Zinc (Zn\(^{2+}\)) deficiency is associated with increased severity and duration of diarrhea in children from developing countries, and optimal Zn\(^{2+}\) supplementation is essential for recovery and prognosis of affected individuals. Administration of oral rehydration solution (ORS) is available and used in target populations, utilization of oral Zn\(^{2+}\) is limited due to a decreased absorptive capacity in intestinal diseases associated with villous atrophy. Recent studies have shown that mice treated with a select set of five amino acids (AA-ORS) have increased villus height, and increased expression and protein levels of key transporters of electrolyte and nutrient absorption. The amino acids selected, threonine, tyrosine and valine were selected based on their ability to maximize electrolyte absorption, thinning the mucosal barrier and increase proliferation of intestinal epithelial cells by expanding the number of Lgr5\(^+\) crypt stem cells (Yin, et al. 2016).

Objective and Hypothesis

The objective of the study was to determine the effect of AA-ORS on the expression and function of the main apical Zn\(^{2+}\) transporter, ZIP4 (SLC39A4) in small intestinal epithelial cells (Fig. 1). In irradiated mice, an experimental model known to cause profound intestinal barrier dysfunction and decreased absorption. Our hypothesis is that AA-ORS increases Zn\(^{2+}\) absorption in compromised intestinal epithelial cells after irradiation, and therefore could also be used to optimize Zn\(^{2+}\) utilization, and to treat Zn\(^{2+}\) deficiency in humans with similar underlying conditions.

Methods

Eight week-old male NIH Swiss mice were irradiated at 50Gy, and gavaged daily with AA-ORS (300 μL/day) or water as control for 6 days. Zinc absorption was analyzed in different intestinal segments (proximal and distal duodenum, ileum and colon, and mid and distal jejunum) using Ussing chamber flux experiments with Na\(^{2+}\) as isotope. Zn\(^{2+}\) serum levels were measured by Inductively Coupled Plasma Atomic Emission Spectroscopy, and western blot (WB), RT-PCR and immunohistochemistry (IHC) were used to determine ZIP4 gene and protein expression, and histological location in ascribed intestinal segments. One-way ANOVA was used for statistical analysis, and P≤0.05 was considered significant.

Introduction

AA-ORS enhances Zn\(^{2+}\) absorption by re-establishing the expression of ZIP4 on villus epithelial cells in intestines of irradiated mice. In addition to improving gastrointestinal function following radiation, AA-ORS can be used to optimize Zn\(^{2+}\) utilization in intestinal pathologies that cause Zn\(^{2+}\) deficiency such as environmental enteropathy, infectious diarrhea or inflammatory bowel disease.

Results

Basal Zn\(^{2+}\) absorption was maximal in mid jejunum (5.3 ± 1.9 μg/cm\(^2\)-h), followed by distal ileum (1.3 ± 0.2 μg/cm\(^2\)-h) and proximal ileum (1.2 ± 0.6 μg/cm\(^2\)-h) in normal mice, and irradiation decreased Zn\(^{2+}\) absorption (Fig. 2; Tab. 1). A similar pattern was seen in ZIP4 protein expression with the highest protein levels present in mid jejunum (Fig. 3). Irradiation decreased ZIP4 protein expression in mid and ileum resulting in decreased Zn\(^{2+}\) absorption, and with AA-ORS resulted in recovery of the values (Tab. 1; Fig. 4). Similarly, reduced ZIP4 mRNA expression increased after treatment with AA-ORS in irradiated tissues (Fig. 5). Histologically, ZIP4 was located in the apical crypt, stored in basolateral vesicles, and minimally expressed in the brush border and basolateral membranes of normal villus and crypt epithelial cells (Fig.1.6). The intracellular ZIP4 staining pattern faded with irradiation, and treatment with AA-ORS resulted in restoration of cytoplasmic ZIP4 staining (Fig. 7). Decreased ZIP4 mRNA levels, protein expression and Zn\(^{2+}\) absorption levels were associated with reduced ZIP4 protein concentrations in irradiated mice (0.8 ± 0.1 vs 1.1 ± 0.03 mg/mL), and values returned to normal after treatment with AA-ORS (Fig. 6).

Conclusions

AA-ORS enhances Zn\(^{2+}\) absorption by re-establishing the expression of ZIP4 on villus epithelial cells in intestines of irradiated mice. In addition to improving gastrointestinal function following radiation, AA-ORS can be used to optimize Zn\(^{2+}\) utilization in intestinal pathologies that cause Zn\(^{2+}\) deficiency such as environmental enteropathy, infectious diarrhea or inflammatory bowel disease.

References


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