In Vitro Protective Effects of Cyanidin-3-O-glucoside on Human Intestinal Epithelial Cells Exposed to Palmitic Acid

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The intestinal mucosal barrier plays an important role in the body's protection against luminal pathogens and antigenic molecules. A dysregulation of this, resulting in a paracellular permeability alteration, can lead to severe intestinal disorders, including inflammatory bowel diseases (IBD), the collective name for Crohn's disease and ulcerative colitis, characterized by symptoms such as weight loss, diarrhoea, rectal bleeding, abdominal pain, fever, and anemia. Recently, epidemiological studies reported obesity and metabolic syndrome as risk factors in IBD development. Increased serum free fatty acids (FFA) or adipokines and cytokines, taken up into the enterocyte via the basolateral membrane, are supposed to be potential modulators of intestinal inflammation. In particular, increased FFA uptake is correlated to conspicuous morphological changes in the intestinal cells and also to release of certain cytokines and chemokines that activate and recruit immune cells. However, the mechanisms by which fatty acids influence intestinal inflammation remain unclear and it represents a research focus in pathogenesis of IBD. It has been suggested that FFA promote pro-inflammatory cytokines via metabolism of arachidonic acid with resultant increased derivatives (PGE2, TBXs, etc), altered cell membrane fluidity and protein binding capability, and activation of NF-κB transcription factor and its nuclear targets. Furthermore, FFA can affect the mitochondrial generation of reactive oxygen species (ROS) so inducing intracellular oxidative stress.

Recent studies support beneficial effects of anthocyanins, a class of flavonoid compounds widely distributed in mediterranean diet, in various chronic inflammatory diseases, such as IBD. It has been reported that anthocyanins are able to inhibit the release of proinflammatory cytokines, acting via reduced NF-κB expression and translocation, and finally to modulate apoptosis and oxidative stress via activation of cellular adaptive responses triggered by the Nrf2 transcription factor.

The aim of this work was to determine some of the intracellular mechanisms involved in fatty acid modulation of intestinal epithelial inflammation by using an in vitro intestinal epithelial system consisting of filter grown Caco-2 monolayers and the effects exerted by cyanidin-3-O-glucoside (C3G) pretreatment. Caco-2 cells basolateral exposure to palmitic acid (PA) for 6 h activated NF-κB proinflammatory pathway, and induced IL8 gene expression. Interestingly, cells pretreatment for 24h with C3G (20 μM) was effective in preventing PA-induced changes. Furthermore, C3G was able to improve cellular redox status altered by PA by reducing intracellular ROS. Furthermore, PA exposure for 6 h induced Caco-2 cells apoptosis, as confirmed by reduced Bcl2/Bax ratio. Also in this case, C3G pretreatment prevented the activation of apoptosis in Caco-2 cells probably by modulating intracellular redox status.

In conclusion, C3G showed anti-inflammatory properties through the modulation of NF-κB pathway and improved intracellular redox status altered by PA in Caco-2 cells. Moreover, C3G reduced apoptosis induced by PA that seems to be a new relevant therapeutic mechanism in IBD resolution. These data suggest that anthocyanins could contribute, as complementary approaches to the conventional already existing therapeutic approaches (i.e. non-steroidal anti-inflammatory drugs) to the management of IBD.

Keywords: Inflammatory Bowel Diseases, inflammation, free fatty acid, NF-κB,

oxidative stress.