Azone® Decreases the Buccal Mucosal Permeation of Diazepam in a Concentration-Dependent Manner via a Reservoir Effect

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ABSTRACT: The purpose of this study was to examine concentration-dependent effects of Azone® (AZ) on the buccal absorption of diazepam (DIAZ). Porcine buccal mucosa was placed in modified Ussing chambers and pretreated with 10 μL of 0%, 5%, 20%, and 50% (w/v) AZ in ethanol. DIAZ was administered to the donor chamber either in solution or a chitosan-based gel. The donor chamber disappearance, receptor chamber appearance, and tissue retention of DIAZ were monitored over 2 h by HPLC, with AZ tissue disposition also measured by liquid chromatography–mass spectrometry profiling of tissue cryosections. DIAZ steady-state flux values significantly ($p < 0.05$) decreased 1.4- and 2.4-fold in 20% and 50% AZ-pretreated tissues, respectively. Only 20% and 50% AZ-pretreated tissues were also accompanied by an increased loss of DIAZ from the donor chamber, suggesting DIAZ was forming a reservoir in the buccal mucosa with higher AZ concentrations. Indeed, the percentage of the initial DIAZ dose remaining in the mucosa following a 2 h experiment was increased 3.0-fold with a 50% AZ pretreatment compared with control. AZ provided a concentration-dependent reservoir for DIAZ in buccal mucosa, resulting in retarded release into the receptor chamber, an approach that may be exploited for controlled release of DIAZ. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1133–1141, 2014
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INTRODUCTION

Diazepam (DIAZ) is a benzodiazepine acting in the central nervous system with therapeutic activity in a wide range of disorders, such as anxiety, multiple sclerosis, alcohol withdrawal, epilepsy, and seizures.[1–4] Given the varied indications of DIAZ, it is sometimes necessary to ensure a rapid onset of effect when treating conditions such as epilepsy and seizures, whereas daily dosing is required in chronic treatment of other diseases, for example, multiple sclerosis. One major disadvantage with chronic treatment of DIAZ is the risk of dependency, as peak plasma concentrations following rapid absorption are often associated with euphoria.[3] Given that this issue of dependency is limiting the use of this very useful pharmacological agent, devising drug delivery approaches to retard the systemic absorption of DIAZ may provide benefits to this serious clinical problem. Although the use of oral controlled release formulation approaches could achieve this goal, alternative sites of drug administration can often be more easily manipulated to achieve a slower rate of absorption, in addition to avoiding first pass metabolism.

The buccal mucosa is considered an attractive site for systemic drug absorption and the potential for this route as an alternative site of drug delivery has received increased attention during the last two decades. As an alternative to the often preferred oral route of administration, delivery of drugs across the buccal mucosa has a number of advantages including; (1) avoidance of presystemic metabolism (due to direct drainage of blood from the buccal mucosa into the internal jugular vein), (2) prevention of gastrointestinal degradation, which may result in low oral bioavailability of many drugs including both small, and in particular, large molecules, (3) a rapid onset of action, and (4) easy accessibility with good patient acceptance and compliance.[5–7] Because of a limited surface area available for absorption, however, one of the key issues limiting buccal drug delivery has been the inherent permeability of compounds across the buccal mucosa. Therefore, most drugs successfully delivered to the systemic circulation by the buccal route have been low-molecular-weight drugs resulting in a fast onset of action. Many approaches to enhance the permeability of drugs across the buccal mucosa have been attempted, including the use of chemical penetration enhancers such as bile salts, surfactants, fatty acids, and chitosan, with various mechanisms of actions proposed.[5,8–10] For assessing drug permeability across the buccal mucosa, many *in vitro* models have been developed,[11–15] but only a few have been correlated to *in vivo* buccal absorption studies.[14,15] Porcine buccal mucosa has been the preferred *ex vivo* model for many years, as this type of tissue has many similarities to human tissue in terms of thickness of epithelium, degree of keratinization, and intercellular lipid content.[16,17]

Azone® [1-dodecylazacycloheptan-2-one or laurocapram (AZ)] is a chemical penetration enhancer shown to be beneficial in transdermal and keratinized oral mucosal drug delivery.[18,19] AZ has previously demonstrated varying effects on buccal drug delivery in different studies, including no effect, increased permeability, and decreased permeability depending on the

Abbreviations used: DIAZ, diazepam; AZ, Azone®; SS_{flux}, steady-state flux.

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physicochemical properties of the drug.[19–23] The enhancing effects of AZ in keratinized tissue (such as palatal and gingival mucosa or skin) are believed to result from a disruptive effect of AZ on the intercellular lipids and thereby increasing diffusivity through the membrane.[24] In nonkeratinized tissue (such as the buccal and sublingual mucosa), the lipids are in a much less tight and ordered state,[25] and therefore, the barrier function might be less able to be disturbed by AZ. Instead, the mechanism by which AZ alters buccal mucosal penetration is thought to be via increasing the partitioning of drugs into the mucosa, where AZ is deposited in the buccal tissue, and lipophilic drugs are rapidly taken up by the tissue. Interestingly, whether this enhancement in partitioning leads to reduced or increased permeability appears to be dependent on the drug.[20,21] For example, pretreatment with AZ appears to significantly increase uptake of estradiol into the tissue, leading to a 4.3-fold reduction in permeability into the receptor chamber,[21] whereas the same treatment leads to a 3.8-fold increase in permeability of triamcinolone acetonide across the buccal mucosa.[20] However, predicting what drug-related properties result in the enhanced or reduced permeability effects of AZ pretreatment remain to be exploited. Furthermore, whether these effects of AZ are concentration-dependent has not been investigated, as all previous studies have been conducted with an AZ pretreatment concentration of 5% (w/v). Decreasing the permeability of a drug (such as DIAZ) through exploiting AZ pretreatment could be a beneficial *in situ* controlled release platform. The rapid uptake into the tissue would be considered an advantage, as one of the practical concerns when applying a prolonged release formulation to the oral cavity is the inconvenience caused by long exposure to the dosage form as both water and food consumption is usually prohibited. In addition, the rapid uptake into the tissue minimizes the potential for loss of drug through swallowing. Furthermore, the subsequent slow absorption into the systemic circulation can assist in minimizing the euphoric effects of DIAZ, thus, minimizing the risk for dependency.

Therefore, the aims of this study were to assess the effect of AZ on the buccal mucosal absorption of DIAZ, and to identify whether any permeability altering effects of AZ were concentration dependent. Furthermore, the impact of incorporating AZ into a mucoadhesive DIAZ formulation (closely resembling what might be employed in the clinical setting) on the permeability of DIAZ was assessed using a gel formulation containing the bioadhesive polymer chitosan, a natural polymer consisting of alternating glucosamine and N-acetyl-glucosamine units.[26,27]

MATERIALS AND METHODS

Materials

AZ was obtained from Yick Vic Chemical and Pharmaceuticals (HK) Ltd. (Hong Kong, China). Sodium hydrogen carbonate was purchased from Merck (Darmstadt, Germany). Sodium chloride was obtained from Chem Supply Pty Ltd. (Gillman, South Australia, Australia). DIAZ, D(+)glucose, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), magnesium sulfate heptahydrate, 2-(N-morpholino)ethanesulfonic acid, and 4-morpholineethanesulfonic acid (MES) were all purchased from Sigma–Aldrich (St. Louis, Missouri). Calcium chloride dihydrate, potassium chloride, sodium dihydrogen phosphate dehydrate, and o-phosphoric acid-85% (v/v) were obtained from Thermo Fisher Scientific Inc. (Waltham, Massachusetts). Chitosan was purchased from Primex ehf (Siglufjordur, Iceland). Hexane was obtained from Ajax Finechem (Seven Hills, New South Wales, Australia). [^{3}H]-DIAZ (specific activity 73.3 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, Missouri) and Utima Gold™ scintillation fluid was purchased from PerkinElmer Life Sciences (Boston, Massachusetts). Deionized water was obtained from a Milli-Q water purification system (Millipore Corporation, Billerica, Massachusetts).

Solubility of DIAZ in AZ and Krebs Bicarbonate Ringer Buffer

Krebs bicarbonate Ringer buffer (KBR) was prepared with 6.75 g/L NaCl, 0.31 g/L KCl, 1.84 g/L $NaHCO_3$, 2.20 g/L glucose, 0.95 g/L HEPES, 0.29 g/L $MgSO_4 \bullet 7H_2O$, 0.25 g/L $NaH_2PO_4 \bullet 2H_2O$, and 0.37 g/L $CaCl_2 \bullet 2H_2O$ in water. The buffer was carbogenated (95% O_2 and 5% CO_2) for 30 min before pH adjustment to 7.4. Solubility measurements were performed using the shake flask method. An excess of DIAZ was added into an Eppendorf vial followed by addition of 1 mL KBR or AZ ($n = 3$ each) and shaken at 37°C for 3 days. Aliquots of 100 μL were taken from each sample at 24, 48, and 72 h, and centrifuged at 14,000*g* for 5 min. A 50-μL aliquot of the supernatant was removed and diluted to an appropriate concentration in mobile phase and analyzed by HPLC, as described below. If the variation between the sample points was less than 5%, equilibrium was considered to be obtained. Samples with AZ were initially diluted with ethanol (EtOH) to dissolve AZ.

Permeability Studies

Buccal tissue from freshly slaughtered domestic pigs was obtained at a local abattoir (Australian Food Group, Melbourne, Australia) and transported in ice-cold KBR. The buccal mucosa was carefully isolated from the underlying connective tissue with surgical scissors and forceps within 2 h postmortem. All tissues were isolated and kept in ice-cold carbogenated KBR before commencing the permeability experiment. Porcine buccal mucosa was placed on one half of the modified Ussing chamber (as previously described[22]), and except for the diffusional area, the tissue was covered with Parafilm® to ensure the subsequently administered pretreatment solution remained within the diffusional area (0.64 cm^2). The pretreatment solution [10 μL of EtOH (control) or 5%, 20%, or 50% (w/v) AZ in EtOH] was administered onto the diffusional area, and after 5 min (enough time for the EtOH to evaporate), the Parafilm® was removed before the donor and receptor chambers were clamped together and filled with 1.5 mL of KBR. The modified Ussing Chambers were kept at 37°C and bubbled with carbogen at a rate of 3–4 bubbles/s. For short-term incubation studies (5 min), the donor chamber was filled with 20 μg/mL DIAZ immediately after evaporation of the EtOH from the AZ/EtOH formulation, whereas long-term incubation studies (125 min) were performed by adding KBR to the donor chamber for 120 min after the 5 min evaporation time. After the 125 min pretreatment time (for long term incubation studies), KBR was removed from both chambers and filled with either 1.5 mL of KBR (receptor chamber) or 20 μg/mL DIAZ in either KBR solution or chitosan gel as described below. During the 2 h permeability study, 20 μL samples were taken from the donor chamber and 200 μL samples were taken from the receptor chamber