Exploiting the Buccal Mucosa as an Alternative Route for the Delivery of Donepezil Hydrochloride

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ABSTRACT: The potential of the buccal mucosa as an alternative route for the systemic delivery of donepezil (DPZ) hydrochloride, and the impact of various skin penetration enhancers on DPZ buccal permeability, was assessed using an *in vitro* model. DPZ was applied to porcine buccal mucosa in modified Ussing chambers either alone (20 μg/mL) or with different treatment protocols of various enhancers including Azone® (AZ), deoxycholic acid (DA), polyethylene glycol (PEG) 400, and oleic acid (OA)–PEG 400. DPZ permeated the buccal mucosa very rapidly with a permeability coefficient of $35.6 \pm 4.9 \times 10^{-6}$ cm/s, which was not significantly affected by AZ pretreatment. Coapplication of DA 0.6% (w/w), but not DA 0.01% (w/w), reduced the buccal permeation of DPZ (3.5-fold), and PEG 400 reduced the absorption of DPZ in a concentration-dependent manner (1.6- and 18.0-fold reduction at 5% and 50%, w/w, PEG 400, respectively). Coapplication of a combination of OA 1% (v/w) and PEG 400 5% (w/w) further reduced DPZ permeability (5.5-fold), which was demonstrated to result from excipient-induced DPZ precipitation as assessed by light microscopy analysis. These results confirm the feasibility of a novel buccal delivery system for Alzheimer's disease, and suggest various approaches that may be exploited for controlled buccal delivery of DPZ.
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INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that impairs memory and cognitive function mainly in the elderly.[1] Given that AD is a multifactorial disease, various approaches have been proposed to either slow down the progression or prevent the onset of AD; however, the cholinergic hypothesis is still the only hypothesis on which currently approved treatments are based.[2,3] According to this hypothesis, the degradation of cholinergic neurons in the basal forebrain and the loss of cholinergic neurotransmission in the cerebral cortex and other brain regions contribute significantly to cognitive decline.[4] Consequently, therapeutic agents that inhibit acetylcholinesterase have shown beneficial effects in improving cognitive function, albeit they do not prevent the progression of the disease.[5] Different acetylcholinesterase inhibitors are currently approved for use in AD treatment such as donepezil (DPZ), galantamine, rivastigmine, and tacrine. DPZ has shown significant advantages over the other inhibitors because it is approximately 10 times more potent than tacrine, 500–1000-fold more selective for acetylcholinesterase over butyrylcholinesterase,[6] and it also exhibits a longer plasma elimination half-life (70–80 h) relative to other acetylcholinesterase inhibitors, resulting in longer dosing intervals.[7]

The most commonly reported adverse effects of orally administered DPZ occur in the gastrointestinal tract, including nausea, vomiting, and diarrhea.[8,9] These effects are dose-dependent[9] and are more prominent in patients who exhibit poor metabolism (~50% of this population cluster), resulting in adverse events even at low doses.[3] Furthermore, there may be large fluctuations in plasma concentrations after oral administration[10,11] as a result of the rapid absorption characteristics of DPZ, and the interpatient variability in metabolism. For these reasons, and the fact that patients with memory deficits may be at risk of forgetting to self-medicate (further impacting on steady-state plasma concentrations of DPZ), alternative routes of DPZ delivery could significantly enhance therapeutic options available for AD patients. Although one report suggests the possibility of subcutaneous injection of poly lactic-*co*-glycolic acid microparticles for controlled DPZ delivery,[12] this administration route can be inconvenient for many patients, and thus, alternative routes for drug delivery should be investigated. To this end, a recent preclinical study has evaluated the transdermal absorption of DPZ across hairless mouse skin.[13] However, the barrier nature of the skin resulted in an extremely low permeability coefficient of DPZ (25.0×10^{-9} cm/s), requiring a large surface area to achieve the desired plasma levels.

The buccal mucosa represents another route for the delivery of therapeutic agents, and has been exploited clinically for the systemic delivery of fentanyl citrate, miconazole nitrate, and midazolam.[14] The buccal mucosa exhibits high permeability and appreciable bioavailability of therapeutics as it is highly vascularized, with direct access to the systemic circulation through the internal jugular vein and avoidance of hepatic first-pass metabolism.[15] Not only would this route allow for a

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reduction in the dose and, consequently, plasma fluctuations of DPZ, but would minimize the potential for the gastrointestinal side effect profile associated with DPZ. Furthermore, this route is a more convenient route (relative to the proposed subcutaneous injection paradigm), allowing for easy administration and removal of a DPZ dosage form in the case of emergency or overdosage.[14] The potential of the buccal mucosa as an alternative route of delivery for DPZ has not been investigated; however, given the higher permeability of the buccal mucosa relative to the skin,[16] such a delivery route may be of extreme benefit to AD patients. However, as the buccal mucosa still exhibits some barrier properties, chemical enhancers may be necessary to improve the buccal delivery of DPZ. This may be particularly important for DPZ, given the p*K*a of DPZ of 8.8, and with experimental studies being undertaken at pH 7.4 (where DPZ will be approximately 96% ionized),[17] the permeability of DPZ may be limited. Therefore, the purpose of this study was to investigate the potential of the buccal mucosa for the systemic delivery of DPZ and the impact of different skin chemical penetration enhancers [Azone®, AZ; deoxycholic acid, DA; polyethylene glycol (PEG) 400; and oleic acid (OA)–PEG 400 combination] on DPZ permeability using an *in vitro* porcine buccal mucosa model previously established in our laboratory.[18–20]

MATERIALS AND METHODS

Materials

DPZ was purchased from EMD Chemicals (San Diego, California). Krebs bicarbonate Ringer (KBR) buffer was prepared with 115.5 mM NaCl, 4.2 mM KCl, 21.9 mM $NaHCO_3$, 12.2 mM glucose, 4.0 mM HEPES, 1.2 mM $MgSO_4{\cdot}7H_2O$, 2.5 mM $CaCl_2{\cdot}2H_2O$, and 1.6 mM $NaH_2PO_4{\cdot}2H_2O$, and adjusted to pH 7.4 with carbogen (95% O_2 + 5% CO_2) bubbling. AZ was purchased from Yick-Vic Chemicals and Pharmaceuticals (HK) Ltd. (Hong Kong, China). Ammonium acetate, OA, PEG 400, and DA were obtained from Sigma–Aldrich (St. Louis, Missouri). Acetonitrile (Mallinckrodt, Paris, Kentucky) was of HPLC grade and all other chemicals were of analytical grade and were used as received. Water was obtained from a Milli-Q water purification system (Millipore, Milford, Massachusetts).

Methods

DPZ Transport Studies

Porcine buccal tissue was obtained from a local abattoir immediately after slaughter and was transported in ice-cold KBR (pH 7.4). Within 2 h of slaughter, the buccal epithelium was carefully separated from the underlying connective tissue using forceps and surgical scissors. The separated epithelial tissue (~500 μm in thickness) was kept in ice-cold KBR and supplied with carbogen bubbling until being placed in modified Ussing chambers (diffusional area 0.64 cm^2). To determine the impact of AZ, DA, PEG 400, and combinations of PEG 400 and OA on the transport of DPZ, different treatment protocols were considered (pretreatment and coapplication) and compared with that of control (DPZ in KBR). Different pretreatment approaches were considered depending upon the type of penetration enhancer used.

For pretreatment protocols, porcine buccal mucosa was placed in the modified Ussing chambers, which were clamped together, and the donor chamber was filled with 1.5 mL of either KBR alone or KBR containing DA 0.6% (w/w) or OA 1% (w/w)–PEG 400 5% (w/w) for 30 min. The receptor chambers were filled with 1.5 mL of KBR and the chambers were kept at 37°C for 30 min and supplied with carbogen bubbling (95% O_2 and 5% CO_2). For the AZ pretreatment, a 10-μL aliquot of AZ 50% (w/w) in ethanol (EtOH) 95% (v/v) or EtOH 95% (v/v) alone was applied to the exposed buccal mucosa area and the Ussing chambers were clamped together after 30 min. After all pretreatment approaches, solutions were removed and the donor and receptor chambers were filled with 1.5 mL of DPZ solution (20 μg/mL in KBR) and KBR, respectively, and the permeability study was commenced (described in the following paragraph). A donor chamber concentration of DPZ of 20 μg/mL was used for all experiments based on the sensitivity of the HPLC assay and aqueous solubility of DPZ.

For cotreatment approaches, the modified Ussing chambers were clamped together immediately after the porcine buccal mucosa was inserted, and both the donor and receptor chambers were incubated with 1.5 mL of KBR for 30 min at 37°C and supplied with carbogen bubbling. After this equilibration period, both donor and receptor solutions were removed and replaced with either 1.5 mL of DPZ (20 μg/mL in KBR) with or without enhancers [DA 0.01 or 0.6% (w/w), PEG 400 5% (w/w) or 50% (w/w), or OA 1% (w/w)–PEG 400 5% (w/w)] in the donor chamber and KBR in the receptor chamber. From this step forward, similar procedures were considered for both the pretreatment and coapplication protocols. Immediately after addition of the DPZ solution (alone or in the presence of penetration enhancer), a 20-μL aliquot of the donor solution was taken to determine the initial concentration of DPZ present in this chamber. At time intervals of 30 min over 4 h, samples were taken simultaneously from the donor chamber (20 μL) and receptor chamber (200 μL). Although the receptor chamber was replenished with 200 μL of fresh KBR after each receptor sample was taken, the donor chamber was not replenished to prevent continual dilution of the donor chamber solution. Donor and receptor chamber samples were diluted with an equal volume of acetonitrile to precipitate buffer salts and proteins (which may have been extracted from the buccal tissue over the 4-h period). Samples were vortexed and centrifuged for 5 min at 13,147*g* and the supernatant was then transferred into a vial and analyzed by HPLC (according to the method described below). All experiments were conducted using the buccal mucosa of at least two pigs with six replicates.

The steady-state permeation flux (J_{ss}) was determined from the linear slope of the cumulative amount of DPZ permeated versus time curve. The apparent permeability coefficient (P_{app}) of DPZ was then determined by dividing J_{ss} by the initial donor chamber concentration, and P_{app} values from different treatments were compared using a one-way analysis of variance followed by a Tukey's post-hoc test. All statistical analyses were performed using GraphPad Prism software (version 5; GraphPad, San Diego, California).

HPLC Analysis

Chromatographic conditions were evaluated and optimized to obtain good resolution, a narrow peak shape without tailing, adequate sensitivity, and a short retention time of DPZ in KBR (which had been previously exposed to buccal mucosal tissue for 4 h). The concentration of both samples and standard solutions (0.1–10 μg/mL) was assessed by HPLC using a Shimadzu