Cyanidin-3-O-glucoside modulates intestinal inflammatory response induced by TNF-α: an approach based on in vitro epithelial and epithelial-endothelial co-culture models

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Inflammatory Bowel Diseases (IBD) are chronic intestinal inflammatory disorders characterized by an excessive release of pro-inflammatory mediators, intestinal barrier dysfunction and altered permeability, and excessive activation of NF-κB cascade. IBD conventional therapies are insufficiently selective and associated to severe side effects. Phenolic compounds are considered to possess antioxidant and anti-inflammatory activities and are a great hope in prevention and treatment of chronic intestinal inflammation. In fact, the discovery of specific genes regulated by the Antioxidant Responsive Element (ARE) affected by antioxidants/electrophiles, led to the hypothesis that some phytochemicals may act as modulators of signal transduction pathways.

The aim of the present study was to evaluate the beneficial effects of Cyanidin-3-O-glucoside (C3G), an anthocyanin widely distributed in mediterranean diet, and the underlying mechanisms of action, in an vitro model of acute phase of intestinal inflammation using differentiated Caco-2 cells exposed to the proinflammatory cytokine TNF-α. Caco-2 cells exposure to TNF-α for 6 h activated IKK/NF-κB proinflammatory pathway, and induced COX-2 and IL6 expression. Interestingly, cells pretreatment for 24 h with C3G (20-40 μM) was effective in preventing TNF-α-induced changes. Our results also demonstrated that C3G improved intracellular redox status altered by TNF-α, and activated Nrf2/ARE pathway, at baseline and after TNF-α treatment. C3G increased Nrf2 nuclear translocation, and HO-1 and NQO1 expression and these effects can be associated to NF-κB pathway inhibition.

Epithelial cells are positioned in close proximity to various cell types, which places them in the unique position of providing signals to neighbouring cells located in the underlying mucosa, e.g. endothelial cells (EC), thereby possibly influencing the immunological response of the gut. A non-contact coculture system was used to investigate whether Caco-2 cells activated with TNF-α were able to provide signals that can induce EC dysfunction, and if C3G was able to prevent Caco-2-induced EC activation. Our results demonstrated that coculture of human umbilical vein endothelial cells (HUVECs) with TNF-α-stimulated Caco-2 cells led to a significant up-regulation of endothelial VCAM-1 and E-Selectin expression, accompanied by an increase in EC nuclear NF-κB p65 accumulation. Such evidence suggests that epithelial cells might activate neighboring EC to support leukocyte recruitment. Caco-2 pre-treatment with 20 μM C3G was effective in preventing EC adhesion molecules expression, and NF-κB p65 nuclear localization. Furthermore, inhibition of endothelial NF-κB activation using the IKK inhibitor wedelolactone resulted in a similar significant decrease of adhesion molecules expression. These data support the hypothesis that Caco-2 cell-produced factors exert their effects on endothelial dysfunction through activation of the NF-κB signaling pathway.

In conclusion, our data suggest that C3G may have protective effects against TNF-α-mediated intestinal mucosal damage acting as cell signaling modulator and inducing activation of the Nrf2/ARE pathway. Furthermore, inhibition of epithelial cells inflammation can protect from epithelial-induced EC activation, thus preventing the colonic inflammatory response and the subsequent immune cell recruitment during inflammation.