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The antioxidant activity of chlorogenic acid isomers is positively correlated with capacity to up-regulate nuclear factor-κB (NFκB) signaling in Caco-2 cells

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Statement of the Problem: Chlorogenic acids (CGAs) are the most abundant phenolic compounds present in coffee beans. Major chlorogenic acids (CGAs) in coffee include; caffeoylquinic acid (3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA)) and dicaffeoylquinic acid (3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), 4,5-dicaffeoylquinic acid (4,5-diCQA)). In this study, we measured the antioxidant activity of major CGA isomers using chemical and cell-based antioxidant assays. Furthermore, we explored the relationship between the antioxidant activity capacity with affinity to modulate nuclear factor-κB (NFκB) signaling in an in vitro human intestinal inflammation model.

Methodology & Theoretical Orientation: The antioxidant activity of CGA isomers were evaluated by ORAC assay and intracellular oxidative assay in Caco-2 cells. Then the capacities of CGA isomers in modulating reactive oxygen species (ROS) and nuclear factor-κB (NFκB) signaling were studied in an in vitro human intestinal inflammation model which is the differentiated Caco-2 cells treated with a cocktail of human interferon and phorbol 12-myristate 13-acetate (IFN+PMA).

Findings: The results obtained from ORAC assay showed that antioxidant capacity of 5-CQA was 3.5±0.1 mM Trolox Equivalent/mmol, similar to 4-CQA and 3-CQA, respectively; whereas 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA all had significantly higher capacity to scavenge peroxyl radical as evidenced by higher ORAC values ($P<0.05$). The relative antioxidant activity of CGA isomers in AAPH challenged Caco-2 cells was also determined. In this assay. AAPH was used to generated ROS and initiate oxidative stress in Caco-2 cells, with the DCFH-DA probe used as the measure for quantifying intracellular ROS. A dose-response reduction in fluorescence intensity was observed for all six CGA isomers. At low (2 mM) equimolar concentrations tested, caffeylquinic acids was ineffective at suppressing fluorescence intensity, whereas dicaffeylquinic acids suppressed around 20% fluorescence intensity compared to the control. Increasing molar concentrations by 10-fold showed caffeylquinic acids and dicaffeylqunic acids to reduce 40% and 60% AAPH induced ROS, respectively, compared to the control ($P<0.05$). Differentiated Caco-2 cells treated with IFN+PMA in vitro was used to study human intestinal inflammation. An increase in ROS level was observed in cells exposed to IFN+PMA compared to cells without any treatment (blank), indicating that inflammation status was accompanied with an increased ROS generation. Pre-incubation with CGA for 24 hours before IFN+PMA treatment significantly reduced ROS generation compared to the cells without CGA treatment but with IFN+PMA exposure. This result suggested that CGA isomers alleviated oxidative stress in inflamed Caco-2 cells. To further investigate the mechanisms behind the intracellular antioxidant of CGA isomers in IFN+PMA treated Caco-2 cells, the cytosolic fraction and nuclear fraction of were extracted and separated and the amount of p65 (the subunit of NFκB) was quantified by ELISA in the nuclear fraction. The result showed that 24 hour pre-incubation with CGA before IFN+PMA treatment significantly increased p65 nuclear translocation, when compared to the control cells ($P<0.05$). This result indicates that CGA isomers can up-regulate NFκB signaling in inflamed Caco-2 cells.

Conclusion & Significance: CGA isomers showed free radical scavenging capacity both in a cell free and cell based assays using either AAPH or IFN+PMA to challenge Caco-2 cells. In addition, induced oxidative stress corresponded to up-regulation of NFκB signaling in IFN+PMA challenged Caco-2 cells. Hence, antioxidant capacity of chlorogenic acid isomers is positively correlated with affinity to up-regulate nuclear factor-κB (NFκB) signaling in human intestinal cells. We conclude that CGA isomers exert intracellular antioxidant activity through both direct free radical scavenging action and indirect modulating inflammatory pathways.

Biography

Ningjian Liang received her bachelor degree in Biology Engineering from China Agriculture University in 2009. Then she went to the University of Hawaii at Manoa and got her master degree in Food Science under the supervision of Dr. Yong Li. During the master thesis program, Ningjian invented a novel detection method to specifically detect viable *Salmonella Typhimurium* in vegetables. After completing her master degree, Ningjian joined Dr. David D. Kitts' lab at the University of British Columbia to pursue the Ph.D in Food Science. Ningjian's research focuses on the effects of bioactive compounds in coffee on modulating oxidative and inflammatory responses in human intestine

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