Anthocyanins as inflammatory modulators and the role of the gut microbiota

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Abstract

The health benefits of consuming fruits that are rich in polyphenols, especially anthocyanins, have been the focus of recent in vitro and in vivo investigations. Thus, greater attention is being directed to the reduction of the inflammatory process associated with the intestinal microbiota and the mechanism underlying these effects because the microbiota has been closely associated with the metabolism of these compounds in the gastrointestinal tract. Further interest lies in the ability of these metabolites to modulate the growth of specific intestinal bacteria. Thus, this review examines studies involving the action of the anthocyanins that are present in many fruits and their effect in the modulating the inflammatory process associated with the interaction between the host and the gut microbiota. The findings of both in vitro and in vivo studies suggest a potential antiinflammatory effect of these compounds, which seem to inhibit activation of the signaling pathway mediated by the transcription factor NFκB. This effect is associated with modulation of a beneficial gut microbiota, particularly an increase in Bifidobacterium strains.

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1. Introduction

The gastrointestinal tract is the first organ exposed to components of the diet. Thus, its functionality, integrity and modulation of the complex microbiota could have important implications for local and systemic health [1,2]. As recently proposed, intestinal microbiota modification can either attenuate or contribute to the inflammatory process [3], weight gain [4], adiposity and metabolic disorders [5,6].

Unfavorable changes in the microbiota may lead to the development of dysbiosis and damage in colonic barrier integrity, causing increased translocation of bacterial products, such as the lipopolysaccharide (LPS), a component from membrane surface of the gram-negative bacteria and results in Toll-like receptor 4 (TLR4)-mediated inflammatory responses involving the activation of the transcription factor nuclear factor NFκB [7–11].

Accordingly, the intestinal microbiota is now widely studied, with topics ranging from its influence on the preservation of the intestinal barrier and its possible association with the risk of developing various diseases [1,12].

Moreover, recent strategies for reducing the inflammatory process involve the use of phenolic compounds [13,14]. Phenolic compounds are phytochemicals that are found in a variety of fruits and vegetables because they are secondary metabolites of plants. These compounds include simple phenols or polyphenols [15]. Polyphenols are a large group of compounds characterized by the presence of various hydroxyl groups in aromatic rings. These compounds are divided into two main categories: nonflavonoid and flavonoid compounds; the latter form an important class because of their wide distribution in plants [16].

In this review, we highlight pigments in the flavonoid family, anthocyanins, which are responsible for the red, blue and purple colors of fruits and vegetables [17]. These compounds have high antioxidant capacity [18,19] and play an important role in modulating the inflammation related to chronic diseases [20–23].

It is speculated that the antiinflammatory effect of this class of phytochemicals involves mechanisms related to their intestinal functions and their interaction with the intestinal microbiota. Thus, polyphenols might promote intestinal colonization by specific groups of bacteria because the microbiota plays an important role in the metabolism of these compounds in the gastrointestinal tract [13,14]. Thus, we investigate the effect of anthocyanins in the inflammatory process through its interaction with the complex intestinal microbiota.

2. Inflammatory process mediated by LPS and TLR4

The low-grade chronic inflammation involved in the pathophysiology of inflammatory and metabolic diseases has been associated with a condition known as endotoxemia, which is characterized by increased levels of circulating LPS [7–11,24]. The LPS can reach the blood circulation through the intracellular transport facilitated by the chylomicrons, by binding TLR4 expressed on the apical surface of enterocytes and/or through paracellular intestinal epithelium due to impaired tight junction (TJ) integrity [42–44]
During the intracellular transport involving chylomicrons, LPS is internalized by intestinal epithelial cells and transported to the enterocyte Golgi apparatus, where chylomicrons are stored prior to release to the circulation [25–27].

Researchers have reported that high-fat diets can change the TJ, increasing the gut permeability to high-molecular-weight molecules, such as LPS, possibly involving modification in the intestinal microbiota composition [25,26]. Thus, high-fat diets are associated with increased translocation of LPS, both through increased intestinal permeability and increased release of chylomicrons [11,25–27] (Fig. 1).

LPS plays a key role in the relationship between gut microbiota changes, inflammation and metabolic disorders. Consequently, studies linking chronic low-grade inflammation to obesity indicate that the proportion of the two main phyla bacteria differs in obese individuals. This change seems to alter intestinal permeability by increasing the absorption of LPS, leading to increased activation of inflammatory pathways and impaired insulin signaling. Obese individuals appear to have a lower ratio of gram-negative Bacteroidetes (e.g., species of Bacteroides) to gram-positive Firmicutes (e.g., \textit{Lactobacillus}, \textit{Clostridium}, \textit{Bacillus} and \textit{Mycoplasma}) [26,28].

Under normal conditions, low serum concentrations of LPS are detected, but LPS is essential for modulating the immune system. However, at high concentrations, the LPS forms a complex containing LPS binding proteins and the CD14 coreceptor, recognized by TLR4 that triggers intracellular signaling [8,9].

Toll-like receptor TLRs belong to a family of type I transmembrane receptors comprising at least 11 types in humans and 13 types in rodents, and TLRs are an important class of pattern recognition receptors, which are present in different types of cells, such as epithelial and immune cells. These cells are both involved in the tolerance to the complex microflora residing in the gut and in the induction of inflammatory responses against pathogens [8,11,29].

TLRs play a crucial role in the recognition of invader pathogens and the activation of immune responses that protect the host, ranging from the recognition of a variety of pathogen-associated molecular patterns, among which are the LPS, peptidoglycans and lipoproteins [29–31].

In particular, TLR4 is a subtype of TLRs that can be activated by LPS and nonagonist bacterial compounds, such as saturated fatty acids, which in turn activate the NF-\kappaB and the production of proinflammatory cytokines, including IL-6, IL-\beta and TNF-\alpha [8,24,31].

The TLR4 signaling pathway involves phosphorylation and ubiquitination reactions of several adapter proteins. Briefly, after activation, TLR4 dimerizes itself and can induce two different signaling pathways, one myeloid differentiation factor 88 (MyD88)-dependent pathway and one MyD88-independent pathway, which induce the production of proinflammatory cytokines and interferon type 1, respectively (Fig. 2) [8,9].

In the dependent pathway, MyD88 is recruited to activate phosphorylation of interleukin receptor-associated kinases IRAK1 and IRAK4, which in turn activates the tumor necrosis-associated factor TRAF-6 adapter protein. TRAF-6 forms a complex with enzymes involved in the ubiquitination process, activating transforming growth factor beta activated kinase 1, which then phosphorylates the inhibitor kinase complex IKK\beta, triggering the decoupling of NF-\kappaB in NF-\kappaBp50 and NF-\kappaBp65 dimers through degradation of its inhibitory protein IkB. Therefore, NF-\kappaB translocates into the nucleus and controls the expression of an array of inflammatory cytokine genes [8,29].

3. Gut microbiota, intestinal permeability and the inflammatory process

The gut microbiota is characterized by several types of commensal and symbiotic microorganisms, including bacteria, which are located at the host mucosal surface. The mammalian gastrointestinal tract,
particularly its distal portion, holds a microbial community known as the gut microbiota, which is involved in the regulation of gut physiology and morphology [12,32] and in a variety of intestinal functions, such as the production of short-chain fatty acids, folate and vitamin K [32,33].

In healthy subjects, there are 10 times more bacteria in the gut microbiota relative to the number of cells in the body; these bacteria are distributed over 1000 species that contribute to gastrointestinal homeostasis [34,35]. All these microorganisms produce a combined genome, known as the microbiome, which exceeds the human genome by approximately 100 times [36,37].

Evidence shows that age, diet, use of antibiotics and probiotics affect the composition of the intestinal microbiota [1–6]. This microbiota plays an important role in the integrity of the colonic barrier, the translocation of LPS and the inflammatory response [39–41]. In addition, the microbiota performs key functions in regulating different host metabolic pathways [6,7,38–40].

The lumen provides an intestinal barrier against microorganisms and food antigens, and this function is largely performed by TJ. TJ is formed by a complex of proteins localized at the apical side of epithelial cell membranes, such as claudin and occludin; this complex is associated with peripheral membrane proteins, which are known as zonula occludens (ZOIs) and are involved in the regulation of paracellular permeability [42–44].

The ZOs have three isoforms (ZO-1, ZO-2 and ZO-3) that interact with occludin and claudin proteins; this interaction governs the formation and maintenance of TJ. Functional analyses indicate a specific structural function for ZO-1, which is an important molecule in the formation of TJ and affects cell barrier properties [41,45].

Caricilli et al. suggested that microorganisms, particularly Bifidobacterium strains, could regulate ZO-1 expression [46]. In fact, Cani et al. found that higher expression of proteins involved in the TJ was associated with the presence of Bifidobacterium spp. in the rodent intestine [40]. However, pathogenic bacteria and proinflammatory cytokines may alter the structure of these proteins and thereby impair the function of the intestinal barrier, increasing the LPS translocation to the circulation [26,47,48].

Cani et al. demonstrated that the modulation of intestinal bacteria caused by the consumption of a high-fat diet increases the intestinal permeability by reducing the expression of genes that encode TJ proteins, such as occludin and ZO-1 [39]. Kim et al. also reported significantly lower expression levels of claudin-1 and occludin in response to impaired intestinal permeability following the administration of a high-fat diet in mice over a period of 8 weeks [23].

In addition, Cani et al. showed that the high-fat diet increased endotoxemia and the gene expression of proinflammatory cytokines (TNF-α and IL-1β) in visceral adipose tissue; these changes were accompanied by a decrease in Bifidobacterium in the mouse intestine. The authors suggested that Bifidobacterium levels are negatively correlated with the endotoxemia caused by a high concentration of LPS [10].

Regarding Lactobacillus, different species can have a distinct effect on intestinal permeability, inflammation, body weight and adiposity [49,50].
A metaanalysis of 17 randomized clinical trials in humans, 51 studies in farm animals and 14 experimental models showed that *Lactobacillus fermentum* and *Lactobacillus invernue* were associated with weight gain in animals and that the use of *Lactobacillus acidophilus* resulted in significant weight gain in both animal and human studies. In contrast, *Lactobacillus plantarum* was associated with weight loss in animals, and *Lactobacillus gasseri* was associated with weight loss in both humans and obese animals [28].

A variety of data support the importance of diet and its influence on the composition of the intestinal microbiota in animals and humans [1, 11–14, 38–40, 51]. A diet, particularly rich in fat and low in fiber, has been associated with overgrowth of pathogenic microorganisms, causing an imbalance known as intestinal dysbiosis [5–8]. Studies have shown that dysbiosis may be responsible for increased endotoxemia, which induces the chronic low-grade inflammation involved in the pathophysiology of diseases, such as diabetes and obesity [8–12, 26].

In this sense, treatment with beneficial bacteria strains, including *Bifidobacterium* spp, and *Lactobacillus* spp, has been associated with the control of insulin resistance, obesity and/or hepatic steatosis in rodents fed a high-fat diet [52–55]. The greater presence of *Bifidobacterium* has been inversely correlated with the development of fat mass, glucose intolerance, adipose tissue inflammation and LPS levels [10,56].

Wang et al. also demonstrated the benefit of two species of *Lactobacillus* and *Bifidobacterium* for alleviating the inflammation and obesity induced by a high-fat diet; these benefits have been associated with the modulation of rodent intestinal microbiota [57].

The use of probiotics, such as *Lactobacillus rhamnosus* GC, is associated with beneficial effects on intestinal epithelial cells and a proinflammatory response. *In vitro* studies have shown that *L. rhamnosus* reduces the negative effects of proinflammatory cytokines on the integrity of the epithelial barrier, in part through inhibition of NF-κB signaling [49]. Orlando et al. also reported that the administration of *L. rhamnosus* in cell culture was able to restore paracellular permeability, affecting the expression of different TJ proteins, particularly ZO-1 [58].

Moreover, literature reports indicate that *Lactobacillus reuteri*, *L. rhamnosus*, *L. planatarum*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *B. longum* subsp. *infantis* have a potential anti-inflammatory effect [24,49,58,59]. In fact, the gut microbiota was connected to the anti-inflammatory functions performed by intestinal macrophages through the induction of anti-inflammatory cytokine IL-10 production, which is important for maintaining gastrointestinal tract homeostasis [60].

In a model of the human colon microbiota that used different *Lactobacillus* and *Bifidobacterium* strains, the administration of *B. bifidum* and *L. rhamnosus*, among others, reduced the LPS concentration, indicating an ability to decrease TNF-α [24]. Another study conducted by D’Argenio et al. showed that treatment with a specific symbiotic formulation involving *Lactobacillus paracasei* promoted the attenuation of inflammation and hepatic fibrosis in rodents [50].

Overall, the literature clearly indicates a role of *Lactobacillus* and *Bifidobacterium* in the modulation of inflammation, possibly through changing the intestinal permeability by increasing the TJ protein expression.

### 4. Anthocyanins

Anthocyanins are water-soluble pigments that are widely distributed in flowers and fruits, and they can be used as possible alternatives to artificial food colorants. These pigments are glycosides of anthocyanidins; aglycone possesses a fundamental skeleton of 2-phenylbenzopyrylium, which is known as the flavylum cation [17,18,61–63].

More than 90% of all anthocyanins isolated in nature are based only on the following six anthocyanidins (Fig. 3): cyanidin (cyd), pelargonidin (pld), peonidin (pnd), delphinidin (dpd), petunidin (ptn) and malvidin (mvd), which are differentiated by the substitution pattern on the B ring. Depending on the degree of hydroxylation and methylation, the dominant anthocyanin color varies from orange (pelargonidin) to violet (delphinidin). Anthocyanins contain one or more monosaccharide units, which most commonly occur at position 3, sometimes at position 5 and rarely at position 7. The most common sugar moieties are glucose, galactose, rhamnose and arabinose. Glycosylation confers solubility and a certain degree of stability to these compounds and is sometimes accompanied by acylation [17,64].

In addition to the coloring properties of anthocyanins, their bioactive properties have caused the growing interest in the consumption of anthocyanin-rich foods. Quantities of 200 g of eggplant or black grapes can provide up to 1500 mg of anthocyanins and 100 g portions of berries can provide up to 500 mg [65]. Brazilian berries, such as açai (*Euterpe oleracea* Mart.), and jucara (*Euterpe edulis* Mart.), can provide between 239 and 409 mg of anthocyanins per 100 g [61–63].

The most widespread anthocyanin in foods is cyanidin (50%) and then pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%) and malvidin (7%) [17]. The average daily intake in Europe has been 19.83 mg/day in Holland and 64.88 mg/day in Italy for men. Among women, the observed consumption ranged from 18.73 mg/day in Spain to 44.08 mg/day in Italy [66]. Already in Finland, the consumption was estimated to be 82 mg/day, with the main sources being berries, red wine, juices and the coloring agent E163 [65].

Several food processing and storage factors, such as pH, temperature, light, oxygen and enzymes changes, can lead to degradation and can influence the color and stability of anthocyanins. The interactions with food components, such as ascorbic acid, metal ions and sugars, can also cause these changes. Nevertheless, some alternatives such as freeze drying or encapsulation by a spray dryer allow minimization of nutritional and sensory losses and increase the shelf life [67,68].
5. Anthocyanins: Interaction with microbiota and inflammatory process

Studies indicate an important role of anthocyanins in the modulation of inflammation and oxidative stress and in reducing the risk of developing chronic diseases [14,20–23]. Indeed, a high antioxidant capacity is attributed to anthocyanins, mainly due to their phenolic structure; these compounds act by inhibiting or decreasing free radicals by donating or transferring electrons from hydrogen atoms [19,67]. The potential of anthocyanins for lowering the risk of disease development is also attributed to its antiestrogenic, antiinflammatory and cell proliferation inhibitor effects [14,20,21,69] and more recently to its reduction of lipid accumulation during adipocyte differentiation [22,70].

A study by Song et al. demonstrated that cyanidin 3-glycoside exerted a protective role against oxidative stress. The authors found inhibition of the cytoxicity induced by acrylamide and decreased activity of the glutathione-S-transferase and glutathione peroxidase in breast cancer cells [71].

Dietary supplementation with 2% açai pulp improved the oxidative stress biomarkers and lipid profile in the serum of hypercholesterolemic mice treated with high-fat diets, reduced the superoxide dismutase activity and increased the paraoxonase activity [72]. Additionally, anthocyanins have shown an effect in reducing the weight gain and adiposity in animals [22]. In vitro investigations also revealed that anthocyanins influence the function of adipocytokines and thus may play a role in preventing obesity and metabolic syndrome [21,70].

Indeed, anthocyanins can suppress the lipid accumulation in fat cells due to extensive inhibition of transcription factors that regulate lipogenesis [70]. Likewise, experiments using rodents fed for 12 weeks with a high-fat diet showed that supplementation with 40 and 200 mg/kg of anthocyanins isolated from cherries attenuated weight gain by 5.2% and 11.2%, respectively. The authors also reported lower levels of plasma leptin, glucose, triacylglycerol, total cholesterol and LDL cholesterol, as well as reductions in the size of adipose cells and the IL-6 and TNF-α gene expression in this tissue [22].

Another study showed that açai promoted human endothelial cell protection against oxidative stress and LPS-induced inflammation by reducing the expression of inflammatory cytokines (IL-6 and IL-8), inhibiting adhesion molecule expression and NF-κB [73]. Inhibition of the nuclear translocation of NF-κB is an important mechanism of action associated with the antiinflammatory effects of the different anthocyanins present in fruits [20,21] (Fig. 2).

The antioxidant and antiinflammatory activities of anthocyanins have been demonstrated in vitro in macrophages incubated with 0–20 μg/mL of blueberry, blackberry or currants and LPS-induced inflammation. In this study, the antiinflammatory effect of the anthocyanins found in blueberry (mainly malvidin 3-glucoside), blackberry (mainly cyanidin 3-glucoside) or blackcurrant (mainly delphinidin 3-rutinoside) was mediated, at least in part by the inhibiting nuclear translocation of NF-κB p65 subunit [20].

However, Graf et al. have reported that the consumption of 15 mg/day of anthocyanin during a treatment with blueberry and grape juice (80:20) did not affect gut-associated immunity and inflammatory status in healthy rodents. The authors suggested that the antiinflammatory role of the anthocyanins found in several studies may not be a general effect of this compound but the result of the interaction of several factors, including the source, the relationship with diet and the inflammatory status [74].

Moreover, studies have considered the bioavailability of phenolic compounds, specifically anthocyanins, and the participation of the microbiota in the metabolism of these compounds in the colon. Anthocyanins have a rapid biotransformation, reaching a maximum concentration in human plasma at approximately 1.5 h [65]. Hassimotto et al. verified that, in a period of up to 8 h, the cyanidin absorption corresponded to 0.11% of the administered dose of blackberry extracts in rodents. However, there was a significant increase in antioxidant activity in the plasma despite the low anthocyanin absorption observed [75].

In humans, a clinical trial involving 12 healthy subjects revealed that the consumption of açai (7 ml/kg body weight) in different forms after an overnight fast improved the serum concentration of anthocyanins, with a peak of 2321 ng/L 2.2 h after the consumption in the form of pulp and of 1138 ng/L 2.0 h after juice consumption [76].

Evidence suggests that high concentrations are found in the distal intestine, cecum and colon because most anthocyanins are not absorbed in the upper gastrointestinal tract [77,78]. Data indicate that approximately 85% of blueberry anthocyanins reach the colon. Thus, the polyphenols that are not metabolized and absorbed in the proximal intestine reach the colon and undergo the most degradation by the local microbiota into phenolic acids for subsequent absorption [14,64,65,77].

Anthocyanins are metabolized by certain types of bacteria, including Bifidobacterium spp. and Lactobacillus spp., which have the enzymes, such as polyphenol oxidase, needed to catalyze reactions and thus could encourage gut colonization by that bacterial species [78]. These factors suggest that the mechanisms underlying the antiinflammatory effect of phenolic compounds may involve their interaction with the local microbiota and its functions in the intestine [14].

Although Graf et al. reported no changes in the rodent intestinal microbiota after treatment for 10 weeks with grape juice and blueberry (80:20), several studies indicated that these compounds stimulate the intestinal colonization by beneficial strains of bacteria, particularly Bifidobacterium spp. [13,14,79] (Fig. 1).

Similarly, Espley et al. demonstrated an association between a treatment with fruit that has a high content of flavonoids, including anthocyanins, and a reduction in inflammation markers and changes in the intestinal microbiota. The study showed an increase in Bifidobacterium spp. related to the total bacterial count in the colon and decreases in the levels of leukotriene LTB4, prostaglandin PGE2 and TNF-α that were associated with the consumption of flavonoids by healthy mice [13].

The bifidogenic potential of polyphenols suggests the involvement of the gut microbiota in the antiinflammatory effects caused by these compounds. Researchers found that the supplementation of 6 mg/day of pomegranate peel extract, which is rich in phenolic compounds, in obesity induced by a high-fat diet associated with hypercholesterolemia and inflammatory disorders, increased the cecal content weight and pool of Bifidobacterium spp. in the cecum of animals. Consumption of pomegranate extract reduced cyclooxygenase COX-2 in the colon and visceral adipose tissue, mRNA levels of IL-1β in the visceral adipose tissue and IL-6 in the colon compared with those in mice that were only fed a high-fat diet [14].

Metabolic programming model also indicated that the supplementation with anthocyanin-rich fruit in the maternal diet rich in trans fatty acids restored the expression of Lactobacillus spp. and Bifidobacterium spp. genomics DNA, increased the 20-1 gene expression and reduced inflammatory markers by down-regulation of NF-κB signaling in the offspring [80,81].

Furthermore, a controlled, randomized, crossover clinical trial led by Boto-Ordóñez et al. confirmed the important role of polyphenols, particularly anthocyanins, in the modulation of the microbiota. The ingestion of red wine without alcohol (dealcoholized red wine) for 20 days by nine participants was able to increase the fecal concentration of Bifidobacterium, Enterococcus and Enterobacteria lenta compared with that for the consumption of distilled liquor (Gin) and for the baseline. Additionally, the urinary concentrations of phenolic acids (syringic, p-coumaric, 4-hydroxybenzoic and vanillic acids), which are all anthocyanin metabolites, were positively correlated with the Bifidobacterium [79].
Indeed, a recent review discussed evidence for interplay between anthocyanins and the gut microbiota. The authors discussed the microbiota role in anthocyanin metabolism and bioavailability, the anthocyanin metabolites role in the colon as potential bioactive molecules and their function as prebiotics agents. Finally, despite the methodological difficulties and the limited studies available, there is a consensus that polyphenols, particularly anthocyanins, can modulate bacterial growth in the colon [78].

6. Conclusion

Phenolic compounds, especially anthocyanins, are associated with a favorable modulation of microbiota and inflammatory markers, and this modulation involves the down-regulation of transcription factor NF-κB signaling pathway. This effect may be related to the interplay between anthocyanins and the gut microbiota, which possibly results in improved activity of the intestinal barrier and reduced translocation of LPS into the circulation. Such factors may contribute to the diet therapeutic approaches that have been developed for controlling inflammation and preventing chronic diseases through modulation of the microbiota.

Nevertheless, more consistent studies are necessary, particularly clinical research on the great potential for controlling the inflammation associated with chronic diseases. Furthermore, studies have indicated that the beneficial effects of anthocyanins depend on many factors, including the food source, the relation and synergy with other bioactive compounds of the diet, eating habits and the inflammatory status.

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References


