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Research paper

Enhancement of transdermal apomorphine delivery with a diester prodrug strategy

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ABSTRACT

Diester prodrugs of apomorphine, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) were synthesized, and their partition coefficients, capacity factor ($\log K'$), enzymatic hydrolysis, and in vitro permeation across nude mouse skin were characterized. The lipophilicity of the diesters was between that of apomorphine HCl and the apomorphine base. The prodrugs were chemically stable, but enzymatically unstable in esterase medium, skin homogenate, and human plasma. DAA showed a faster hydrolysis in plasma compared to DIA. Total fluxes (nmol/cm^2/h) of the parent drug and prodrug were significantly greater after topical treatment with the diesters in aqueous solutions (water, 30% polyethylene glycol in water, and 30% glycerol in water) compared to treatment with HCl and base forms of apomorphine. DIA flux from deionized water was 51 nmol/cm^2/h, which exceeded the flux of apomorphine HCl by 10-fold. The extent of parent drug regeneration after topical application ranged 51–88% and 34–61% for DAA and DIA, respectively, depending on the vehicles selected. Permeation measurements using intact and stratum corneum-stripped skins demonstrated that the viable epidermis/dermis was an important barrier to prodrug permeation. Nano-sized lipid emulsions were also used as carriers for apomorphine and its prodrugs. Diester prodrugs exhibited superior skin permeation compared to the parent drug when formulated into the emulsions. DAA and DIA fluxes from lipid emulsions were 11- and 3-fold higher than that of apomorphine HCl. The results in the present work suggest the feasibility of diester prodrugs for the transdermal delivery of apomorphine.

1. Introduction

Parkinson's disease affects approximately 1% of people over 65 years old and approximately 3% of those over 85 years old [1]. Apomorphine is considered to be a classical mixed type of dopamine D_1 and D_2 receptor agonists. It is used in the therapy of Parkinson's disease [2]. However, orally administered apomorphine in advanced Parkinson's disease patients is not successful due to the requirement of high doses as a result of metabolic constraints and the first-pass effect. High oral doses of apomorphine can cause gastrointestinal complications and are associated with nephrotoxicity [3]. Apomorphine is most commonly administered by repeated subcutaneous infusions or injections, which invariably result in the appearance of subcutaneous nodules. So far, none of the administration routes has resulted in a delivery system suitable for the widespread clinical application of apomorphine.

Transdermal delivery of apomorphine could be an ideal route of administration due to the advantages of elimination of first-pass metabolism, a reduction in gastrointestinal side effects, and sustained release to resolve the short half-life (32 min) of apomorphine. Although the skin as a route for drug delivery can offer several advantages, the barrier nature of the skin enables only a few molecules to penetrate into and permeate through it. According to the previous study [4], transdermal apomorphine delivery only shows a bioavailability of ~10%. The in vitro permeation of apomorphine by passive diffusion also suggests a negligible flux of near zero [5]. The prodrug approach represents an alternative and very promising method of enhancing skin permeation of drugs. The prodrug concept involves the chemical modification of a drug into a bioreversible form in order to change its pharmaceutical and pharmacokinetic properties and thus enhance its delivery [6,7]. A prodrug strategy to prolong the duration of action in astrocyte

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cultures was reported in the literature [8], but no information exists about the use of apomorphine prodrugs transdermally. The main purpose of this investigation was to develop and evaluate prodrugs for the transdermal delivery of apomorphine. The skin shows high enzymatic activity, mainly due to esterase activity. Ester prodrugs were designed and thoroughly investigated to increase transdermal drug delivery [9,10]. In the present work, two diester prodrugs, diacetyl apomorphine (DAA) and diisobutyryl apomorphine (DIA), were synthesized and evaluated along with apomorphine HCl and apomorphine base. The physicochemical properties and enzymatic hydrolysis of the synthesized compounds were characterized.

The second goal of the study was to utilize nano-sized lipid emulsions as carriers for apomorphine and its derivatives. This work used an in vitro Franz cell to evaluate the skin permeation of apomorphine and the prodrugs using nude mouse skin as the barrier. The contents of both the prodrug and parent drug were determined after transdermal delivery of diester prodrugs into and across the skin. The possible pathways of apomorphine and its prodrugs via the skin were explored to elucidate the transdermal transport mechanisms of the drug/prodrug from lipid emulsions and other vehicles such as water, polyethylene glycol (PEG), glycerol, and mineral oil.

2. Materials and methods

2.1. Materials

Apomorphine HCl, esterase from porcine liver, glycerol, mineral oil, *n*-octanol, and Pluronic F68 were purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA). PEG400 was supplied by Kanto Chemical (Tokyo, Japan). Myverol 18–04 K was obtained from Quest (Naarden, the Netherlands). All other chemicals and solvents were of analytical grade and were used as received.

2.2. Preparation of apomorphine base

Apomorphine base was obtained using a method of precipitation [11]. After a saturated solution of Na_2CO_3 (1.4 g/ml) was added drop-wise to an apomorphine HCl solution in deionized water, apomorphine base was precipitated. The precipitate was then filtered and washed several times with deionized water to remove the Na_2CO_3. After drying, the residual apomorphine base was obtained and verified by infrared (IR) and nuclear magnetic resonance (NMR) analyses.

2.3. Preparation of diester prodrugs

The synthesis, purification, and proof structure of DAA were described previously [12]. The general procedure of the DAA preparation was the use of carboxylic acid anhydride for esterification of apomorphine by utilizing pyridine as the catalyst. This method of DAA to prepare DIA by using isobutyric anhydride in place of acetic anhydride was conveniently extended in this study. Apomorphine hydrochloride (1.00 g, 3.20 mmol), acetic anhydride or isobutyric anhydride (6 ml), and pyridine (1 ml) were mixed in a three-necked round bottom flask with a magnetic stirrer bar under nitrogen. When the mixture was stirred at room temperature for 4 h, the reaction was complete as monitored by thin-layer chromatography. Then, this reaction mixture was diluted by ethyl acetate (70 ml) and a solution of potassium carbonate (7.60 g, 0.055 mol) in water (60 ml). The organic phase was washed by water (20 ml) and brine (10 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a yellow crude oil which was chromatographed through a short column of silica gel to afford DIA (0.99 g, 2.83 mmol) in 88% yield, mp 124–125 °C MS(EI) *m*/*z* (% relative intensity) 266(100), 351(M+, 95), 350(78), 308(70), 265(25), 43(21), 224(21), 352(21), 267(20), 309(17), 206(17). DIA (1.10 g, 2.70 mmol) was also obtained in 84% yield, mp 105–106 °C MS(EI) *m*/*z* (% relative intensity) 266(100), 407(M+, 79), 336(76), 406(61), 43(40), 265(23), 267(21), 408(20), 337(20), 224(17), 294(17).

2.4. Partition coefficients (log P)

A predetermined amount of a methanolic solution of apomorphine or its prodrugs was placed in a glass tube. After completely evaporating the methanol, 1 ml of deionized water and *n*-octanol or mineral oil was added to the tubes. The mixture was shaken reciprocally in an incubator at 32 °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/prodrug concentrations in both the organic solvent and water were determined by high-performance liquid chromatography (HPLC). Partitioning was calculated as the log $P_{octanol/water}$ or log $P_{MO/water}$ (compound concentration in the *n*-octanol or mineral oil phase/compound concentration in the water phase).

2.5. Capacity factor (log K′)

The K' values of apomorphine and its prodrugs were determined isocratically using HPLC. The HPLC system included a Hitachi L-2130 pump (Tokyo, Japan), a Hitachi L-2200 sample processor, and a Hitachi L-2400 ultraviolet (UV)–visible detector. A 25-cm-long, 4-mm-inner-diameter C18 column (Merck, Darmstadt, Germany) was used. The mobile phase consisted of an acetonitrile: pH 2.5 aqueous solution adjusted with phosphoric acid (20:80) at a flow rate of 1.0 ml/min. The UV wavelength was set to 212 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 (methanol), which was about 1.9 min from the sample injection time.

2.6. Hydrolysis of apomorphine prodrugs

The enzymatic hydrolysis of diester apomorphine was carried out using esterase, nude mouse skin homogenate, and human plasma. The female nude mouse (ICR-Foxn1nu strain, 8 weeks old) was used in accordance with a protocol approved by the Institutional Animal Care and Use Committee at Chang Gung University. After sacrifice, nude mouse skin (at 150 mg) was cut into pieces and placed into a test tube. pH 7.4 buffer (850 μl) was added to the tissue sample and homogenized for 5 min. The homogenate was centrifuged at 9500*g* for 10 min and filtered through a PVDF membrane to obtain the incubation medium. Blood samples were obtained from healthy donors by venipuncture and collected into test tubes containing 124 mM sodium citrate (one volume of sodium citrate solution + nine volumes of blood), which was approved by the Institutional Review Board at Chang Gung Memorial Hospital. The plasma was obtained as described previously [13]. Stock solutions were prepared by dissolving a weighed amount of each prodrug in methanol to give a concentration of 0.5 mg/ml. A volume of 1 ml of this solution was added to the test tube, followed by evaporation of the organic solvent. A volume of 2 ml of esterase (2.02 IU/ml) in pH 7.4 buffer, skin homogenate, or human plasma was added to the test tube. The resulting solution was incubated and shaken at 37 °C. At predetermined intervals, the reaction mixture was withdrawn, and a 400 μl acetonitrile was rapidly added to stop the enzymatic reaction. After thawing and