Development of a Transmucosal Technique for Erythromycin Delivery to Treat Gastroparesis

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Received 25 August 2009; revised 27 October 2009; accepted 31 October 2009

Published online 28 December 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22037

ABSTRACT: Gastroparesis is a serious condition that limits meal or medication emptying from the stomach, resulting in a variety of symptoms, altered nutrition, and inconsistent medication delivery. Our aim was to develop a transmucosal system to deliver erythromycin (EM), a gastric prokinetic agent, to bypass intestinal absorption. Humans and Sprague–Dawley rats were given EM by injection, gavage, or transmucosal gel with or without permeation enhancers. Pharmacokinetics were compared between subjects and across different delivery modalities. Drug concentrations in blood were measured using a bioassay. Design of Experiment techniques were used to optimize transmucosal antibiotic delivery in the Sprague–Dawley Model. Finally, we examined the scale-up of transmucosal delivery to human patients. Transmucosal delivery of EM increased with addition of ursodeoxycholate. While EM release from gels with ursodeoxycholate was significant, it was less than by injection. Scale-up to a human model indicated that delivery of EM using this transmucosal delivery system is insufficient for clinical need. The transport of EM seems limited by its solubility in water and thickness of the epithelial cell layers. Providing successful transmucosal delivery of EM and similar molecules to humans will require more aggressive techniques to disrupt the cellular layer, or pro-drug strategies to increase lipid solubility. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2905–2913, 2010

Keywords: pharmacokinetics; oral (buccal) mucosa; permeation enhancer; bioassay; Carbopol/Carbomer; Sprague–Dawley rat

INTRODUCTION

Gastrointestinal tract motility regulates the orderly movement of ingested material through the gut to ensure adequate digestion and absorption of nutrients, electrolytes, fluid, and medications. Many diseases can interfere with this process leading to gastrointestinal dysfunction, occasionally resulting in poor nutrition, hydration, and inconsistent medication absorption. Gastroparesis is an uncommon, yet serious and well recognized, disorder characterized by symptoms related, in part, to prolonged emptying of contents from the stomach in the absence of mechanical obstruction.[1] It may occur acutely, for example, in response to surgery or medicines, or chronically, such as a result of long-standing diabetes mellitus, surgery, or in an idiopathic form. Individuals with chronic gastroparesis may present with a wide range of symptoms including bloating, abdominal pain, anorexia, early satiety, nausea, and vomiting. These symptoms may be severely disabling causing weight loss and malnutrition requiring nutritional supplementation or even gastric resection.

Gastroparetic patients are frequently administered medications orally to enhance movement of ingested material through the stomach and to improve absorption of other medications necessary for treatment of their underlying condition.[2] The medications used to stimulate gastric motility (i.e., prokinetic agents), however, may not be absorbed consistently, thereby limiting their usefulness. A method to deliver prokinetic agents that bypasses the gastrointestinal tract could provide more consistent levels of these drugs. This would, theoretically, lead to better regulation of gastrointestinal motility and, thus, absorption. Once absorption (and serum levels) of the prokinetic agent becomes more predictable, patients who once had inconsistent motility may have more predictable absorption of medications necessary for their particular disease. Currently

We have no conflicts of interest to disclose.

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Journal of Pharmaceutical Sciences, Vol. 99, 2905–2913 (2010)

available alternatives to the oral delivery of prokinetic medications include the intravenous and subcutaneous injection routes. While these methods of drug delivery have demonstrated clinical benefits, they are not practical for long-term use in the patient with chronic gastroparesis.

We hypothesized that transmucosal delivery would be an ideal alternative to the oral or parenteral delivery of prokinetic agents. Buccal delivery was postulated as the route with the highest probability of success as buccal tissue is much thinner than skin, thereby increasing the ability to deliver consistent amounts of drug continuously as compared with transdermal techniques. Several drugs have been successfully delivered through the oral buccal mucosa.[3] Poly(acrylic acid)s, with the trade name Carbopol, have been available for use for transmucosal drug delivery for many years, in part, due to their muco-adhesive and biocompatible properties and their proven safety in humans.[4,5] To determine the feasibility of buccal delivery, we performed several pharmacokinetic (PK) studies using erythromycin (EM), a macrolide antibiotic with potent prokinetic effects, in cellular, rat, and human models, and compared the various distributions. Subsequently, we examined the effectiveness of sodium glycodeoxycholate (SGDC), a well-recognized permeation enhancer, and a similar clinically approved drug, ursodeoxycholic acid, to increase the delivery of EM across buccal tissue.

MATERIALS AND METHODS

Materials

Kirby Bauer Bioassay

Kocuria rhizophila was obtained from ATCC (Manassas, VA) (9934). Difco™ Nutrient Broth, Antibiotic Medium 11, and bioassay dishes (REF 351040) were obtained from Becton Dickinson (Sparks, MD). Antibiotic assay discs were obtained from Whatman International Ltd (Kent, UK) (England) (CAT No. 2017-006). Acetonitrile was obtained from EMD Chemicals (Gibbstown, NJ) (LOT No. 47226). Images of each assay plate were taken with a Nikon DX camera and analyzed using measurement tools in ImageJ. Fetal bovine serum (FBS) was obtained from Gibco (Carlsbad, CA) (Cat. No. 16000-044).

Caco-2 Cell Preparation and Monolayer Growth

Caco-2 cell lines (HTB-37) and Eagle's Minimum Essential Medium (EMEM) (Cat. No. 30-2003) were obtained from ATCC. Penicillin/streptomycin was obtained from Mediatech (Manassas, VA) (10,000 IU/mL/10,000 μg/mL; #30-002-C1). L-glutamine was obtained from Irvine Scientific (Santa Ana, CA) (#9317). Inserts were obtained from BD Falcon (Franklin Lakes, NJ) (Cat. No. 353097). The 24-well plates were obtained from Corning (Corning, NY) (No. 3526).

Animal Pharmacokinetic Study

Erythromycin lactobionate (EML) was obtained from Hospira (Lake Forest, IL) (LOT No. 47 767Z7). Carbomer 940 was obtained from Spectrum Chemical (Gardena, CA) Mfg. Corp. (C1477). Heparinized vials were obtained from Sarstedt (Newton, NC) (REF 41.1393.105). SGDC was obtained from Sigma (St. Louis, MO) (G9910). Preformulated erythromycin ethylsuccinate (EME) (Abbott Laboratories (Abbott Park, IL) NDC 0074-6305-13) was obtained suspended in water (80 mg/mL). Ursodeoxycholate (Ursodiol), which is FDA approved for use in humans, was obtained from Spectrum Chemical Mfg. Corp. (Lot No. XD 1029). Plastic syringes were obtained from National Scientific (Rockwood, TN) Company (S7510-1).

Methods

Kirby Bauer Bioassay: Quantification of Samples

Detection of EM in the blood samples was accomplished via bioassay against *Kocuria rhizophila* in a procedure described by Fernandes et al.[6] Bacteria were streaked across agar dishes and grown for 3 days. Eighteen hours prior to the assay, a single competent colony was removed from the plate and grown in 5 mL of Nutrient Broth. Approximately 50 μL of the overnight culture was used to inoculate 500 mL of Antibiotic Medium 11 that had been autoclaved and cooled to 55°C. Approximately 70–100 mL of inoculated agar was poured into a large bioassay dish, allowing a uniform lawn of bacteria to form on the assay plate. Upon agar/broth solidification, 25 discs were spread evenly across the top of the agar.

Blood samples were extracted with an equal volume of acetonitrile to release bound EM. Samples were vortexed momentarily, centrifuged at 14,000 rpm for 20 min, and the supernatant was removed. Twenty-five microliters of the 5 standards and 20 samples were pipetted on the discs in the assay plate in a predetermined fashion. The plate was inverted and allowed to incubate for 30 h at 37°C. Zones of inhibition formed around each disc based on the EM concentration of each sample. A semi-log relationship exists between EM concentration and zone diameter.[7] The unknown concentrations were determined from a standard curve created by plotting the zone diameters of inhibition against the log of the antibiotic concentration of each standard.[7] Excel software (Microsoft, Redmond, WA) was used to plot a line of best fit, or standard curve, from the semi-log