Select Amino Acids Alter DMT1 Abundance on the BBM of Intestinal Enterocytes and Enhance or Reduce Iron Flux in Duodenal Organ Cultures

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Introduction
- Approximately 1.6 billion people worldwide are anemic, with iron deficiency (ID) as the most common cause.
- Iron overload (IO), most commonly associated with the genetic disease Hereditary Hemochromatosis (HH), is characterized by inappropriate high iron (Fe) absorption, leading to Fe loading in organs and subsequent damage.
- About 1 million Americans have the common HH mutation, which can lead to HH.
- Both conditions warrant better treatment options, as iron supplementation is not always effective for individuals with ID, and current treatments for IO (chelation therapy) are non-specific and have unwanted side effects.
- Our previous studies demonstrated that select amino acids (AA) can influence trafficking of membrane transport proteins in enterocytes and thus influence electrolyte absorption in the gut.
- We used to determine if AAs can influence trafficking of the predominant intestinal iron transporter, duodenal metallic transporter 1 (DMT1), since and off of the duodenal brush border membrane, potentially increasing or decreasing iron absorption, respectively.

Hypothesis
Certain AAs can influence trafficking of the predominant intestinal iron transporter, DMT1, thus modulating intestinal iron absorption.

Methods

Long Studies
- For initial screening of the effect of 4 AAs on DMT1 trafficking, or ex vivo duodenal loops excised from adult mice were filled with a buffer containing individual AAs and incubated at 37°C in an aerated bath for 45 min. Luminal fluid was removed, the duodenal mucosa was scraped, and brush border membrane vesicles (BBMV) were purified. DMT1 protein expression levels were then determined by Western blot analysis.

Formulation Design
- Amino acids were ranked based on how they influenced DMT1 protein expression on the BBM. Two formulations were created that contained mixtures of AAs that either increased or decreased DMT1 protein expression on the BBM.

Using Chamber Flux Studies
- To test the formulations, ¹⁵Na flux studies were performed with mouse duodenal organ cultures. Using chamber technology in the presence of the AA formulations. Studies were conducted in wild-type mice and heterozygous (Hamp⁶⁻⁻) knockout mice (a model of HH in which intestinal Fe absorption is appropriately elevated). Tissues were paired based on conductances, and for each chamber and samples were acquired from the opposite side every 15 min for 60 min. Net flux (¹⁵Na) was calculated by subtracting unidirectional (maximal) (entorhinal) flux from maximal (to-wanel) (absorption) flux.

AA Gavage Study
- Daily oral gavage of control and AA formulations was performed in adult male Hamp⁶⁻⁻ mice for 6 weeks, after a 2-hour evening fast (before dark period). Mice were maintained on an Fe-deficient diet (3.6 ppm Fe). Ex vivo studies (TO23,0302) throughout the study. Iron parameters were assessed after sacrifice including hemoglobin, serum ferritin, non-heme Fe in tissue, DMT1 protein expression, etc.

Statistical Analysis
- Student’s t-test and 1-way ANOVA with Tukey’s multiple comparisons test were performed, when appropriate.

Results

Future Studies
- In vivo experiments in which mice will be weaned on to an Fe-deficient diet for 2 weeks, then replenished with Fe alone or Fe + 4 AA via daily gavage, to determine if the 4 AA formulation causes a more effective repletion.
- In vivo experiments in which Hamp⁶⁻⁻ mice are given control and 5 AA formulations in the drinking water for 3-6 weeks to determine if iron status has improved by assessing serum ferritin, serum iron, and tissue non-heme iron, etc.
- To further test the AA formulations, using chamber ¹⁵Na flux studies will be conducted with human intestinal samples from patients undergoing the Whipple procedure, in collaboration with Shands Hospital at the University of Florida.

Conclusions
- The 4 AA formulation (on-trafficking) increased ¹⁵Na flux by ~4-fold (p<0.05). Interestingly, this AA formulation significantly decreased conductance (p<0.0001), indicating a tightening of the epithelial barrier.
- ¹⁵Na flux studies in duodenal sheets isolated from Hamp⁶⁻⁻ mice showed an approximate 10-fold reduction in iron flux (p<0.05) when exposed to the 5 AA formulation (off-trafficking).
- The 4 AA gavage after 6 weeks in Hamp⁶⁻⁻ mice significantly increased HB (p<0.01) and serum ferritin (p<0.01).
- The AA gavage data shows that diet, AA concentration, duration and frequency of dosing are all important factors to be considered for future in vivo studies.
- These initial findings could lead to the development of specific AA formulations that could be used to mitigate topological changes in intestinal iron transport associated with common disease states.

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References

Figure 1: DMT1 protein expression in the presence of single amino acids. Representative Western Blot images (A) and quantified relative DMT1 protein expression (normalized to Caspase-3) protein first and then normalized to control levels (B) from ileal lysis in adult mice (C).