

Translational Approaches for Pharmacotherapy Development for Acute Diarrhea

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A report is presented of a National Institute of Diabetes and Digestive and Kidney Diseases-sponsored single topic conference designed to determine scientific advances needed to encourage development of pharmacotherapy for diarrheal diseases.

Acute diarrheal diseases are a global public health problem in developing and developed countries. They are a leading cause of mortality and morbidity for children under the age of 5 worldwide and have a major economic impact in developed countries. Although oral rehydration solution (ORS) along with oral zinc therapy has reduced mortality owing to acute diarrheal diseases, there are essentially no other approved, safe, and effective drugs to decrease stool volume and prevent fluid loss. On September 25–27, 2011, the National Institute of Diabetes and Digestive and Kidney Diseases convened a workshop on Translational Approaches for Pharmacotherapy Development for Acute Diarrhea to assess current treatments for acute diarrhea, to identify common host pathways targeted by infectious agents associated with acute diarrhea, to evaluate which pathophysiologic mechanisms are most clinically relevant as potential targets, and to identify promising areas for translational research.

What Are the Current Understanding and Challenges for Treating Acute Diarrhea?

Worldwide, childhood mortality from diarrhea has remained at approximately 1.2 million per year for the past 5 years. Diarrhea remains the second leading cause of death of children <5 years old and accounts for 15% of childhood deaths worldwide.¹ In addition, there are new epidemics of infectious diarrhea—such as the 2011 European Shiga-toxin-producing *Escherichia coli* and ongoing Haitian cholera outbreaks. Most diarrheal disease-related deaths occur in the aged in the United States; the overall mortality of diarrhea in this population has not been established.

The decrease in worldwide diarrhea-related mortality from 4.6 million in 1980 to present levels is associated, at least in part, with the use of ORS. Despite improvements in ORS formulation and the increased effectiveness with addition of zinc to the regimen, its use has dropped from a peak in the 1990s and is stalled at a level of <33% of children with diarrhea under the age of 5.

The decrease in ORS use may be attributed to access, but also to the difficulty in administration for care-givers and the failure of treatment to significantly reduce the duration or volume of diarrhea. Increasing ORS usage could further reduce diarrhea mortality and morbidity, and it is likely that therapeutic measures (some in research and development) to significantly decrease fluid loss and duration of diarrhea would help to drive the use of this life-saving therapy.

Although ORS has reduced childhood mortality, repeated diarrheal episodes in children have been linked to malnutrition, stunting, and impaired physical and mental development.² These outcomes of diarrheal disease have declined slightly, if at all, and the underlying pathogenic mechanisms need to be better defined. Further research must also determine whether treatment for dehydration is sufficient to avoid these consequences or if additional, novel therapies are needed to stop the diarrhea and promote epithelial repair.

Beyond the use of ORS to prevent dehydration, there are few safe and effective pharmaceutical options for treating acute diarrhea. New, safe, and effective drug therapies that complement ORS/zinc for treating acute diarrhea are needed, particularly in developing countries where the disease burden is the greatest. Such therapies may also be of benefit in developed countries, where ORS treatment is uncommon despite significant morbidity, public health, and economic impact of traveler's diarrhea and chronic diarrheal disease. Even with growing use of

Abbreviations used in this paper: AQP, aquaporins; CaCC, calcium-activated Cl⁻ channels; cAMP, adenosine 3',5'-cyclic monophosphate; CaSR, calcium-sensing receptor; CFTR, cystic fibrosis transmembrane conductance regulator; cGMP, guanosine 3',5'-cyclic monophosphate; DRA, down-regulated in adenoma; ENaC, epithelial Na⁺ channel; ENS, enteric nervous system; Epac, exchange protein directly activated by cAMP; GC-C, guanylyl cyclase C; GI, gastrointestinal; Kcna1, large conductance, Ca²⁺-activated K⁺ channel; LPA, lysophosphatidic acid; MLCK, myosin light chain kinase; NHERF1-4, Na⁺/H⁺ exchanger regulatory factor 1-4; NHE2, Na⁺/H⁺ exchanger 2, SLC9A2; NHE3, Na⁺/H⁺ exchanger 3, SLC9A3; NHE8, Na⁺/H⁺ exchanger 8, SLC9A8; ORS, oral rehydration solution; PKA, protein kinase A; SGLT1, Na⁺/glucose cotransporter 1, SLC5A1; TNF, tumor necrosis factor; STAT, signal transducer and activator of transcription.

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vaccines against enteric pathogens such as rotavirus and cholera, an unfulfilled requirement for drug therapy to shorten duration and lessen volume of diarrhea remains.

Do Diverse Mechanisms of Pathogenesis Lead to Final Common Pathways?

Multiple enteric pathogens, including bacteria, viruses, and parasites, affect the intestinal epithelium and cause diarrhea. They exploit the human host's epithelial plasma membrane receptors and intracellular signaling and trafficking pathways in a variety of ways to facilitate infection. However, the diversity of host-microbial interactions mediating initial infection may not offer a "common" mechanism that can be targeted for all infectious diarrheal diseases. Nevertheless, the host response to pathogenesis includes some potentially common features, such as altered electrolyte and water transport (as described below), alterations in tight junction function and regulation, and interactions with the nuclear factor- κ B and mitogen-activated protein kinase pathways (via Toll-like receptors). Therapeutic utility of the nuclear factor- κ B pathway, however, would have to consider its complex regulation of multiple cellular processes and potentially unwanted consequences.

Recent advances have identified human genetic variations associated with increased susceptibility to several types of acute diarrheal diseases. These include polymorphisms of leptin, CD14, lactoferrin, osteoprotegerin promoter, interleukin-10 promoter haplotypes, and apolipoprotein E4. These polymorphisms should be confirmed as reliable markers of susceptibility. Further, new screening approaches are needed to determine additional genetic markers of altered susceptibility and responses to treatment for diarrheal diseases in developed and developing countries.

The intestinal microbiome should also be investigated for its role in altering susceptibility to enteric infections and diarrheal diseases. The role of commensal bacteria and of probiotics in preventing and reversing acute diarrhea is a developing area,³ with *Lactobacillus* GG treatment of rotavirus diarrhea in children being 1 successful example.

Infectious diarrhea can be grouped into 2 classes based on alterations in electrolyte and fluid movement⁴: (1) Secretory diarrhea is most often caused by enterotoxigenic pathogens, and it is characterized by stimulation of Cl^- , HCO_3^- , and K^+ secretion and a reduction of Na^+ and Cl^- absorption. With rotavirus as an exception, these diarrheas in general do not significantly inhibit other nutrient transporters, such as Na^+ /glucose cotransporter 1, SLC5A1 (SGLT1; Table 1). (2) Inflammatory diarrhea is often caused by enteropathogenic and invasive organisms, and is characterized by reduced Na^+ and Cl^- absorption but without stimulated Cl^- or HCO_3^- secre-

tion.⁴ In some inflammatory diarrheas, there is even reduction in intestinal Cl^- secretion. The brush border Na^+ / H^+ exchanger 3, SLC9A3(NHE3) is the major absorptive transporter inhibited in inflammatory diarrheal diseases. The SLC26a gene family of $\text{Cl}^-/\text{HCO}_3^-$ exchangers member SL26A3, down-regulated in adenoma (DRA), which together with NHE3 mediates the majority of small intestinal NaCl absorption, is also inhibited in inflammatory diarrhea.

Although the myriad enteric pathogens signal through a variety of pathways to infect the host epithelium, the repertoire of alterations in electrolyte movement seems to be more limited. Thus, drug development for diarrheal diseases might focus on stimulating Na^+ (and perhaps Cl^-) absorptive processes in enteropathogenic, inflammatory, and enterotoxigenic diarrheas and, in enterotoxigenic diarrheas, inhibition of anion secretory processes. Other potential therapies correct defects in specific transport proteins or common, upstream pathways that regulate transporter function. Factors linked to electrolyte transport, including paracellular permeability, smooth muscle contractility, enteroendocrine function, and the enteric nervous system (ENS) may also be effective therapeutic targets. Focused therapies directed against a common host response may allow a single pharmacologic approach to be applied to almost all diarrheal diseases, both acute and chronic. In addition to electrolyte movement, mucosal destruction may also be evident in some acute diarrheal diseases. Thus, mechanisms for temporarily increasing epithelial proliferation and restitution should also be considered in these instances.

What Host Pathophysiologic Mechanisms Can Be Targeted for Treating Acute Diarrhea?

Transporters and Channels

Several of the main transport proteins and channels implicated in the pathophysiology of diarrheal diseases are listed in Table 1 and Figure 1. For inflammatory diarrheas involving Na^+ absorptive cells, NHE3, DRA, and large conductance, Ca^{2+} -activated K^+ channel may be potential drug targets based on current studies, which include whole animal studies. Na^+ / H^+ exchanger 2, SLC9A2, and epithelial Na^+ channel (distal colon only) might be considered based on cell models or nonintestinal studies. For secretory diarrheas, which involve Cl^- secretory cells, cystic fibrosis transmembrane conductance regulator (CFTR), the calcium sensing receptor, guanylyl cyclase C receptor and basolateral (KCNQ1, KCNN4) or apical (large conductance, Ca^{2+} -activated K^+ channel) K^+ channels are considered important components of Cl^- secretion and could be explored as drug targets based on current studies, which include whole animal studies. Calcium-activated Cl^- channels (CaCC)

Table 1. Potential Host Targets and Biological Role in Acute Diarrhea

Potential host target	Alternative name(s)	Biological role in acute diarrhea	References
Transporters and channels Aquaporins (AQPs)		Family of water channels involved in transepithelial water transport; the relative contribution of AQPs to transcellular water transport compared with other cellular and paracellular routes in intestinal physiology and acute diarrhea is unclear.	J Physiol 1999;517:317–326
CaCC	Calcium activated chloride channels	CaCC may be important for Cl ⁻ secretion owing to rotavirus (which increases intracellular Ca ²⁺), AIDS-related, and drug-induced diarrhea; CaCC Cl ⁻ conductance is well-documented in intestinal epithelium, but the molecular identity is unknown; TMEM16A is present in enterocytes but no major role in intestinal Cl ⁻ secretion has been identified; several small molecules and natural products have been identified that inhibit CaCC activity.	Gastroenterology 2009;136:1939–1951 Nat Rev Drug Discov 2009;8:153–171 FASEB J 2010;24:4178–4186 J Biol Chem 2011;286:2365–2374
CFTR	Cystic fibrosis transmembrane conductance regulator	CFTR is a Cl ⁻ channel present predominantly in crypt cells, with some presence in villus cells of the intestinal epithelium; it is the major intestinal channel for Cl ⁻ secretion in cholera; regulation is complex, involving posttranslational modifications, membrane trafficking and macromolecular interactions with other receptors (eg, LPA ₂ receptor) and regulatory factors (eg, NHERF2); several CFTR inhibitors, including active components of natural products, have been identified and are being developed as drugs.	Science 1994;266:107–109 Am J Physiol Cell Physiol 2003;285:C1–C18 J Clin Invest 2002;110:1651–1658 Gastroenterology 2004;126:511–519 Nat Rev Drug Discov 2009;8:153–171 Am J Physiol Gastrointest Liver Physiol 2011;300:G82–G98
DRA	Down-regulated in adenoma, SLC26A3	DRA is the major Cl ⁻ /HCO ₃ ⁻ exchanger linked to NHE3 as part of neutral NaCl absorption through some part of the human small intestine and colon; often inhibited in inflammatory diarrheal diseases; trafficking is lipid raft- and second messenger-dependent; <i>Enteropathogenic Escherichia coli</i> effector proteins alter DRA trafficking; probiotic <i>Lactobacillus acidophilus</i> and butyrate increase amount of DRA expression and its presence in brush border membranes.	J Clin Invest 2007;117:428–437 Gastroenterology 2008;135:1645–1653 J Nutr 2008;138:1355–1359 Gastroenterology 2009;136:893–901 Am J Physiol Gastrointest Liver Physiol 2010;298:G395–G401
ENaC	Epithelial Na ⁺ channel	Heteromeric tetrameric channel that is the rate-limiting factor for electrogenic Na ⁺ absorption in the colon; present in the distal colon; regulated by trafficking, hormones, and intracellular second messengers; short chain fatty acids (ie, butyrate) stimulate ENaC-dependent electrogenic Na ⁺ absorption; role in normal GI physiology and in acute diarrhea is unclear; a small molecule activator of ENaC has been developed.	Gastroenterology 2007;132:236–248 J Biol Chem 2008;283:11981–11994
Kcnma1	Large conductance, Ca ²⁺ -activated K ⁺ channel	Localized on the apical membrane of surface and crypt cells in the distal colon; mediates cAMP-induced K ⁺ secretion that may contribute to driving force for water secretion.	
NHE2	Na ⁺ /H ⁺ exchanger 2, SLC9A2	NHE2 is found in brush border of human small intestine absorptive cells and surface and crypt cells of the colon; most expressed in the distal colon where it contributes to Na ⁺ absorption; electroneutral NHE2 Na ⁺ absorption is linked to short chain fatty acid/OH ⁻ exchange; can be stimulated by butyrate.	Annu Rev Physiol 2005;67:411–443

Meeting Summary, *continued*

Table 1. Continued

Potential Host Target	Alternative Name(s)	Biological Role in Acute Diarrhea	References
NHE3	Na ⁺ /H ⁺ exchanger 3, SLC9A3	NHE3 is the major protein responsible for Na ⁺ absorption in the small intestine; absorptive function inhibited in inflammatory diarrhea; regulation is by changes in endocytosis/exocytosis plus dynamic changes in association with microvillar cytoskeleton via NHERF2; SGLT1 and NHE3 are linked whereby glucose-stimulation of SGLT1 increases exocytosis of NHE3 to the brush border apical membrane; a small peptide mimicking the C-terminal regulatory region of NHE3 can be taken up into cells to stimulate NHE3 exchange activity.	Annu Rev Physiol 2005;67:411–443 Physiol Rev 2007;87:827–872 Gastroenterology 2011;140:560–571 J Biol Chem 2011;286:34486–34496 J Cell Sci 2010;123:2434–2443 J Exp Biol 2009;212:1628–1646
NHE8	Na ⁺ /H ⁺ exchanger 8, SLC9A8	May be important for Na ⁺ absorption during early development but not in adult intestine; expression and protein levels decrease with age and in the presence of inflammation.	Am J Physiol Gastrointest Liver Physiol 2005;289:G36–G41 Am J Physiol Cell Physiol 2009;296:C489–C497
SGLT1	Na ⁺ /glucose cotransporter 1, SLC5A1	Primary transporter responsible for Na ⁺ -linked glucose transport across the brush border membrane of enterocytes in the small intestine; target of ORS, where glucose in ORS promotes the absorption of Na ⁺ (and fluid) to prevent dehydration; recent study demonstrates that activation of SGLT1 is also coupled to activation of NHE3-mediated Na ⁺ absorption.	Gastroenterology 2011;140:560–571
Receptors and regulatory factors			
AS160	TBC1D4	AS160 is a 14-3-3 binding protein, an Akt/SKG substrate (with increased phosphorylation due to elevated aldosterone levels) and possibly a Rab-GAP that acts with aldosterone to increase the expression and localization of ENaC.	Mol Biol Cell 2010;21:2024–2033
Caveolin-1		Required for TNF-induced occludin endocytosis and barrier loss; genetic knockout of caveolin-1 or pharmacologic inhibition of endocytosis block TNF-induced barrier dysfunction.	J Cell Biol 2010;189:111–126
CaSR	Calcium-sensing receptor	G protein-coupled receptor that monitors Ca ²⁺ levels and other signals in the extracellular space along the GI tract; stimulation of CaSR by small molecule calcimimetic agents inhibit cAMP-, cGMP-, Ca ²⁺ -, and osmotic-induced intestinal Cl ⁻ secretion in both animal models and in isolated colonic crypts.	Proc Natl Acad Sci U S A 2006;103:9390–9397
Epac	Exchange protein directly activated by cAMP	Rap1 guanine-nucleotide exchange factor that is a downstream target of cAMP; involved in PKA-independent, cAMP-stimulated Cl ⁻ secretion in model intestinal cell lines; Epac activation increases intracellular Ca ²⁺ and Cl ⁻ conductance that is distinct from CFTR; demonstrates cross talk between cAMP and Ca ²⁺ regulation of intestinal Cl secretion.	J Gen Physiol 2010;135:43–58
GC-C	Guanylyl cyclase C	Member of the membrane bound guanylyl cyclases, predominantly expressed on the apical surface of intestinal epithelial cells; serves as receptor for endogenous ligands guanylin and uroguanylin and heat-stable enterotoxin (STa) from enterotoxigenic <i>E. coli</i> ; ligands stimulate cGMP production, activate cGMP- and cAMP-dependent protein kinases, increase Cl ⁻ secretion via CFTR, and inhibit NHE3; pyridopyrimidine derivatives identified as GC-C inhibitors that block cGMP synthesis in cell lines and suppress STa-induced fluid secretion in in vivo intestinal loop models.	Cell 1990;63:941–948 J Biol Chem 1993;268:19598–19603 Gastroenterology 2000;118:108–114 Proc Natl Acad Sci 2008;105:8440–8445 Bioorg Med Chem Lett 2009;19:3067–3071 Mol Cell Biochem 2010;334:67–80

Table 1. Continued

Potential Host Target	Alternative Name(s)	Biological Role in Acute Diarrhea	References
Leptin receptor		Leptin receptor signaling in intestinal epithelial cells is part of the innate immune response that protects from, and repairs epithelial damage (eg, in the case of <i>Entamoeba histolytica</i> infection)	Mucosal Immunol 2011;4:294–303 J Clin Invest 2011;121:1191–1198
LPA receptor	Lysophosphatidic acid (LPA) receptor	Family of G protein coupled receptors; LPA ₂ receptor forms a macromolecular complex with CFTR and NHERF2; LPA signals through this complex to inhibit Cl ⁻ secretion in a cholera toxin-induced mouse model of secretory diarrhea; specific LPA ₂ receptor agonists might be developed as anti-secretory agents to treat acute diarrhea; LPA also stimulates NHE3 and fluid absorption via LPA ₅ receptor and NHERF2 and DRA via LPA2 receptor.	J Exp Med 2005;202:975–986 Gastroenterology 2010;138:649–658; Am J Physiol Gastrointest Liver Physiol. 2010;298:G182-G189
MLCK	Myosin light chain kinase	MLCK phosphorylates myosin II regulatory light chain to regulate cytoskeletal contraction; epithelial MLCK is required for tight junction barrier disruption in a mouse model of T cell activation-induced diarrhea; MLCK inhibition can prevent cytokine-induced barrier dysfunction and diarrhea; membrane-permeant peptide inhibitors of MLCK have been developed.	Gastroenterology 2002;123:163–172 J Clin Invest 2005;115:2702–2715 J Clin Invest 2006;116:2682–2694 Gastroenterology 2009;136:551–563 J Biol Chem 2010;285:12037–12046
NHERF1-4	Na ⁺ /H ⁺ exchanger regulatory factor 1–4	Family of PDZ domain-containing adaptor proteins that bind to and regulate function of various transport proteins; NHERF1 binds NHE3 and regulates abundance on the outer surface of microvilli in the brush border membrane; NHERF2 binds NHE3 and regulates LPA- and second messenger-stimulated trafficking; NHERF2 binds CFTR and links to LPA receptor.	Proc Natl Acad Sci U S A 1995;92:10723–10727 Ann NY Acad Sci 2009;1165:249–260 J Clin Invest 2009;119:540–550 Am J Physiol Cell Physiol 2011;300:C771–C782 J Exp Biol 2009;212:1638–1646 Gastroenterology 2010;138:649–658 J Cell Biol 2011;193:565–582
Protein kinase CK2		CK2 phosphorylates occludin (S408) to regulate tight junction protein–protein interactions and barrier function; pharmacologic inhibition of CK2 blocks occludin phosphorylation, remodeling of tight junction complexes, and IL-13-mediated barrier loss.	
STAT3 signaling	Signal transducer and activator of transcription 3	STAT3 transcription factor is activated by gp130, the common receptor for the IL6 cytokine family. STAT3 activation is essential in the process of intestinal epithelial wound healing	Cancer Cell 2009;15:103–113 Cell Cycle 2010;15:652–655

cAMP, adenosine 3',5'-cyclic monophosphate; cGMP, guanosine 3',5'-cyclic monophosphate; GI, gastrointestinal; ORS, oral rehydration solution; PKA, protein kinase A; TNF, tumor necrosis factor.

and exchange protein directly activated by cAMP may also be important for Cl⁻ secretion and for integrating adenosine 3',5'-cyclic monophosphate (cAMP) and Ca²⁺ effects on intestinal transport based on cell models or nonintestinal studies.

In addition to these well-studied transporters and channels, several newly described host pathways regulating transporter function are of interest (Table 1). These include molecular factors regulating the trafficking and apical/basolateral distribution of membrane transporters, soluble factors (both pathogenic and probiotic) regulating transporter expression and localization, macromolecular complexes that regulate transporter function, the calcium sensing receptor that inhibits cyclic nucleo-

side- and calcium-induced intestinal secretion, sodium-linked chloride absorption via DRA and nutrient-dependent Na⁺ absorption (via SGLT1) stimulation of neutral NaCl absorption whereby stimulation of SGLT1 increases the amount of NHE3 on the apical surface.

Although many of the individual transporters in the small intestine and colon have been identified and characterized in normal physiology and, in some cases, disease pathophysiology in animal models, further research is needed to develop a catalogue and map of the transporters and regulatory factors present along the horizontal (small bowel/colon) and vertical (crypt/villus) axes in the human intestine. In addition, it is important to understand how the array and distribu-

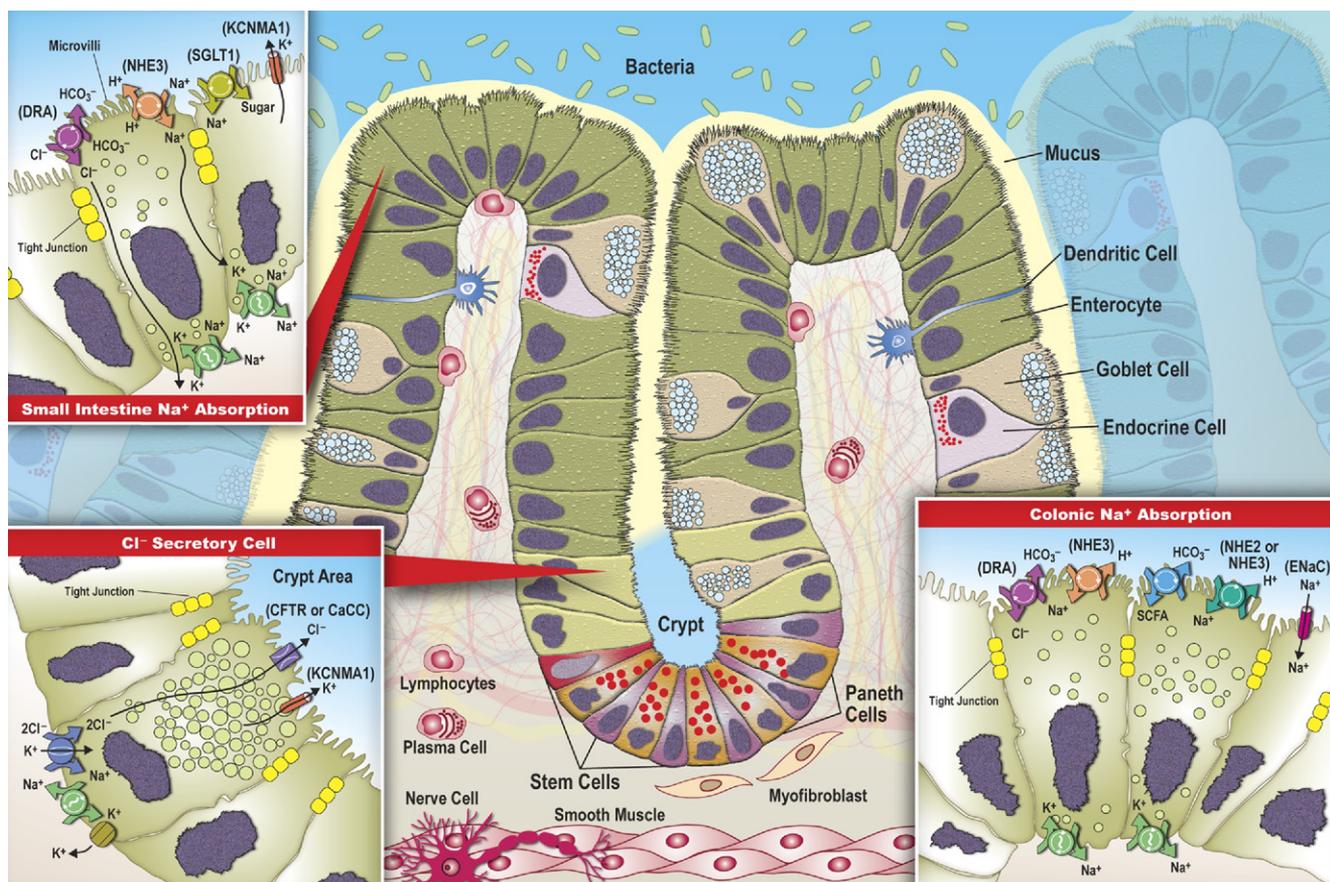


Figure 1. Host physiologic processes contributing to acute diarrhea susceptibility and pathophysiology. Although the colon has a distinct morphology and function, similar processes and factors are present. The epithelial barrier, with its glycocalyx and mucus layer, serves to separate both the commensal and pathogenic microbiota from the underlying lamina propria. This epithelium is maintained and renewed continually from stem cells. In addition, the epithelial cells interact with immune cells and myofibroblasts from the lamina propria. The immune interactions with the epithelium and in the lamina propria are tightly controlled to allow commensal bacteria to colonize the gut, but combat infections that cause diarrhea. Electrolyte transport by the epithelium drives fluid movement. Transport is mediated by cell-surface transport proteins on the apical and basolateral surfaces of the enterocytes. *Insets* illustrate ion channel transport in representative jejunal sodium absorptive cells (*inset*: small intestinal Na^+ absorptive cell), a small intestinal and colonic crypt chloride secretory cell (*inset*: Cl^- secretory cell [in crypt of small intestine and colon]) and a colonic sodium absorptive cell (*inset*: colonic Na^+ absorptive cell). Specific transporters and/or their regulatory factors may be considered as targets for therapy development. Tight junctions (TJ), also play an important role in regulating paracellular transport of electrolytes and water. Specialized enteroendocrine cells may also contribute to control of electrolyte and fluid transport. The enteric nervous system innervates both the epithelium, influencing transport, and the smooth muscle, influencing motility. Each villus is supplied with blood vessels and lymphatics, in addition to the other components of the lamina propria. Research to understand not only the individual components, but the integrated roles of these many cells and tissues in health and in acute diarrhea will likely be needed to achieve development of effective anti-diarrheal therapies. Figure derived from Binder HJ. Causes of chronic diarrhea. *N Engl J Med* 2006;355:236–239.

tion of transporters change during normal development and aging and under pathophysiologic conditions such as acute diarrhea.

Given the profusion of transport proteins and regulatory pathways, a systems biology approach may be useful for characterizing the transporter proteome of the human small intestine and colon under normal physiology and disease pathophysiology. A systems-level view of how individual transporters and regulatory pathways are integrated may provide a better understanding of the pathophysiology of acute diarrheal diseases and identify novel targets for intervention. To facilitate a systems approach, research resources are needed, including anti-

bodies, a catalogue of miRNAs, siRNAs, maps of epigenetic modifications, and access to biospecimens. The systems-wide approach could be coupled with structural studies of key transporters and macromolecular complexes to provide additional insight into transporter function/regulation, and to aid in the development of small molecules that modulate these properties.

In all studies of transporter function, careful consideration should be given to the types of model systems used. Although valuable in many instances, the standard cell models do not always faithfully reproduce the presence and location of native transport proteins. For example, CFTR was recently shown to be present not only in

crypt cells, as implied by cell models, but also in patches of villous cells in the mouse duodenum. In addition, Caco-2 cells are often used as a model of an Na⁺-absorptive small intestinal cell. However, many clones of these colonic adenocarcinoma-derived cells fail to express significant amounts of the major small intestinal Na⁺ absorptive protein, NHE3. Finally, epithelial cell models cannot capture the importance of nonepithelial intestinal components in physiology and disease. For example, approximately 50% of volume losses from cholera- and rotavirus-induced diarrheal diseases are neurally mediated. Understanding the neural regulation of fluid loss can only be gained in whole animal or co-culture experiments. Similarly, alterations in motility and the role of the enteroendocrine system can best be appreciated in organ-level or whole animal models.

Translational research for acute diarrhea could benefit from the development of at least 2 types of models: (1) Animal models of inflammatory diarrhea to supplement the mouse models of secretory diarrhea due to cholera toxin and rotavirus and (2) human intestinal models for the validation of targets and studies of efficacy of promising drug candidates. Testing of efficacy in a human model is important for validating the presence of the particular target and other apical membrane components in human intestinal segment(s). It is possible that “humanized” animal models can be developed in which the abundance and distribution of specific transporters more closely mimics that seen in humans.

The ability to culture intestinal “organoids” containing crypts and surface absorptive cells is a potentially important advance.⁵ These structures, which can be derived from human or mouse single Lgr5⁺ small intestinal and colonic stem cells and isolated crypts, seem to express CFTR in their crypt cells and NHE3 in their surface cells and demonstrate CFTR-dependent secretion. This system might be useful for early screening of and/or validation of epithelial cell mechanisms in human tissue. Further work is needed to confirm other aspects of epithelial function (eg, Na⁺ absorption) in these models and to develop strategies to incorporate the neural, muscular, endocrine, and inflammatory contributions to diarrhea.

Paracellular Transport, Motility, and Enteroendocrine and Enteric Nervous Systems

Tight junctions, which are important regulators of intestinal barrier function and paracellular transport,⁶ should be explored further as potential targets for treating diarrhea. The expression of some tight junction proteins, such as some claudins, is altered in many infectious and inflammatory diarrheal diseases. Butyrate and inhibitors of myosin light chain kinase, caveolin-1, and CK2 have been shown in animal models to alter tight junctions and merit further consideration in drug development. However, it will be important to confirm that

mechanisms in rodent models translate to human disease. Although changes in permeability can be detected in humans, research is needed to determine whether, as has been shown in animals, these changes are sufficient to allow abnormal fluid, electrolyte, or nutrient movement. A better understanding of how transcellular and paracellular water movement are normally regulated and altered in diarrheal diseases is also needed.

Current motility agents are considered to be only relatively effective and to have significant side effects at high doses. Further development of therapies targeting motility will benefit from additional research to define the role of intestinal motor function in diarrhea and to determine whether motility changes are primary in disease pathogenesis, or secondary to distention and increased fluid flow. Motility changes may be caused by pathogenic bacteria via activation of the ENS. The ENS also innervates the epithelium and can influence transcellular and paracellular transport. Agents that modulate neuronal function could be useful for reversing neurally mediated fluid loss in cholera and rotavirus-induced diarrhea, and probably would be helpful in other causes of diarrhea in which a role of the ENS can be expected but is not yet demonstrated. The influence of the endocrine system, through activity of the enteroendocrine cells, may be a further fruitful avenue of exploration.⁷ Genetic studies have implicated neurogenin and enteroendocrine function in chronic diarrhea.

New approaches are needed to quantitatively measure changes in paracellular permeability and motor function in human patients with diarrhea, with consideration for how these measures may be used as surrogate outcomes in studies of treatment of diarrhea. Although measurement of stool volume, which is difficult to do in practice, can be used to assess severity of diarrhea, biomarkers should be developed to assess the magnitude of secretory and inflammatory diarrheas.

The contribution of tight junctions, barrier function, motility, enteroendocrine cells, and the ENS to diarrheal diseases should also be integrated with a systems analysis of transporter function to create a “synthetic gastrointestinal tract” under normal and pathophysiologic conditions of acute diarrheal diseases.

How Can Pathophysiologic Mechanisms Be Translated Into Potential Treatment Options?

Further development of anti-diarrheal drugs should take into account (1) patentability (unless repurposing an approved drug), (2) validated mechanism(s) in man, (3) validated safety, (4) chemical tractability, and (5) ability to complete phase II/III studies in a reasonable period of time (ie, short-term outcomes). In addition, to help increase the likelihood of success of treatments

for acute diarrhea in developing countries, practical consideration should be given to costs, oral administration, stability during distribution and storage, and ease of use by non-medically trained caregivers.

For some of the potential drug targets identified, model compounds have already been identified by high-throughput screening and modeling based on structure/function and regulatory studies of transporters. Among these are CFTR inhibitors, including thiazolidinones, glycine hydrazides, pyrimid-pyrrolo-quinolinediones, and some natural products.⁸ Of these, pyrimid-pyrrolo-quinolinedione compounds are effective in cell models at <5 nmol/L. CaCC inhibitors have also been identified. However, the role of CaCCs in human diarrheal diseases requires further validation. In addition, preliminary animal studies suggest that a peptide mimetic of the NHE3 C-terminus and an inhibitor of the K_{cnm1} K⁺ channel warrant further evaluation. Another promising approach is to identify active components and molecular mechanisms of natural products known to be effective against acute diarrhea. Recent advances include identification of active components in red wines, green teas, and other “local remedies” that inhibit chloride secretion.

Additional research could help develop existing candidates that have varying extents of clinical application. Zinc seems to be effective in reducing the duration and recurrence of diarrhea in children, but the mechanism(s) of its effect and even precise measures of whole body status are unavailable. Clotrimazole is US Food and Drug Administration approved and shown to inhibit fluid secretion in intact rabbit colon and in a mouse model of cholera by blocking Ca²⁺- and cAMP-induced basolateral K⁺ conductances. Further research is needed to better define the mechanism of action and to test it as an antidiarrheal agent. Probiotics, as mentioned, have been partially developed for their application to treat diarrhea.^{rad} ORS clearly improves mortality in childhood acute diarrhea, but the subsequent consequences of treating too late or not enough (effects on height, growth rate, cognition, intestinal physiology, etc) need better definition. Such information would be a powerful driver to produce an improved ORS with formulations such as fermentable starch-ORS, or small molecule antisecretory drugs. Modification of antimotility agents to restrict side effects could make these more useful therapies.

Translational research would also benefit from development of noninvasive biomarkers, chemical probes, and imaging tools that can be applied to humans. Biomarkers should have a direct relationship to the mechanisms resulting in diarrhea. Development of such markers is, thus, dependent on further mechanistic studies as outlined. Chemical biology approaches and the development of chemical probes could help to further dissect the molecular pathways and identify novel targets regulating normal phys-

iology and pathophysiology of acute diarrhea. This depends on development of high-throughput assays for screening modulators of epithelial transport and suitable available systems for preclinical lead optimization and candidate selection.

Summary and Conclusions

Despite the gains in treatment of acute diarrhea with the implementation of ORS and zinc therapy, additional agents that reduce the duration and amount of diarrhea should prove to be valuable treatment options. In addition to reducing morbidity and mortality from the diarrhea, it will be important to understand whether such treatments can also prevent long-term effects, including changes to the intestinal epithelium and altered growth and development in children. To develop such treatments, a firm foundation is needed for understanding the components that are intrinsic to development of diarrhea and that can be therapeutically targeted; a broad array of components should be considered including epithelial membrane molecules (especially transport proteins) and, as well, other cells and tissues that make up the gut. Future research should emphasize understanding of human physiology, gastrointestinal microbiology, and nutrient metabolism and use models that lend understanding to the human system.

Summary of Recommendations to Advance Development of Diarrheal Disease Pharmacotherapy

- Use in vivo methods in animal models and, when possible, human tissue, to determine the best approach to new drug discovery, whether this involves increasing sodium absorption, inhibiting anion secretion, or restoring barrier function, and considering other epithelial and nonepithelial components involved in acute infectious diarrhea.
- Develop a modified oral rehydration solution that will be associated with reduced duration and decreased amount of diarrhea.
- Improve understanding of pathophysiologic mechanisms of human diarrheal diseases, in part by developing a systems approach toward mapping and cataloging transport proteins, integrated with research on motor function, endocrine function, and the role of the enteric nervous system, in the small and large intestine in health and disease.
- Understand the changes in these pathogenetic factors during early development/childhood and aging, the ages at most risk for severe consequences of diarrhea.
- Increase knowledge of genetic risk factors (host and microbiome) for development of acute infectious diarrhea.

- Develop in vivo animal models of inflammatory diarrhea and in vitro human models for testing drug efficacy.
- Develop techniques to quantify clinically relevant markers of diarrhea (eg, fluid/paracellular movement, motor function) in humans.

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Appendix

Meeting Participants

The following speakers, moderators, and discussants contributed to the research presentations and dis-

cussions described in this meeting summary: David Alpers, Nadia Ameen, James Anderson, Kim Barrett, Henry Binder, David Brown, Michael Camilleri, Hugo de Jonge, Mark Donowitz, Pradeep Dudeja, Mary K. Estes, Olivier Fontaine, Raymond Frizzell, John Geibel, Fayez Ghishan, Richard Guerrant, Gail Hecht, Stephen James, Olga Kovbasnjuk, Wayne Lencer, Ronald Margolis, Sean Moore, Anjaparavanda Naren, James Nataro, Tue Nguyen, William A. Petri, Vazhaikkurichi Rajendran, David A. Sack, Joerg-Dieter Schulzke, Terez Shea-Donohue, Jerrold Turner, Alan Verkman, and James Versalovic.

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The views expressed by the authors are their own and do not necessarily represent the views of the National Institutes of Health or the United States Government.

Conflicts of interest

These authors disclose the following: Mark Donowitz is partial owner of Tranzmembrane, Inc, which holds the patent for the human NHE3 gene. The remaining authors disclose no conflicts.