Oral delivery of macromolecular drugs: where we are after almost 100 years of attempts.

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Abstract

Since the first attempt to administer insulin orally in humans more than 90 years ago, the oral delivery of macromolecular drugs (>1000 g/mol) has been rather disappointing. Although several clinical pilot studies have demonstrated that the oral absorption of macromolecules is possible, the bioavailability remains generally low and variable. This article reviews the formulations and biopharmaceutical aspects of orally administered biomacromolecules on the market and in clinical development for local and systemic delivery. The most successful approaches for systemic delivery often involve a combination of enteric coating, protease inhibitors and permeation enhancers in relatively high amounts. However, some of these excipients have induced local or systemic adverse reactions in preclinical and clinical studies, and long-term studies are often missing. Therefore, strategies aimed at increasing the oral absorption of macromolecular drugs should carefully take into account the benefit-risk ratio. In the absence of specific uptake pathways, small and potent peptides that are resistant to degradation and that present a large therapeutic window certainly represent the best candidates for systemic absorption. While we acknowledge the need for systemically delivering biomacromolecules, it is our opinion that the oral delivery to local gastrointestinal targets is currently more promising because of their accessibility and the lacking requirement for intestinal permeability enhancement.

Keywords

Oral drug delivery, oral bioavailability, permeation enhancers, protease inhibitors, high molecular weight, biologics

Abbreviations

ADH: anti-diuretic hormone, AON: antisense oligonucleotide, 5-CNAC: *N*-(5-chlorosalicyloyl)-8-aminocaprylic acid, EDTA: ethylenediaminetetraacetate, FDA: Food and Drug Administration, GC C: guanylyl cyclase C, GI: gastrointestinal, GLP-1: glucagon-like peptide 1, IBD: inflammatory bowel diseases, ICAM-1: intercellular adhesion molecule 1, MG: myasthenia gravis, NDA: New Drug Application, SNAC: *N*-(8-[2-hydroxybenzoyl]amino)caprylic acid, tkRNAi: transkingdom RNA interference platform

1. Introduction

The first attempt to deliver insulin orally in humans was undertaken as early as 1922, only one year after the discovery of insulin by Drs. Frederick Banting and Charles Best[1], when increasing doses of insulin were given orally to a single diabetes patient. The results were negative, and already in this first study, the critical challenges of oral protein delivery became apparent: poor and variable absorption, and low efficacy compared with subcutaneous injection. Although the interest and efforts in the oral delivery of biomacromolecules have intensified over the past two decades, safely and effectively delivering high-molecular-weight substrates *via* the oral route remains highly challenging for formulation scientists[2,3].

The gastrointestinal (GI) tract is a hostile environment for biomacromolecules because it is evolutionarily optimized to break down nutrients and deactivate pathogens. The highly acidic pH in the stomach results in the protonation of proteins and their unfolding, which exposes more motifs that are recognized by protein-degrading enzymes[4]. The enzymes in the stomach (pepsin) and small intestine (*e.g.*, chymotrypsin, amino- and carboxypeptidases, RNases and DNases) cleave proteins and nucleic acids into smaller fragments and single units[4]. In the colon, enzymatic fermentation processes further degrade biomacromolecules[4]. Because therapeutically active biomacromolecules are equally affected by these processes, the fraction surviving these degradation processes is generally low and variable, especially in the presence of food[5]. The macromolecular drug needs to overcome multiple barriers designed to prevent the entry of dietary and bacterial antigens in order to reach the systemic compartment. To access the epithelial cell layer, the biomacromolecule firstly needs to diffuse through the mucus layer covering the intestinal epithelium[5]. The latter is another important barrier, as the tight junctions which seal the epithelial cells restrict the paracellular transport (*i.e.*, the passage between cells) to small molecules and ions (<600 Da)[6]. The transcytotic pathway (*i.e.*, the passage across the cell in an endocytotic vesicle) is mediated by lumenally expressed endocytotic receptors (*e.g.*, vitamin B12 receptor, transferrin receptor), and therefore necessitates conjugation to the respective ligands in order to be exploited in drug delivery[7]. Another access point to the systemic compartment is the phagocytotic M-cells of Peyer's patches which sample luminal antigens and can take up particular substrates in the low micrometer range[8]. However, the proportion of M-cells in the gut epithelium is small and varies greatly between species, which complicates predictions of absorption in humans based on animal data[9].

Not surprisingly, only six biomacromolecules have been approved by the Food and Drug Administration (FDA) for oral delivery: two locally and two systemically delivered peptides, one locally delivered non-peptidic macrocycle, and one locally delivered protein mixture. However, several orally applied formulations of proteins, peptides, and nucleic acids are currently under clinical evaluation. Often, these formulations contain at least one of the following excipients (**Figure 1**): an enteric coating and/or protease inhibitors to prevent drug degradation and permeation enhancers to enable

paracellular transport of macromolecules[10,11]. Mechanistically, absorption enhancement can be achieved by mechanically disrupting tight junctions or the plasma membrane, lowering mucus viscosity, and modulating tight junction-regulating signaling pathways[2]. Additional strategies for the oral delivery of biomacromolecules under clinical development include buccal delivery, utilizing carrier-mediated transcytosis, and local delivery to GI targets.

The overwhelming majority of currently approved oral drugs and clinical candidates exhibit a molecular weight of <1000 Da[12]. Above this threshold, low bioavailability, inter- and intraindividual variability, food effects, and long-term safety concerns of bioavailability-enhancing excipients remain important challenges of oral delivery despite clear advances in knowledge after nearly 90 years of trial and error. In this review, we address orally applied biomacromolecular therapeutics (>1000 Da) already marketed or under clinical investigation for local or systemic delivery with an emphasis on the drug formulations and the biopharmaceutical aspects. The oral delivery of vaccines will not be covered in this manuscript, and the readers are referred to other recent reviews for more information on this topic[13–16].

2. Local delivery

Macromolecular drugs that act on GI targets increasingly move into focus because local delivery avoids the challenges of reaching the systemic compartments. Advantages of locally delivering high-molecular-weight drugs include fewer restrictions regarding drug size and a potentially more favorable safety profile due to minimal systemic exposure, reduced immunogenicity, and the absence of permeation-enhancing excipients[17,18]. To conserve the therapeutic activity in the GI tract, several formulation strategies have been employed, such as enteric/colon-targeting capsules that protect against the harsh GI environment, supplementation with sacrificial proteins that compete for degradation, and hindering enzymatic access using polymer conjugation or protective antibodies which bind to known cleavage epitopes [4,17,19]. The ailments that are targeted by locally acting macromolecules include inflammatory diseases (Crohn's disease, ulcerative colitis), metabolic disorders (e.g., exocrine pancreas insufficiency), constipation, and infections (Table 1)[4,17].

2.1. *Peptides*

2.1.1. *Vancomycin and fidaxomicin*

Isolated in 1953 from a soil sample in the jungle of the island Borneo, the antibiotic vancomycin is produced by the bacterium *Amycolatopsis orientalis*[20]. Vancomycin is a glycosylated tricyclic heptapeptide (1449 Da) which contains modified amino acid residues (e.g., chlorinated tyrosine)[20–22]. Due to its highly hydrophilic (log P -3.1) and large cyclic structure, vancomycin is only marginally absorbed and metabolized in the GI tract[23,24]. Intravenously applied vancomycin was initially approved by the FDA to treat penicillin-resistant bacterial infections in 1958, and it is still indicated for severe infections caused by susceptible strains of methicillin-resistant staphylococci, *Clostridium difficile*-associated diarrhea, and staphylococcal enterocolitis and for patients who are allergic to beta-lactam antibiotics. Vancomycin is formulated as gelatin capsules containing 125 or 250 mg of active compound (package leaflet). In 2011, a non-peptidic macrocyclic macrolide antibiotic fidaxomicin (1058 Da, Dificid®, Cubist Pharmaceuticals) with minimal systemic absorption and GI metabolism was approved for the treatment of *C. difficile* infections. Fidaxomicin showed lower relapse rates compared with vancomycin. In view of its much higher costs, fidaxomicin treatment may be most suitable for patients who are at highest risk of relapse[25]. Further cyclic peptidic compounds in clinical trials for the local treatment of *C. difficile* infections include the lipopeptide surotomycin (1681 Da, phase III, Cubist Pharmaceuticals/Merck), the lipoglycopeptide ramoplanin (2554 Da, NTI-851, phase IIb, Nanotherapeutics), the thiazolyl peptide LFF-571 (1367 Da, phase II but currently removed from pipeline, Novartis), and the lantibiotic NVB302 (2115 Da, phase I, Novacta Biosystems Limited)[26,27].

2.1.2. *Linaclotide*

Linaclotide (1525 Da) is a truncated derivative of *Escherichia coli* heat-stable (ST) enterotoxin that consists of 14 amino acid residues and three disulfide bonds[28]. It acts as a guanylyl cyclase C (GC C) agonist locally in the small intestine (**Figure 2**) [28–30]. In 2012, it received FDA approval (Linzess®, Forest Labs LLC) for the treatment of chronic idiopathic constipation and irritable bowel syndrome with constipation. After oral administration, linaclotide is minimally absorbed, and its plasma

concentrations are below the limit of quantification[31]. Because linaclotide is stable in the stomach, it is formulated as hard gelatin capsules containing 0.145 or 0.290 mg of the active compound (package leaflet). However, it is cleaved in the small intestine to a 13-amino acid active metabolite[31], which is eventually degraded in the intestine[32].

2.1.3. Plecanatide and derivatives

The GC C agonist plecanatide (1682 Da, Synergy Pharmaceuticals) is a synthetic analog of uroguanylin, a naturally occurring GI regulator of GC C signaling, with one amino acid substitution that results in stronger receptor binding[33]. It is a bicyclic peptide consisting of 16 amino acid residues with two disulfide bonds[33]. Plecanatide completed the clinical phase III for the treatment of chronic idiopathic constipation, and it is currently being evaluated in phase III for irritable bowel syndrome with constipation. It showed a statistically significant increase in the number of complete spontaneous bowel movements per week compared with placebo (approximately 20% of durable responders at a dose of 3 mg per day vs. 12% in the placebo group). In comparison to linaclotide, plecanatide showed reduced diarrhea incidence (10% vs. 20%)[34]. In addition to plecanatide, Synergy Pharmaceuticals is developing a more stable analog, dolcanatide (SP-333), to treat opioid-induced constipation. Dolcanatide has two D-amino acid substitutions at C- and N-terminus in order to improve stability in simulated gastric and intestinal fluids (www.synergypharma.com). Dolcanatide has completed a randomized, double-blind, placebo-controlled phase II clinical trial in patients with constipation who take opioid analgesics for chronic pain (NCT01983306). Despite the increased stability in the intestinal environment, the dose of dolcanatide needed to achieve a therapeutic effect was comparable with that for plecanatide. Furthermore, the dolcanatide-mediated activation of GC C ameliorated inflammation in a colitis mouse model, promoting its development in the treatment of ulcerative colitis[35].

2.2. Antibodies

2.2.1. Antibody delivery

The vast majority of clinically tested antibodies with GI luminal targets inactivate undesired molecules, such as bacterial toxins, cytokines, viruses, and virulence factors[17]. In the treatment of inflammatory bowel diseases (IBD), capturing TNF- α in the intestinal lumen is a promising alternative to systemic treatment with anti-TNF- α antibodies (e.g., adalimumab, infliximab and certolizumab pegol) with regard to systemic immunosuppression, development of neutralizing antibodies, and needle-free administration[36–38]. AVX-470 (Avaximab™-TNF, Avaxia Biologics) is an orally administered polyclonal antibody which targets luminal TNF- α in the intestine[39]. It is produced by purifying total antibodies from the milk of dairy cows that are immunized with human TNF- α , which results in approximately 0.3-0.9% (m/m) TNF- α -specific and mainly IgG₁ subtype antibodies (www.avaxiabio.com). Providing early immunity to neonates, high amounts of colostrum antibodies seem to be partially resistant to GI digestion in adults with fully developed digestive systems[40–42]. AVX-470 is developed as an enteric-coated capsule for treating pediatric ulcerative colitis and is currently in phase II of clinical trials. According to the company's website, a phase Ib trial (NCT01759056) in 36 ulcerative colitis patients showed that the treatment resulted in consistent encouraging trends across multiple disease parameters (colonic TNF- α levels, endoscopic index of

severity, serum C-reactive protein levels) after four weeks, and no allergic reaction or human anti-bovine antibodies in serum were observed. Bovine immunoglobulin was detected in stools and displayed TNF- α -binding activity, suggesting that the large excess of decoy antibodies in the formulation (>99%) protected a fraction of the anti-TNF- α antibodies.

2.2.2. *Genetically modified antibody-secreting bacteria*

Intrexon's (formerly ActoGeniX) approach for luminal antibody therapy consists of delivering attenuated bacteria to the GI tract, where the antibody is secreted *in situ*. Food-grade *Lactococcus lactis* was genetically modified to secrete certolizumab, an anti-TNF- α Fab fragment (47.8 kDa). In an open-label clinical trial in healthy volunteers, lyophilized bacteria administered in enteric capsules were recovered in both the small and large intestine by endoscopic sampling, and the antibody was secreted by living bacteria in the colon lumen (www.dna.com). The treatment was safe and well-tolerated. In addition, preclinical studies demonstrated the treatment's efficacy in multiple colitis models and showed that *Lactococcus lactis* adhere especially to the inflamed mucosa, which increases the concentration of the antibody in the close proximity of target cells[43]. However, questions remain regarding the concentration variability and the intestinal stability of the secreted antibody.

2.3. Oral enzyme therapy

Enzyme replacement therapy *via* the oral route is an important therapeutic option in metabolic disorders in which a certain enzymatic action in the GI tract is absent. Numerous oral enzyme products are on the market for a variety of disorders such as exocrine pancreatic insufficiency due to pancreatitis, cystic fibrosis and other conditions, and lactose, fructose, sucrose and histamine intolerance (e.g., Creon®, Lacteeze®, Sucraid®, DAOSiN®)[4]. Whereas the only FDA-approved enzymatic drug is pancreatin (a mixture of bovine or porcine pancreatic amylase, proteases, and lipases), the other oral enzyme products are generally under the legal framework of dietary supplements, which usually do not require thorough efficacy studies.

Because these therapeutic enzymes are inactivated in the stomach by low pH and pepsin, one or a combination of stabilization strategies are generally needed. Creon® (Abbvie), for instance, consists of pancreatin formulated in enteric-coated capsules, whose dissolution is triggered by the pH increase in the small intestine. An uncoated formulation of pancreatin (Viokace®, Aptalis Pharma) which is used to treat exocrine pancreatic insufficiency is co-administered with proton pump inhibitors to decrease gastric degradation. As an exception, sacrosidase and the acid lactase (tilactase) produced by *Aspergillus oryzae* are intrinsically stable and active in acidic conditions[4], and they do not require sophisticated delivery systems. However, average residence time of lactase in the stomach might be insufficient for complete digestion of high amounts of lactose[44]. Therefore, a formulation of a lactase, which is active in neutral to basic pH of the small intestine, was recently developed as a capsule containing enteric-coated pellets (Lactosolv®, Sciotec Diagnostic Technologies)[44]. The same approach has been successfully applied for other enzyme deficiencies (Xylosolv® for fructose and DAOSiN® for histamine intolerance). As an alternative, the administration of probiotics that secrete β -galactosidase, which is capable of lactose hydrolysis, has been proposed; however, different clinical trials in patients with lactose intolerance gave inconsistent efficacy results[45]. Oral formulations of

polymer-conjugated enzymes and enzymes in enteric-coated capsules are under preclinical investigation for other pathologies such as celiac disease and phenylketonuria[4,46–50].

2.4. Cytoprotective/anti-inflammatory proteins

As a consequence of chemotherapy and radiation, cancer patients often develop a complication called oral mucositis in which the mouth's mucosal lining is destroyed and ulcers form. AG013 (Intrexon Corporation) is a bacteria-based system which delivers a mucosa-healing protein for the treatment of oral mucositis. The protein, called human trefoil factor 1, is involved in mucosal healing and tissue protection *via* multiple mechanisms, including inhibiting apoptosis during cell migration and stabilizing the protective mucus layer[51]. The formulation consists of an oral rinsing suspension containing attenuated (*i.e.*, unable to replicate due to thymidylate synthase deficiency[51]), genetically engineered *Lactococcus lactis* which adhere to the buccal mucosa and secrete the mucosa-healing factor locally for up to 24 h after administration[52]. A phase Ib study in head and neck cancer patients undergoing chemotherapy produced positive results, showing a 35% reduction in the duration of oral ulcers[51].

A similar *in situ* system based on *Lactococcus lactis* which secretes the anti-inflammatory cytokine interleukin-10 (AG011, Intrexon) was proposed for ulcerative colitis. However, a combination of enema and enteric capsules containing interleukin-10-secreting *Lactococcus lactis* did not result in mucosal healing in a phase IIa, double-blind, placebo-controlled study with 60 ulcerative colitis patients[53]. Recently, another genetically modified strain of *Lactococcus lactis* was engineered to deliver the endogenous serine protease inhibitor elafin. The expression of this anti-inflammatory peptide is lowered in patients with active celiac disease, and elafin treatment was effective in decreasing inflammation and intestinal permeability in a mouse model of celiac disease[54].

2.5. Nucleic acids

Antisense oligonucleotides (AONs) are short, single-stranded, synthetic sequences of nucleotides which can hybridize with complementary mRNA and consequently decrease the expression of the encoded protein[55]. Several GI tract pathologies hold attractive targets for local nucleic acid therapy (*e.g.*, IBD or familial adenomatous polyposis)[56,57]. Because nucleic acids are readily degraded by the RNases and DNases in the GI tract and depurinated in the acidic stomach environment, they require stability-enhancing chemical modifications of the backbone or bases[58]. Various modifications of the ribose ring (*e.g.*, 2'-O-(2-methoxyethyl) or 2'-F-ANA) not only exhibit enhanced nuclease resistance but also show higher affinity to complementary mRNAs and thus improved knockdown efficacy[59,60].

Mongersen (GED-0301, Celgene) is a 21-mer phosphorothioate AON targeting the mRNA of the Smad7 protein, which is overexpressed in IBD mucosal tissues and leads to suppression of anti-inflammatory TGF- β signaling[61]. Mongersen acts on epithelial and lamina propria cells, and its bioavailability after oral administration is minimal[62]. An enteric-coated oral formulation of mongersen was evaluated in 166 patients with active Crohn's disease in a phase II trial[61]. Participants received 10, 40, or 160 mg of mongersen once daily for 2 weeks. In the mongersen groups that received 40

and 160 mg, significantly more patients reached clinical remission, the primary end point, after four weeks of treatment (55% and 65%, respectively), compared with 10% in the placebo group. According to the company, follow-up data of this trial (e.g., on mucosal healing by endoscopy) will be presented in the future.

Alicaforsen (Atlantic Healthcare) is a 20-mer phosphorothioate AON targeting the mRNA of intercellular adhesion molecule 1 (ICAM-1), which recruits immune cells to the site of inflammation and is overexpressed in the gut epithelium of IBD patients[63]. Although a four-week treatment with injectable alicaforsen fell short of inducing clinical remission in two phase II trials (NCT00048113; NCT00048295), a six-week regimen of nightly 60 mL alicaforsen enemas containing hydroxypropyl methylcellulose as a thickening agent was beneficial in treating moderate to severe ulcerative colitis and pouchitis (*i.e.*, an ileal pouch inflammation in colectomy patients)[63,64]. According to the company's website (www.atlantichc.com), alicaforsen enema is undergoing phase III clinical trials for the treatment of moderate to severe ulcerative colitis and pouchitis.

Because the cellular absorption of naked oligonucleotides is limited, Marina Biotech is developing a novel strategy to enhance oligonucleotide cell uptake using a transkingdom RNA interference platform (tkRNAi) based on genetically modified *Escherichia coli*. The most advanced tkRNAi candidate, CEQ508, is designed to express and deliver short hairpin RNA silencing β -catenin, which is overexpressed in gut epithelial cells of familial adenomatous polyposis patients and is responsible for uncontrolled cell growth[65]. The genome of CEQ508 has been altered to allow for whole bacterium endocytosis by the epithelial cells and, ultimately, β -catenin silencing[66]. In 2010, CEQ508 received orphan drug status from the FDA, and a phase Ib clinical study was initiated; however, due to financial problems, the development program was halted. Recently, the FDA granted Fast Track designation to CEQ508, and clinical trials are expected to begin again (www.marinabio.com). Other approaches for nucleic acid delivery to the mucosa are under investigation, including nano/microparticles that target mucosal macrophages and colon-activatable peptide nucleic acids [57,67].

3. Systemic delivery

Delivering macromolecules systemically after oral intake is highly challenging because, in addition to the stability issues due to GI degradation, the limited permeability of the GI mucosa generally leads to low and erratic absorption. Therefore, inter- and intraindividual differences in pharmacokinetic parameters are generally high, especially in the presence of food. This chapter provides an overview of oral macromolecular drugs that reached clinical trials or received market approval (Table 2).

3.1. *Peptides*

3.1.1. *Octreotide*

Octreotide is a synthetic analogue of the endogenous hormone somatostatin, a regulator of the neuroendocrine system[68]. It was approved by the FDA in 1998 as a subcutaneous or intramuscular treatment for acromegaly (usually a result of growth hormone-secreting tumors), carcinoid tumors and vasoactive intestinal peptide-producing tumors. In comparison with its mother compound, the cyclic octapeptide possesses a lower molecular weight (1019 vs. 1638 Da), a higher logP (-1.69 vs. -8.50), and two D-amino acids. Additionally, the terminal carboxyl group is reduced to an alcohol[69]. The higher stability of octreotide over somatostatin in pepsin-containing simulated gastric fluid results in part from the inclusion of a D-amino acid in the pepsin-vulnerable amide bond of octreotide, rendering octreotide non-recognizable by this enzyme[68]. Despite these structural improvements, orally administered aqueous octreotide showed an unfavorable pharmacokinetic profile (low bioavailability, highly variable plasma concentrations) in pigs and humans[70,71]. To improve the pharmacokinetics upon oral administration, octreotide (Mycapssa™, formerly Octreolin™, Chiasma) was formulated as an oily suspension with the permeation enhancer sodium caprylate, which transiently increases paracellular permeability for approximately 1.5 h[72]. The absorption of octreotide in the oily suspension was dose-dependent and independent of the intestinal region[72]. The passage through the stomach, however, led to low bioavailability such that in the subsequent clinical studies, the suspension was formulated in an enteric-coated capsule[72]. As reported in a publication of four phase I and phase II studies, a 20-mg dose of Octreolin™ in fasting healthy volunteers led to a similar pharmacokinetic profile as a subcutaneous injection of 0.1 mg (apart from a lag phase of 20 min)[73]. A high-fat meal decreased the bioavailability of oral octreotide by 90%, probably due to the premature dissolution of the enteric-coated capsule caused by increased stomach pH[73]. Furthermore, the presence of food in the intestine may slow down diffusion to the intestinal mucosa, hindering octreotide absorption[73]. A baseline-controlled phase III clinical trial was conducted in acromegaly patients who had previously received somatostatin receptor ligand injections and were switched to a twice-daily regimen of oral octreotide. The intervention met its primary end point in that it resulted in controlled levels of insulin-like growth factor-1 and growth hormone in 65% of the subjects after seven months of the treatment, compared with 89% at baseline[74]. In 6.5% of the subjects, adverse GI events such as nausea, diarrhea and abdominal pain led to early termination of the study[74]. Hoffmann-La Roche bought the rights for development and commercialization of Octreolin™ but

terminated the development in 2014 and transitioned the drug back to Chiasma, which has filed a New Drug Application (NDA) in 2015.

3.1.2. *Desmopressin*

The cyclic nonapeptide desmopressin (1-desamino-8-D-arginine vasopressin, 1128 Da) is a synthetic derivative of the peptide hormone vasopressin (anti-diuretic hormone, ADH)[68,75]. In comparison to the endogenous vasopressin, desmopressin exhibits desirable features such as a significantly longer antidiuretic effect and strongly diminished vasopressor action[75]. Desmopressin was approved by the FDA in 1978 for the treatment of post-hypophysectomy polyuria/polydipsia, central diabetes insipidus, and nocturnal enuresis. The oral formulation (DDAVP®, Ferring Pharmaceuticals) was approved in 1992 by the FDA for the latter two indications. A variety of formulations of desmopressin are currently marketed for parenteral, nasal, and oral applications. Desmopressin exhibits exceptionally high stability in human gastric fluid, presumably due to its rigid cyclic structure and the absence of pepsin cleavage motifs[68]. In contrast, the stability of desmopressin in intestinal fluids is limited but enhanced compared with that of vasopressin[68]. This finding may be due to the replacement of the L-arginine at position 8 with its D-enantiomer in desmopressin, which seems to hinder the recognition by trypsin[68]. Because of its hydrophilicity (logP -1.95), the permeability of desmopressin across a Caco-2 monolayer is low and concentration-dependent, which suggests passive permeation[76]. The oral bioavailability of a standard-dose desmopressin acetate tablet (0.2 mg) is only approximately 0.1%[77]. In 2005, a lyophilisate tablet for sublingual administration of desmopressin was approved (DDAVP® Melt, Ferring Pharmaceuticals). Despite its circumventing the harsh conditions of the GI tract, the bioavailability of this formulation is only approximately 0.25%[78]. With regard to the broad therapeutic window of desmopressin, the antidiuretic action does not seem to be influenced by the low and variable oral bioavailability. Indeed, food intake did not change the efficacy (urine production and osmolality) or safety (plasma sodium concentrations) of desmopressin tablets despite significantly altering the pharmacokinetic parameters[79]. Furthermore, the incidence of water intoxication-induced hyponatremia, a serious adverse drug reaction related to desmopressin overdose, was lower in oral than in nasal application, suggesting a low risk of this adverse drug reaction due to erratic absorption[80].

3.1.3. *Cyclosporine*

Originally isolated in 1969 from the fungus *Tolypocladium inflatum* in a Norwegian soil sample, cyclosporine (Cyclosporine A) has become a widely used immune-suppressive peptide approved by the FDA for the prophylaxis of organ transplant rejection and the second-line treatment of the autoimmune diseases rheumatoid arthritis and psoriasis. Cyclosporine is a cyclic peptide composed of 11 amino acid residues (1202 Da). The exceptionally high stability in gastric and intestinal fluids is most probably related to cyclosporine's high structural rigidity and low potential for H-bonding-mediated interaction with peptidases[68]. The high hydrophobicity (logP 3.6) of cyclosporine enables its transcellular passage by passive diffusion, but it also makes it susceptible to p-glycoprotein-mediated expulsion on the apical side of enterocytes[81]. Interestingly, structural motifs such as the β -turns of cyclosporine seem to be important features in cell permeability[82]. The low solubility in

aqueous solutions due to the high hydrophobicity led to the development of a self-emulsifying formulation in soft gelatin capsules or as an oral solution, approved by the FDA in 1995 (Neoral®, Novartis)[83]. Compared with the originally developed oil-in-water emulsion (SandImmun®, Novartis) this formulation is characterized by lower intra- and interindividual variability, a more linear dose-exposure relationship, and a markedly decreased influence of food fat content[83–85]. Nonetheless, the narrow therapeutic window generally necessitates monitoring cyclosporine plasma concentrations to determine the optimal dose.

3.1.4. *Acyline*

The linear decapeptide acyline (MER 104, Merrion Pharmaceuticals, 1533 Da) competitively inhibits the receptor binding of gonadotropin-releasing hormone, thereby inhibiting the secretion of luteinizing and follicle-stimulating hormone and interfering with the production of downstream sex hormones (e.g., testosterone, estrogen). It is a candidate for treating hormone-dependent conditions (breast and prostate cancer, endometriosis) and male oral contraception. Acyline contains a variety of modifications (e.g., five D-amino acids, two acylated 4-aminophenylalanines) enhancing its stability and receptor-binding properties[86]. It is formulated with the Gastrointestinal Permeation Enhancement Technology (GIPET, Merrion) as an oral dosage form. GIPET is composed of an enteric-coated matrix tablet that contains the permeation enhancer sodium caprate[10,87]. In a phase I clinical trial, acyline tablets (10, 20, and 40 mg) were administered in eight healthy men after overnight fasting and under continued fasting for 4 h [88]. High variability was observed among the subjects, and no dose effect on pharmacokinetic parameters (maximum concentration and time of maximum concentration, half-life, and area under the curve, *AUC*) was observed[88]. After 12 h, all three doses suppressed luteinizing and follicle-stimulating hormone serum concentrations by approximately 70% and 30%, respectively[88]. Suppression of the downstream hormone testosterone was also observed, and the effects of the lowest and highest doses could be discriminated[88]. One study participant reported a GI-related adverse event (nausea and vomiting)[88]. While acyline is still listed as being under active development by the company's website, no trials have been published since 2009.

3.1.5. *Calcitonin*

Calcitonin is a natural peptide hormone regulator of calcium homeostasis[89]. It was approved by the FDA in 1978 and is indicated for the treatment of hypercalcemia and symptomatic Paget's disease and as a second-line treatment of postmenopausal osteoporosis. Marketed calcitonin products are administered *via* the parenteral and nasal routes and contain salmon calcitonin as the active ingredient because this peptide exhibits forty times higher potency compared with the human version[89]. Salmon calcitonin is a 32 amino acid peptide (3432 Da) with a highly negative logP (-28.49)[68]. Due to its size and peptidase-recognizable motifs, it is rapidly degraded in gastric and small intestinal fluids *in vitro*, and it only marginally diffuses across Caco-2 monolayers[68,90]. The bioavailability of salmon calcitonin in a rat model ranged from 0.0011% after intrajejunal to 1% after colonic administration[90]. SMC021 (Novartis) is an oral salmon calcitonin formulation which includes the permeation enhancer *N*-(5-chlorosalicyloyl)-8-aminocaprylic acid (5-CNAC, Eligen® Technology,

Emisphere Technologies). In two phase I trials, oral salmon calcitonin tablets were tested in healthy postmenopausal women and patients with osteoarthritis, and it reached the systemic compartment and suppressed bone resorption markers[91,92]. However, the amount of water administered with the tablet and the time of administration with respect to food intake were identified as influencing factors (**Figure 3**)[91,92]. These studies presumably contributed to the decision to terminate SMC021 development.

Another oral calcitonin formulation (TBRIA™, formerly Ostora, Tarsa Therapeutics) that is currently in clinical development consists of an enteric-coated tablet, which releases its content at a pH of 5.5 in the duodenum[93]. The tablet contains citric acid to decrease the small intestinal pH and thus diminishes the activity of small intestinal peptidases whose pH optimums are in the neutral to basic range[93]. Despite reports that citrate's calcium chelating properties may increase paracellular absorption by disrupting tight junction complexes by depleting intracellular calcium, permeation enhancement was low in Caco-2 monolayers[94]. In a phase III trial, oral or nasal calcitonin or placebo were administered to 565 postmenopausal women who were also receiving vitamin D and calcium supplements[93]. The oral salmon calcitonin formulation given at a dose 6 times higher than the nasal one met the primary endpoint of this clinical trial in that it was superior to nasal calcitonin and placebo in increasing lumbar spine bone mineral density after 48 weeks of treatment[93]. Adverse events were mainly limited to the GI tract[93]. Nausea and dyspepsia were significantly more common in the group receiving the calcitonin tablet compared to the nasal formulation which could have been associated with the administration route and the higher dose in the oral treatment group[93]. In a recently reported phase II trial, no food effect on therapeutic efficacy was observed[95]. Following the phase III clinical trial, Tarsa has filed an NDA with the FDA (under review). The FDA's benefit-risk assessment of this formulation will likely be influenced by their warning about a higher incidence of cancer in patients who received calcitonin compared with placebo, even though a causal relationship could not be established[96].

3.1.6. *Semaglutide*

Semaglutide (Novo Nordisk, 4114 Da) is a glucagon-like peptide 1 (GLP-1) analogue consisting of 31 amino acid residues. GLP-1 derivatives are used to treat type 2 diabetes because they improve glucose control by three mechanisms: stimulating insulin release in a glucose-dependent manner, suppressing glucagon activity under hyperglycemia, and delaying gastric emptying leading to slower glucose absorption[97]. Semaglutide has more favorable pharmacokinetics than its rapidly inactivated mother compound owing to several structural modifications. An amino acid substitution at position 8 (alanine to α -aminoisobutyric acid) impairs dipeptidyl peptidase-4-mediated degradation[97]. Furthermore, the conjugation of C18 fatty diacid *via* a spacer to the lysine in position 26 prolongs plasma half-life by increasing binding to albumin and reducing renal clearance, making semaglutide suitable for once weekly subcutaneous dosing[97]. Oral semaglutide (OG217SC, formerly NN9924, Novo Nordisk) was formulated as a tablet containing the permeation enhancer *N*-(8-[2-hydroxybenzoyl]amino)caprylic acid (SNAC, Eligen® Technology, Emisphere Technologies)[10]. In a phase II trial involving 600 type II diabetes patients, OG217SC tablets were administered once daily

(2.5 to 40 mg) for 26 weeks (www.novonordisk.com). HbA_{1c} improvements were dose-dependent from 0.7 to 1.9%, making the 40 mg oral dose comparable with the 1 mg subcutaneous dose. Oral semaglutide was well tolerated and adverse events were largely dose-dependent, transient, and confined to the GI tract. According to the company's website, Novo Nordisk will initiate phase IIIa development of OG217SC.

3.2. *Proteins*

3.2.1 *Insulin*

Human insulin consists of 51 amino acids (5800 Da) organized in two chains (A chain 21 aa, B chain 30 aa) linked with two disulfide bridges[68]. Although insulin formulations have only been approved for subcutaneous and pulmonary administration, their application *via* the oral route remains an attractive and vital field of research. In addition to the greater convenience of orally applying insulin, absorbing insulin from the gut into the portacaval vein and the liver mimics the pancreatic insulin secretion better than the subcutaneous route, and promises to decrease peripheral hyperinsulinemia[98]. Multiple insulin formulations for oral administration have entered clinical trials, ranging from enteric-coated capsules and formulations with permeability enhancers, liposomes, and polymer conjugates to buccal sprays[99]. Referring the readers to three comprehensive reviews[98–100] on oral insulin delivery, we will present here the most clinically advanced insulin-containing drug delivery systems for oral or buccal administration with published results from clinical trials.

3.2.1.1. *Oral insulin*

Several oral insulin delivery systems are based on enteric-coated capsules with bioavailability-improving adjuvants. Unfortunately, the exact compositions of the formulations were generally not communicated[98]. Capsulin™ (Diabetology) is formulated as an enteric-coated capsule that contains insulin, a non-disclosed “dissolution aid”, and an aromatic alcohol (e.g., phenoxyethanol, benzyl alcohol and phenyl alcohol, according to the patent[101]) for absorption enhancement[102]. In a randomized controlled crossover phase IIa study, Capsulin™ was administered 60 min before breakfast and the evening meal in sixteen patients with type II diabetes[102]. Although significant hypoglycemic action of Capsulin™ was shown, postprandial blood glucose remained above baseline within 2 h after administration. Moreover, a dose-dependent increase in plasma insulin levels upon Capsulin™ administration was not observed in the reported clinical trials, and the effect of food remained unclear[99]. Capsulin™ is supposedly starting phase IIb clinical trials after Diabetology entered a licensing agreement with the Indian pharmaceutical company USV Limited in 2012 (www.diabetology.co.uk)[100].

ORMD-0801 (Oramed) is another insulin-containing enteric capsule[103]. According to the filed patent, the undisclosed formulation likely contains one or several protease inhibitors (e.g., soya bean trypsin inhibitor, ethylenediaminetetraacetate, EDTA), permeation enhancers (e.g., EDTA), and lipoidal carriers (e.g., vegetable, fish or synthetic omega-3 fatty acid)[101]. In a pilot study involving eight type I diabetes patients, ORMD-0801 capsules were administered 45 min before meals as an add-on to the standard insulin treatment and dietary regimen[104]. The add-on significantly decreased the frequency

of high blood glucose readings and glucose AUC, but the response varied among the patients, as reported in other early-phase clinical trials of ORMD-0801[99,104]. The ORMD-0801 is currently in phase II clinical trials for type I and type II diabetes.

An insulin formulation that used the permeation enhancer SNAC was tested in sixteen healthy fasting volunteers[105]. Plasma glucose decreased 20 – 40 min after insulin administration in a dose-dependent manner[105]. Despite the high amount of administered SNAC (2.1 g), no adverse events were reported[105]. In the meantime, the development of this formulation was terminated by Emisphere Technology[100]. Furthermore, an oral insulin formulation OI338GT (formerly NN1953, Novo Nordisk), that is based on GIPET is currently in phase I and II clinical development for diabetes type I and II, respectively, but published results were not available at the time of submission of this review.

IN-105 (Biocon) is an insulin derivative based on conjugating a methoxy-triethylene glycol propionyl moiety to a lysine on the B chain of human insulin which aims at improving stability in the GI tract[106,107]. In a cross-over study, 20 diabetes type II patients received IN-105 as tablets (10 to 30 mg) 20 min before ingesting a standardized high-carbohydrate meal[108]. The postprandial blood glucose levels were significantly decreased for all IN-105 doses in a dose-dependent manner, but insulin concentration levels varied, and hypoglycemic events were reported for all doses except the lowest[108]. In an unpublished six-month phase III clinical trial, IN-105 failed to meet the primary efficacy endpoint of achieving an HbA_{1c} (glycated hemoglobin) reduction of 0.7% after placebo adjustment[99]. In 2012, Biocon and Bristol-Myers Squibb signed a license option agreement for worldwide development and marketing of IN-105 (except India), but the exact state of the current development is unclear.

Insulin was further encapsulated in “hepatocyte-directed vesicles” (Diasome Pharmaceuticals). The unilamellar liposomes (25 to 125 nm) are composed of the structural phospholipid distearoyl lecithin, negatively charged dicetyl phosphate, cholesterol, and a liver-targeting agent (biotin-phosphatidylethanolamine), which aims at decreasing peripheral hyperinsulinemia[109]. However, scientific publications that demonstrate the uptake and liver-targeting effect are not available. The formulation is dried using a proprietary procedure and formulated as an oral gelatin capsule[110]. In a placebo-controlled clinical trial, liposomal insulin was given 30 min before every meal as an add-on therapy to six type II diabetes patients[110]. It was significantly more effective in lowering mean postprandial plasma glucose concentrations and AUC than the placebo but no dose-response relationship was observed[110].

3.2.1.2. *Buccal insulin*

Oral-lyn™ (Generex Biotechnology Corporation) is an oral formulation for buccal delivery that contains recombinant human insulin and non-disclosed absorption enhancers[98]. According to the patent, these GRAS status excipients are likely to be sodium alkyl sulfate, EDTA, sodium salicylate, and a lecithin-based or bile salt-derived agent, claimed by the company to form insulin-containing mixed micelles and enhance absorption[101,111]. The insulin liquid formulation is propelled into the mouth

as a fine-particle aerosol by the RapidMist™ device[111,112]. In a study with 31 subjects who suffered from impaired glucose tolerance, six puffs of Oral-lyn™ right before and six puffs 30 min after a standard oral glucose tolerance test decreased plasma glucose levels approximately 30% after 2 and 3 h compared with no treatment, and hypoglycemia was not observed in this time frame[112]. However, an increase in plasma insulin levels was only observed 30 min after the administration of the first six puffs[112]. According to an announcement by Generex, the company has improved the formulation to increase insulin bioavailability and the insulin amount per puff, and is planning to update its IND on file at the FDA.

In conclusion, the goal of reproducibly delivering insulin *via* the oral route has not yet been achieved. Inter- and intraindividual variability often undermines reliable dosing, especially in the presence of food. Presumably, non-buccal insulin formulations will have to be taken at specific times before food is ingested, and the meals cannot be fully or partially skipped to avoid hypoglycemia. These restrictions in eating habits and the limited reliability counterbalance the advantages of needle-free insulin delivery. The most appropriate role of the currently tested oral insulin formulation may be as an add-on to improve blood glucose control, provided that the risk of hypoglycemic episodes is small.

3.3. *Nucleic acids*

Despite the polyanionic nature and high molecular weight (>5000 Da) of AONs [113], several attempts at systemic AON delivery *via* the oral route have been undertaken. Mipomersen (formerly ISIS 301012, Genzyme/Isis Pharmaceuticals) is a 20-mer phosphorothioate AON stabilized with 2'-O-(2-methoxyethyl) modifications at the five terminal bases at both ends. It is used to treat homozygous familial hypercholesterolemia because it targets the liver apoB mRNA which encodes apolipoprotein B, an essential component of several fat carriers (e.g., chylomicrons, LDL, VLDL). The AON was formulated with the permeability enhancer sodium caprate, and upon its administration once daily for 90 days, the average plasma bioavailability was 6%[114]. A maximum reduction of apoB of 12-15% was observed on day 55 of the treatment. The formulation for subcutaneous administration of mipomersen was further developed and finally approved by the FDA in 2013 (Kynamro®, Genzyme). In 1999, Isis Pharmaceuticals and Elan formed Orasense to develop an oral formulation of ISIS 104838, a 20-mer phosphorothioate AON that targeted the TNF- α mRNA. It contains 2'-O-(2-methoxyethyl) modifications at the five terminal bases at both ends[11]. Several enteric-coated capsules with varying durations of sodium caprate release (total 660 mg of sodium caprate and 100-140 mg of AON per capsule) were evaluated in healthy humans for the oral delivery of ISIS 104838[11]. Each subject received five capsules per treatment, and all formulations were well-tolerated. However, the inter-individual variability in AON oral bioavailability was high, ranging from 0.7% to 27.5% in ten fasting subjects and with 12% being the average bioavailability of the best formulation[115]. In 2005, Isis Pharmaceuticals terminated the development of ISIS 104838.

Monarsen (EN 101, Amarin Corporation) is an AON under investigation for myasthenia gravis (MG), a neuromuscular autoimmune disorder. It downregulates a pathologic mRNA splicing variant of acetylcholinesterase, which constitutes an established target in MG[116]. Monarsen is a 20-mer phosphodiester oligonucleotide with 2'-O-methyl modifications at the three terminal bases of the 3'

end[116]. It was given orally for four days as an aqueous solution to 16 patients who suffered from MG [117]. Thirteen patients showed improved Quantitative MG scores; however, the study was not placebo-controlled. In the following phase IIa study, oral monarsen given for 7 days at a maximum daily dose of 40 mg decreased Quantitative MG scores by 20.3% compared to baseline[118]. Further development of monarsen for MG was discontinued. Recently, monarsen was reported to exert an off-target anti-inflammatory effect through activation of Toll-like receptor 9, which additionally decreased AChE activity. Therefore, the observed therapeutic effect might be attributable to the indirect action of AON rather than the target knockdown[119]. This finding led to further development of monarsen for the oral treatment of ulcerative colitis by BioLineRx under the name BL-7040. This drug has completed phase II clinical trial with positive results, showing reduced neutrophil levels and histological improvement (www.biolinerx.com).

4. Safety of bioavailability-increasing excipients

To address the challenges posed by the low stability and intestinal absorption of orally applied macromolecular drugs, excipients such as permeation enhancers and/or protease inhibitors are often added to oral drug delivery systems. Given that the body is exposed to the excipients as much as it is exposed to the drug, their safety profiles are essential features of the final product, especially in case of long-term use. This section reviews protease inhibitors and permeation enhancers with a focus on excipient toxicity. Enteric coatings will not be covered due to their widespread use and established safety profiles.

4.1. Protease inhibitors

A major obstacle to the local and systemic delivery of peptides and proteins is their susceptibility to enzymatic degradation in the GI tract[120]. Protease inhibitors reversibly or irreversibly inactivate luminal and/or brush border membrane-bound enzymes in the GI tract[120]. A conceptual drawback of protease inhibitors is the impaired digestion of dietary proteins[120]. In rodents, protease inhibitors led to the activation of a feedback loop that resulted in enhanced secretion of proteases, and pancreas hypertrophy and hyperplasia[121–124]. Inhibition of protease activity should therefore be restricted to the area of the liberation and absorption of the macromolecular drug.

Protease inhibitors are grouped into amino acid-based, (poly)peptidic, and non-amino acid-based inhibitors[120]. Those based on amino acids, (poly)peptides, and their derivatives competitively bind to the enzyme active site[120]. However, their efficacy is often limited due to generally low inhibitory activity and rapid dilution, digestion, and absorption in the GI tract. For instance, in a small short-term clinical study, the peptidic serine protease inhibitor aprotinin was co-administered with human calcitonin in the colon, and although no adverse events related to aprotinin were reported[125,126], the excipient failed to increase the bioavailability of human calcitonin. In rats, aprotinin and several other protease inhibitors (soybean trypsin inhibitor, sodium glycocholate, camostat mesilate, and bacitracin) fell short in enhancing insulin absorption upon small-intestinal administration[126]. In view of the low potency and presumably high doses needed to generate an effect, concerns regarding the safety profiles of the excipients and their potential systemic availability have been raised. The systemic

exposure of peptidic aminopeptidase-inhibitor bacitracin, for instance, was associated with nephrotoxicity[127]. Non-amino acid-based chelating agents inactivate proteases by scavenging enzyme cofactors (calcium, zinc, cobalt, manganese, magnesium). The chelating agent EDTA is widely used in the pharmaceutical and food industries, but its binding capacity for calcium is not sufficiently high to inhibit the activity of the calcium-dependent luminal endoproteases trypsin and chymotrypsin[120]. Due to its considerably higher affinity to zinc, EDTA may be more useful in targeting zinc-dependent exonucleases, provided that it is not excessively diluted. However, the binding to essential trace elements may lead to nutritional deficiencies, especially upon chronic administration. Furthermore, small-molecule protease inhibitors can be associated with local and systemic adverse reactions. The synthetic trypsin inhibitor camostat mesylate, for instance, increased insulin bioavailability upon co-administration in the colon but was associated with pancreas hypertrophy[122,126]. This serine protease inhibitor further showed cross-reactivity with other proteases, leading to off-target effects (e.g., enhanced pulmonary mucociliary clearance due to inhibition of airway channel-activating proteases)[128,129].

4.2. Permeation enhancers

A variety of compounds with different mechanisms of action are grouped under the term permeation enhancer[130]. They enhance membrane permeability by disrupting the mucus layer on the intestinal epithelium, transiently opening tight junctions, disrupting the membrane bilayer packing, and directly binding to the substrate to form an absorbable complex[130]. The most important and clinically advanced permeation enhancers are salts of medium-chain fatty acids[131]. Sodium caprate, the salt of decanoic acid, opens tight junctions, and thus enhances permeability *via* the paracellular pathway[132]. Even though cytotoxic effects of sodium caprate were observed in Caco-2 monolayers, repeated oral administrations of sodium caprate did not result in morphological changes in the gastrointestinal mucosa of several animal species[115,133–135]. Orally administered sodium caprate were investigated in several clinical studies and, even upon long-term administration, generally well-tolerated apart from being associated with GI adverse events in some patients[131]. Rectal administration of sodium caprate-containing suppositories, however, led to transient mucosal damage in healthy subjects[136]. Furthermore, the safety profile of sodium caprate remains insufficiently investigated in certain high-risk populations, notably in patients with increased GI permeability at baseline (e.g., irritable bowel diseases) or subjects exposed to GI-irritant drugs such as nonsteroidal anti-inflammatory drugs and ethanol[131].

Another strategy for enhancing membrane permeation is the formation of a membrane-permeable complex between the substrate and a complexing agent. SNAC and 5-CNAC are claimed to form a non-covalent complex with the substrate, protecting it from intestinal degradation and increasing its hydrophobicity in order to enable passive permeation of the epithelium, after which the complex disassembles[137]. However, this mechanism of action has been the subject of controversial discussions[10,130]. Interestingly, SNAC damaged Caco-2 cells in various cytotoxicity assays but the toxicity profile in rats was favorable apart from the appearance of histopathological changes in the stomach which the authors partly related to the application method (gavage)[137–139]. It was

generally well-tolerated in clinical studies, with occasional reports of adverse GI events (nausea, vomiting)[105,140–142].

Conceptually, enhancing intestinal permeation entails the risk of increasing the exposure to dietary antigens, which potentially increases the risk of triggering chronic autoimmune diseases[143]. However, currently published clinical trials have not confirmed this hypothesis. Furthermore, surfactant-type permeation enhancers impair the protective properties of the mucus layer, facilitating the diffusion of luminal bacteria to the intestinal epithelium and ultimately disturbing the host-microbiota relationship. For instance, it was recently found that the ubiquitous pharmaceutical excipients and food additives polysorbate-80 and carboxymethyl cellulose induced a microbiome composition shift, inducing low-grade inflammation and obesity in wild-type mice and promoting colitis in a predisposed mouse model[144]. While the clinical relevance of these intriguing *in vivo* findings has yet to be established, alterations in the composition of the gut microbiota were also reported in healthy humans upon long-term administration of the permeation enhancer chitosan[145]. These studies show that even widely used excipients with GRAS status may lead to local and systemic pathophysiologic effects, underlining the need for more thorough long-term preclinical and clinical testing.

5. Conclusions

In spite of the considerable progress made in pharmaceutical technology since the first oral insulin application in 1922, the administration of biomacromolecules *via* the oral route remains one of the greatest challenges for formulation scientists. In the systemic delivery of orally administered biomacromolecules, the harsh environment of the GI tract and the intestinal epithelial barrier often result in considerable luminal degradation, low and erratic absorption and bioavailability, and highly variable plasma concentrations. Food effects and drug interactions (e.g., proton pump inhibitors) may further influence the pharmacokinetic profile. Therefore, the oral administration is currently restricted to potent low-molecular-weight peptides with large therapeutic windows or with plasma concentration monitoring. Although novel approaches such as buccal delivery are under clinical investigation, it remains to be seen if the systemic delivery of orally applied macromolecules above 5000 Da, notably insulin, can be achieved sufficiently reproducibly to reach a favorable efficacy and safety profile. In the current trials, permeation enhancers in combination with protease inhibitors are often used to overcome the absorption and enzymatic barriers. However, serious risks might be associated with the long-term application of these excipients, including increased exposure to dietary antigens and pathogens due to the sustained increased permeability of intestinal mucosa and potentially pathogenic changes caused by alterations in the intestinal microbiome. Therefore, thorough long-term toxicity studies of these excipients are needed.

In contrast, the local delivery to GI targets avoids the challenges of crossing the intestinal epithelium. GI resistance is often conferred by chemical stabilization strategies, which are especially straightforward for peptides and short nucleic acids. Furthermore, novel approaches such as genetically modified probiotics that produce and secrete the desired proteins *in situ* are on the horizon. However, luminal degradation, potential immunogenicity, and the risk of inadvertent systemic exposure (e.g., in patients with increased intestinal permeability) remain challenging. Moreover, the

therapeutic areas of local delivery are mainly restricted to diseases of the GI tract (GI infections, IBD, and certain metabolic diseases).

In view of these challenges, we believe that a better understanding of the molecular interactions between targets and their biomacromolecular substrates and ligands will eventually yield low-molecular-weight drugs, which are more suitable for oral delivery than their macromolecular parent compounds.

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Figure captions

Figure 1. Strategies for oral delivery of macromolecular drugs that act on local or systemic targets. In buccal or sublingual delivery, the drug targets the buccal or sublingual mucosa, which avoids degradation pathways in the GI tract. Often, permeation enhancement is necessary to cross the multi-layered buccal epithelium. Mucoadhesive bacteria that secrete the desired protein *in situ* are a novel means of achieving sustained release in the oral cavity. To enhance stability against degrading enzymes, macromolecular drugs can be chemically modified by polymer conjugation (proteins), backbone and base modifications (nucleic acids), and cyclization as well as by introducing D-amino acids (peptides). Sacrificial proteins, protease inhibitors, and enteric coatings can also be included in the formulation to further improve GI resistance. To achieve meaningful systemic exposure, absorption enhancement by tight junction-disrupting excipients is often needed. A novel approach is the *in situ* production and secretion of therapeutic biomacromolecules by genetically modified bacteria.

Figure 2. Schematic representation of the mechanisms of action, primary structure, and diarrhea incidence of the GC C agonists linaclotide, plecanatide, and dolcanatide. The sequences of these peptides are based on the physiological agonist uroguanylin and the structurally similar heat-stable *Escherichia coli* enterotoxin (ST) but include sequence adaptations such as unnatural D-amino acids (dN = D-asparagine, dL = D-leucine). Lines above and below the peptide sequences denote the disulfide bonds. Upon binding of an activating ligand, GC C converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), increasing the intracellular cGMP concentration and activating the cystic fibrosis trans-membrane conductance regulator (CFTR). The resultant efflux of chloride ions out of the epithelial cell leads to a water influx into the intestinal lumen, which normalizes bowel movements. Extracellular cGMP further inhibits intestinal nociceptors. Thus, GC C agonists improve constipation and alleviate chronic abdominal pain in patients suffering from inflammatory bowel syndrome with constipation. The incidence of diarrhea, a common treatment-emergent adverse event associated with excessive treatment response, is also indicated for the three peptides (dose and regimen in parentheses). The information was collected from [29–34] and the website of Synergy Pharmaceuticals.

Figure 3. Influence of water and food on the plasma profile of SMC021 (salmon calcitonin formulated with the permeation enhancer 5-CNAC). The higher amount of water co-administered with the tablet resulted in lower calcitonin plasma concentrations (60 min before a meal, a). The time span between tablet administration and meal intake further influenced systemic exposure to calcitonin (b). In a twice-daily dosing scheme, the morning dose resulted in higher calcitonin plasma concentrations than the evening dose, potentially due to the fasting before and after the morning dose in contrast to meals ingested 1.5 h before and 1 h after tablet administration. Figures adapted from [91,92] with permission.

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GC-C activators' structure

ST	NSSNY	CCELCCNPACTGCT
Linacotide		CCEYCCNPACTGCT
Uroguanylin	NDD	CELCVNVACTGCL
Plecanatide	NDE	CELCVNVACTGCL
Dolcanatide	dNDE	CELCVNVACTGCdL

	<i>Diarrhea incidence, %</i>
Linacotide	20 (0.29 mg once daily)
Plecanatide	9.7 (3 mg once daily)
Dolcanatide	5.4 (3 mg once daily)

Table 1. Macromolecular treatments for local GI therapy in clinical development (as of January 2016, source: Thomson Reuters Integrity®, ClinicalTrials.gov, and company press releases)

Type	Generic and trade name, molecular weight	Modification/Formulation	Indication	Development phase (NCT#)
Peptide	Vancomycin (Vancocin®), 1449 Da	Cyclic structure, modified amino acid residues; gelatin capsule	<i>Clostridium difficile</i> -associated diarrhea (CDAD) and staphylococcal enterocolitis including methicillin-resistant strains	Marketed
	Fidaxomycin (Dificid®), 1058 Da	Non-peptidic macrocycle; tablet	CDAD	Marketed
	Surotomylin, 1681 Da	Cyclic structure	CDAD	Phase III (NCT01597505, NCT01598311)
	Ramoplanin, 2554 Da	Cyclic structure	CDAD	Phase IIb (N.A.)
	LFF-571, 1367 Da	Cyclic structure, thiazolyl peptidic	CDAD	Phase II (NCT01232595)
	NVB302, 2115 Da	Cyclic structure, thioether bonds	CDAD	Phase I (N.A.)
	Linacotide (Linzess®), 1525 Da	Cyclic structure; gelatin capsule	Chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C)	Marketed
	Plecanatide, 1682 Da	Cyclic structure	CIC and IBS-C	CIC phase III completed in 2015 (NCT01919697) IBS-C phase III ongoing (NCT02493452)
	Dolcanatide (SP-333), 1682 Da	Cyclic structure, two D-amino acid substitutions	Opioid-induced constipation (OIC) and ulcerative colitis (UC)	OIC phase II completed in 2014 (NCT01983306)

				UC phase Ib ongoing (N.A.)
Antibody	AVX-470 (Avaximab™-TNF), broad	Polyclonal antibody; enteric capsule	Pediatric UC	Phase II ongoing (N.A.)
	AG014, 47.8 kDa	Lyophilized <i>Lactococcus lactis</i> secreting certolizumab; enteric capsule	Inflammatory bowel disease	Phase Ia completed in 2014 (N.A.)
Enzyme	Creon® (porcine protease, amylase, lipase)	Enteric capsule	Exocrine pancreatic insufficiency	Marketed
Protein-secreting bacteria	AG013, 6.5 kDa	Oral rinsing solution containing 2.0×10^{11} colony-forming units (CFU)/15 mL of <i>Lactococcus lactis</i> secreting human trefoil factor 1	Chemotherapy- or radiation-induced oral mucositis	Phase Ib completed in 2012 (NCT00938080)
	AG011, 18 kDa	<i>Lactococcus lactis</i> secreting anti-inflammatory cytokine IL-10; enteric capsule in combination with enema	UC	Phase IIa completed in 2009, failed (NCT00729872)
Nucleic acid	Mongersen (GED-0301), 6952 Da	Phosphorothioate AON; enteric coating	Moderate to severe Crohn's disease	Phase II completed in 2014 Phase I ongoing (NCT02367183)
	Alicaforsen, 6368 Da	Phosphorothioate AON; nightly enema with hydroxypropyl methylcellulose	UC, pouchitis	Phase III ongoing (NCT02525523)
	CEQ508	Suspension of genetically modified <i>E. Coli</i> expressing invasin, listeriolysin, and short hairpin RNA targeting β -catenin	Familial adenomatous polyposis	Phase I/II ongoing since 2011 (N.A.)

N.A: unknown or non-existent NCT number

Table 2. Macromolecular treatments for oral administration in clinical development (as of January 2016, source: Thomson Reuters Integrity®, ClinicalTrials.gov, and company press releases)

Type	Generic and trade name, molecular weight	Formulation	Indication	Development phase (NCT#)
Peptide	Octeotride (Mycapssa™), 1019 Da	Oily suspension containing sodium caprate	Acromegaly	New Drug Application under review
	Desmopressin (DDAVP®), 1128 Da	Tablet, lyophilizate	Central diabetes insipidus, nocturnal enuresis	Approved
	Cyclosporine (Neoral®), 1202 Da	Self-emulsifying system	Organ transplant rejection, autoimmune diseases	Approved
	Acyline (MER-104), 1533 Da	GIPET tablet (sodium caprate)	Prostate cancer, male oral contraception	Phase I/II completed in 2008 (NCT00603187)
	Calcitonin (TBRIA™), 3432 Da	Citric acid-containing enteric coated tablet	Postmenopausal osteoporosis	New Drug Application under review
	Semaglutide (OG217SC), 4114 Da	Eligen®-based tablet (SNAC)	Diabetes Type II (T2D)	Phase III ongoing (N.A.)
Protein	Insulin, 5800 Da			
	Human insulin (Capsulin™)	Enteric coated tablet with hydrophilic aromatic alcohol	Diabetes Types I (T1D), T2D	Phase IIb ongoing (N.A.)
	Human insulin (ORMD-0801)	Enteric coated capsule containing several protease inhibitors, permeation enhancers, lipoidal carriers	T1D, T2D	Phase II ongoing (NCT02496000)
	Long-acting insulin analogue	GIPET tablet	T1D, T2D	Phase I/II ongoing

	(OI338GT)			(NCT02470039)
	Insulin-containing hepatocyte-directed vesicles	Liposomes decorated with biotin	T1D, T2D	Phase III ongoing (NCT00814294)
	Insulin-triethylene glycol conjugate (IN-105)	Tablet	T1D, T2D	Phase III ongoing (N.A.)
	Human insulin (Oral-lyn™)	Spray for buccal delivery	T1D, T2D	Updating IND
Nucleic acid	Monarsen (EN 101, BL-7040), 6139 Da	2'O-methyl modifications at the three terminal bases at 3' end; aqueous solution	Myasthenia gravis (MG), ulcerative colitis (UC)	MG discontinued UC phase II completed in 2013 (NCT01506362)
	ISIS 104838 sodium salt, 7701 Da	2'O-(2-methoxyethyl) modifications at 5 terminal bases of both ends; enteric-coated capsule containing sodium caprate	Rheumatoid arthritis, Crohn's disease	Discontinued
	Mipomersen sodium (ISIS 301012), 7595 Da	2'O-(2-methoxyethyl) modifications at 5 terminal bases of both ends; enteric-coated capsule containing sodium caprate	Homozygous familial hypercholesterolemia	Discontinued

N.A: unknown or non-existent NCT number

Oral delivery of macromolecular drugs