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Secretory diarrhoea: mechanisms and emerging therapies

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Abstract

Diarrhoeal disease remains a major health burden worldwide. Secretory diarrhoeas are caused by certain bacterial and viral infections, inflammatory processes, drugs and genetic disorders. Fluid secretion across the intestinal epithelium in secretory diarrhoeas involves multiple ion and solute transporters, as well as activation of cyclic nucleotide and Ca^{2+} signalling pathways. In many secretory diarrhoeas, activation of Cl^- channels in the apical membrane of enterocytes, including the cystic fibrosis transmembrane conductance regulator and Ca^{2+}-activated Cl^- channels, increases fluid secretion, while inhibition of Na^+ transport reduces fluid absorption. Current treatment of diarrhoea includes replacement of fluid and electrolyte losses using oral rehydration solutions, and drugs targeting intestinal motility or fluid secretion. Therapeutics in the development pipeline target intestinal ion channels and transporters, regulatory proteins and cell surface receptors. This Review describes pathogenic mechanisms of secretory diarrhoea, current and emerging therapeutics, and the challenges in developing antidiarrhoeal therapeutics.

Introduction

Diarrhoeal diseases have been a major health problem throughout history.[1] In the past, diarrhoeal diseases were often fatal and disease outbreaks spread quickly, affecting large populations. Today, despite the success of interventions such as oral and intravenous rehydration therapy, secretory diarrhoea remains a substantial cause of mortality and morbidity worldwide, particularly in children and the elderly. In 2015, it is estimated that worldwide 577,000 children aged <5 years and 502,000 adults aged >70 years will die from diarrhoeal diseases.[2] For these vulnerable populations, the mortality risk due to diarrhoeal disease is often further increased by associated risk factors such as malnutrition and pre-existing enteric infections.[3] In addition to the mortality risk, repeated diarrhoeal episodes are

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associated with long-term impairment of physical and mental development, with an estimated global loss of ~1,400 years of healthy life due to disability per 100,000 population.[2,4]

The prevalence of diarrhoeal disease, as for other important global causes of childhood mortality such as pneumonia and malaria, is correlated closely with climate and economic development. The most severely affected regions include developing countries in sub-Saharan Africa and South Asia.[2] The major causes of diarrhoeal diseases in developing countries are infectious, including enterotoxin-producing bacteria, such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli*; viruses, such as rotavirus; enteroinvasive bacteria, such as *Shigella* and *Salmonella*; and parasites, such as *Entamoeba histolytica* and *Cryptosporidium parvum*.[5] The most common cause of severe diarrhoea worldwide is rotavirus (28% of diarrhoeal episodes), with *V. cholerae* producing at least 1–2% of cases.[6] Severe diarrhoea outbreaks that rapidly affect large populations are often associated with complex humanitarian emergencies such as the displacement of people into refugee camps, natural disasters such as earthquakes, and armed conflict, leading to the loss of health and sanitation infrastructure. Examples include the refugee crisis in Rwanda in 1994, the conflict in Zimbabwe in 2008, and the earthquake in Haiti in 2010.

Many noninfectious causes of diarrhoea are prominent in developed countries. Diarrhoea is associated with adverse effects of drugs, particularly certain cancer and HIV therapeutics.[7,8] Up to 28% of patients with HIV treated with protease inhibitors report more than four loose or watery stools per day.[7] Intestinal inflammatory and autoimmune conditions, such as ulcerative colitis, Crohn's disease and coeliac disease, can have a substantial diarrhoeal component.[9,10] IBS is prevalent in developed countries; 10–20% of the adult population in the US are estimated to have IBS, of which around one-third suffer from chronic diarrhoea.[11] Severe secretory diarrhoea is also caused by rare congenital disorders, such as microvillus inclusion disease, familial diarrhoea syndrome and tufting enteropathy, as well as by peptide-secreting neuroendocrine tumours.[12–15] However, infectious causes of diarrhoea still represent a large proportion of the disease burden in developed countries. The incidence of diarrhoea caused by rota-viruses has fallen dramatically over the past 5 years with the widespread administration of the rotavirus vaccine, although the incidence of diarrhoea caused by noroviruses has increased and become the leading cause of disease outbreaks from contaminated food in the US.[16] The main bacterial causes of food-related diarrhoeal disease in the US are *Salmonella enterica* (19,000 hospitalizations per year) and *Campylobacter jejuni* (8,000 hospitalizations per year),[16] and there have been annual enterohaemorrhagic *E. coli* outbreaks since the early 1990s.

In this Review, we describe the major pathogenic mechanisms of secretory diarrhoea, discuss currently available pharmacological therapies and therapies that are being developed, and examine the major challenges in the development of diarrhoeal therapeutics.

Mechanisms of diarrhoeal disease

Diarrhoea results from excessive secretion and/or impaired absorption of fluid and electrolytes across the intestinal epithelium (Figure 1). The movement of fluid between the

intestinal lumen and blood is driven by the active transport of ions, mainly Na^+, Cl^-, HCO_3^-, and K^+, and solutes, mainly glucose. Fluid absorption or secretion involves the coordinated activity of membrane transporters located on the apical (lumen-facing) and basolateral (circulation-facing) epithelial membranes.[17] The intestinal epithelium is structurally configured into long, finger-like projections (villi) and glandular, tube-like structures (crypts), with the relative villus-to-crypt ratio differing along the intestine. Functionally, both absorption and secretion can occur in the same epithelial cells, although secretory processes predominate in crypts and absorptive processes in villi.

Fluid absorption is driven by the active transport of Na^+ across the epithelium with parallel Cl^- or HCO_3^- absorption. The electrochemical driving force for this process is the basolateral Na^+/K^+-ATPase. In the small intestine, fluid absorption is facilitated by the sodium/hydrogen exchanger 3 (NHE3, also known as SLC9A3), sodium/glucose cotransporter 1 (SLC5A1), and Cl^-/HCO_3^- exchanger (DRA [SLC26A3] and PAT1 [SLC26A6]).[18–20] Electroneutral fluid absorption is carried out by the coordinated activity of NHE3 with Cl^-/HCO_3^- exchangers (PAT1 for HCO_3^- absorption and DRA for Cl^- absorption in the jejunum and colon).[21,22] Substrate-specific transporters such as SLC5A1 facilitate cotransport of Na^+ across the apical membrane together with D-glucose (or D-galactose), with the electroneutral glucose transporter SLC2A2 facilitating glucose exit across the basolateral membrane.[23,24] In the colon, in addition to electroneutral Na^+ transport by Na^+/H^+ exchange (proximal colon), absorption is facilitated by the epithelial Na^+ channel and short-chain fatty acid transporters.[25,26] Intracellular messengers, including Ca^{2+} and cyclic nucleotides such as cAMP and cGMP, inhibit the activity of apical Na^+ transporters.[27–30] There is considerable cross-activation of the intestinal epithelial second messengers cAMP and Ca^{2+}.[31,32]

Intestinal fluid secretion is driven by transepithelial Cl^- secretion through basolateral and apical Cl^- channels and transporters. Cl^- is transported into the cell at the basolateral membrane by a Na/K/Cl symporter (NKCC1, also known as SLC12A2), which is driven by the Na^+ concentration gradient produced by the Na^+/K^+-ATPase.[33] Basolateral K^+ channels (KCNQ1/KNE3 and KCNN4) provide the electrochemical driving force for apical Cl^- exit across Cl^- channels,[34] primarily the cyclic-nucleotide-activated cystic fibrosis transmembrane conductance regulator (CFTR) and Ca^{2+}-activated Cl^- channels (CaCCs).[33] Enteric nerves and cell surface receptors such as the calcium-sensing receptor (CaSR) are thought to modulate intracellular signalling pathways and hence electrolyte absorption and secretion.[35,36]

Bacterial diarrhoeas

Bacteria such as *V. cholerae* and enterotoxigenic *E. coli* secrete specific enterotoxins (for example cholera toxin and heat-stable enterotoxin, respectively) that increase levels of intracellular cyclic nucleotides, resulting in activation of apical CFTR Cl^- channels and hence Cl^- secretion (Figure 1b).[37,38] Data from immortalized and primary human intestinal cells show that elevation of cAMP, cGMP and Ca^{2+} concentrations by bacterial enterotoxins also inhibits NHE3.[39,40] Bacteria can also increase various humoral agonists, neurotransmitters or neuropeptide receptors such as 5-hydroxytryptamine, VIP peptides and

the galanin receptor type 1,[41] thus activating Cl^- secretion and inhibiting Na^+ absorption.[42] Invasive bacteria such as *Salmonella* and *Shigella* cause a tissue inflammatory response involving recruitment of immune cells and release of cytokines, resulting in intra-cellular Ca^{2+} signalling.[43] Enteropathogenic and invasive bacteria also result in alterations in transport protein expression, with several studies showing evidence of impaired Na^+ and Cl^- absorption.[40,44,45]

Viral diarrhoeas

Enteric rotavirus infection causes fluid secretion as well as structural changes in the intestinal epithelium,[46] producing an age-related secretory diarrhoea.[47,48] An elaborated rotaviral protein (NSP4) is thought to act as an enterotoxin causing elevation of cytoplasmic Ca^{2+} concentration by binding to a membrane receptor (integrin $\alpha_1\beta_2$), the neuropeptide galanin and/or by activation of enteric nerves (Figure 1c).[49–51] Rotaviral NSP4 also inhibits NHE3 and SLC5A1.[52] The molecular mechanisms by which other enteric viruses such as norovirus cause diarrhoea is not known at present.[53] Multiple pathogenic mechanisms for drug-induced diarrhoeas are likely to exist, but there is evidence that certain drugs such as HIV protease inhibitors and chemotherapy agents induce diarrhoea through intra-cellular Ca^{2+}-dependent mechanisms similar to those in rotaviral diarrhoea.[54,55]

Congenital diarrhoeas

Congenital diarrhoeas can result from rare inherited genetic mutations in several intestinal proteins (Figure 1d). The target genes in many of these familial enteropathies have been discovered with the advent of low-cost gene sequencing. Mutations in the Na^+ transporter SLC5A1 underlie glucose and galactose mal-absorption, and mutations in the Cl^-/HCO_3^- exchanger DRA result in congenital Cl^- diarrhoea.[56,57] A mutation in the guanylin receptor GUCY2C, which causes familial diarrhoea syndrome, results in constitutive cGMP activation with consequent CFTR-mediated Cl^- secretion and NHE3 inhibition.[14] Alterations in the integrity of intestinal structure caused by mutations in proteins such as MYO5B and the epithelial cell adhesion molecule also result in altered signalling with putative activation of electrolyte secretion.[58,59] Additional details about congenital diarrhoeas can be found elsewhere.[60,61]

Inflammatory diarrhoea

Intestinal inflammation is seen in autoimmune diseases such as IBD and coeliac disease. Although the predominant feature of these diseases is chronic tissue damage secondary to inappropriate immune cell activation, several overlapping signalling pathways affect intestinal fluid transport homeostasis.[62] Activation of epithelial inflammatory signalling pathways such as NF-κB result in Ca^{2+} or cyclic nucleotide signalling and stimulation of Cl^- secretion or inhibition of Na^+ absorption (Figure 1e). The release of inflammatory mediators such as TNF and IL-6 by activated T cells and neutrophils, which causes degranulation of mucosal mast cells and release of histamine and prostaglandins, can also stimulate Cl^- secretion.[63,64] Epithelial Na^+ absorption is also probably affected by inflammation, with studies showing down-regulation of NHE1 (also known as SLC9A1) and NHE3 in IBD, and defective NHE3 function and regulation by adaptor proteins in an IL-10-

deficient mouse model of colitis.[65–67] Predominantly inflammatory diarrhoea is seen in response to some bacterial pathogens, such as *Clostridium difficile*, which is the most common cause of nosocomial antibiotic-associated diarrhoea. *C. difficile*-associated diarrhoea and colitis occurs secondary to elaborated toxins that activate epithelial inflammatory signalling pathways and recruit immune cells.[68]

Current management and treatment

The acute treatment of infectious diarrhoeal diseases centres around prompt and complete amelioration of dehydration. In hospitals, particularly in developed countries, this treatment is often achieved through intravenous fluid replacement.[69] In developing countries and non-hospital settings oral rehydration solution (ORS) is the mainstay of treatment.[70] Although ORS effectively treats dehydration when administered appropriately, it does not change fluid losses, diarrhoeal output or duration of illness. Most of the current and historical therapeutics used for symptomatic treatment are classed as antimotility agents or antisecretory agents based on their mechanism of action (Table 1). For diarrhoeas caused by enteric infections, various antibiotics are also used depending on the pathogenic organism.[71] Antibiotics shorten illness duration and are used particularly for dysentery (bloody diarrhoea), but selection of appropriate antibiotics requires laboratory diagnosis of the pathogenic organism, which often is not available. Although antibiotics are effective in reducing symptoms and the duration of infectious diarrhoeas, their delayed onset of action means they do not prevent immediate dehydration. Also, concerns about antibiotic resistance and the fact that they are contra-indicated in specific enteric infections have prevented their recommendation for widespread use.[72,73]

ORS

ORS is an orally ingested solution that stimulates intestinal Na^+ absorption by SLC5A1 and Na^+-coupled amino acid transporters. The current WHO-recommended ORS is hypo-osmolar (245 mOsm/l) to increase water absorption.[74] The widespread use of ORS over the past four decades has remarkably reduced the mortality associated with diarrhoeal disease by ~70%.[75] However, ORS administration in developing countries has stagnated since 1995, with ORS only used in 30–35% of children with diarrhoea aged <5 years.[76,77] In the past decade, alternative forms of ORS have been developed. In one form, the substrate linked to Na^+ absorption is nonabsorbable starch, which is relatively resistant to digestion by pancreatic amylase and is metabolized by bacteria to short-chain fatty acids in the colon where it stimulates Na^+ absorption by NHE2 (also known as SLC9A2).[74,78] In clinical trials, patients with cholera who receive nonabsorbable starch ORS have a shorter duration of diarrhoea and require less parenteral fluid support than patients who receive conventional ORS.[79] Zinc has been shown to shorten the duration of acute diarrhoea and is part of the WHO-recommended ORS.[80] The mechanism of this effect of zinc is unclear, and whether zinc deficiency plays a role has not been established. *In vitro*, zinc blocks Cl^- secretion by inhibiting K^+ channels in the basolateral membrane and stimulating NHE3.[81,82]

Antimotility agents

Drugs that inhibit intestinal motility have been used extensively to treat diarrhoea. The putative mechanism of action for antimotility drugs is increased Na^+ and fluid absorption as a result of slow intestinal transit. Loperamide and diphenoxylate are μ-opioid agonists that are widely used for mild, nonspecific diarrhoea. They are not recommended in bacterial diarrhoeas primarily owing to the risk of paralytic ileus, and diphenoxylate also has substantial central opioid effects.[83] 5-hydroxytryptamine$_3$ antagonists such as alosetron have shown efficacy in chronic diarrhoea related to IBS;[84] however, their use has been limited by concerns of ileus and ischaemic colitis. Although loperamide is widely used and effective in mild acute diarrhoea,[85] the potential serious adverse effects of antimotility drugs together with a narrow therapeutic index has limited their recommendation and use, particularly for infectious diarrhoeas.

Antisecretory agents

Reducing intestinal fluid secretion has been a relatively underexploited area for antidiarrhoeal therapeutics. Historically, bismuth subsalicylate was shown to have antidiarrhoeal efficacy and early mechanistic studies indicated that salicylates such as aspirin inhibit enterotoxin-induced Cl^- secretion and promote Na^+ absorption.[86] Racecadotril, an encephalinase inhibitor, or its active metabolite thiorphan, initially showed promise as an antidiarrhoeal. Inhibition of the breakdown of endogenous encephalins could exert anti-secretory effects through encephalin-stimulated activation of epithelial μ-opioid receptors. Small clinical studies initially showed efficacy in cholera-toxin-mediated fluid secretion; however, a large double-blind, placebo-controlled trial showed no improvement in diarrhoeal output or duration.[87,88] More recently, a natural-product antisecretory agent, crofelemer, has been approved for use in HIV-related diarrhoeas based on a clinical trial showing efficacy in improving chronic diarrhoea in patients with HIV.[89] Crofelemer is a heterogeneous proanthocyanidin oligomer extracted from the bark latex of the South American tree *Croton lechleri*. The putative mechanism of action for crofelemer is inhibition of Cl^- channels in the apical membrane. However, *in vitro* studies showed crofelemer to be a weak and partial antagonist of CFTR, although a relatively strong inhibitor of CaCCs.[90] It is unclear whether this inhibition of CaCCs underlies the efficacy of crofelemer in HIV-related diarrhoea.

Probiotics

Several meta-analyses and clinical studies in developed countries suggest that probiotics prevent or reduce the duration of diarrhoea.[91–93] However, these effects seem to be highly strain-dependent and dose-dependent. Studies using specific bacterial strains have shown effects on expression and/or function of DRA, CFTR and NKCC transporters, suggesting that probiotics affect mechanisms of Cl^- secretion and electroneutral absorption.[94–96] A double-blind, placebo-controlled study in children aged 1–6 years showed that a specific probiotic (*Lactobacillus reuteri*) significantly reduced the incidence of acute diarrhoea, particularly in children with poor nutritional status.[97] Currently, probiotics are not routinely recommended in community settings in developing countries, although increasing evidence shows that they might be beneficial as adjunctive antidiarrhoeal therapies.

New antidiarrhoeal therapies

Most of the currently used antidiarrhoeal therapies are decades old and only in the last 5 years has there been renewed interest in development of antidiarrhoeal therapeutics,[98] with several novel drug candidates targeting intestinal fluid transport (Table 2). Some of these new therapeutic candidates have emerged from greater understanding of the mechanisms of intestinal fluid secretion and the use of high-throughput screening technology. Figure 2 depicts the molecular targets for antidiarrhoeal therapeutics at various stages of development.

CFTR inhibitors

Activation of CFTR Cl^- channels in the small intestine and colon occurs in secretory diarrhoeas caused by bacterial enterotoxins secreted in cholera and Traveller's diarrhoea.[99] Intestinal Cl^- and fluid secretion are absent in CFTR-knockout mice and in patients with cystic fibrosis,[100,101] and the use of CFTR inhibitors blocks colonic Cl^- transport in human tissue.[99] High-throughput screening has revealed three chemical classes of small-molecule CFTR inhibitors. The thiazolidinone $CFTR_{inh}$-172 (Figure 3) inhibits CFTR by binding at or near Arg347 and stabilizes the channel in its closed state.[102] Studies in mouse models of cholera and intestinal fluid secretion induced by heat-stable toxin have demonstrated efficacy of $CFTR_{inh}$-172.[99,103] Another class of CFTR inhibitors, which probably interfere with ATP gating, are the PPQ/BPO compounds;[104,105] the most potent of these compounds, (R)-BPO-27, inhibits CFTR Cl^- conductance with IC_{50}~4 nM and has shown efficacy in models of polycystic kidney disease,[104] but has not yet been tested in models of diarrhoea.

Glycine hydrazides such as GlyH-101 are another class of CFTR inhibitors that bind to the CFTR pore on its extracellular surface as demonstrated by patch clamp, molecular modelling and efficacy of a membrane-impermeant polyethylene glycol-hydrazide conjugate.[106] The GlyH-101 analogue iOWH032 has completed a safety clinical trial,[107] although a planned phase II trial (NCT02111304) has been terminated before patient enrolment. iOWH032 is unlikely to be effective because it is a weak CFTR inhibitor and can undergo rapid convective washout in the intestine.[108] To address the potency and washout limitations, non-absorbable macromolecular conjugates have been synthesized containing a malonic acid hydrazide (MalH) moiety that inhibits CFTR. One such conjugate, MalH-lectin, inhibited CFTR with an IC_{50} down to 50 nM, remained bound to CFTR for >6 h, and reduced mortality in a neonatal mouse model of cholera.[109] The high potency of the MalH-lectin conjugate and its resistance to washout is possibly due to trapping in the glycocalyx on enterocytes. Multivalent MalH-polyethylene glycol conjugates have also been synthesized and shown to be CFTR inhibitiors with nanomolar potency,[110] greater chemical stability and lower cost than lectin-containing CFTR inhibitor conjugates.

CaCC inhibitors

CaCCs provide a second route for Cl^- secretion by enterocytes and might also be involved in enterotoxin-mediated secretory diarrhoeas by cross-talk in signalling mechanisms. CaCCs probably represent the primary pathway for apical membrane Cl^- secretion in rotaviral and perhaps other viral diarrhoeas,[48] as well as in secretory diarrhoeas induced by some HIV

protease inhibitors[54] and chemotherapeutics.[55] The molecular identity of the major CaCC(s) in enterocytes remains unclear. The CaCC anoctamin-1 is expressed in interstitial cells of Cajal and has an important role in intestinal motility,[111] but its role in Cl^- conductance in enterocytes remains uncertain.[112]

Small-molecule and natural-product CaCC inhibitors have been identified. A phenotype-based screen in HT-29 cells identified several small-molecule CaCC inhibitors, the most potent being the 3-acyl-2-aminothiophene $CaCC_{inh}$-A01.[113] $CaCC_{inh}$-A01 prevented watery diarrhoea in a neonatal mouse model of rotavirus.[114] Screening of natural products to find potential CaCC inhibitors identified tannic acid, which motivated the discovery that polyphenolic gallotannins strongly inhibit CaCCs. Remarkably, oral administration of an alcohol-free red wine extract prevented rotaviral diarrhoea in neonatal mice, without effect on the rotaviral infection.[114] The red wine extract did not inhibit CFTR or prevent cholera-toxin-induced intestinal fluid secretion. In another natural-product study, diarrhoea remedies from sources around the world were screened for inhibition of Cl^- channels. A commonly used Thai herbal remedy fully inhibited both CFTR and CaCC Cl^- conductance *in vitro*, and was efficacious in mouse models of cholera and rotaviral diarrhoea.[115] Natural products represent inexpensive and readily available potential therapies for serious secretory diarrhoeas.

NKCC1 inhibitors

The electroneutral NKCC1 transporter is the major pathway for Cl^- entry into enterocytes from the blood side and is therefore an attractive target for development of inhibitors that are predicted to block both CFTR-mediated and CaCC-mediated secretory diarrhoeas. NKCC inhibitors such as bumetanide are approved for use as diuretics, as the kidney expresses the NKCC2 isoform. Although novel NKCC1 inhibitors are emerging from screening efforts, challenges in their development for diarrhoeal therapy include obtaining high selectivity over NKCC2 (to prevent diuretic action) or confining their action to the intestine, and preventing potential off-target effects such as hearing impairment seen in NKCC1-knockout mice.[116]

CaSR agonists

The CaSR is a G-protein-coupled receptor expressed throughout the small intestine and colon.[117] In the small intestine the CaSR is expressed on the basolateral membranes of crypt and villus epithelial cells and on the apical surface of villus cells,[117] while in the colon it is present on the apical and basolateral membrane of crypt cells and on cells of the enteric nervous system and some entero-endocrine cells. Extracellular signals that activate the CaSR include divalent (Ca^{2+}, Mg^{2+} and Cd^{2+}) and trivalent (gadolinium^{3+} and La^{3+}) cations, and some L-amino acids. Activation of the CaSR seems to have multiple actions on electrolyte transport, including decreasing enterotoxin-induced Cl^- secretion and reversing inhibition of Na^+ absorption.[35] This effect may in part involve breakdown of cyclic nucleotides by phosphodiesterase activation[35] and regulation of tight junction assembly.[118] CaSR activation by calcimimetic drugs such as R-568 reduces fluid losses stimulated by bacterial enterotoxins and has been proposed as a potential antidiarrhoeal, although to date no clinical studies have investigated CaSR activation by calcimimetics or Ca^{2+} itself.

K^+ channel inhibitors

Several studies have shown that inhibition of basolateral K^+ channels reduces enterotoxin-mediated fluid secretion.[119] The antifungal clotrimazole blocks both cAMP and Ca^{2+}-sensitive K^+ channels in enterocytes with IC_{50} ~5 μM and has emerged as a potential antidiarrhoeal drug candidate.[120] Clotrimazole is approved by the FDA for other indications, with its primary adverse effects related to dose-dependent inhibition of cytochrome P450 enzymes. Other K^+ channel inhibitors, including clotrimazole analogues[121] and more potent new chemical entities, could be potential antisecretory development candidates.

NHE3 agonists

Studies in cell culture and rodent models suggest that NHE3 is inhibited in secretory diarrhoeas, which seems to be an exaggeration of the NHE3 inhibition that occurs physiologically early in the postprandial state.[122] NHE3 activity in enterocytes is inhibited following acute elevations in cAMP, cGMP or Ca^{2+} concentrations, as produced by bacterial enterotoxins, ionophores or humoral agonists including 5-hydroxytryptamine and carbachol.[28,123,124] These mechanisms probably apply in human secretory diarrhoeas. NHE3 activity is reduced in congenital Na^+ diarrhoea as seen in intestinal biopsies,[125] with mutations in the transport domain of NHE3 accounting for this autosomal recessive condition (A. Janecke *et al.*, unpublished data). Human enteroids generated from proximal small intestine of healthy individuals show NHE3 activity under basal conditions, which is acutely stimulated by dexamethasone and inhibited by cAMP, cGMP, Ca^{2+}, bacterial enterotoxins and rotaviral infection.[126] A peptide that mimics part of the NHE3 C-terminal domain and prevents NHE3 inhibition by cAMP, Ca^{2+} and cholera toxin has potential utility as a proabsorptive therapeutic.

Antisecretory factor

A secreted, 41 kilodalton protein named antisecretory factor released mainly from the pituitary gland [127] has been shown in rodent models to prevent intestinal fluid secretion induced by cholera toxin, heat-stable toxin, *C. difficile* toxin A, *Campylobacter* toxin and prosta-glandins.[128] The active component of antisecretory factor consists of seven amino acids in its N-terminus. The antisecretory factor-16 peptide, consisting of the active N-terminal domain of the antisecretory factor, also blocks intestinal fluid secretion. The exact mechanism of action for antisecretory factor is unclear, but the antisecretory effect of antisecretory factor-16 was abolished by vagotomy, suggesting its action is on nerves and perhaps on capillary permeability. A modified egg-yolk product enriched with antisecretory factor (Salovum®, AS-Faktor AB, Sweden) reduced the duration of acute and prolonged (>7 days) diarrhoea in a randomized, placebo-controlled trial in children aged 7–60 months.[129] Further clinical and mechanistic studies are needed for this promising drug.

Lysophosphatidic acid

Lysophosphatidic acid is a naturally occurring phospho-lipid present in many foods. Several studies have suggested that interaction of lysophosphatidic acid with its intestinal receptor (lysophosphatidic acid receptor 2) results in the formation of a macromolecular complex that modulates CFTR activity. Lysophosphatidic acid receptor agonists prevent enterotoxin-

mediated fluid secretion in rodents, suggesting that LPA-based nutritional supplements could be used as adjunct therapy in diarrhoea.[130] Lysophosphatidic acid also stimulates NHE3 in the small intestine through lysophosphatidic acid receptor 5 on Na^+-absorptive cells.[131]

Other potential therapies

Several other potential antidiarrhoeal therapeutics with specific indications are currently in phase II or phase III clinical trials (Figure 2). Eluxadoline is a mixed μ-opioid and δ-opioid inhibitor with antimotility and analgesic properties developed primarily for diarrhoea-predominant IBS. Results from phase II trials showed some clinical improvement in stool consistency and abdominal pain, and little rebound constipation.[132] Another compound aimed at diarrhoea-predominant IBS showing efficacy in phase II trials is LX1033, an inhibitor of tryptophan hydroxylase that affects intestinal motility by inhibition of 5-hydroxytryptamine synthesis.[133] A semi-synthetic bile acid analogue, obeti-cholic acid, which activates the nuclear bile acid receptor (farnesoid X receptor), relieves symptoms in patients with bile acid diarrhoea, which has been suggested to contribute to diarrhoea in some patients with IBS.[134] The action of obeticholic acid is thought to involve a feedback mechanism with an increase in the bile acid receptor, which results in increased intestinal production of fibroblast growth factor 19 and uptake into the portal system, thus reducing hepatic bile acid synthesis and intraluminal bile salt concentration.

Conclusions and future challenges

Although diarrhoeal diseases remain a major global health challenge, there has been a remarkable lack of effective new therapeutics. Part of the reason for the lack of progress is that global and regional health policy has rightly focused on approaches targeted at prevention, such as vaccines, improved sanitation, and better health delivery including the use of ORS. As described herein, there is now an emerging pipeline of potential antidiarrhoeal agents. In developing countries where diarrhoeas are primarily caused by infection, these new agents might be useful as adjuncts or additives to ORS, reducing diarrhoeal output and hence protecting against severe dehydration particularly in the early stages of disease, and secondarily promoting ORS usage. In complex medical emergencies, where availability of rehydration fluids might be limited, such therapeutics could provide life-saving alleviation of fluid loss. To be beneficial in developing countries the ideal therapeutic should have a wide therapeutic index, be stable in harsh environments and be extremely low-cost. Major challenges in moving forward include funding, logistics and design of informative clinical trials. The development of drugs that can be used safely in children and the elderly, where the disease burden is greatest, poses additional challenges in terms of drug adverse effects and patient recruitment for clinical trials. For indications such as diarrhoeas related to chronic inflammatory diseases, IBD or drug-induced diarrhoeas, the development pathway for antisecretory or proabsorptive compounds is clearer, as in the case of crofelemer. Progress towards new antisecretory agents has been accelerated with the elucidation of new regulatory pathways in intestinal fluid transport, raised awareness of natural-product remedies, and emergence of new tools such as ion-sensitive fluorescent proteins and human enteroid models for drug screening. The next and more challenging

process will be translating these discoveries into safe, low-cost therapies to reduce the global health and economic burden of diarrhoeal diseases.

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Key points

- Diarrhoeal disease remains a major global health burden
- Secretory diarrhoea results from abnormal fluid and electrolyte absorption and/or secretion
- Ion channels and transporters such as the cystic fibrosis transmembrane conductance regulator and sodium/hydrogen exchanger 3 are targets for antisecretory antidiarrhoeal drugs
- New compounds targeting intestinal transporters are in development for antidiarrhoeal therapy, including small molecules, natural compounds and existing drugs
- Antidiarrhoeal therapies might be useful as stand-alone therapy or together with oral rehydration solutions

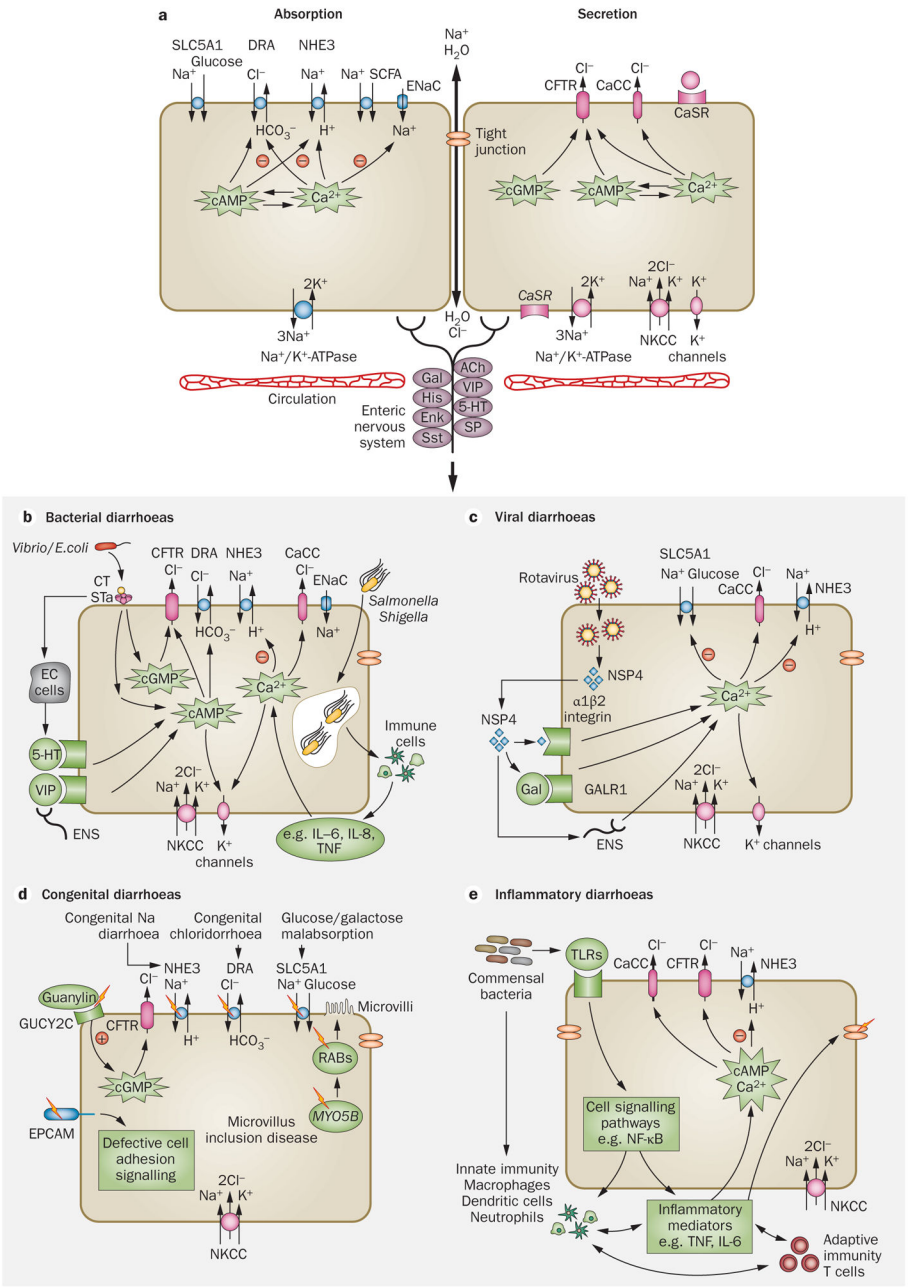

Figure 1.

Mechanisms of intestinal fluid absorption and secretion in secretory diarrhoeas. **a** | Luminal and basolateral membrane transporters and intracellular signalling mechanisms are involved in intestinal fluid absorption and secretion by enterocytes. **b** | Some bacteria secrete enterotoxins that increase intracellular cyclic nucleotides, resulting in Cl^- secretion and inhibition of NHE3 and Na^+ absorption. Invasive bacteria cause a tissue inflammatory response involving recruitment of immune cells and release of cytokines, resulting in intracellular Ca^{2+} signalling. **c** | The rotaviral protein NSP4 causes elevation of cytoplasmic Ca^{2+} concentration by binding to integrin-$\alpha_1\beta_2$, galanin and/or by activation of enteric

nerves. Rotaviral NSP4 also inhibits NHE3 and SLC5A1. **d** | Congenital diarrhoeas result from rare inherited genetic mutations in several intestinal proteins. **e** | Activation of inflammatory signalling pathways such as NF-κB result in Ca^{2+} or cyclic nucleotide signalling and stimulation of Cl^- secretion or inhibition of Na^+ absorption. The release of inflammatory mediators such as TNF and IL-6 by activated T cells and neutrophils can also stimulate Cl^- secretion. Abbreviations: CaCC, calcium-activated chloride channel; CaSR, calcium-sensing receptor; CFTR, cystic fibrosis transmembrane conductance regulator; CT, cholera toxin; DRA, down regulated in adenoma $Cl^-/HCO3^-$ exchanger; EC, enterochromaffin; ENaC, epithelial Na^+ channel; ENS, enteric nervous system; EPCAM, epithelial cell adhesion molecule; Gal, galanin; GALR1, galanin receptor 1; GUCY2C, guanylate cyclase C (heat stable enterotoxin receptor); 5-HT, 5-hydroxytryptamine; MYO5B, unconventional myosin-5b; NHE, sodium/hydrogen exchanger; NKCC, Na/K/Cl symporter; NSP4, nonstructural protein 4; RABs, Ras-related proteins; SCFA, short-chain fatty acid; SLC5A1, sodium/glucose cotransporter; STa, heat-stable toxin; TLR, toll-like receptor; VIP, vasoactive intestinal peptide.

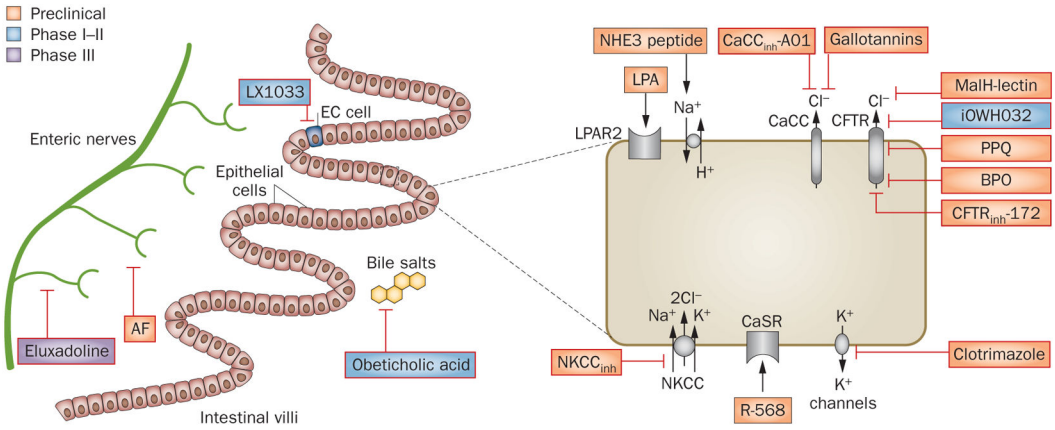

Figure 2.

Potential therapies for secretory diarrhoeas at various stages of development and their molecular targets. Various molecular targets including intestinal ion channels and transporters, regulatory proteins and cell surface receptors are found on epithelial cells lining the intestine, enteric nerves and enterocytes. Novel therapeutics targeting these molecules are in the development pipeline. Abbreviations: AF, antisecretory factor; BPO, benzopyrimido-pyrrolo-oxazinedione; CaCC, calcium-activated chloride channel; CaSR, calcium-sensing receptor; CFTR, cystic fibrosis transmembrane conductance regulator; EC, enterochromaffin; LPA, lysophosphatidic acid; LPAR2, LPA receptor 2; NHE, sodium/hydrogen exchanger; NKCC, Na/K/Cl symporter; PPQ, pyrimido-pyrrolo-quinoxalinedione.

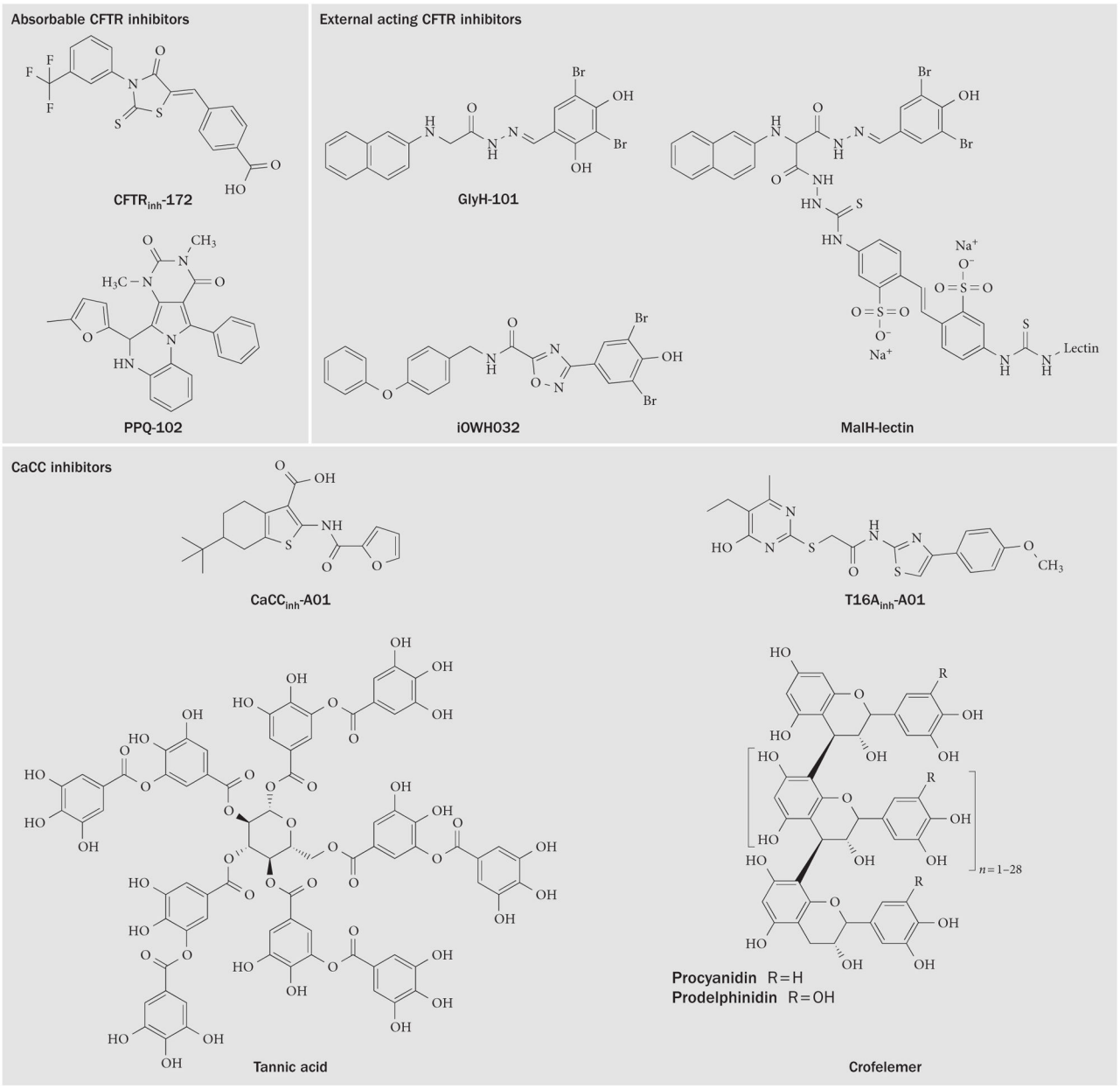

Figure 3.

Chemical structures of CFTR and CaCC inhibitors for secretory diarrhoeas. Abbreviations: CaCC, calcium-activated chloride channel; CFTR, cystic fibrosis transmembrane conductance regulator; PPQ, pyrimido-pyrrolo-quinoxalinedione.

Table 1

Current antidiarrhoeal therapies

Therapy (dose, administration) [Brand name]	Mechanism of action	Indications	Notes and adverse effects	References
Alosetron (1–2 mg daily, oral) [Lotronex®, Prometheus Laboratories, USA]	5-hydroxytryptamine$_3$receptor antagonist; antimotility, antisecretory	Refractory diarrhoea-predominant IBS	Contraindicated for patients with constipation; intestinal ileus, obstruction, perforation, ischaemic colitis	Koch *et al.* (2004)[135]
Cholestyramine resin (4–12 g daily, oral) Questran®, Bristol–Myers Squibb, USA] Colesevelam (4–12 g daily, oral) [Welchol®, Daiichi-Sankyo, USA; Cholestagel®, Genzyme, Netherlands]	Sequesters bile salts; antisecretory, proabsorption	Bile acid malabsorption diarrhoea;ileal resection	Unlabelled indication; impaired absorption of other drugs; malabsorption of fat-soluble vitamins	Koch *et al.* (2004)[135]
Crofelemer (250 mg daily, oral) [Fulyzaq®, Salix Pharmaceuticals, USA]	CFTR and CaCC channel inhibitor; antisecretory	Diarrhoea associated with antiretroviral therapy for HIV/AIDS	Not approved for infectious or other diarrhoeas	Macarthur *et al.* (2013)[89]
Diphenoxylate and atropine (5–20 mg daily, oral) [Lomotil®, AMCo, UK]	μ-opioid receptor agonist; antimotility	Acute nonspecic diarrhoea	Contraindicated in diarrhoea associated with enterotoxigenic bacteria, pseudomembranous colitis and ulcerative colitis; CNS depression, dizziness, paralytic ileus	Karim *et al.* (1972)[83]
Loperamide (4–16 mg daily, oral) [Imodium®, Johnson & Johnson, USA]	μ-opioid receptor agonist; antimotility	Acute nonspeci c diarrhoea, chronic diarrhoea associated with IBD	Contraindicated in bacterial enterocolitis, pseudomembranous colitis and ulcerative colitis; constipation, nausea	DuPont *et al.* (1990)[85]; Koch *et al.* (2004)[135];Ericsson *et al.* (1990)[137]
Racecadotril (100–300 mg daily, oral) [Hidrasec®, Tiorfan®, Bioproject, France]	Enkephalinase inhibitor; antisecretory	Acute nonspeci c diarrhoea	Not approved in USA; headache	Hamza *et al.* (1999)[87]
Bismuth subsalicylate (0.5–4 g daily, oral) [Pepto-Bismol®, Procter & Gamble, USA;Bismatrol]	Inhibition of prostaglandin synthesis; antisecretory; antimicrobial	Acute mild nonspecic diarrhoea	Faecal discoloration, tinnitus, CNS depression, dizziness, Reyes syndrome in children	DuPont *et al.* (1990)[85]

Abbreviations: CaCC, calcium-activated chloride channel; CFTR, cystic fibrosis transmembrane conductance regulator; CNS, central nervous system.

Table 2

Potential antidiarrhoeal therapies

Name/class	Target and mechanism	Potential indication	Development stage
Antisecretory factor	Inhibition of enteric nervous system; increased epithelial permeability	Acute secretory diarrhoea	Phase III
$CaCC_{inh}$-A01	Absorbable CaCC inhibitor	Acute secretory diarrhoea	Preclinical
iOWH032	Extracellular-acting CFTR inhibitor	Acute secretory diarrhoea	Phase I
$CFTR_{inh}$-172	Absorbable CFTR inhibitor	Acute secretory diarrhoea	Preclinical
Clotrimazole	K^+ channel inhibitor	Acute secretory diarrhoea	FDA-approved for other indication
Eluxadoline	μ-opioid receptor agonist	Diarrhoea- predominant IBD	Phase III
Gallotannins	CaCC inhibitors	Acute secretory diarrhoea	Preclinical Nutritional supplement
LX1033	Tryptophan hydroxylase inhibitor; decreased 5-hydroxytryptamine synthesis	Diarrhoea- predominant IBD	Phase II
Lysophosphatidic acid	Lysophosphatidic acid receptor 2 antagonist	Acute secretory diarrhoea	Preclinical Nutritional supplement
MalH-lectin	Inhibitor of the external pore of CFTR	Acute secretory diarrhoea	Preclinical
NHE3 C-terminal peptide	NHE3 regulatory complex agonist	Acute secretory diarrhoea	Preclinical
Obeticholic acid	Semi-synthetic bile acid	Bile acid malabsorption diarrhoea	Phase II
PPQ/BPO	Absorbable CFTR inhibitors	Acute secretory diarrhoea	Preclinical
R-568	Calcium-sensing receptor agonist	Acute secretory diarrhoea	Preclinical

Abbreviations: BPO, benzopyrimido-pyrrolo-oxazinedione; CaCC, calcium-activated chloride channel; CFTR, cystic fibrosis transmembrane conductance regulator; NHE, sodium/hydrogen exchanger; PPQ, pyrimido-pyrrolo-quinoxalinedione.