Iron Chlorophyllin Bio-efficacy and Metabolites Following Simulated Digestion and Incubation with Caco-2 Cells

Siqiong Zhong[1], Amanda Bird[1,3], Rachel Kopec[1,2]
[1]Human Nutrition Program (OSUN), Department of Human Sciences, The Ohio State University, [2]Foods for Health Discovery Theme, [3]Department of Molecular Genetics, The Ohio State University

INTRODUCTION

Iron is an essential trace mineral for humans, and is a necessary cofactor for energy metabolism and immune function. Developing countries rely on poorly absorbable plant-based sources of iron (i.e.$FeSO_4$), leading to a higher rate of iron deficiency. Heme iron can be more efficiently absorbed, but is predominantly found in red meat. Iron chlorophyllin (IC) utilizes the base porphyrin structure of plant-based chlorophyll to bind iron (**Figure 1**). As a heme analogue, IC has previously been shown to survive digestion, and to be capable of delivering iron to human small intestinal cells.[1] However, the dose-response of increasing concentrations of IC has not been evaluated.

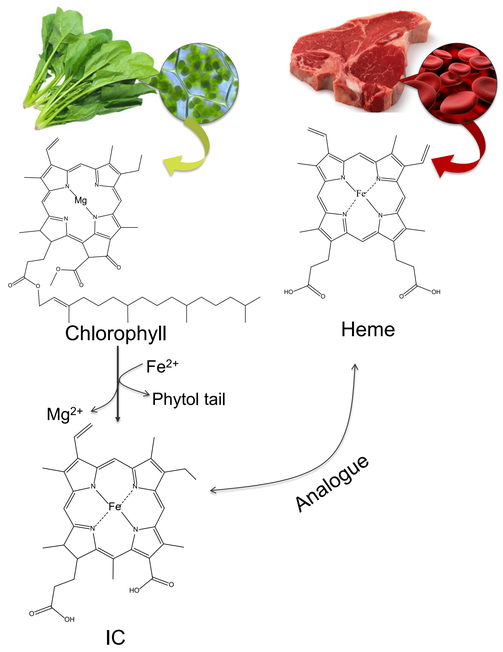

Figure 1. Structure of chlorophyll, heme and IC.

AIMS

1) To determine whether increasing IC concentrations increase intestinal cell iron in a dose dependent manner.

2) To identify novel IC metabolites observed following simulated digestion and intestinal cell absorption.

METHODS

Experimental Design

- Negative control: water
- Positive control: $FeSO_4$ and hemoglobin
- Doses of dependent testing group: IC (1.5, 8, 34, 81 ppm)

Simulated *in vitro* digestion and Caco-2 small intestinal cell uptake[2] (Figure 2)

Iron concentration analysis

- Furnace atomic absorption spectrometry (AAS)

Statistical analysis

- One-way ANOVA followed by Tukey's-post-hoc test (P <0.05)

Metabolite analysis

- Ultra high-performance liquid-chromatography- diode array-high resolution-time-of-flight mass spectrometry (UHPLC-DAD-TOF)
- Agilent Mass Profiler molecular feature extraction
- Agilent Biotransformation Mass Defects software predicts cell phase I and phase II metabolites

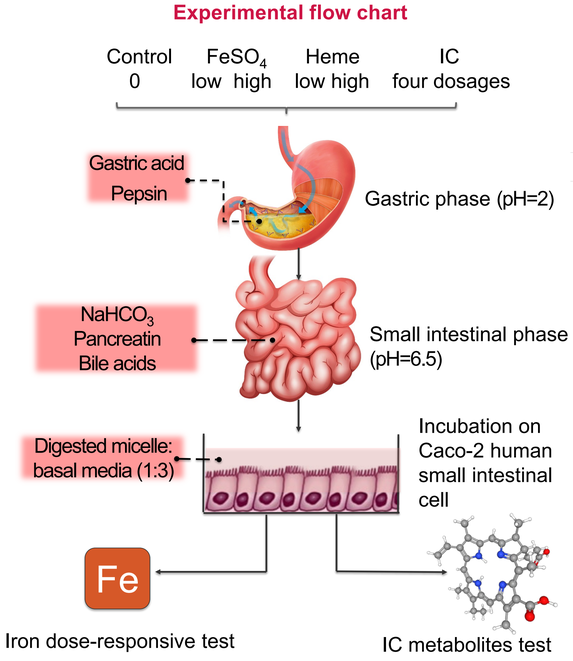

Figure 2. In vitro digestion and Caco-2 small intestinal cell uptake experimental design.

RESULTS

Iron Chlorophyllin bio-efficacy

- Iron concentrations increased through the 8 ppm IC dose, but higher doses did not result in greater concentrations of cellular iron. (**Figure 4**)
- IC delivered as much iron to the cells as heme, and trended toward increased iron delivery relative to $FeSO_4$ (P = 0.068) when comparing across the low dose concentrations. (**Figure 4**)

Iron Chlorophyllin Metabolites

- Following digestion, Fe-chlorin e4 and e6 were totally converted to IC derivatives. (**Figure 3**)
- Dehydrogenated and demethylated IC metabolites were also detected in the cell.

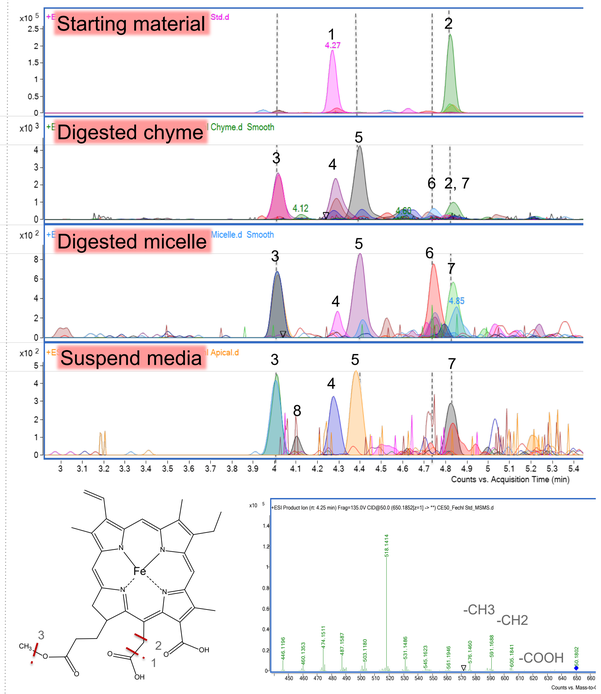

Figure 3. An untargeted MS scan was conducted in +ESI mode. Above are peaks selected out of the total ion chromatogram which had associated peaks in the diode array detector with chlorophyll-like spectra. The iron isotopic pattern was used to identify IC derivatives.

Fe-chlorin e6 and e4 ($[M]^+$=650.1802, 606.1961, peak #1, 2), were the major compounds in the IC starting material. *In vitro* digestion modified IC side chains and formed IC derivatives. Peaks 1 to 8 represent IC identified in different compartments of the *in vitro* digestion experiment. 1) m/z= 650.1802, 2) m/z= 606.1961, 3) m/z= 620.1753, 4) m/z= 604.1822, 5) m/z= 576.1840, 6) m/z= 610.1876, 7) m/z= 590.1647, 8) m/z= 648.1694. The chemical structure shows MS2 fragmentation patterns.

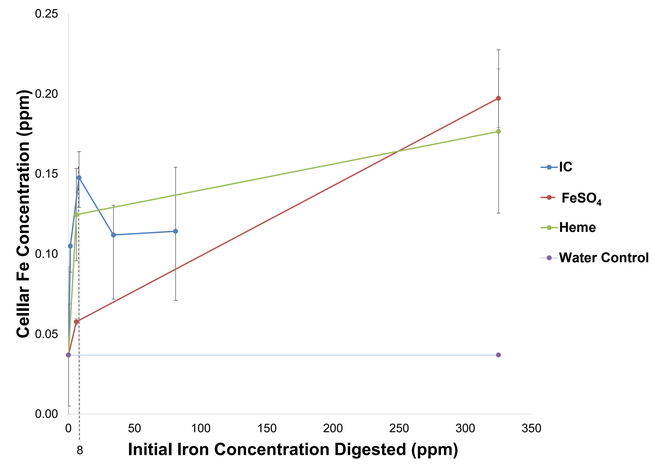

Figure 4. Iron concentration in Caco-2 human small intestinal cells after incubating with IC, $FeSO_4$, heme and deionized water for 4 h. Data points represent the mean, error bars represent standard error of the mean (n=3) at each dose.

CONCLUSIONS

Results suggest that IC may better deliver iron to Caco-2 cells as compared to $FeSO_4$, and should be further explored as a strategy for iron supplementation in those who rely on plant sources for their needs (i.e. vegetarians, those in developing countries who rely on staple crops for iron).

BIBLIOGRAPHY

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