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Apomorphine and its esters: Differences in Caco-2 cell permeability and chylomicron affinity

Nrupa Borkar[a], Zhizhong Chen[a], Lasse Saaby[b], Anette Müllertz[a,b], Anders E. Håkansson[c], Christian Schönbeck[d], Mingshi Yang[a], René Holm[a,d], Huiling Mu[a,*]

[a] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark
[b] Bioneer: FARMA, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark
[c] Process Chemistry, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark
[d] Pharmaceutical Science and CMC Biologics, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark

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ABSTRACT

Oral delivery of apomorphine via prodrug principle may be a potential treatment for Parkinson's disease. The purpose of this study was to investigate the transport and stability of apomorphine and its esters across Caco-2 cell monolayer and their affinity towards chylomicrons. Apomorphine, monolauroyl apomorphine (MLA) and dilauroyl apomorphine (DLA) were subjected to apical to basolateral (A-B) and basolateral to apical (B-A) transport across Caco-2 cell monolayer. The stability of these compounds was also assessed by incubation at intestinal pH and physiological pH with and without Caco-2 cells. Molecular dynamics (MD) simulations were performed to understand the stability of the esters on a molecular level. The affinity of the compounds towards plasma derived chylomicrons was assessed. The A-B transport of intact DLA was about 150 times lower than the transport of apomorphine. In contrast, MLA was highly unstable in the aqueous media leading to apomorphine appearance basolaterally. MD simulations possibly explained the differences in hydrolysis susceptibilities of DLA and MLA. The affinity of apomorphine diesters towards plasma derived chylomicrons provided an understanding of their potential lymphatic transport. The intact DLA transport is not favorable; therefore, the conversion of DLA to MLA is an important step for intestinal apomorphine absorption.

1. Introduction

The physicochemical properties of drug molecules as well as their oral absorption can be altered by chemical derivatization yielding bio convertible prodrugs. Apomorphine, a drug used in the treatment of Parkinson's disease, is conventionally administered sub-cutaneous as it undergoes an extensive hepatic metabolism upon oral administration. In our previous work, apomorphine was chemically modified with fatty acyl groups to obtain lipophilic diester prodrugs aiming at lymphatic transport after oral administration (Borkar et al., 2015). In order to attain lymphatic transport of apomorphine prodrugs, it is crucial that the prodrugs are permeable across the intestinal membrane retaining a sufficient lipophilic character. Hence, the permeability across the intestinal mucosa must be considered a prerequisite for achieving a potential lymphatic transport of apomorphine prodrugs.

The transport across Caco-2 monolayer grown on a filter support can serve as a good model for assessing intestinal

Abbreviations: ANOVA, analysis of variance; CHARMM, chemistry at Harvard Macromolecular Mechanics; CM, chylomicron; DLA, dilauroyl apomorphine; DPA, dipalmitoyl apomorphine; HBSS, Hank's buffered salt solution; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanosulfonate]; ^{1}H-NMR, proton nuclear magnetic resonance; HPLC, high performance liquid chromatography; LC/MS, liquid chromatography-mass spectrometry; LOD, Limit of detection; LOQ, Limit of quantification; MD, molecular dynamics; MES, 2-(*N*-morpholino)ethanesulfonic acid; MLA, monolauroyl apomorphine; NAMD, nanoscale molecular dynamics; Papp, apparent permeability; SEM, standard error of the mean; TEER, trans epithelial electrical resistance; TFA, trifluoroacetic acid; TIP3P, transferable intermolecular potential 3P; VMD, visual molecular dynamics.

* Corresponding author.
E-mail addresses: nrupa.borkar@sund.ku.dk (N. Borkar), zzchern@foxmail.com (Z. Chen), lasse.saaby@sund.ku.dk (L. Saaby), anette.mullertz@sund.ku.dk (A. Müllertz), aeh@lundbeck.com (A.E. Håkansson), cbec@lundbeck.com (C. Schönbeck), mingshi.yang@sund.ku.dk (M. Yang), rhol@lundbeck.com (R. Holm), huiling.mu@sund.ku.dk (H. Mu).

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permeability (Hilgers et al., 1990), which can be used as a predictive tool for *in vivo* absorption of drugs (Hilgers et al., 1990; Yun et al., 2004). Caco-2 cells are derived from human colonic carcinoma cells and are known to express various transporters and secrete endogenous brush border/metabolic enzymes, which mimic human intestinal barrier functionally and morphologically (Sun et al., 2008). It has been demonstrated that several drugs undergo enzymatic degradation in the intestinal epithelium posing a limitation to their oral absorption (Imai et al., 2005). Specifically, the permeability of prodrugs containing ester linkages has been shown to be limited by the presence of esterases (Okudaira et al., 2000; Ruiz-Balaguer et al., 2002). Hence, in this case, investigating the stability, as well as permeability of apomorphine and its esters becomes an important step leading towards an understanding of a potential oral absorption.

It has previously been demonstrated that diisobutyryl and diacetyl apomorphine were transported across skin tissue for transdermal delivery (Liu et al., 2011). However, to the best of our knowledge, the understanding of lipophilic apomorphine prodrugs intended for oral drug delivery is absent with respect to enzymatic stability and their transport across Caco-2 monolayer. Therefore, this study aimed to provide an understanding of the permeability of apomorphine prodrugs and to measure the lymphatic transport potential. This study also aimed to investigate the prodrug stability in order to investigate if this may affect their absorption potential. In addition, molecular dynamics (MD) simulations of apomorphine esters was used as a tool to understand their stabilities on a molecular level.

2. Materials and methods

2.1. Materials

Apomorphine (HCl salt, hemihydrate (≥98.5%)), lauroyl chloride (98%), trifluoroacetic acid (TFA), bovine serum albumin, 2-(*N*-morpholino)ethanesulfonic acid (MES) and *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanosulfonate] (HEPES), soybean oil, hydroquinone (99%), boron trifluoride (50% w/v in methanol), pentadecanoic acid (99%), sodium bromide (99%) and pancreatin from porcine pancreas were all purchased and used without further purification from Sigma–Aldrich (St. Louis, MO, USA). The pancreatic lipase activity was at least equivalent to 3× U.S.P. specifications. Halofantrine hydrochloride was purchased from APAC Pharmaceutical LLC (Hangzhou, China). Halofantrine free base was prepared from halofantrine hydrochloride as previously reported (Porter et al., 1996). Cell culture media and Hanks balanced salt solution (HBSS) were purchased from Life Technologies (Taastrup, Denmark). Sodium chloride (99.5%) and sodium bicarbonate (99.5%) were purchased from Merck (Darmstadt, Germany). ^{14}C-mannitol with a specific activity of 55.0 mCi/mmol and Ultima Gold™ scintillation fluid was obtained from PerkinElmer Life (Boston, MA, USA). Maleic acid (99%) and bovine bile extract were obtained from Fluka Chemi AG (Buchs, Switzerland). Phosphatidylcholine (99%) was purchased from Lipoid (Ludwigshafen, Germany). Maisine 35-1 (glycerol monolinoleate) was obtained from Gattefossé (Lyon, France). Kolliphor RH-40 was purchased from BASF (Ludwigshafen, Germany). Methanol (99.8%), acetonitrile (99.9%) and absolute ethanol (100%) were purchased from VWR (Radnor, PA, USA). Water for the experiments was from a SG Ultraclear 2002 purification system (Barsbüttel, Germany). All other chemicals were of analytical grade and used as received.

2.2. Synthesis and purification of apomorphine esters

The synthesis and purification of apomorphine diesters (dilauroyl apomorphine (DLA) and dipalmitoyl apomorphine (DPA)) was carried out as previously reported by Borkar et al. (2015). The synthesis of monolauroyl apomorphine (MLA) was based on the synthesis for diesters with some modifications (Borkar et al., 2015). Briefly, to synthesize MLA, apomorphine·HCl·$^1/_2H_2O$ (1.2 g, 3.7 mmol) was dissolved in TFA (10 mL) followed by the addition of lauroyl acid chloride (7.5 mmol). The mixture was heated on a water bath at 70 °C for 5 min. After 5 min, the reaction mixture was cooled immediately over ice and then evaporated to dryness over a stream of nitrogen. Column chromatography was used to purify the residue using ethyl acetate:heptane (1:1, v/v) containing 5% v/v triethylamine as the eluent. Fractions were collected and evaporated to dryness. The purity was determined by LC/MS and ^{1}H NMR as previously described in Borkar et al. (2015).

The crude MLA product was dissolved in 2-propanol and was subjected to purification by preparative HPLC/MS using Waters® SunFire C18 preparative column (5 μm, 30 mm × 100 mm). Solvent A was milli-Q water with 0.05% TFA while solvent B was acetonitrile with 0.05% TFA. Method: 0.00–5.00 min: 20% → 100% solvent B in solvent A, 5.00–5.50 min: solvent B, 5.50–6.00 min: 100% → 20% solvent B in solvent A, flow rate: 90 mL/min. With positive ion mode of atmospheric pressure photo ionization, the mass/charge ratio of monoesters 449 *m*/*z* was monitored and the fraction was collected. The fractions were pooled and concentrated *in vacuo*. The product was obtained as a mixture of the two monoesters with a ratio of 1.4:1, which was quantified based on HPLC-UV method. The purity was determined by LC/MS and ^{1}H NMR as previously described in Borkar et al. (2015). The log P value of MLA was calculated to be 7.2 (Chem Draw software, PerkinElmer, USA).

2.3. Transport of compounds across Caco-2 monolayers

Caco-2 cells at passage 20 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and were seeded in T-75 culture flasks and grown in Dulbecco's Modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin/streptomycin (90 U/mL and 90 μg/mL, respectively), 1% L-glutamine, and 1% non-essential amino acids. The cells were grown in an atmosphere of 5% CO_2 (v/v) at 37 °C.

For transport experiments, the cells were seeded onto tissue-culture treated Transwell® filters (1.1 cm^2, 0.4 μm pore size) at a density of 10^5 cells/cm^2 17–21 days prior to experimentation. The Transwell® filter inserts were placed in T-12 culture trays with 0.5 mL culture medium in the apical compartment and 1 mL culture medium in the basolateral compartment. Transepithelial electrical resistance (TEER) values were measured at ambient temperature before and after the transport experiments using a voltohmmeter containing Endohm measurement chambers (EVOM, World Precision Instruments, Florida, USA). Caco-2 cell monolayers displaying values between 200 and 500 Ω cm^2 were used for the transport study.

The transport of apomorphine was investigated in both, the apical to basolateral (A-B) and the basolateral to apical (B-A) direction. HBSS supplemented with 0.05% bovine serum albumin (added to prevent drug adsorption on to plastic surfaces), 4.45 mM sodium bicarbonate and 10 mM MES (adjusted to pH 6.5) or 10 mM HEPES (adjusted to pH 7.4) was used in the transport study. HBSS media adjusted to pH 6.5 was used on the apical side, while HBSS media adjusted to pH 7.4 was used on the basolateral side. The compounds were pre-dissolved in absolute ethanol and added to the respective buffer solutions resulting in a final ethanol concentration of 1% (v/v) and a compound concentration of 30 μg/mL. Caco-2 cells were equilibrated for 10 min prior to the transport study by incubating with 0.5 mL and 1 mL buffer on the