ANTHOCYANIN RESEARCH
THE EFFECT OF ANTHOCYANIN INTAKE ON INTESTINAL CELLS IN VITRO

The peer-reviewed study results summarized below were published in the journal *Food & Function* in 2017.

BACKGROUND

Anthocyanins are water-soluble compounds responsible for many of the red to blue-violet pigments found in plants and fruits like blueberries, bilberries, black rice, and black currants. In the past, anthocyanins were only known as colorants, but current research indicates they in fact have numerous health benefits.

Anthocyanins are studied for a wide array of benefits, including metabolic health. There are many metabolic factors that are affected by anthocyanins in ways that are similar to the effects of active lifestyle, which is known for health improvements in many tissues throughout the body. These metabolic changes can have positive effects on the body and impact other important mechanisms including gut health, metabolism, and inflammatory balance. One of the ways anthocyanins are thought to provide health benefits is through their impact on the gastrointestinal tract. Therefore, we began our research with intestinal cells.

The intestinal epithelial cells are single-layered cells that constitute the largest and one of the most important physical barriers against the external environment. These cells act as a selectively permeable barrier that allows good things like nutrients and water in, while defending against toxins, oxidants, and other damaging compounds. These cells are linked by special proteins including tight junction proteins that hold these cells together. If these cells are damaged by known disrupters such as inflammation, it can cause increased permeability of these cells, which leads to increased passage of damaging substances to the body and contributes to adverse consequences. The effect of anthocyanins on intestinal cells is poorly understood, so this study was carried out to better understand the potentially protective and beneficial qualities of anthocyanins.

METHODS

As an initial step to determine the impact of anthocyanins on intestinal health, we investigated the capacity of different types of anthocyanins *in vitro* using Caco-2 cells. Caco-2 cells are widely used as a model of intestinal health. In this study, they were differentiated into intestinal epithelial cells. The purpose of this study was to determine if anthocyanins can effectively protect against inflammation-induced increased permeability. The cells were treated with tumor necrosis alpha (TNF-α) as a source of inflammation which causes increased permeability, and then permeability was assessed by measuring changes in transepithelial electrical resistance (TEER) and paracellular transport of fluorescein isothiocyanate (FITC) dextran. TEER is the measurement of electrical resistance across the cell as a reliable method to confirm the integrity and permeability of the cell, while FITC dextran is a substance used to measure the transport or passage of this compound from one side of the epithelial cell to the other. TNFα causes a significant decrease in TEER and an increase in FITC-dextran paracellular transport. Both methods are important to determine the protective effect of anthocyanins against inflammation-induced permeability.

RESULTS

Different anthocyanins were analyzed for their chemical structures and spatial configurations in order to assess the effects of the pure anthocyanins tested (Figure C). Cyanidin, delphinidin, malvidin, peonidin, and petunidin were all tested, as they are major sources of anthocyanins in the human diet. The results showed that cyanidin and delphinidin both inhibited the TNF-α-induced decrease in TEER. On the contrary, malvidin, peonidin, and petunidin mostly did not protect against the TNF-α-induced TEER decrease (Figure A).

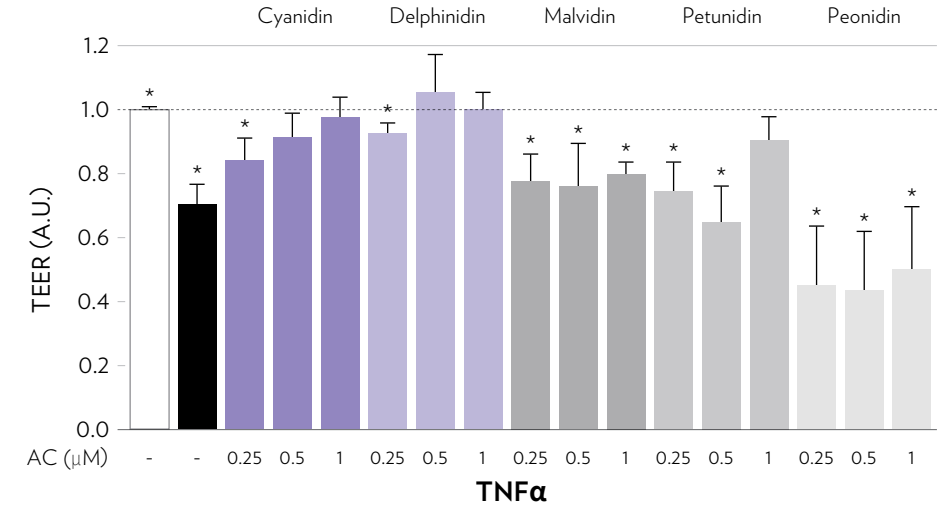

Figure A

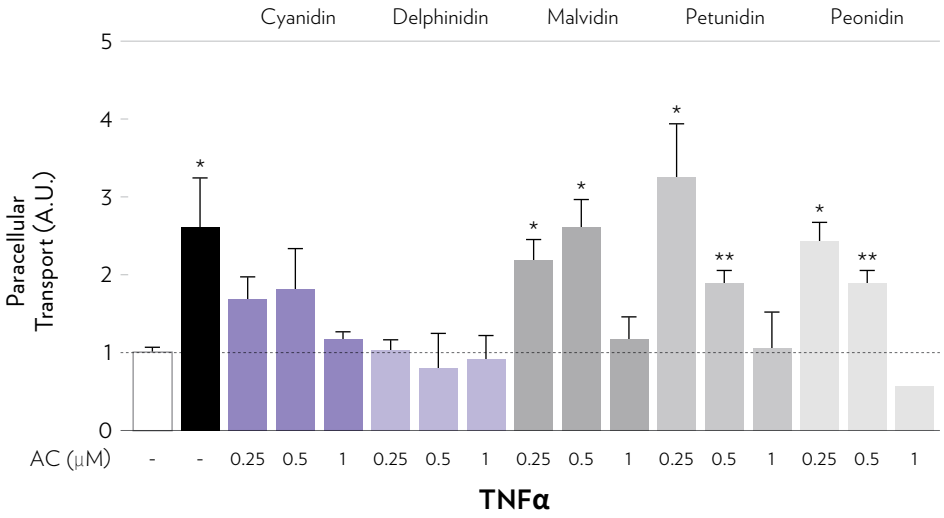

Figure B

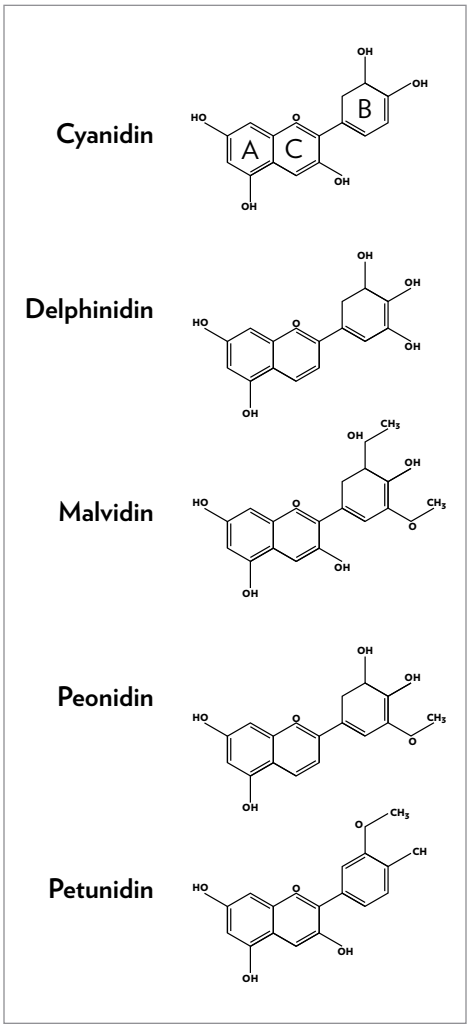

Figure C

Both cyanidin and delphinidin inhibited the increase in FITC transport induced by TNF-α at all concentrations tested. However, petunidin, peonidin, and malvidin had no protective effects at the lower dose tested and had various degrees of protection at the higher doses tested (Figure B).

After analyzing the effects of pure anthocyanins, anthocyanin-rich extracts were tested on TNF-α-induced permeabilization of Caco-2 cells. Anthocyanin-rich extracts had different amounts of total and individual anthocyanins. Of these anthocyanin-rich extracts, black chokeberry, black rice, and domesticated blueberry extracts most effectively inhibited TNF-α-induced alterations on TEER, whereas black rice, bilberry, and crowberry extracts most effectively inhibited TNF-α-induced alterations on FITC-dextran paracellular transport.

We did further analysis to evaluate the contribution of the anthocyanins present in these anthocyanin-rich extracts on the protective effects on cell barrier integrity. Cyanidin and delphinidin had positive correlations between TEER and anthocyanins, yet conversely, no significant correlations were found between TEER and the total anthocyanins. This finding indicates that anthocyanins differentially protected Caco-2 cells from inflammation-induced permeability, and that cyanidin- and delphinidin-rich extracts had the best results in this study.

DISCUSSION

The major finding of this study is that the protective action of anthocyanins on intestinal epithelial cells depends on their unique chemical structure. Cyanidin and delphinidin were selectively stronger compared to malvidin, peonidin, and petunidin in the prevention of TNF-α-induced loss of TEER and increase of paracellular transport. The most effective extracts in protecting Caco-2 cells from TNF-α-induced TEER loss were those containing the highest cyanidin and delphinidin content. This discovery motivated the formulation of a product focused on anthocyanins.

In summary, this study shows that cyanidin and delphinidin anthocyanins are highly effective in protecting intestinal epithelial cells against TNF-α-induced barrier permeabilization. Their action was observed within a range of concentrations that would be realistically expected in the gut after consumption of anthocyanin containing foods. These findings support the concept that consumption of berries, plants, or supplements containing anthocyanins, especially cyanidins and delphinidins, can provide beneficial health effects.

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REFERENCE

Cremonini E, Mastaloudis A, Hester SN, et al. Anthocyanins inhibit tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity. Food Funct. 2017;8(8):2915-2923. doi:10.1039/c7fo00625j.

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