical peeling combined in one unique entity, we synthesized co-drugs by coupling HQ and SA via covalent linkage. Two ester co-drugs were designed by combining HQ and SA in 1:1 (4-hydroxyphenyl 2-hydroxybenzoate, HPH) and 1:2 (1,4-phenylene *bis*(2-hydroxybenzoate), PBH) stoichiometric ratios, respectively (Fig. 1). The physicochemical properties of co-drugs, such as the partition coefficient (log *P*), solubility, and melting point, were characterized in this report. That the HQ–SA conjugation may exhibit some pharmacological actions cannot be ignored. The tyrosinase inhibition activity of co-drugs was examined to confirm this possibility. Molecular modeling was also utilized to assess the possible docking between co-drugs and tyrosinase. Esterases, skin homogenate, and plasma were used to test the co-drug hydrolysis. The comparison of skin absorption between the conjugates and parent drugs was carried out by Franz cells. Both pig and nude mouse skins were used as permeation barriers. The cutaneous tolerance of topically applied co-drugs was demonstrated by evaluating skin physiology and histopathology after in vivo administration in nude mice.

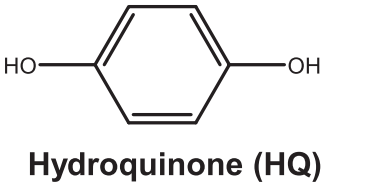
2. Materials and methods

2.1. Materials

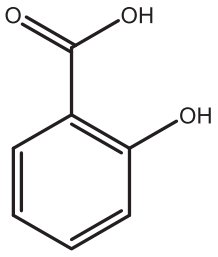
HQ, SA, polyethylene glycol (PEG)400, esterases from porcine liver, 4-dimethylaminopyridine (DMAP), and mushroom tyrosinase were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N,N′*-dicyclohexylcarbodiimide (DCC) was supplied by Alfa Aesor (Ward Hill, MA, USA). Tetrahydrofuran (THF) was obtained from Mallinckrodt (Hazelwood, MO, USA).

2.2. Synthesis of 4-hydroxyphenyl 2-hydroxybenzoate (HPH)

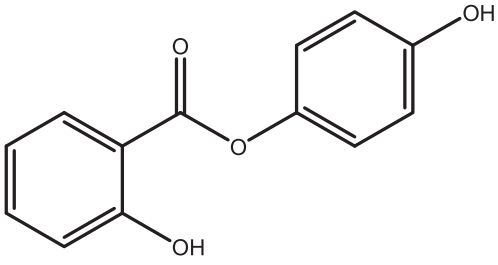
A dispersion of HQ (7.8 mmol), SA (7.2 mmol), and DCC (7.3 mmol) in THF (45 ml) was stirred at room temperature in the presence of nitrogen gas. DMAP (0.8 mmol) was added and



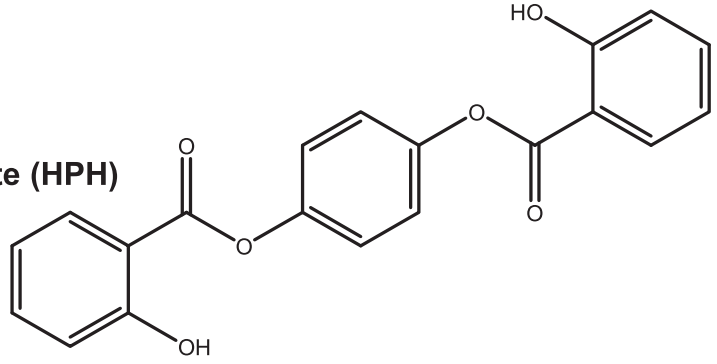
Hydroquinone (HQ)



Salicylic acid (SA)



4-Hydroxyphenyl 2-hydroxybenzoate (HPH)



1, 4-Phenylene bis(2-hydroxybenzoate) (PBH)

Fig. 1. Chemical structures of hydroquinone (HQ), salicylic acid (SA), 4-hydroxyphenyl 2-hydroxybenzoate (HPH), and 1,4-phenylene *bis*(2-hydroxybenzoate) (PBH).