Select Amino Acids Alter DMT1 Abundance on the BBM of Duodenal Enterocytes and Modulate Iron Flux

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Abstract

Over 1.5 billion people worldwide are anemic, with iron deficiency (ID) as the most common cause. Nevertheless, Hematological Hemorrhage (HH), affects some 1 million individuals in the US. We previously demonstrated that select amino acids (AA) can influence trafficking of membrane transport proteins in enterocytes and thus influence nutrient absorption in the gut. We thus hypothesized that AAs could also influence trafficking of intestinal divalent metal ion transporter 1 (DMT1). For an initial screen of the effect of AAs on DMT1 trafficking, an in vitro duodenal loop was excised from adult mice, filled with a buffer containing individual AAs and incubated at 37°C in an oxygenated bath for 45 min. Subsequently, brush-border membrane vesicles (BBMV) were purified from mucosal scrapes and DMT1 protein expression was determined by immunoblotting. Amino acids were ranked based on how they influenced DMT1 expression on the BBM, and two formulations were made: one that induced DMT1 expression and another that decreased DMT1 expression. To extend these observations to a functional level, the in vitro flux studies were performed with AA-exposed mouse duodenal epithelial cell cultures. For these experiments, initially showed that one AA formulation increased PFe flip by 4-fold. To test the other AA formulation, we utilized a model of iron (Fe) transport in mice, in which intraluminal iron absorption is inappropriate elevated. Importantly, the in vitro flux studies in HAmp™ mice showed a 10-fold reduction in iron transport after exposure to the 4- AA mixture. Furthermore, 6-day Gavage studies were also conducted daily for 6 days with single AAs in WT mice and daily for 6 weeks with 4- AA and 5 AA formulations inAMP KO mice. Additional gavage fluxes are currently underway. We thus conclude that select AAs can influence DMT1 trafficking to and from the BBM and thus alter intestinal iron flux. These findings could lead to the development of specific AA formulations that could be used to mitigate pathological changes in intestinal iron transport.

Hypothesis

Certain AAs can influence trafficking of DMT1, thus modulating intestinal iron absorption.

Methods

Loop Studies

• For initial screening of the effect of AAs on DMT1 trafficking, an in vitro duodenal loop excised from adult mice was filled with buffers containing individual AAs and incubated at 37°C in an oxygenated bath for 45 min. Luminal fluid was released, the duodenal mucosa was scraped, and brush-border membrane vesicles (BBMV) were purified. DMT1 protein expression levels were then determined by Western blot analysis.

6 Day Single AA Gavage Study

• 8-week-old mice were gavaged daily with single AA buffer for 6 days. On day 7, mice were sacrificed and 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, and two formulations were created that contained AAs that either increased or decreased DMT1 expression.

Using Chamber Flux Studies

• To test the formulations, the in vitro flux studies were performed with mouse duodenal organ cultures using Pseudobombyx technology in the presence of the AA formulations. Studies were conducted in wild-type mice and hemizygous (Hamp™) knock-out mice (a model of HH in which intestinal Fe absorption is inappropriately elevated). Tissues were paired based on conductance, and Fe was added to one side of the chamber and samples were acquired from the opposite side every 15 minutes for 60 minutes. Net fluxes were calculated by subtracting serum-to-mucosal in suctioned flux from mucosal-to-serosal in absorption flux.

3 and 5 week AA Gavage Study

• Daily oral gavage of control and AA formulations was performed in adult male Hamp™ mice for 3 and 6 weeks, after a 2-hour fasting period (before the dark period). Mice were maintained on an ad libitum diet (20% protein, Envigo TD.121200S) throughout the study. Iron parameters were assessed after sacrificing including hemoglobin, serum ferritin, non-heme Fe in tissues, and DMT1 protein expression.

Results

• The 4 AA formulation (on-trafficking) increased PFe flux by 4-fold (p<0.05). Interestingly, this AA formulation significantly decreased conductance (p<0.0001), indicating a tightening of the epithelial barrier. 6 Fe fluxes in Hamp™ 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 fluxes in Hamp™ mice revealed no flux differences when AAs is present, suggesting iron transport via AA-transporters is not occurring (i.e. Fe-α-chelidonic). Loop and 6-day gavage results are consistent among 4 of the AAs in the 5 AA formulation.

• The 4 AA gavage after 6 weeks in Hamp™ mice significantly decreased 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 findings could lead to the development of AA formulations that could be used to mitigate pathological changes in intestinal iron transport associated with common disease states.

Conclusions

Acknowledgements

References