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Research paper

Evaluating and optimizing oral formulations of live bacterial vaccines using a gastro-small intestine model

João M.S. de Barros [a], Adele Costabile [b,1], Dimitrios Charalampopoulos [b], Vitaliy V. Khutoryanskiy [a], Alexander D. Edwards [a,*]

[a] School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AD, UK
[b] Food and Nutritional Sciences, University of Reading, Whiteknights, Reading RG6 6AD, UK

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ABSTRACT

Gastrointestinal (GI) models that mimic physiological conditions *in vitro* are important tools for developing and optimizing biopharmaceutical formulations. Oral administration of live attenuated bacterial vaccines (LBV) can safely and effectively promote mucosal immunity but new formulations are required that provide controlled release of optimal numbers of viable bacterial cells, which must survive gastrointestinal transit overcoming various antimicrobial barriers. Here, we use a gastro-small intestine gut model of human GI conditions to study the survival and release kinetics of two oral LBV formulations: the licensed typhoid fever vaccine Vivotif comprising enteric coated capsules; and an experimental formulation of the model vaccine *Salmonella* Typhimurium SL3261 dried directly onto cast enteric polymer films and laminated to form a polymer film laminate (PFL). Neither formulation released significant numbers of viable cells when tested in the complete gastro-small intestine model. The poor performance in delivering viable cells could be attributed to a combination of acid and bile toxicity plus incomplete release of cells for Vivotif capsules, and to bile toxicity alone for PFL. To achieve effective protection from intestinal bile in addition to effective acid resistance, bile adsorbent resins were incorporated into the PFL to produce a new formulation, termed BR-PFL. Efficient and complete release of 4.4×10^7 live cells per dose was achieved from BR-PFL at distal intestinal pH, with release kinetics controlled by the composition of the enteric polymer film, and no loss in viability observed in any stage of the GI model. Use of this *in vitro* GI model thereby allowed rational design of an oral LBV formulation to maximize viable cell release.

1. Introduction

Live bacterial cells are administered orally as attenuated vaccines [1], for *in situ* production of biopharmaceuticals [2], or as probiotics to improve gastrointestinal (GI) health [3]. Attenuated live bacterial vaccines (LBV) administered orally (e.g. Vivotif, Cholera-Garde), closely mimic natural infection and promote potent, long-lasting protective immunity [1]. The small intestine mucosa, particularly the Peyer's patches found in the ileum, is typically the target site for oral LBV. Although many formulations have been developed for oral delivery of therapeutic live bacteria ranging from exotic functional foods to yoghurt [4], solid dosage forms such as capsules or tablets containing dried live bacteria offer the most control of both dose and site of delivery while drying is essential to achieve stability and shelf life [5]. However, even in late stage clinical trials LBV are still being administered in bicarbonate buffer suspension reconstituted from lyophilized stocks (e.g. attenuated cholera strains Peru-15 [6] and CVD 103-HgR [7]) – a formulation that did not gain large scale adoption for Ty21a in spite of the greater immunogenicity of buffered liquid administration over capsules in clinical trials [8,9]. The poor palatability of bicarbonate buffers has led to the suggestion of alternate liquid formulations such as protein shakes [10] which provide effective protection of attenuated vaccine cells from gastric acid.

Like other biopharmaceuticals, LBV present unique formulation challenges not only during manufacture but also after

Abbreviations: LBV, live bacterial vaccine; GI, gastro-intestinal; SIF, USP simulated intestinal fluid (pH 7.0); SGF, USP simulated gastric fluid (pH 2.0); BioSGF, complete simulated gastric fluid; BioSIF, complete simulated intestinal fluid; CFU, colony forming unit; PFL, polymer film laminate; BR-PFL, bile resin polymer film laminate.

* Corresponding author.
E-mail address: a.d.edwards@reading.ac.uk (A.D. Edwards).
[1] Current address: Health Sciences Research Centre, University of Roehampton, SW15 4JD, UK.

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administration, and dried bacterial cells must survive many antimicrobial defenses during GI transit including gastric acid, antimicrobial, gastric and pancreatic enzymes, and bile surfactants. Although non-viable bacterial cells can promote immunity, viable cells are significantly more immunogenic [11]. These challenges are exacerbated by an elevated sensitivity to acid and bile when dried [12–14]. Acid-labile conventional pharmaceuticals are protected from gastric acid by encapsulation using gastro-resistant coatings such as acid-insoluble enteric polymers [15–17], and Vivotif is formulated in a capsule coated with hydroxypropymethylcel lulose-phthalate. In contrast live bacterial vaccines in recent clinical trials utilize an unconventional oral delivery form, with lyophilized cells resuspended in a bicarbonate buffer in an effort to neutralize gastric acid following oral administration (e.g. [6]). Delivery to the intestine using enteric coatings releases tablet or capsule content to intestinal microbicides including bile. Although the main function of bile acids is to solubilize dietary lipids, many microbes are intolerant to detergents and bile represents a major microbicidal barrier to the survival of bacteria in the gut. Drying even highly bile adapted enteric organisms such as *Salmonella* bacteria increases susceptibility to bile, raising the possibility that dry cells released from enteric coated formulations may then be rapidly killed by bile following intestinal release, dramatically reducing dose of viable cells. This temporary bile sensitivity of dried cells is rapidly reversed after rehydration and bile adsorbing resins can be added to oral formulations to protect dried cells from bile toxicity [12–14].

Understanding the fate of food and drugs after ingestion is both vital to achieve efficient delivery and uptake of pharmaceuticals and to understand the impact of diet on gut health, and *in vitro* modeling remains a critical tool for the study of food and oral formulations in the GI tract. *In vivo* studies are complex and expensive and although critical to understand vaccine efficacy, have not been used to assess the delivery of live bacterial vaccine cells; instead, preclinical studies focus on immunogenicity as a downstream consequence of administration of formulated cells. In some studies fecal shedding can be monitored as an indirect indicator of GI bacterial load but immune responses can be generated without fecal shedding proving this is a poor indicator of live cell delivery. Small animal models cannot be used to test human dosage forms as capsules and tablets are too large for oral administration, and there are no published reports of the release kinetics and location or number of viable cells released into the GI tract of large monogastric mammals from solid oral formulations of therapeutic live cells. As a result we know very little about the fate of live therapeutic cells administered orally, or the influence of cell viability and release location on downstream immunogenicity, and so carefully designed *in vitro* studies remain vital.

Conventional dissolution testing utilizes very simple simulated GI fluids, typically a simple acidic simulated gastric fluid (SGF; often 0.1 M HCl, pH ~ 2.0) followed by a simple phosphate buffer for simulated intestinal fluid (SIF; typically pH 6.8) to simulate intestinal transit, with some minor variations such as the pH or the addition of enzymes [18]. Although carefully defined simple dissolution testing protocols are adequate for testing small molecule drugs and essential for manufacturing quality control, these conditions poorly reflect the dynamic and complex physiological conditions encountered during GI transit *in vivo*. Simple SGF and SIF are particularly inappropriate for complex biological therapeutics such as live therapeutic bacteria since they lack antimicrobial components such as bile acids. Several validated gastrointestinal models have been developed [19] such as the three-stage compound continuous culture system that simulates the nutritional and environmental conditions in the human large intestine [20]; TIM, a multi-compartmental, dynamic computer-controlled model that simulates the GI tract [21]; and the simulator of the human microbial ecosystem (SHIME) which operates in sequential batch mode and simulates the entire human gastrointestinal system [22]. Here, we adapted an established gut model designed and validated to reproduce the spatial, temporal, nutritional, and physicochemical characteristics of the microbiota in the human colon [23] to the study of oral formulations of live bacterial vaccines. This three-stage continuous culture system comprises of three glass fermenters simulating the ascending, transverse, and distal colon, and contains a complete microbiota from a fecal inoculum.

In this study, upstream gastric and small intestinal stages were added to this model to study viability and release of LBV from oral formulations through simulated GI transit. We evaluated the fate of LBV for both an established licensed oral vaccine formulation in enteric coated capsules (Vivotif), and a novel polymer film laminate formulation developed recently by our laboratory [17]. These formulations were previously tested in simple SGF and SIF but the effect of complete simulated gastrointestinal fluids that mimic physiological conditions has not been reported. We found that dried *Salmonella* typhi strain Ty21a cells in the enteric coated Vivotif capsules were sensitive to both gastric acid and bile, suggesting that major losses in viable cell numbers are likely to be released *in vivo* from this widely used vaccine formulation. Likewise, although our novel enteric polymer film laminate (PFL) formulation was effective at protecting live bacteria of a model vaccine strain of *Salmonella* typhimurium from gastric acid, dried cells in this formulation remained highly susceptible to bile. We therefore incorporated bile sequestrants into the polymer films to produce a bile resistant polymer film laminate (BR-PFL) formulation, which delivered maximal viable cells to the simulated distal intestinal site.

2. Materials and methods

2.1. Materials

Eudragit L100 55 (Eudragit L, methacrylic acid–ethyl acrylate copolymer 1:1), was from Evonik, Germany. Cholestyramine, triethyl citrate, sodium chloride, potassium phosphate buffer, pancreatin, pepsin, LB broth and LB agar were purchased from Sigma Aldrich (Gillingham, UK). Peptone and bile salts were from Oxoid (Basingstoke, UK). The oral typhoid fever vaccine Vivotif consisting of *Salmonella* Typhi strain Ty21a formulated in a capsule coated with hydroxypropymethylcellulose-phthalate, was from Crucell (the Netherlands). The model live bacterial vaccine strain was *Salmonella enterica* serovar Typhimurium strain SL3261.

2.2. In vitro gastro-small intestine model system

A semi-continuous gastro-small intestine model system was constructed (Fig. 1) consisting of four (V1, V2, V3 and V4) jacketed glass vessels (Soham Scientific, UK), with operating volumes of 50, 100, 100 and 100 mL representing the stomach, duodenum, jejunum and ileum, respectively. Each vessel was completely sealed and had a cover designed to accommodate a sample glass holder (design to keep PFL form in place without affecting dissolution), pH electrode, temperature probe, and entry parts for N_2, 0.5 M HCl, 0.5 M NaOH, and digesta if necessary, and contents continuously mixed by magnetic stirrer bar. The temperature was maintained by circulating water at 37 °C through the jacketed vessels. pH controller pumps (Fermac 260; Electrolab, Tewkesbury, United Kingdom) were used to automatically control the pH of the simulated fluid in vessels V2, V3 and V4 to pH 5.5, 6.5 and 7.2, respectively. An anaerobic environment was maintained by sparing all vessels with O_2-free N_2 (15 mL/min).