Radiation decreased mineral absorption by increasing intracellular secretagogues in the crypt
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BACKGROUND
Ionizing radiation (IR) is known to cause mineral loss, decreasing sodium (Na⁺) and chloride (Cl⁻) absorption while stimulating electrolytic Cl⁻ secretion. However, little is known about the mechanism by which IR decreases mineral absorption. We hypothesize that increased secretagogues in the crypt and/or villus decrease mineral absorption.

MATERIALS AND METHODS
Animal model: NIH Swiss 8-week-old mice were irradiated using ¹³⁷Cs at doses of 0 Gy and 5 Gy. Experiments were performed 6 days following irradiation.
Using chamber: Mice ileal and jejunal sections were mounted in Ussing chambers to measure transepithelial short-circuit current (Isc) conductance (G), and unidirectional Jms & Jsm net fluxes (Jn) of Na⁺ and Zn²⁺.
Immunohistochemistry and western blot: Mice ileal and jejunal tissues were collected to study protein levels and expression of NHE3, Anoctamin-1 (Ano1), and ZIP4.
Villus and crypt isolation: Villus and crypt cells were isolated using a calcium chelation technique.
Intracellular calcium levels: Measured using confocal intracellular Ca²⁺ fluorescence microscopy by loading cells with Fluo-8 AM dye.
Cyclic AMP levels: cAMP levels in villus and crypt were determined using colorimetric direct cAMP immunoassay kit (Calbiochem, EMD Millipore, Billerica, MA).

RESULTS

Fig 1. Net sodium absorption decreased following irradiation (1.8 ± 0.3 μeq.cm⁻².h⁻¹ vs 0.4 ± 0.2 μeq.cm⁻².h⁻¹) P < 0.001; n = 9.

Fig 2. Net zinc absorption in different segments of normal mice small intestine. Proximal duodenum (PD), distal duodenum (DD), middle jejunum (MJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI), proximal colon (PC), and distal colon (DC). Zinc absorption was the highest in mid jejunum (5.33 ± 1.89 μeq.cm⁻².h⁻¹) compared to other segments (P < 0.05); n = 6.

Fig 3. ZIP4 protein expression in different segments of GI tract. Proximal duodenum (PD), distal duodenum (DD), middle jejunum (MJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI), proximal colon (PC), and distal colon (DC).

Fig 4. 5 Gy radiation caused a decreased zinc absorption (10.6 ± 11.3 neq.cm⁻².h⁻¹) in mid jejunum when compared with 0 Gy mice (38.6 ± 10.5 neq.cm⁻².h⁻¹). Similarly, in ileum radiation decreased zinc absorption (-14.5 ± 8.5 neq.cm⁻².h⁻¹) in comparison to control tissue (-11.2 ± 14.4 neq.cm⁻².h⁻¹).

Fig 5. NHE3 and ZIP4 protein levels decreased while ANO1 protein levels increased following radiation in brush border membrane isolated from mouse small intestine.

Fig 6. Representative immunofluorescence and immunohistochemistry staining pattern for ZIP4 in mid jejunum of 0 Gy and 5 Gy irradiated mice. ZIP4 is mainly located in the apical cytoplasm and basolateral vesicles, and expressed in the apical and basolateral membrane of villus and crypt epithelial cells. Radiation decreased ZIP4 staining pattern in villus epithelial cells.

Fig 7. Intracellular cAMP levels in crypt and villus cells: Radiation showed a significant increase in intracellular cAMP levels (13.7 ± 2.4 pmol/mg) in crypt cells from mid jejunum in comparison to 0 Gy mice (5.64 ± 2.5 pmol/mg). Crypt cells from ileum showed no significant changes in cAMP levels. Following radiation, villus cells isolated from mid jejunum showed a decrease in intracellular cAMP.

Fig 8. Intracellular calcium fluorescence: irradiation increased intracellular calcium levels in 5 Gy mice when compared to 0 Gy mice.

Summary
- Irradiation decreased net sodium absorption.
- Isotope flux studies showed that maximum zinc absorption occurred in mid jejunum.
- Western blot analysis using brush border membrane from mouse small intestine showed highest protein levels in mid jejunum.
- Radiation decreased zinc absorption.
- Radiation decreased NHE3 and ZIP4 protein levels and increased ANO1 protein levels.
- Intracellular calcium fluorescence increased in small intestinal cells from 5 Gy irradiated mice with a significant increase in cAMP levels in crypt cells.

Conclusion
Decrease in zinc absorption parallels the secretagogue-induced anion secretion in mouse small intestine following radiation.

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