Mechanisms of action of zinc in acute diarrhea

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Purpose of review

For over a decade, the importance of zinc in the treatment of acute diarrhea has been recognized. More recently, the mechanisms of action of zinc are becoming clearer. This review is focused on the new evidence on the mechanisms of action of zinc in acute diarrhea.

Recent findings

The vast majority of data derive from in-vitro studies using intestinal cell lines or from animal model. The positive action by zinc in acute diarrhea derives from a regulation of intestinal fluid transport, mucosal integrity, immunity, gene expression, and oxidative stress. A complex homeostatic network is also able to regulate zinc status at cellular and extracellular level.

Summary

All these data support the use of zinc in the treatment of acute diarrhea, but further clinical studies are needed to explore the selective effects of zinc against specific pathogens responsible for diarrhea.

Keywords

enterocyte growth and differentiation, intestinal inflammation, intestinal ion transport, intestinal pathogens, oxidative stress, zinc homeostasis

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Introduction

Diarrhea causes almost 1.3 million deaths annually in children less than 5 years of age, mainly in the poorest regions of the world. Reduction in this mortality burden is crucial to achieve the UN's Millennium Development Goal 4 to decrease the child mortality rate by two-thirds between 1990 and 2015 [1•]. Recent evidence has demonstrated that zinc supplementation has a considerable beneficial effect on the clinical course of acute diarrhea, and more recently, the mechanisms of action of zinc are becoming clearer [2••]. As reported in a recent systemic review, zinc supplementation reduced the mean duration of acute diarrhea by approximately 20% and persistent diarrhea by 15–30% [3••]. Recently, another systemic review of the effects of zinc for diarrhea treatment was designed to meet the needs of the Lives Saved Tool (LiST). In LiST, increases in coverage of an intervention result in a reduction of one or more causes of mortality. The mentioned review was designed to develop estimates of the effect of an intervention in reducing death due to diarrhea and it reported that zinc for the treatment of diarrhea will reduce diarrhea mortality by 23%. In addition, zinc has been shown to decrease diarrhea prevalence in both 24-h and 2-week recall survey [4•]. Introduction of zinc to community programs resulted in increased use of oral rehydration solution (ORS), decreased use of unnecessary antibiotics, and a reduced need for medical visits for acute diarrhea [5]. Zinc is now included in the WHO essential medicine list for diarrhea treatment, and in the 2008 Copenhagen Consensus, a group of leading global economists ranked zinc supplementation as the most effective intervention for advancing human development [6]. Zinc is now widely used in the treatment of acute diarrhea in developing countries where it is responsible for saving more than 400 000 lives a year [7]. Moreover, a universal zinc-containing super ORS has been proposed by various authors [1•,8•]. This review is focused on the new evidence on the mechanisms of action of zinc in acute diarrhea.

Toward a better definition of the mechanisms of zinc in acute diarrhea

Zinc is one of the most important trace elements for human health. It serves over 300 biological functions and elicits effects on multiple systems, including the gastrointestinal tract [9]. Zinc is not stored in the body, so its level is determined by the balance of dietary intake, absorption, and losses. A zinc deficiency state may exist in children with acute diarrhea as a result of intestinal

loss, and chronic zinc deficiency may increase susceptibility to diarrhea [10]. The positive action by zinc in acute diarrhea derives from several possible mechanisms: first, zinc regulates intestinal fluid transport and mucosal integrity; second, zinc plays a substantive role in immunity; third, zinc can modify expression of genes encoding several zinc-dependent enzymes, such as metalloproteases, cytokines and uroguanylin; fourth, zinc may modulate oxidative stress [11••,12••,13]; and lastly, zinc is able to resist its potential loss in diarrhea. The intestine is the first tissue confronted with zinc. Sufficient absorption of zinc from the small intestine is essential for body health, but high levels of zinc are deleterious. Thus, maintaining an appropriate zinc level is crucial. Zinc homeostasis is coordinated via regulation by proteins involved in uptake, excretion, and intracellular storage or trafficking. These proteins are metallothioneins and transmembrane transporters, which include zinc-regulated transporter (ZRT) iron-regulated transporter (RT)-like protein (ZIP) and cation diffusion facilitator (CDF) families. Metallothioneins belong to a family of low molecular weight, cysteine-rich intracellular proteins that bind zinc, and their biological roles include the detoxification of harmful metals and the homeostasis of essential metals. The ZIP family plays prominent roles in zinc uptake and transport into the cytoplasm. ZIP transporters have also been found to mobilize stored zinc by transporting the metal from an intracellular compartment into the cytoplasm. The CDF family transports zinc in the direction opposite to that of the ZIP proteins, promoting zinc efflux or compartmentalization by pumping zinc from the cytoplasm out of the cell or into the lumen of an organelle. Zinc transporter proteins (ZnT) are other members of the CDF families. ZnT1, a ubiquitous ZnT located in the plasma membrane, transports zinc out of cells. In addition, divalent metal transporter (DMT1) is located in the apical membrane of enterocytes and takes iron into enterocytes. It is predominantly an iron transporter, with lower affinity for other metals such as zinc. Some data indicate that zinc transport is upregulated by DMT1 based on protein levels and mRNA expression [14•]. In addition, during the stage of acute inflammation, monocytes secrete proinflammatory chemokines that stimulate ZIP4 in the hepatocytes, thereby arresting and storing zinc by fusing it with metallothioneins. However, circulating zinc ions also have the ability to decrease hyperfunctional monocytes and, thus, reduce a precipitous fall of zinc ions in circulation [15].

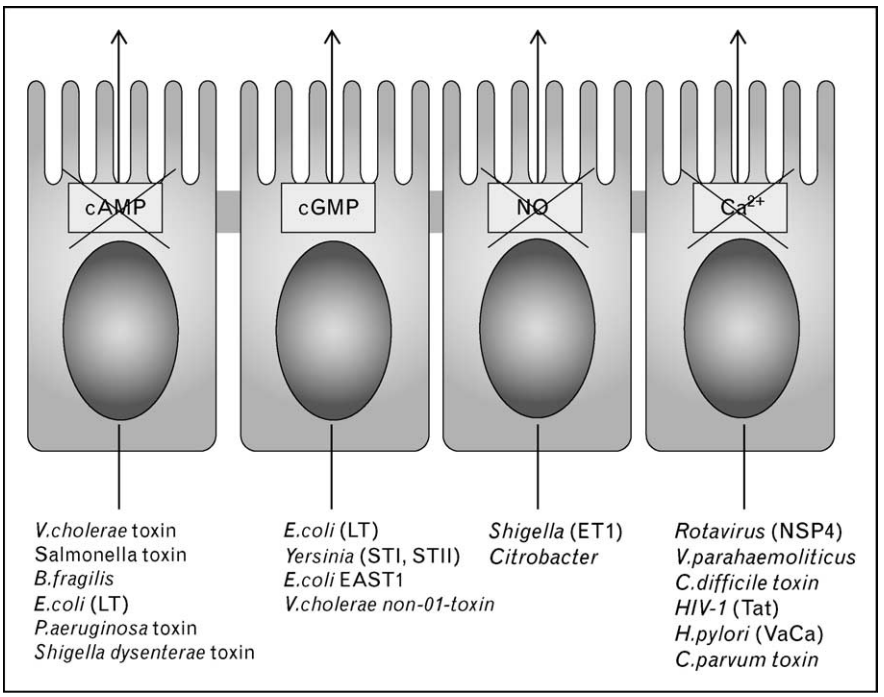
Effects of zinc on intestinal transepithelial ion transport

Zinc induces ion absorption in enterocytes in basal conditions. Zinc addition to the mucosal side of a monolayer of human enterocyte cell line (Caco-2 cell) mounted in Ussing chambers induced a decrease in short-circuit current (Isc), indicating ion absorption with a maximal effect at 25 min. Zinc addition to the basolateral side of enterocytes induced a decrease in Isc entirely similar to that observed with mucosal addition, although the magnitude of the response was slightly reduced compared to that observed with mucosal addition [16]. These results indicate that zinc exerts a direct proabsorptive effect on intestinal transepithelial ion transport.

A number of organisms cause diarrhea by producing and secreting enterotoxins that affect the absorptive and/or secretory processes of the enterocyte without causing considerable acute inflammation or mucosal destruction. Some enterotoxins trigger signaling molecules such as cyclic AMP (cAMP) or cyclic GMP (cGMP), which, in turn, activate apical Cl^- channels, leading to an increase in secretion of Cl^- and consequently of water [16, 17••,18]. Using an in-vitro model, we have demonstrated that zinc prevents active ion secretion induced by cholera toxin by directly inhibiting the rise in intracellular cAMP concentration, but that it does not affect *Escherichia coli* heat-stable enterotoxin-induced and cGMP-mediated ion secretion [16]. These findings suggest that zinc exerts a selective effect against intestinal pathogens (Fig. 1). Several toxins, including Ciguatera fish toxin, *Clostridium difficile* toxin, *Cryptosporidium parvum* toxin, *Rotavirus* enterotoxin (the nonstructural protein 4, NSP4), *Helicobacter pylori* vacuolating toxin, and *Vibrio parahaemolyticus* enterotoxin induce a direct secretory effect in the enterocyte modifying intracellular Ca^{2+} [11••,17••,18]. We have reported that the transactivator factor peptide (Tat) produced by HIV-1, functioning as an enterotoxin, induces ion secretion by a Ca^{2+}-mediated mechanism. Subsequently, we demonstrated that the preincubation of human enterocytes with zinc resulted in almost total inhibition of the Tat-induced ion secretion interacting directly with a specific mechanism of HIV-1-related diarrhea. These data, providing an explanation for the observed reduction of diarrhea in patients with HIV infection during zinc supplementation, suggest that zinc could act also in Ca^{2+}-mediated diarrhea [19]. These effects are particularly interesting for childhood diarrhea. In fact, *Rotavirus*, the major agent of acute diarrhea in infancy, stimulates Cl^- secretion through a phospholipase C-dependent Ca^{2+} signaling pathway induced by the NSP4 [20].

The importance of nitric oxide as a second messenger involved in intestinal ion secretion has become fully recognized only recently. The pathogenic effects exerted by nitric oxide are strictly concentration-dependent and associated with an increased nitric oxide production triggered by the activity of inducible nitric oxide synthase isoform (iNOS), an enzyme involved in selected infectious and noninfectious diarrheal diseases. Zinc is an established nitric oxide scavenger, and these data suggest

Figure 1 Main intracellular pathways of intestinal ion secretion modulated by zinc



In-vitro evidence suggests that zinc is able to inhibit three out of the four main intracellular pathways of intestinal ion secretion: cyclic adenosine monophosphate (cAMP), calcium (Ca^{2+}), and nitric oxide. But it is unable to affect cyclic guanosine monophosphate (cGMP)-induced ion secretion. In the figure are also reported the main agonists of these pathways.

that zinc could be effective in nitric oxide-induced ion secretion. We showed that zinc significantly inhibits intestinal Cl^- secretion induced by interferon-γ, a well established agonist of this mechanism, and this was associated with a decrease in nitric oxide production by the enterocytes [11••]. These results confirm previous observations, obtained in in-vivo animal models, showing that zinc is able to inhibit lipopolysaccharides and IL-1-induced diarrhea through a reduction in nitric oxide production and by removing chemically nitric oxide generated in the gut. Previous data have suggested that zinc and nitric oxide play an active regulatory role in intestinal ion transport. A low nitric oxide concentration, sustained by constitutive nitric oxide synthase, maintains the physiological intestinal ion pro-absorptive tone. On the contrary, an excess of nitric oxide production stimulated by pro-inflammatory cytokines or microbial toxins induces intestinal fluid secretion through an upregulation of iNOS. This mechanism may be activated in intestinal infections and inflammation and an overexpression of iNOS gene have been demonstrated in zinc-deficient animal model. The results of our study suggest that zinc is part of this network in the enterocyte. The number of intestinal pathogens that utilize nitric oxide to induce diarrhea is limited, but not negligible. *Shigella* enterotoxin 1 exerts an irreversible, dose-dependent enterotoxic effect mediated by an increased nitric oxide intestinal concentration that is partially blocked by iNOS inhibitors. However, high nitric oxide production has been shown to contribute to diarrhea by acting as a secretagogue, in particular in inflammatory diarrhea. In light of this fact, the effects of zinc on the nitric oxide pathway open new perspectives in the treatment of intestinal inflammatory diseases.

Effects of zinc on intestinal mucosa integrity, cell growth, and differentiation

Previous studies have shown that zinc plays a key role in the maintenance of membrane barrier function and in controlling inflammatory reactions by showing that the depletion of zinc causes phosphorylation-mediated disruption of junctional complexes and cytoskeleton disorganization, thus promoting the migration of neutrophils [21•,22]. The presence of high zinc concentration (>200 μmol/l) or zinc chelating agent in cells could induce apoptosis. High or low (<10 μmol/l) zinc concentration inhibits enterocyte proliferation [15]. Remarkably, these doses correspond to that observed in ion transport experiments [11••], suggesting a possible link between transepithelial ion transport and cell growth processes. Cell cycle progression involves the activation of the intracellular MAP kinase cascade [23] that leads to DNA synthesis in the nuclei. We investigated whether the extracellular signal-regulated kinase (ERK) pathway, a member of the MAP kinase family, was involved in the

Table 1 Effects of zinc deficiency on immune system

T-cell subpopulation	Th1 cytokines	Th2 cytokines	NK cell	Others
$CD4^+$ to $CD8^+$ ratio decreased	IL-2 production and IL-2 mRNA decreased	No change in IL-4, IL-6, and IL-10	Binding of NF-κB to DNA and its translocation from cytosol to nucleus decreased	Thymulin activity decreased
$CD4^+$ $CD45RA^+$ to $CD4^+$ $CD45RO^+$ ratio decreased	IL-2R-α and IL-2-β production and mRNAs decreased		IκB phosphorylation decreased, accounting for decreased activation of NF-κB	Increase in the levels of TNF-α, IL-1-β, and cytokines and mRNA
	IFN-γ decreased		$CD8^+$ $CD73^+$ decreased	Increased lipid peroxidation by nitric oxide
			Increased NF-κB-DNA binding in LPS-stimulated cells	Decreased production and mRNA of A-20

IFN, interferon; IL, interleukin; NF-κB, nuclear factor-κB; NK, natural killer.

zinc-induced cell response. Upregulation of ERK1/2 was induced by zinc in undifferentiated Caco-2 cells. In addition, cell differentiation is an important process at intestinal level. Genetic programming and selected factors play a role in intestinal differentiation in modulating brush border disaccharidase expression. Sucrase and lactase are markers of enterocyte differentiation and increase during cell migration from the crypt to villus tip. Under zinc stimulation, both sucrase and lactase activities were significantly increased, suggesting that zinc induces enterocyte differentiation (personal observation).

Effects of zinc on intestinal defense mechanisms

The intestinal defense mechanisms against pathogens determining diarrhea are numerous; among these, the most important are the epithelial barrier and the cellular and humoral constituents of innate and acquired immunity. These parts interact in complex networks protecting against acute diarrhea and zinc has been shown to be a crucial player in immunonutrition [24]. Several recent reviews have documented the immune-related functions of zinc [12••,13,25].

Both primary and secondary antibody responses have been reported to be depressed in zinc-deficient animal models. Zinc is considered as a key player in innate and acquired immunity and zinc homeostasis influences the development and function of immune cells, particularly T cells. In humans even a mild deficiency of zinc may be accompanied by an imbalance of Th1 and Th2 cells, decreased recruitment of T-naive cells, decreased percentage of T cytolytic cells, decreased natural killer (NK) cell lytic activity, and decreased serum thymulin activity (see Table 1). Studies on cell culture models showed that the activation of many zinc-dependent enzymes and transcription factors is adversely affected due to zinc deficiency. In HUT-78 [T helper 0 (Th0) cell line], it has been shown that a decrease in gene expression of interleukin-2 (IL-2) and IL-2 receptor-α (IL-2R-α) were due to decreased activation of nuclear factor-κB (NF-κB) in zinc-deficient cells. Decreased NF-κB activation in HUT-78 due to zinc deficiency was due to decreased binding of NF-κB to DNA, decreased level of NF-κB p105 (the precursor of NF-κB p50) mRNA, decreased κB inhibitory protein (IκB) phosphorylation, and decreased Iκκ. These effects of zinc were cell-specific. In HL-60 cells (promyelocytic leukemia cell line), zinc enhances the upregulation of A20 mRNA, which, via TRAF pathway, decreases NF-κB activation, leading to decreased gene expression and generation of tumor necrosis factor-α (TNF-α), IL-1-β, and IL-8.

The ability of zinc to function as an antioxidant and to stabilize membranes suggests that it has a role in the prevention of free radical-induced injury during inflammatory processes. Together O_2, H_2O_2, and OH are known as reactive oxygen species (ROS), and these are produced continuously *in vivo* under aerobic conditions. The NADPH oxidase is a group of plasma membrane-associated enzymes, which catalyze the production of O_2^- from oxygen by using NADPH as the electron donor. Zinc is an inhibitor of this enzyme. The dismutation of O_2^- to H_2O_2 is catalyzed by an enzyme super oxide dismutase (SOD), which contains both copper and zinc. Zinc is known to induce the production of metallothioneins and is an excellent scavenger of OH [14•]. Iron and copper ions catalyze the production of OH from H_2O_2. Zinc is known to compete with both iron and copper for binding to cell membrane, thus decreasing the production of OH [14•].

Conclusion

Zinc is a multipotent agent at intestinal level: it modulates ion transport, stimulates enterocyte growth and differentiation, reduces intestinal permeability, and positively regulates oxidative stress and inflammation. Few other substances exert such a variety of beneficial effects in the intestine. As an adjunct to ORS, zinc has the potential to improve the management of acute diarrhea, determined by different mechanisms, at affordable costs. Recently, main in-vitro advances have been obtained in our knowledge on cellular mechanism elicited by zinc that could be potentially useful to support its use in the treatment of acute diarrhea. Further clinical studies are

needed to explore the selective effects of zinc against specific pathogens responsible for diarrhea.

References and recommended reading

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- •• of outstanding interest

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