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Peptide inhibitors of chloride channels for treating secretory diarrhea

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1. ABSTRACT

Morbidity and mortality associated with diarrheal diseases remain significant burdens on global health. In the developing world, the major sources of secretory diarrhea are infectious, including those caused by bacteria such as enterotoxic *Escherichia coli*, and viruses such as rotavirus. In many cases of secretory diarrhea, activation of pathways for cyclic nucleotides and/or Ca^{2+} signaling in the apical membrane of enterocytes increases the conductance of Cl^- channels at the enterocyte lumen-facing membrane. Those channels include the cystic fibrosis transmembrane conductance regulator (CFTR) and Ca^{2+}-activated Cl^- channel (CaCC). Inhibition of enterocyte Cl^- channels is an effective strategy for anti-secretory drug therapy. Small molecules and natural peptides with Cl^- channel inhibitory activity have shown efficacy in diarrhea models. Screening of natural peptides via the patch-clamp technique provides evidence that such channel inhibition by an extract of black tea may be responsible for its anti-diarrhea benefits.

2. INTRODUCTION

Diarrheal diseases have always been a major global health challenge. Secretory diarrhea is a leading cause of mortality and morbidity in children under age 5 and adults above age 70 (1-3). For these susceptible populations, the risk is often further enhanced by associated enteric infections (4, 5). In developing countries, secretory diarrhea is primarily caused by bacteria such as enterotoxic *Escherichia coli* (ETEC), whereas in developed countries the leading causes are viruses such as rotavirus (6-8). By secreting enterotoxins, ETEC activates apical membrane cystic fibrosis transmembrane conductance regulator (CFTR), a Cyclic Adenosine monophosphates(cAMP)-activated Cl^- channel, resulting in chloride secretion (9-11). In rotaviral diarrhea, non-structural rotaviral protein 4 (NSP4) induces the Ca^{2+}-activated Cl^- channel (CaCC) at the lumen-facing membrane of intestinal epithelial cells (12-14). Excessive chloride secretion drives fluid and electrolytes into the intestinal lumen, thereby causing secretory diarrhea (15-19).

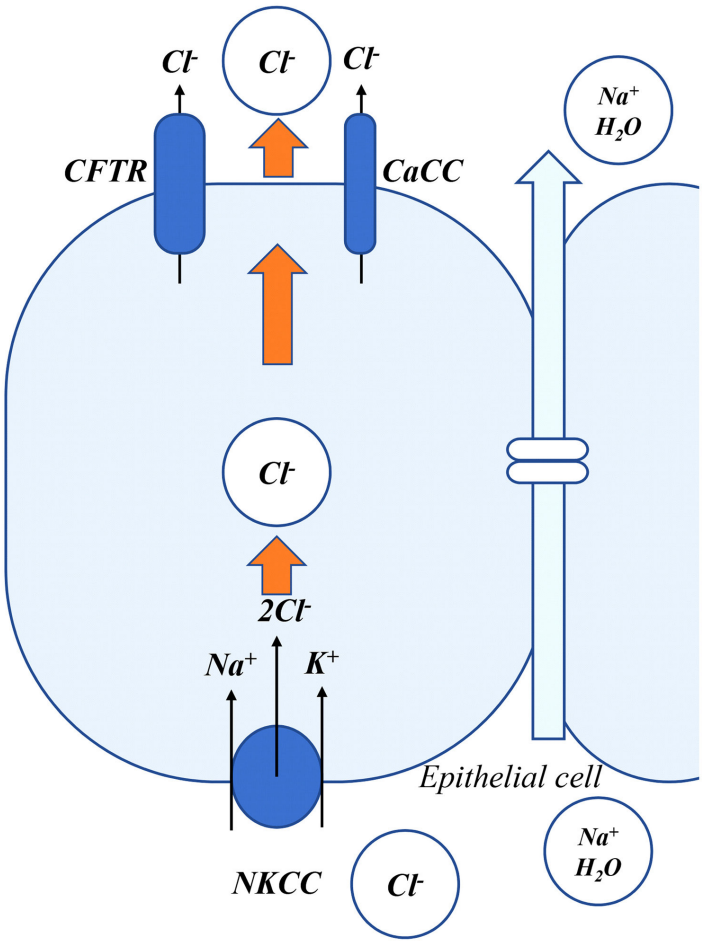

Figure 1. Intestinal ion and water transport. Fluid secretion involves active cross-cell Cl^- transport from basolateral side via NKCC transporter and apical Cl^- channels, with corresponding passive Na^+ and water flux.

Current anti-diarrheal therapies center around prompt improvement of dehydration, such as through intravenous fluid replacement and oral rehydration solution (20, 21). Although symptomatic treatment effectively ameliorates dehydration, it does not change the secretion of chloride or electrolyte losses, and most contemporary methods for managing and treating infectious secretory diarrhea are decades old (22-25). In the past five years, CFTR and CaCC have emerged as novel drug candidates based on greater understanding of the mechanisms of Cl^- secretion and intestinal electrolyte movement (26). Several Cl^- channel inhibitors have been identified from screening of small-molecule collections or natural peptides (14, 27). The latter are often recognized as an excellent starting point for drug development and, when compared with traditional small molecules, peptides are relatively safe, highly selective and efficacious, and easy to synthetize (27, 28).

Here, we describe the major pathophysiology mechanisms of secretory diarrhea and discuss the opportunities that have been revealed from basic research to clinical applications for Cl^- channel inhibitors. We also report that a black tea extract containing peptides strongly inhibits intestinal CaCC, and we present several ways in which peptide inhibitors offer tremendous growth potential as new therapeutic strategies.

3. CFTR AND CACC ARE ANTI-SECRETORY TARGETS FOR TREATING DIARRHEA

Secretory diarrhea results from excessive secretion of fluid and electrolytes into the intestinal lumen (24, 29-31). The movement of sodium and water between the lumen and blood vessel is driven by active trans-epithelial Cl^- secretion. This involves the activation of chloride channels located on the apical (lumen-facing) epithelial membranes (32). The electrochemical driving force for chloride secretion across the luminal membrane through CFTR and CaCC is established by basolateral (circulation-facing) membrane transporters (Figure 1). Thus, CFTR and

CaCC are potential membrane channel targets to reduce intestinal fluid and the secretion of electrolytes.

3.1. Targeting CFTR

As a cAMP-activated chloride channel, CFTR occurs on the apical surface of many mammalian epithelia and fluid-transporting tissues (33-35). In 1989, The CFTR gene was unexpectedly identified when researchers were searching for the cystic fibrosis gene through positional cloning (13, 36, 37). Mutations in that gene cause cystic fibrosis, a hereditary, lethal disease. This transmembrane regulator contains the ATP binding cassette (ABC) transporter structural motif. Members of the ABC superfamily have two six-helix membrane-spanning alpha helices, each followed by a homologous nucleotide binding domain (NBD) (38-40). However, unlike other ABC proteins, CFTR has a regulatory (R) domain linking the first NBD and the second membrane-spanning domain. Activation of CFTR involves ATP binding and hydrolysis at its NBDs, as well as cAMP-dependent phosphorylation of multiple R-domain sites, the details of which remain unknown.

Under normal physiological conditions, CFTR is expressed at the apical epithelial membrane, and plays a major role for chloride and, hence, fluid secretion into the intestine lumen (9). After ETEC infection, enterotoxins up-regulate intracellular cyclic nucleotide levels of intestinal epithelial cells, resulting in activation of the CFTR channel. Pharmacological inhibition of CFTR Cl^- conductance has potential therapeutic value in secretory diarrhea initiated by ETEC.

3.2. Targeting CaCC

The CaCC is widely distributed in various tissues of vertebrates and invertebrates, such as neurons, myocardium, skeletal muscle, smooth muscle, epithelial cells, olfactory cells, photoreceptor cells, uterine muscle cells, breast cells, and lymphocytes, where it has a wide range of physiological functions, including neuronal and cardiac excitation, smooth muscle contraction, trans-epithelial fluid secretion, olfactory and sensory signal transduction, and oocyte fertilization (34, 41, 42). Therefore, investigating the molecular basis of CaCC is of great interest to many scientists. The characteristics of early candidates are not consistent with endogenous CaCC properties (43-45). In 2008, three laboratories independently demonstrated that transmembrane protein 16A (TMEM16A) is the molecular basis for CaCC. Strong evidence for this included the similarity of electrophysiological properties between TMEM16A and native CaCC, the presence of CaCC currents in various transfected cell systems, a decrease in CaCC currents after RNAi knockdown, and its tissue distribution (46-48). Therefore, identification of TMEM16A may be helpful to our discovery of targeted inhibitors of CaCC.

Intracellular calcium is a major factor influencing CaCC permeability. Chemical, electrical, or sensory signals can elevate the level of calcium in intestinal epithelial cells and then induce the opening of CaCC, causing chloride outflow to produce an inward current.

4. MECHANISMS OF INFECTIOUS SECRETORY DIARRHEA

Secretory diarrhea that arises from ETEC and rotavirus infections is widespread and prevalent in gastrointestinal diseases. In developing countries, the incidence of infectious diarrhea leads all infectious diseases. Diarrhea is also the main factor in malnutrition, stunted growth, and developmental disorders in children. Therefore, it is important that researchers understand the pathogenesis of infectious secretory diarrhea.

4.1. ETEC diarrhea

ETEC secretes specific adhesin and enterotoxins (heat-liable and heat-stable) that boost the levels of intracellular cyclic nucleotides, resulting in activation of apical CFTR Cl^- channels and, hence, Cl^- secretion (10, 49). After ETEC enters the intestine, secretory adhesin binds to intestinal epithelial cell-specific receptors and then colonizes the intestinal lumen. Enterotoxins are direct pathogenic factors for secretory diarrhea, activating the membrane adenylate cyclase by binding to the intestinal epithelial cell receptor (50). After cAMP activates cAMP-dependent protein kinase, it phosphorylates the CFTR Cl^- channel. That phosphorylated channel remains open and secretes excess Cl^- to cause secretory diarrhea (51).

4.2. Rotaviral diarrhea

Enteric rotavirus infection leads to fluid secretion as well as morphological changes in the intestinal epithelium, resulting in age-related secretory diarrhea. The non-structural protein NSP4 is produced on the basal side of rotavirus-infected intestinal cells and is encoded by the rotavirus gene. This protein is thought to act as an enterotoxin that elevates the concentration of cytoplasmic Ca^{2+} by binding to a membrane receptor (integrin alpha1 beta2) or the neuropeptide galanin, and/or by activating enteric nerves (52). When the concentration of cytoplasmic Ca^{2+} increases, the activated CaCC in intestinal epithelial cells releases chloride ions to produce secretory diarrhea (53).

5. DISCOVERY AND DEVELOPMENT OF CFTR AND CACC INHIBITORS

Secretory diarrhea is caused by the activation of cyclic nucleotide and/or Ca^{2+} signaling pathways in intestinal epithelial cells, which increase the

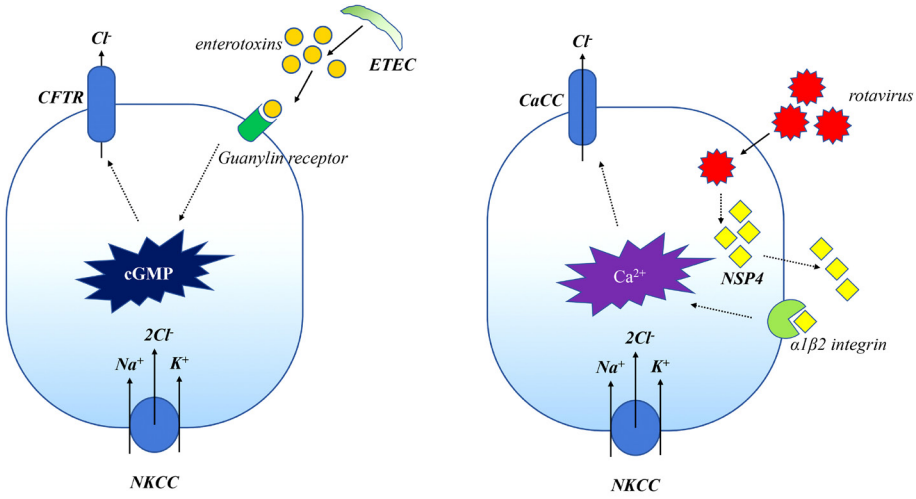

Figure 2. Signal pathways controlling intestinal fluid secretion. *Left*. CFTR signaling pathway activated by ETEC enterotoxins. Enterotoxins bind to membrane receptors that cause increase in cyclic cGMP, resulting in CFTR activation. *Right*. Activation of CaCC signaling pathway by rotavirus. Rotavirus releases NSP4, which causes elevation of cytoplasmic Ca^{2+} via binding to membrane receptor.

conductance of Cl^- channels at the apical membrane. Being able to inhibit those Cl^- channels presents an attractive strategy for treatment via anti-secretory drugs. Screening of small molecule collections and natural peptides has identified several classes of Cl^- channel inhibitors that show efficacy in diarrhea models but must still be tested clinically.

5.1. Small-molecule inhibitors

Three chemical types of small molecules that show CFTR inhibition have been identified from the screening of synthetic small-molecule libraries (14). The prototype of CFTR inhibitors includes the thiazolidinone CFTRinh-172, which prevents CFTR Cl^- conductance by binding at or near arginine-347 on the cytoplasmic side of CFTR and then stabilizing the channel in its closed state (54). In mouse diarrhea models of heat-stable enterotoxin-induced intestinal fluid secretion, CFTRinh-172 has an anti-secretory effect. A second type of absorbable CFTR inhibitor that targets the cytoplasmic surface is the PPQ/BPO compound. Such compounds are effective in polycystic kidney disease models, but have not been tested in models of diarrhea (55). A third chemical type of small-molecule CFTR inhibitors (glycine hydrazides) binds to the extracellular CFTR surface in the channel pore itself, as demonstrated by patch clamp analysis (56, 57). However, by locating an external site on the intestine, the underlying disorder is the accessing of CFTR in the deep intestinal crypts that are resistant to a strong convective rinse in secretory diarrhea.

The initial phenotype-based screen was performed with human colonic cell line HT-29. Those efforts identified several small-molecule CaCC inhibitors, e.g., CaCCinh-A01, which fully inhibits CaCC-dependent halide flux in different intestinal cell lines. Since then, this inhibitor has been shown to prevent secretory diarrhea in a neonatal mouse model of rotavirus (58).

5.2. Peptide inhibitors

Protein-protein interactions are the basis of cellular functions. Most of these interactions involve short peptide motifs, and interest is increasing in the use of peptide-based, targeted therapeutics (59). The advantages of peptides are their specificity, relative safety, ease of production, and their ability to be modified through chemical synthesis and molecular biology techniques (60, 61). Screening the natural peptide inhibitors of Cl^- channels represents an attractive source of antidiarrheal therapeutics because they are generally inexpensive and have the potential for rapid translation to the clinic. Various leaf teas, especially green and black, manifest a wide range of CaCC inhibitory activities. Our laboratory has utilized a peptide inhibitor screen with a patch clamp recording technique to demonstrate strong CaCC inhibition by black tea, which contains numerous natural peptides. Because CaCC activation is involved in rotaviral diarrhea, we found that the black tea extract prevented rotaviral diarrhea in neonatal mice but had no effect on the rotaviral infection. Control experiments showed that a tea extract with minimal *in vitro* CaCC activity did not prevent rotaviral diarrhea. However, the use of black tea extracts for CaCC-dependent diarrhea requires more in-depth study. Natural products, with a defined mechanism of action, represent a possibly inexpensive and readily available therapy for secretory diarrhea.

6. SUMMARY AND PERSPECTIVE

Anti-secretory drug therapy has considerable potential to reduce the morbidity and mortality that are associated with infectious diarrhea. The identification and validation of small molecules and effective natural products, as well as repurposed drugs, that can block infectious diarrhea through Cl^- channels is of great importance. Precise regulation of specific protein–peptide interactions is a promising new therapeutic strategy. The use of peptides as a means of modulation represents an exciting path toward this goal. Several different technologies have been introduced to aid in the discovery of peptide binders that will be suitable for use as drug frameworks. Although the development of anti-secretory drugs still presents multiple challenges, the future for peptide drugs is becoming brighter as more candidates are now approved or in clinical trials. The next decade may bring the prospect of new drugs against secretory diarrhea.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

1. W. H. Organization: GHO | Global Health Observatory Data Repository (African Region) | Life tables by country - Côte d'Ivoire

2. L. Liu, S. Oza, D. Hogan, Y. Chu, J. Perin, J. Zhu, J. E. Lawn, S. Cousens, C. Mathers and R. E. Black: Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*, 388(10063), 3027-3035 (2016) DOI:10.1016/S0140-6736(16)31593-8

3. G. B. D. D. D. Collaborators: Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*, 17(9), 909-948 (2017) DOI: 10.1016/S1473-3099(17)30276-1

4. C. L. Walker, I. Rudan, L. Liu, H. Nair, E. Theodoratou, Z. A. Bhutta, K. L. O'Brien, H. Campbell and R. E. Black: Global burden of childhood pneumonia and diarrhoea. *Lancet*, 381(9875), 1405-16 (2013) DOI: 10.1016/S0140-6736(13)60222-6

5. P. M. Sherman and C. Macarthur: Current controversies associated with Helicobacter pylori infection in the pediatric population. *Front Biosci*, 6, E187-92 (2001)

6. K. L. Kotloff, J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. Faruque, A. K. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acacio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne and M. M. Levine: Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet*, 382(9888), 209-22 (2013) DOI: 10.1016/S0140-6736(13)60844-2

7. G. Lee, M. Paredes Olortegui, P. Penataro Yori, R. E. Black, L. Caulfield, C. Banda Chavez, E. Hall, W. K. Pan, R. Meza and M. Kosek: Effects of Shigella-, Campylobacter- and ETEC-associated diarrhea on childhood growth. *Pediatr Infect Dis J*, 33(10), 1004-9 (2014) DOI: 10.1097/INF.0000000000000351

8. B. R. Lindsay, S. Chakraborty, C. Harro, S. Li, J. P. Nataro, H. Sommerfelt, D. A. Sack and O. Colin Stine: Quantitative PCR and culture evaluation for enterotoxigenic Escherichia coli (ETEC) associated diarrhea in volunteers. *FEMS Microbiol Lett*, 352(1), 25-31 (2014) DOI: 10.1111/1574-6968.12362

9. J. R. Thiagarajah and A. S. Verkman: CFTR inhibitors for treating diarrheal disease. *Clin Pharmacol Ther*, 92(3), 287-90 (2012) DOI: 10.1038/clpt.2012.114

10. M. J. Bijvelds, M. Loos, I. Bronsveld, A. Hellemans, J. P. Bongartz, L. Ver Donck, E. Cox, H. R. de Jonge, J. A. Schuurkes and J. H. De Maeyer: Inhibition of Heat-Stable Toxin-Induced Intestinal Salt and Water Secretion by a Novel Class of Guanylyl Cyclase C Inhibitors. *J Infect Dis*, 212(11), 1806-15 (2015) DOI: 10.1093/infdis/jiv300

11. A. M. Pattison, E. S. Blomain, D. J. Merlino, F. Wang, M. A. Crissey, C. L. Kraft, J. A. Rappaport, A. E. Snook, J. P. Lynch and

S. A. Waldman: Intestinal Enteroids Model Guanylate Cyclase C-Dependent Secretion Induced by Heat-Stable Enterotoxins. *Infect Immun*, 84(10), 3083-91 (2016)
DOI: 10.1128/IAI.00639-16

12. J. M. Ball, P. Tian, C. Q. Zeng, A. P. Morris and M. K. Estes: Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. *Science*, 272(5258), 101-4 (1996)

13. E. A. Ko, B. J. Jin, W. Namkung, T. Ma, J. R. Thiagarajah and A. S. Verkman: Chloride channel inhibition by a red wine extract and a synthetic small molecule prevents rotaviral secretory diarrhoea in neonatal mice. *Gut*, 63(7), 1120-9 (2014)
DOI: 10.1136/gutjnl-2013-305663

14. J. R. Thiagarajah, E. A. Ko, L. Tradtrantip, M. Donowitz and A. S. Verkman: Discovery and development of antisecretory drugs for treating diarrheal diseases. *Clin Gastroenterol Hepatol*, 12(2), 204-9 (2014)
DOI: 10.1016/j.cgh.2013.12.001

15. J. R. Thiagarajah, T. Broadbent, E. Hsieh and A. S. Verkman: Prevention of toxin-induced intestinal ion and fluid secretion by a small-molecule CFTR inhibitor. *Gastroenterology*, 126(2), 511-9 (2004)

16. J. Wang, K. A. Haanes and I. Novak: Purinergic regulation of CFTR and Ca(2+)-activated Cl(-) channels and K(+) channels in human pancreatic duct epithelium. *Am J Physiol Cell Physiol*, 304(7), C673-84 (2013)
DOI: 10.1152/ajpcell.00196.2012

17. S. M. Hendrick, M. S. Mroz, C. M. Greene, S. J. Keely and B. J. Harvey: Bile acids stimulate chloride secretion through CFTR and calcium-activated Cl- channels in Calu-3 airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol*, 307(5), L407-18 (2014)
DOI: 10.1152/ajplung.00352.2013

18. J. R. Martins, P. Kongsuphol, E. Sammels, S. Dahimene, F. Aldehni, L. A. Clarke, R. Schreiber, H. de Smedt, M. D. Amaral and K. Kunzelmann: F508del-CFTR increases intracellular Ca(2+) signaling that causes enhanced calcium-dependent Cl(-) conductance in cystic fibrosis. *Biochim Biophys Acta*, 1812(11), 1385-92 (2011)
DOI: 10.1016/j.bbadis.2011.08.008

19. J. Ousingsawat, P. Kongsuphol, R. Schreiber and K. Kunzelmann: CFTR and TMEM16A are separate but functionally related Cl-channels. *Cell Physiol Biochem*, 28(4), 715-24 (2011)
DOI:10.1159/000335765

20. E. Bruzzese, A. Lo Vecchio and A. Guarino: Hospital management of children with acute gastroenteritis. *Curr Opin Gastroenterol*, 29(1), 23-30 (2013)
DOI:10.1097/MOG.0b013e32835a352f

21. T. A. Sentongo: The use of oral rehydration solutions in children and adults. *Current Gastroenterology Reports*, 6(4), 307-13 (2004)

22. G. Juckett and R. Trivedi: Evaluation of chronic diarrhea. *Am Fam Physician*, 84(10), 1119-26 (2011)

23. M. G. Gareau and K. E. Barrett: Fluid and electrolyte secretion in the inflamed gut: novel targets for treatment of inflammation-induced diarrhea. *Curr Opin Pharmacol*, 13(6), 895-9 (2013)
DOI:10.1016/j.coph.2013.08.014

24. J. Cottreau, A. Tucker, R. Crutchley and K. W. Garey: Crofelemer for the treatment of secretory diarrhea. *Expert Rev Gastroenterol Hepatol*, 6(1), 17-23 (2012)
DOI:10.1586/egh.11.87

25. R. Corinaldesi, V. Stanghellini, G. Barbara, P. Tomassetti and R. De Giorgio: Clinical approach to diarrhea. *Intern Emerg Med*, 7 Suppl 3, S255-62 (2012)
DOI:10.1007/s11739-012-0827-4

26. M. Donowitz, D. H. Alpers, H. J. Binder, T. Brewer, J. Carrington and M. J. Grey: Translational approaches for pharmacotherapy development for acute diarrhea. *Gastroenterology*, 142(3), e1-9 (2012)
DOI:10.1053/j.gastro.2012.01.014

27. C. Corbi-Verge, M. Garton, S. Nim and P. M. Kim: Strategies to Develop Inhibitors of Motif-Mediated Protein-Protein Interactions as Drug Leads. *Annu Rev Pharmacol Toxicol*, 57, 39-60 (2017)
DOI: 10.1146/annurev-pharmtox-010716-104805

28. K. Fosgerau and T. Hoffmann: Peptide therapeutics: current status and future directions. *Drug Discov Today*, 20(1), 122-8 (2015)
DOI: 10.1016/j.drudis.2014.10.003

29. J. R. Thiagarajah, M. Donowitz and A. S. Verkman: Secretory diarrhoea: mechanisms and emerging therapies. *Nat Rev Gastroenterol Hepatol*, 12(8), 446-57 (2015) DOI: 10.1038/nrgastro.2015.111

30. M. Camilleri: Intestinal secretory mechanisms in irritable bowel syndrome-diarrhea. *Clin Gastroenterol Hepatol*, 13(6), 1051-7; quiz e61-2 (2015) DOI: 10.1016/j.cgh.2014.07.020

31. S. Anand, S. Mandal, P. Patil and S. K. Tomar: Pathogen-induced secretory diarrhea and its prevention. *Eur J Clin Microbiol Infect Dis*, 35(11), 1721-1739 (2016) DOI: 10.1007/s10096-016-2726-5

32. V. Singh, J. Yang, T. E. Chen, N. C. Zachos, O. Kovbasnjuk, A. S. Verkman and M. Donowitz: Translating molecular physiology of intestinal transport into pharmacologic treatment of diarrhea: stimulation of Na+ absorption. *Clin Gastroenterol Hepatol*, 12(1), 27-31 (2014) DOI: 10.1016/j.cgh.2013.10.020

33. T. J. Jentsch, V. Stein, F. Weinreich and A. A. Zdebik: Molecular structure and physiological function of chloride channels. *Physiological Reviews*, 82(2), 503-68 (2002)

34. A. S. Verkman and L. J. Galietta: Chloride channels as drug targets. *Nat Rev Drug Discov*, 8(2), 153-71 (2009) DOI: 10.1038/nrd2780

35. S. Resta-Lenert and K. E. Barrett: Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX-2. *Gastroenterology*, 122(4), 1070-87 (2002)

36. J. M. Rommens, M. C. Iannuzzi, B. Kerem, M. L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J. L. Cole, D. Kennedy and N. Hidaka: Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science*, 245(4922), 1059 (1989)

37. R. R. Marchelletta, M. G. Gareau, D. F. McCole, S. Okamoto, E. Roel, R. Klinkenberg, D. G. Guiney, J. Fierer and K. E. Barrett: Altered expression and localization of ion transporters contribute to diarrhea in mice with Salmonella-induced enteritis. *Gastroenterology*, 145(6), 1358-1368 e1-4 (2013) DOI: 10.1053/j.gastro.2013.08.054

38. J. K. Zolnerciks, E. J. Andress, M. Nicolaou and K. J. Linton: Structure of ABC transporters. *Essays in Biochemistry*, 50(1), 43-61 (2011) DOI: 10.1042/bse0500043

39. C. J. Mathews, R. J. MacLeod, S. X. Zheng, J. W. Hanrahan, H. P. Bennett and J. R. Hamilton: Characterization of the inhibitory effect of boiled rice on intestinal chloride secretion in guinea pig crypt cells. *Gastroenterology*, 116(6), 1342-7 (1999)

40. J. Linley, A. Loganathan, S. Kopanati, G. I. Sandle and M. Hunter: Evidence that two distinct crypt cell types secrete chloride and potassium in human colon. *Gut*, 63(3), 472-9 (2014) DOI: 10.1136/gutjnl-2013-304695

41. C. Hartzell, I. Putzier and J. Arreola: Calcium-activated chloride channels. *Annual Review of Physiology*, 67(1), 719-758 (2005) DOI: 10.1146/annurev.physiol.67.032003.154341

42. J. Aoun, M. Hayashi, I. A. Sheikh, P. Sarkar, T. Saha, P. Ghosh, R. Bhowmick, D. Ghosh, T. Chatterjee, P. Chakrabarti, M. K. Chakrabarti and K. M. Hoque: Anoctamin 6 Contributes to Cl- Secretion in Accessory Cholera Enterotoxin (Ace)-stimulated Diarrhea: AN ESSENTIAL ROLE FOR PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP2) SIGNALING IN CHOLERA. *J Biol Chem*, 291(52), 26816-26836 (2016) DOI: 10.1074/jbc.M116.719823

43. L. J. Galietta: The TMEM16 protein family: a new class of chloride channels? *Biophysical Journal*, 97(12), 3047-3053 (2009) DOI: 10.1016/j.bpj.2009.09.024

44. W. Namkung, P. W. Phuan and A. S. Verkman: TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. *J Biol Chem*, 286(3), 2365-74 (2011) DOI: 10.1074/jbc.M110.175109

45. W. Namkung, J. R. Thiagarajah, P. W. Phuan and A. S. Verkman: Inhibition of Ca2+-activated Cl- channels by gallotannins as a possible molecular basis for health benefits of red wine and green tea. *FASEB J*, 24(11), 4178-86 (2010) DOI: 10.1096/fj.10-160648

46. Y. D. Yang, H. Cho, J. Y. Koo, M. H. Tak, Y. Cho, W. S. Shim, S. P. Park, J. Lee, B. Lee and B. M. Kim: TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature*, 455(7217), 1210 (2008)
DOI: 10.1038/nature07313

47. B. C. Schroeder, T. Cheng, Y. N. Jan and L. Y. Jan: Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell*, 134(6), 1019 (2008)
DOI: 10.1016/j.cell.2008.09.003

48. A. Caputo, E. Caci, L. Ferrera, N. Pedemonte, C. Barsanti, E. Sondo, U. Pfeffer, R. Ravazzolo, O. Zegarra-Moran and L. J. Galietta: TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Channels*, 322(5901), 590 (2008)
DOI: 10.1126/science.1163518

49. A. Collaco, J. Marathe, H. Kohnke, D. Kravstov and N. Ameen: Syntaxin 3 is necessary for cAMP- and cGMP-regulated exocytosis of CFTR: implications for enterotoxigenic diarrhea. *Am J Physiol Cell Physiol*, 299(6), C1450-60 (2010)
DOI: 10.1152/ajpcell.00029.2010

50. M. C. Rao, S. Guandalini, P. L. Smith and M. Field: Mode of action of heat-stable Escherichia coli enterotoxin. Tissue and subcellular specificities and role of cyclic GMP. *Biochim Biophys Acta*, 632(1), 35-46 (1980)

51. A. C. Chao, F. J. de Sauvage, Y. J. Dong, J. A. Wagner, D. V. Goeddel and P. Gardner: Activation of intestinal CFTR Cl- channel by heat-stable enterotoxin and guanylin via cAMP-dependent protein kinase. *EMBO J*, 13(5), 1065-72 (1994)

52. M. Riepenhoff-Talty, P. C. Lee, P. J. Carmody, H. J. Barrett and P. L. Ogra: Age-dependent rotavirus-enterocyte interactions. *Proc Soc Exp Biol Med*, 170(2), 146-54 (1982)

53. T. Pham, J. L. Perry, T. L. Dosey, A. H. Delcour and J. M. Hyser: The Rotavirus NSP4 Viroporin Domain is a Calcium-conducting Ion Channel. *Sci Rep*, 7, 43487 (2017)
DOI: 10.1038/srep43487

54. E. Caci, A. Caputo, A. Hinzpeter, N. Arous, P. Fanen, N. Sonawane, A. S. Verkman, R. Ravazzolo, O. Zegarramoran and L. J. Galietta: Evidence for direct CFTR inhibition by CFTR(inh)-172 based on Arg347 mutagenesis. *Biochemical Journal*, 413(1), 135-42 (2008)
DOI: 10.1042/BJ20080029

55. L. Tradtrantip, N. D. Sonawane, N. Wan and A. S. Verkman: Nanomolar Potency Pyrimido-pyrrolo-quinoxalinedione CFTR Inhibitor Reduces Cyst Size in a Polycystic Kidney Disease Model. *Journal of Medicinal Chemistry*, 52(20), 6447 (2009)
DOI: 10.1021/jm9009873

56. Y. Norimatsu, A. Ivetac, C. Alexander, N. O'Donnell, L. Frye, M. S. Sansom and D. C. Dawson: Locating a plausible binding site for an open-channel blocker, GlyH-101, in the pore of the cystic fibrosis transmembrane conductance regulator. *Molecular Pharmacology*, 82(6), 1042-55 (2012)
DOI: 10.1124/mol.112.080267

57. P. Huang, Y. Zhang, X. Chen, L. Zhu, D. Yin, X. Zeng and S. Liang: The activation effect of hainantoxin-I, a peptide toxin from the Chinese spider, Ornithoctonus hainana, on intermediate-conductance Ca2+-activated K+ channels. *Toxins (Basel)*, 6(8), 2568-79 (2014)
DOI: 10.3390/toxins6082568

58. E. A. Ko, B. J. Jin, W. Namkung, T. Ma, J. R. Thiagarajah and A. S. Verkman: Chloride channel inhibition by a red wine extract and a synthetic small molecule prevents rotaviral secretory diarrhoea in neonatal mice. *Gut*, 63(7), 1120-1129 (2014)
DOI: 10.1136/gutjnl-2013-305663

59. P. Wojcik and L. Berlicki: Peptide-based inhibitors of protein-protein interactions. *Bioorg Med Chem Lett*, 26(3), 707-713 (2016)
DOI: 10.1016/j.bmcl.2015.12.084

60. H. Eldar-Finkelman and M. Eisenstein: Peptide inhibitors targeting protein kinases. *Curr Pharm Des*, 15(21), 2463-70 (2009)

61. V. K. Kibirev and T. V. Osadchuk: (Structure and properties of proprotein convertase inhibitors). *Ukr Biokhim Zh (1999)*, 84(2), 5-29 (2012)

Abbreviations: ETEC: enterotoxic *Escherichia coli*; CFTR: cystic fibrosis transmembrane conductance regulator; CaCC: Ca^{2+}-activated Cl^- channel; NSP4: non-structural rotaviral protein

4; ABC: ATP binding cassette; NBD: nucleotide binding domain; TMEM16A: transmembrane protein 16A

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