Despite decades of research on the effects of zinc in infectious diarrhea, there is surprisingly little consensus on how zinc works (or even if it works), on whether zinc’s effects are pathogen-specific, and what patient populations are most likely to benefit from zinc supplementation.1,2 Some recent articles focus on the growth-promoting effects of zinc on the pathogen itself, leading the reader to wonder if zinc supplements might make certain kinds of gastroenteritis worse, not better.3 Indeed, zinc is an essential mineral for all known forms of life, including bacteria, archea, fungi, protists, animals, and plants.

The article by Medeiros et al. in this issue of Virulence focuses on zinc and enteropathogenic E. coli (EPEC), strain 042.4 In this study, concentrations of zinc salts in the range of 0.05 to 0.2 mM inhibited several measures of EPEC virulence, including adherence to cultured host intestinal cells, formation of biofilm on polystyrene plastic, and inhibition of expression of several virulence factors, including aggR, virK, aatA, and so on. Furthermore, zinc-deficient mice challenged with EPEC orogastrically showed weight loss that was reversed by “rescue” with supplemental zinc. The study by Medeiros et al. was conducted with a single EAEc strain, and since EAEC are fairly diverse genetically, follow-up experiments with a collection of EAEC strains would help expand the generalizability of this study.

The article by Medeiros et al. on zinc and EAEC adds to the literature showing a beneficial effect of zinc on infection with diarrheagenic E. coli. For strains of enterotoxigenic E. coli (ETEC) producing the heat-labile toxin LT, there is a theoretical rationale for the use of zinc, based on zinc’s ability to inhibit the cyclic AMP-stimulated potassium channel located in the basolateral aspect of intestinal cells.5,6 Cholera toxin produced by Vibrio cholerae acts via cyclic AMP to stimulate the same K channel, which by K+ efflux maintains a negative intracellular potential in the epithelial cell, allowing chloride secretion via the apical side of the cell to be sustained.

In addition to the theoretical rationale for zinc in ETEC infection, zinc also has anti-virulence effects in enteropathogenic E. coli (EPEC) and Shiga toxigenic E. coli (STEC, also known as enterohemorrhagic E. coli, EHEC).7,8 In the case of EPEC and STEC we also have evidence that zinc supplementation is effective in vivo in rabbit infection models.

Although EPEC and STEC are quite dissimilar to EAEC in terms of genetics and pathogenesis, there is one intriguing parallel in the mode of action of zinc against all three pathogens, which is its ability to induce envelope stress in these E. coli strains.9 Figure 5E of the article by Medeiros et al. shows that degP was strongly induced in EAEC by low concentrations of zinc (0.01 to 0.05 mM). The degP protein is a dual-function protein with chaperone and protease activity involved in degradation of proteins that are misfolded in response to envelope stress. Mellies et al. showed that induction of the envelope stress response was associated with an inhibition of Type III secretion in EPEC, and others have shown that the envelope stress response downregulates other virulence factors.10-12

In his study, Mellies et al. used electron microscopy to visualize the cell surface blebbing and abnormal contour of E. coli cells that accompanied zinc-induced envelope stress. In our laboratory, we wondered if the same changes in the E. coli cells might be visible in the light microscope using structured illumination microscopy (SIM) which is one of several types of super-resolution microscopy techniques developed in the past few years.13 Figure 1 shows the effects of zinc on the size and shape of fluorescently labeled bacterial cells of EPEC strain E2348/69. After exposure to 0.4 mM zinc acetate, some EPEC bacterial cells maintained their normal shape, but many others were misshapen, appearing like deformed clubs, with a lumpy-bumpy outline or a moth-eaten appearance (asterisks in Fig. 1). Other zinc-treated bacteria took on even more bizarre, spheroidal shapes (dagger symbol in Fig. 1), which are atypical of E. coli. Clearly, the envelope stress response is worthy of further study in regard to bacterial pathogenesis.

The beneficial effects of zinc, however, are apparently not limited to diarrheagenic E. coli infections. Zinc oxide nanoparticles showed anti-virulence effects against Campylobacter jejuni in vitro.14,15 Zinc also seems to be effective for children with rotavirus as the etiology of the diarrheal illness.16

The emphasis on the theoretical rationale for zinc therapy for infectious diarrhea may seem like an esoteric, academic debate, but it has important public health policy implications. Zinc-oral rehydration solution (Zn-ORS) is officially recommended by the World Health
Organization (WHO) for rehydration of patients with cholera, such as in the ongoing outbreaks of cholera in Haiti. Use of Zn-ORS is allowed for other etiologies of diarrhoea, but use of zinc-ORS has not been encouraged in developed countries because of the misbelief that zinc is only beneficial in the setting of zinc deficiency. Diarrhoeal illness due to enteraggregative E. coli infection is not uncommon even in developed countries such as the United States. In addition, the STEC O104:H4 strain that caused the large outbreak in Germany in May, 2011, was an EAEC strain that had acquired the Stx toxin gene, reminding us that neglected tropical diseases can sometimes boomerang and show up in unexpected places (think also of dengue fever, West Nile virus, and cholera). The article by Medeiros et al., and others showing direct effects of zinc on pathogenic bacteria, should revive efforts to determine which pathogens are susceptible to zinc therapy, and which, if any, are not. In this context, what would be the effect of zinc against norovirus and Clorstridium difficile? The latter two notorious pathogens are difficult to control and apparently on the increase in developed countries.

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