Advanced Drug Delivery Reviews 64 (2012) 540–556

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Oral colon delivery of insulin with the aid of functional adjuvants☆,☆☆

Alessandra Maroni, Lucia Zema, Maria Dorly Del Curto, Anastasia Foppoli, Andrea Gazzaniga *

Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche "P. Pratesi", Via G. Colombo 71, 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 1 June 2011
Accepted 27 October 2011
Available online 6 November 2011

Keywords:
Oral colon delivery
Formulation
Protein
Peptide
Insulin
Bioavailabilty

ABSTRACT

Oral colon delivery is currently considered of importance not only for the treatment of local pathologies, such as primarily inflammatory bowel disease (IBD), but also as a means of accomplishing systemic therapeutic goals. Although the large bowel fails to be ideally suited for absorption processes, it may indeed offer a number of advantages over the small intestine, including a long transit time, lower levels of peptidases and higher responsiveness to permeation enhancers. Accordingly, it has been under extensive investigation as a possible strategy to improve the oral bioavailability of peptide and protein drugs. Because of a strong underlying rationale, most of these studies have focused on insulin. In the present review, the impact of key anatomical and physiological characteristics of the colon on its viability as a protein release site is discussed. Moreover, the main formulation approaches to oral colon targeting are outlined along with the design features and performance of insulin-based devices.

Contents

Abbreviations: IBD, inflammatory bowel disease; GI, gastrointestinal; GALT, gut-associated lymphoid tissue; CFU, colony-forming unit; SITT, small intestinal transit time; SE, standard error; HPMC, hydroxypropylmethylcellulose; HEC, hydroxyethylcellulose; HPC, hydroxypropylcellulose; CTDC, Colon-Targeted Delivery Capsule; IDE, insulin-degrading enzyme; CYP450, cytochrome P450; ACE, angiotensin-converting enzyme; MW, molecular weight; RIA, radioimmunoassay; HEMA, hydroxyethylmethacrylate; PA, pharmacological availability; Na_2EDTA, disodium ethylenediaminetetraacetate; HPMCAS, hydroxypropylmethylcellulose acetate succinate; W/O/W, water-in-oil-in-water; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LDH, lactate dehydrogenase; GRAS, generally regarded as safe.

☆ In memory of Professor Maria Edvige Sangalli.

☆☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Advances in Oral Drug Delivery: Improved Bioavailability of Poorly Absorbed Drugs by Tissue and Cellular Optimization".

* Corresponding author.
E-mail address: andrea.gazzaniga@unimi.it (A. Gazzaniga).

0169-409X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2011.10.006

1. Introduction

In the area of oral delivery, a growing attention has been focused over the past few decades on the design and manufacturing of advanced formulations intended for release of bioactive compounds to selected regions of the gastrointestinal (GI) tract. By controlling the site of drug liberation throughout the gut, it would be possible to limit the tolerability issues associated with treatments that mainly affect specific GI districts, enhance the bioavailability of drugs that show regional differences in their stability and/or permeability profiles or, alternatively, improve the therapeutic outcome in the management of widespread local pathologies (e.g. phlogosis, ulcers, microbial infections, motility disorders).

In particular, colon delivery appears to be related to a range of either potential or fulfilled interesting applications [1,2]. Indeed, besides its long-lasting exploitation for the topical treatment of intestinal pathologic conditions, such as primarily IBD, it is extensively investigated as a means of achieving therapeutic levels of systemically-acting drugs in the general circulation. This specifically relates to the pursuit of an improved oral bioavailability for degradable and poorly permeable macromolecules of high current relevance and therapeutic value. Among them, insulin is perhaps the most prominent example.

In the present article, the potential of the large bowel as a site for release is preliminarily outlined along with the main targeting approaches that are described in the literature. Subsequently, the rationale behind colonic delivery of insulin is discussed, and an overview of oral delivery devices intended for positioned release of the hormone to the large intestine is provided. Their key design characteristics and the relevant impact on performance are commented. Particular interest is focused on formulations that not only are conceived to protect the conveyed drug molecule and promote its absorption from the colonic environment, but also possess intrinsic mechanisms enabling targeted release. Finally, *in vitro*, *ex vivo* and, when available, *in vivo* results from animal studies are reported.

2. Brief outline of the anatomy and physiology of the colon

The colon is the portion of intestine that starts from the caecum at the ileocaecal valve and ends with the rectum. It comprises the ascending (right), transverse, descending (left) and sigmoid branches. In the adult, it is approximately 150 cm long and 7 cm wide. The colonic wall is formed from overlapping layers, i.e. the mucosa, submucosa, muscularis externa, including a continuous layer of circular fibers and three bands of longitudinal fibers (teniae coli), and the serosa [3,4]. The mucosal layer is further subdivided into the epithelium, lamina propria and muscularis mucosa. The epithelium consists of a single sheet of columnar absorptive enterocytes interconnected by junctional complexes. These enable maintenance of the barrier functions on the one hand, and cellular communication on the other. In addition, they separate the apical brush-border from the basolateral cell membrane, thus ensuring the physiological polarization of the epithelium. Mucus-secreting goblet cells along with endocrine and sporadic Paneth cells are also present, particularly within the crypts of Lieberkühn. Crypts are tubular troughs that extend from the epithelial surface down into the lamina propria. From their base undifferentiated cells migrate to the luminal lining while reaching maturation, thereby supplying replacement for the exfoliated tissue. The colon is rich in gut-associated lymphoid tissue (GALT), which comprises lymphocytes infiltrated into the epithelium and lamina propria, isolated lymphoid follicles and lymphoglandular complexes. M-cells (membranous or microfold cells) are found in the epithelial areas at the interface between the lymphoid tissue and the gut lumen. They are structurally different from the absorptive enterocytes, with wider, shorter and irregular microfolds in place of microvilli, less developed glycocalyx, poor mucus lining, few lysosomes and invaginated basolateral membranes forming interstitial spaces for lymphocytes and macrophages [5]. Indeed, M-cells are specialized cells involved in uptake phenomena that are aimed at mucosal immune surveillance by antigen sampling.

The colon is supplied with blood by the mesenteric arteries and is drained both by the hepatic portal circulation, via mesenteric veins, and the lymphatics [4]. Lymph drainage, however, is by far less efficient. Segmental and peristaltic contractions are accomplished by the smooth muscle layers in order to mix the gut contents while enabling their contact with the epithelium and propel them towards the rectum, respectively [6]. In addition, antiperistaltic movements occur in a distal to proximal direction for fecal retention. Because of the particular motility pattern, with infrequent propulsive waves and the retropulsive contractions, colon transit may take from a few hours to a few days ($8 \div 80$ h) under physiological conditions.

The large bowel is inhabited by the most abundant and diverse microbial population in the gut, ranging from 10^{10} to 10^{12} CFU/ml [4,7]. Fermentation of undigested proteins and, above all, carbohydrates by anaerobic bacteria leads to the production of carbon dioxide, hydrogen, methane and short-chain fatty acids. Their accumulation in the caecum and ascending colon impacts on the luminal pH that normally drops to weakly acidic values in such regions [8].

While the small intestine is primarily responsible for digestion and absorption of nutrients, the colon participates in the maintenance of fluid and electrolyte balance through resorption of water, Na^+ and Cl^- as well as in the formation of feces from waste undigested materials, residues of epithelial cell turnover and bacteria [3,9]. Like the entire GI tract, it also poses effective biophysical and biochemical defensive barriers against the penetration of pathogens, toxins and foreign substances into the body. Consistent with the physiological functions performed, the large bowel shows, as compared with the small intestine, a much reduced surface area exposed due to the presence of semilunar folds instead of circular Kerkring's valves, the lack of villi and less developed microvilli. Moreover, it exhibits a wider lumen that would not promote a close contact between the absorptive epithelium and the contents, narrow tight junctions, which significantly restrain the mucosal permeability to hydrophilic compounds, and a lower blood flow possibly impacting on the concentration gradient that is established between the luminal and basolateral compartments [4]. In addition, the viscous contents and limited volume of free fluid available, which is found in isolated pockets, along with the presence of gas produced through bacterial fermentation activities could overall result in hampered hydration and disintegration of dosage forms as well as dissolution of drug particles, especially in the case of poorly soluble or slowly-dissolving substances [10]. In this respect, it was also highlighted that bacteria tend to adhere to solid surfaces thus forming biofilm barriers that are potentially detrimental to the release process [11].

3. Rationale behind oral colon delivery

Due to the inherent anatomical characteristics and physiological role, the colon has long been considered unsuitable for absorption of substrates other than water or small inorganic ions and, consequently,