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Roles of Zinc in the Pathophysiology of Acute Diarrhea

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Abstract Zinc has caught wide scientific attention for the conceptual promise it has to offer for prevention, control and treatment of acute diarrhea. This review focuses on the mechanisms by which zinc might contribute to the pathogenesis of acute diarrhea and the degree of success achieved in diarrhea control and treatment by zinc supplementation. Animal and in vitro studies have continued to fascinate the scientific fraternity and form a solid basis for the potential use of zinc supplementation against diarrhea. However, emerging evidence in terms of controlled studies in humans beckons a more complete understanding of the mechanistic basis for zinc supplementation. Current evidence indicates that studies specifically addressing the variability in response to zinc supplementation need to be undertaken to better comprehend these mechanisms.

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Introduction

Diarrhea continues to be a persistent threat to health in the developing countries. Annually 10.8 million children die before their fifth birthday of whom 70% die during infancy [1]. In sub-Saharan Africa and South Asia as many as 23% of the child deaths have been attributed to diarrhea [2–4]. Although few would argue about the evident decline in diarrhea mortality in the past decades it is noteworthy that the estimates of mortality and morbidity vary widely around the world and across times [5]. Much of the success achieved in the control of diarrheal morbidity and mortality has been conventionally attributed to two therapeutic interventions: the oral rehydration solution (ORS) and zinc supplementation [6•, 7]. The use of the latter intervention is based on the following three premises: i) there is compelling evidence for the biological role of zinc in etiopathogenesis of diarrhea; ii) there is evidence for a therapeutic benefit ascribable to zinc supplementation in acute diarrhea; iii) there also exists evidence for the prophylactic value of zinc supplementation in the prevention of diarrheal episodes. In this review, we address these three premises in the light of existing and emerging evidence from published literature.

Biological Roles of Zinc in the Context of Diarrhea

Zinc is one the of several important trace elements having far-reaching effects on multiple organs and systems and serves over 300 biological functions [8]. The current understanding of the role of zinc in diarrhea comes from many studies conducted in humans and animals. There are

several independent and interacting mechanisms that together coalesce into an overall influence of zinc on diarrhea. These mechanisms are as follows:

Mucosal Integrity

Zinc is required for general mucosal integrity [9, 10], especially that of the intestinal and respiratory mucosa. The intestinal mucosa acts as the first barrier between the microbes and the host. For this barrier to be robust and impenetrable, it is imperative that the process of enterocyte proliferation be immaculate and reliable. There is evidence to suggest that optimum levels of zinc favor the differentiation and multiplication of enterocytes [11••, 12, 13]. For example, very high (>200 μmol/L) concentration of zinc may induce enterocytic apoptosis while very low zinc levels (<10 μmol/L) are likely to decrease cell proliferation [13]. Two groups have recently reported a possible involvement of the extracellular-regulated kinase (ERK) pathway in mediating the influence of zinc on cell cycle in Caco-2 cell line [11••, 14]. It has also been reported that there is an increased activity of sucrase and lactase consequent to zinc supplementation—fact that indirectly supports the potential influence of zinc on enterocyte proliferation.

Secretion of Chloride Ion

Zinc partially regulates intestinal secretion of the chloride (Cl^-) ion. However a better realization of the exact role of zinc in this context can be gleaned from a brief overview of the mechanisms of infectious diarrhea. As shown in Fig. 1, four main pathways have been identified in diarrheas caused by the infectious agents [11••, 15, 16••]. The major pathway involves the 3′,5′-cyclic adenosine monophosphate (cAMP) driven induction of protein kinase A for phosphorylation of the proteins and subsequent excretion of the Cl^- ion. Carlson et al. [17] and Hoque et al. [18] have demonstrated that zinc can inhibit the cAMP-mediated Cl^- secretion by blocking the potassium channels located at the basolateral aspects of the epithelial cells. These authors have also demonstrated a similar effect for inhibition of Cl^- secretion due to 5-hydroxytryptamine, theophylline and 8-bromoadenosine. Further refining the earlier observations, Carlson et al. [19] have demonstrated that zinc ions on the serosal side are more direct determinants of the Cl^- ion secretion than those on the luminal side. They observed that zinc-induced promotion of ion absorption across the gut is evident in response to the ion secretion caused by *Vibrio cholerae* toxin.[20]

Another pathway implicated in diarrheal pathogenesis involves the cyclic guanine monophosphate (cGMP). Currently there is no evidence to suggest that zinc partakes in this pathway. Indeed, it has been demonstrated that zinc does not inhibit the cGMP-mediated pathway [20]. Yet another mechanism that uses the downstream cGMP pathway but is triggered by nitric oxide (NO) is also implicated in infectious diarrhea. Although zinc may be unable to inhibit the cGMP pathway, it can block the NO release subsequent to toxin attachment for some pathogens (e.g. Shigella ET1, Shigella flexneri and citrobacter spp) [11••, 21–24]. Finally, a fourth pathway that involves Ca^{++} signaling through protein kinase C is another potential mechanistic explanation for diarrhea. Recently, Berni Canani et al. [25] using Caco-2 cells have demonstrated that Cl^- secretion induced by the Tat protein of the human immunodeficiency virus (HIV) can be effectively blocked by zinc. This finding supports the prevailing understanding that zinc may be useful against HIV-associated diarrhea through the Ca^{++} pathway [11••, 15, 21, 26]. This pathway is also interesting because intracellular calcium ion concentrations are known to activate the Na^+/H^+ exchange activator 1 (*NHE1*) gene leading to further regulation of the Cl^- ion secretion [16••, 27, 28]. In addition to these mechanisms of diarrhea initiation and the roles of zinc therein, it has recently been proposed that the enterocytes are equipped with a receptor to specifically pluck zinc ions. This receptor (shown as ZnR in Fig. 1) and its regulation also provide yet another exciting modus for diarrhea prevention and control through zinc supplementation [29•, 30•].

Zinc and Immunity

Zinc plays a substantive and significant role in immunity [31–33]. It should be noted that zinc is a signaling molecule in several immune cells like monocytes, dendritic cells and macrophages and it plays an important role in cell-mediated immune functions and oxidative stress [34••]. Zinc is also an anti-inflammatory agent and improves general immune function [35]. It has been observed that reduced zinc levels are associated with an increased likelihood of malnutrition and diarrhea [36]—conditions indicating a subdued immune function. Zinc has a direct effect (Fig. 1) on the stimulation of thymus, naïve T cell production, clonal expansion, Th1/Th2 cell differentiation and Th1 T cell stimulation [37, 38]. Zinc deficiency in murine models has been shown to be associated with thymic atrophy, decreased splenocyte counts and blunted response to all antigens—T-cell dependent as well as independent [39]. Moreover, the stimulant effect of zinc on immune functions is thought to be beneficial in view of the disturbed T-cell homeostasis in some acute diarrheas [40]. Interestingly, differential immune effects of zinc have been noted on pathogen

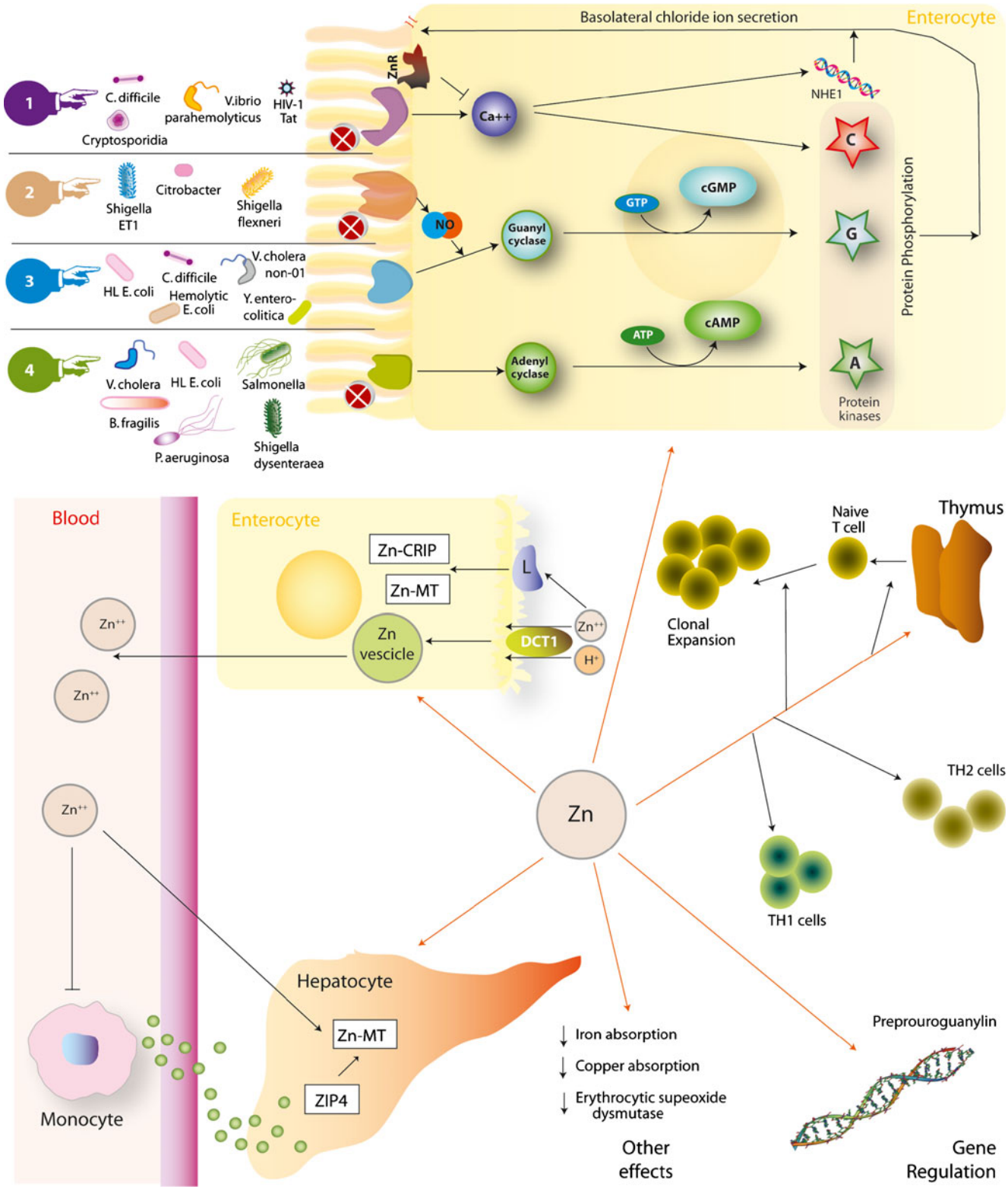

Fig. 1 Pathogenetic contribution of zinc to acute diarrhea. Zinc homeostasis is achieved through zinc transporation and absorption by the enterocytes. The infectious organisms causing acute diarrhea (schematically represented to the left of the enterocyte) use one of the four pathways shown at the top. The white crosses on red circles show the pathways that are inhibited by zinc supplementation. L, dietary ligands; CRIP, cystein-rich intestinal protein; MT, metallothionein; ZIP4, zinc import protein 4; ZnR, zinc receptor; DCT1, divalent cation transporter 1; NHE1, Na^+/H^+ exchange activator 1; NO, Nitric oxide; cGMP, cyclic guanine monophosphate; cAMP, cyclic adenosine monophosphate. Green colored drops passing through the blood vessel into the hepatocytes represent the proinflammatory chemkines. Orange arrows indicate the actions of zinc; black pointed arrows show a positive influence while black blunted lines show an inhibitory influence

specific immune responses. For example, a zinc up-regulated Th1 response is more protective against invasive diarrheal pathogens such as *Salmonella* spp. and *Shigella* spp [41].

Zinc and Chemokines

There is a very intriguing nexus between zinc and chemokines. A possible mechanism by which zinc escapes potential loss in diarrhea is that during the stage of acute inflammation monocytes secrete proinflammatory chemokines that stimulate zinc import protein 4 in the hepatocytes (Fig. 1) thereby arresting and storing zinc by fusing it with metallothionein [23, 42–44]. However, circulating zinc ions also have the ability to decrease hyperfunctional monocytes and thus reduce a precipitous fall of zinc ions in circulation [13]. A similar zinc-wringing mechanism is also observed in the enterocytes [45]. Therefore, by maintaining its circulating levels zinc modulates and moderates the potentially proinflammatory effect of monocytes and other immune cells during acute diarrhea.

Other Mechanisms

Zinc can modify expression of genes [38, 46–48] encoding several zinc-dependent enzymes like metalloproteases and uroguanylin. In cases of zinc deficiency, the activity of these enzymes is decreased. Moreover, zinc may also modulate the cytokine gene expression especially of interleukin-8 and tumor necrosis factor–α [49]. These latter influences of zinc further contribute to its actions on the immune system. Lastly, contradictory to these anti-diarrhea mechanisms of action, zinc reduces the intestinal absorption of other important trace elements like iron and copper. The latter, combined with a reduced activity of the erythrocytic superoxide dismutase can have detrimental effect on diarrheal outcomes [50]. Nevertheless, the net biological effect of zinc on diarrhea is considered to be favorable.

Zinc Supplementation During Acute Diarrhea

Background

Different methods and sources of information contributing to each successive review of diarrhea burden over the past three decades have demonstrated declining mortality but relatively stable morbidity due to this scourge. For example, using 27 published studies Kosek et al. [5] showed that incidence of diarrhea remained remarkably stable over time. The studies varied in their definitions of diarrhoea and the frequency of surveillance. The incidence of diarrhoea was highest among children aged 6–11 months, who experienced a median of 4.8 episodes per child per year. The incidence fell progressively to 1.4 episodes per child per year for 4-year-olds. The median incidence for all children aged less than 5 years was 3.2 episodes per child-year. Diarrheal morbidity therefore continues to be a daunting challenge.

Value of Therapeutic Zinc Supplementation

Encouraged by the biological rationale and enthused by several randomized controlled trials and meta-analyses that initially claimed a strong therapeutic benefit for zinc the World Health Organization and the United Nations Children's Fund took two significant steps in the year 2004 to reduce the diarrheal burden by recommending the use of low-osmolarity oral rehydration solution (ORS) and supplementation with zinc for up to 2 weeks for management of acute diarrhea. However, randomized controlled trials attempting to define the therapeutic benefit of zinc have continued even after this programmatic decision. Moreover, seven meta-analyses [51••, 52••, 53••, 54••, 55••, 56••, 57••] have thus far been conducted addressing this research question and the results from these meta-analyses are summarized in Table 1. These results demonstrate that there is a statistically significant benefit of zinc supplementation to reduce the mean diarrheal duration at the cost of an increased risk of vomiting, however there is no discernible effect on other diarrhea-related outcomes like stool volume and frequency. More importantly, there is a significant heterogeneity of estimated benefit across studies. It is therefore of foremost importance to understand the predictors of zinc efficacy to identify the populations most likely to benefit from supplementation. It appears both intuitive and scientific that to improve the programmatic use of zinc, more studies dealing with the evaluations of the zinc salts used, the dose, the frequency and duration of supplementation, and its acceptability are urgently needed.

Organism Specificity

Another issue that has emerged recently is that given the specific pathways through which zinc acts, it is expected that zinc supplementation may not provide a therapeutic benefit against all the organisms causing diarrhea. In an elegant review, Canani et al. [11••] highlight this point. Recently, we reported the results of a randomized clinical trial that showed an overall lack of benefit of zinc supplementation. However, when we conducted the analyses based on the major isolates,[58••] we found that the diarrheas associated with *Klebsiella* isolates derived significant benefit from zinc supplementation, those with *E. coli* isolates did not show any benefit from zinc supplementation while those with rotavirus isolates showed an increased

Table 1 Reported summary estimates for the influence of therapeutic zinc supplementation on diarrheal outcomes (Adapted from Patel et al., 2010[55••])

Outcome	Review	Ref	*N*	Statistic	ES (95% CI)
Recovery from diarrhea	Bhutta et al., 2000	[51••]	3	RH	0.85 (0.76–0.95)
Diarrhea at day 1	Lukacik et al., 2008	[54••]	5	RR	1.01 (0.99–1.03)
Diarrhea at day 3	Lukacik et al., 2008	[54••]	6	RR	0.97 (0.91–1.03)
	Patro et al., 2008	[56••]	3	RR	0.62 (0.44–0.87)
	Lazzerini et al., 2008	[53••]	3	RR	0.69 (0.59–0.81)
Diarrhea at day 5	Lukacik et al., 2008	[54••]	6	RR	0.94 (0.84–1.05)
	Patro et al., 2008	[56••]	2	RR	0.68 (0.11–4.31)
	Lazzerini et al., 2008	[53••]	2	RR	0.55 (0.32–0.95)
Diarrhea at 7 days	Bhutta et al., 2000	[51••]	3	OR	0.78 (0.56–1.09)
	Patro et al., 2008	[56••]	8	RR	0.71 (0.53–0.96)
	Lazzerini et al., 2008	[53••]	10	RR	0.71 (0.52–0.98)
Mean diarrheal duration	Bhutta et al., 2000	[51••]	5	% Red	15.0*
	Lukacik et al., 2008	[54••]	16	WMD	0.24 (0.21–0.27)
	Patro et al., 2008	[56••]	13	WMD	−0.69 (–0.97–0.40)
	Lazzerini et al., 2008	[53••]	13	WMD	−0.51 (−0.96–0.06)
	Haider and Bhutta, 2009	[52••]	14	WMD	−0.50 (−0.82–0.08)
	Patel et al., 2010	[55••]	19	SMD	−0.25 (−0.35–0.15)
Stool frequency	Lukacik et al., 2008	[54••]	7	% Red	18.0*
	Patro et al., 2008	[56••]	3	WMD	−0.02 (−0.29–0.25)
	Lazzerini et al., 2008	[53••]	7	WMD	−0.02 (−0.19–0.15)
Stool volume	Lukacik et al., 2008	[54••]	3	% Red	30.3*
	Patro et al., 2008	[56••]	3	WMD	−0.38 (−1.04–0.27)
Vomiting	Lukacik et al., 2008	[54••]	11	RR	1.55 (1.30–1.84)
	Patro et al., 2008	[56••]	5	RR	1.22 (1.05–1.43)
	Lazzerini et al., 2008	[53••]	10	RR	1.71 (1.27–2.30)
	Patel et al., 2010	[55••]	9	OR	2.13 (1.37–3.31)

N number of randomized controlled trials included; *Ref* reference number; *RH* relative hazards; *RR* relative risk; *OR* odds ratio; *WMD* weighted mean difference; *SMD* standardized mean difference;*% Red* percentage reduction; *ES* effect size; *CI* confidence interval; *confidence interval not reported

risk of adverse diarrheal outcomes upon receiving zinc supplementation. There is now a burgeoning recognition that universal zinc supplementation in the treatment of acute diarrhea may be less justifiable than thought initially.

Potential Interactions of Zinc with Other Supplements

In clinical scenarios zinc is likely prescribed in the treatment of acute diarrhea along with several other substances that can potentially interact with its absorption or efficacy (similar to iron and copper mentioned earlier). For example, zinc has been used along with vitamin A, vitamin C, multivitamins, ORS, probiotics or combinations of these [44, 50, 59–61]. While zinc down-regulates the absorption of iron and copper, its own absorption by the enterocytes is thought to be improved by vitamins and probiotics [61, 62]. However, controlled trials using factorial designs of the combinations of interactions are currently lacking and required as the net effect of these interactions as well as the effect of zinc in a combinatorial therapeutic context is unknown. Another important aspect of the maintenance of circulating zinc levels is regulation of its absorption through the enterocytes. Zinc needs to be actively transported into the enterocyte, an action mediated by a group of proteins called zinc transporters as well as through dietary ligands [22, 63]. One important zinc transporter that has received some scrutiny is the DCT1 protein (Fig. 1). After the initial spurt of studies almost a decade ago,[22, 63] the field has not observed new discoveries commensurate with the early excitement regarding this molecule. More studies in this direction are required.

Value of Prophylactic Zinc Supplementation

Panda et al. [64] have recently reported that decreased blood levels of zinc are strongly associated with diarrhea in canine models—a finding that corroborates the general view that zinc deficiency can increase diarrhea susceptibility [65]. A logical corollary of this finding is that zinc supplementation—especially in areas where zinc deficiency

is highly prevalent—may reduce the incidence and prevalence of future diarrheal episodes. This possibility has also been evaluated by numerous randomized controlled trials around the world and has been summarized by six meta-analyses [66••, 67••, 68••, 69••, 70••, 71••]. Our recent synthetic review [69••] throws light on some of the nuances of the general paradigm that encourages preventive zinc supplementation.

There is evidence that zinc supplementation reduces the incidence of future diarrheal episodes by 9% (Table 2). Zinc may also reduce the prevalence of diarrhea by 19% and the occurrence of multiple episodes of diarrhea by 28%. Recently, Yakoob et al. [71••] have demonstrated that diarrheal mortality can be reduced by 13% due to preventive zinc supplementation. These numbers can be enticing as they show a substantial preventive benefit of zinc however it must be remembered that the estimates of the summary benefits have shrunk over time and more recent, large and elegant studies have generally not shown a conclusive benefit of zinc prevention. Also, zinc supplementation has been ineffective for prevention and control of diarrhea during infancy or in low birth weight children [68••, 69••, 72]—a finding that indirectly corroborates the observed lack of therapeutic zinc benefit since major cause of diarrhea in infants is rotavirus affliction. Finally, there exists a statistically significant degree of heterogeneity across studies [69••] included in the synthetic reviews making the validity of summary estimates rather questionable. In a nutshell, although biologically relevant, logically intriguing and generally accepted the concept of prophylactic zinc supplementation is yet to be firmly rooted in programmatic practice. Furthermore, programmatic decisions on the prophylactic use of zinc supplementation will need to address additional issues like safety, acceptability, adherence, cost and effectiveness even if zinc supplementation is prophylactically efficacious. Therefore, the issue of wide-spread use of zinc supplementation for prevention and control of diarrhea is currently unresolved.

Conclusions

Convincing biological rationale exists for the role of zinc in the etiopathogenesis of acute diarrhea. This role was supported by earlier controlled trials as well as meta-analyses. However, more recent data have tended to cumulatively blunt some of the early enthusiasm. Additional evidence that has pointed towards possible inter-individual variation in zinc benefit before or during acute diarrhea suggests that other as yet unknown mechanisms might be involved in the overall action of zinc against acute diarrhea. These responses may include organism specificity, dose response, zinc preparations used as well as genetic uniqueness of the host. Whether and to what extent these factors might modify and tailor the beneficial effect of zinc is still unclear. It is also not known why zinc supplementation has a more pronounced effect on diarrheal duration as compared to other outcomes like stool frequency and volume. Well designed preclinical as well as clinical studies are urgently needed to address these important questions with an overall goal to improve the quality and quantity of the benefit of zinc supplementation against diarrhea.

Table 2 Reported summary estimates for the influence of preventive zinc supplementation on diarrheal outcomes (Adapted from Patel et al., 2011[69••])

Outcome	Review	Ref	*N*	Statistic	ES (95% CI)
Incidence of diarrhea	Bhutta et al., 1999	[67••]	7	OR	0.82 (0.72–0.93)
	Aggarwal et al., 2007	[66••]	15	RR	0.86 (0.79–0.93)
	Brown et al., 2009	[68••]	24	RR	0.80 (0.71–0.90)
	Patel et al., 2011	[69••]	31	RR	0.91 (0.86–0.95)
Prevalence of diarrhea	Bhutta et al., 1999	[67••]	7	OR	0.75 (0.63–0.88)
	Patel et al., 2011	[69••]	15	RR	0.81 (0.75–0.88)
Incidence of persistent diarrhea	Bhutta et al., 1999	[67••]	6	OR	0.67 (0.42–1.06)
	Aggarwal et al., 2007	[66••]	3	RR	0.75 (0.57–0.98)
	Patel et al., 2011	[69••]	11	RR	0.89 (0.73–1.09)
Incidence of dysentery	Bhutta et al., 1999	[67••]	3	OR	0.87 (0.64–1.19)
	Aggarwal et al., 2007	[66••]	4	RR	0.85 (0.75–0.95)
	Patel et al., 2011	[69••]	7	RR	0.89 (0.75–1.06)
Mortality	Tielsch et al., 2007	[70••]	4	RR	0.91 (0.82–1.02)
	Brown et al., 2009	[68••]	10	RR	0.94 (0.86–1.02)
	Patel et al., 2011	[69••]	12	RR	0.90 (0.78–1.04)
Diarrheal mortality	Yakoob et al., 2011	[71••]	6	RR	0.91 (0.76–1.09)

CI confidence interval; *ES* effect size; *N* number of randomized controlled trials included; *OR* odds ratio; *Ref* reference number; *RH* relative hazards; *RR* relative risk

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- •• Of outstanding interest

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