Considerations for the Use of Anthocyanins in Canine Chronic Inflammatory Diseases

Nancy Scanlan, DVM, MS

Author contact:
Longer Healthier Life
PO Box 146
Weed, CA 96094
(559) 465-6789
drnancy46@yahoo.com

Abbreviation

NF-κB **Nuclear Factor-κB**

Author's note

There is confusion in the literature regarding the use of the terms a*nthocyanin* and *anthocyanidin*. For the most part, *anthocyanin* is used to indicate the different types of molecules with the basic *anthocyanin* chemical structure, and anthocyanidin is used in reference to specific individual anthocyanin molecules (eg, pelargonidin, cyanidin, petunidin). However, some authors use *anthocyanin* for both purposes, some use *anthocyanidin* for both purposes, and some reverse the most common usage. The terminology in this paper complies to the most common usage, with *anthocyanins* meaning the general class of flavonoids with a flavilium structure and *anthocyanidins* referring to specific molecules.

Abstract

Anthocyanins are a subset of flavonoids, with robust research documenting their benefits in human health. Individual anthocyanidins, anthocyanin-rich extracts of foods, and anthocyanin-rich foods (especially berries) have all shown marked antioxidant properties and the ability to help symptoms of chronic diseases characterized by long-term, low-grade inflammation. No toxicity has been associated with them, although some foods are more likely than others to elicit an allergic reaction in humans. Multiple antioxidant pathways have been identified as the means through which their health benefits are accomplished. A short-term study has shown their ability to do the same for canines without toxic effects. Berries or berry extracts hold promise as another functional food additive that can aid in the treatment of common inflammatory diseases, especially in the geriatric canine.

Introduction

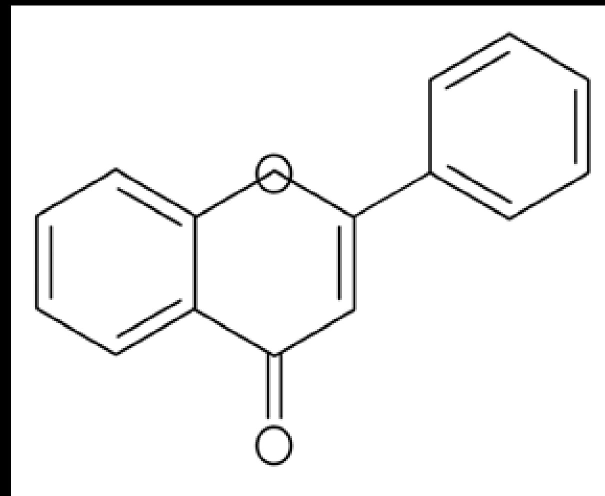
Anthocyanins are a subfamily of flavonoids found in flowers, fruits, seeds, and plant leaves (1). They are pigments, creating the bright red-orange to blue-violet color seen in plants containing them. In human diets, anthocyanins are ingested in amounts up to 9 times greater than other dietary flavonoids (2). Plants produce anthocyanins as a protective mechanism against environmental stress factors, including UV light, cold temperatures, drought, and mold (3, 4). Epidemiological studies in humans have concentrated on the ability of flavonoids and anthocyanins to reduce the incidence of chronic inflammatory diseases such as cardiovascular disease, chronic heart disease, and Alzheimer disease (4–6). Anthocyanins have antimicrobial, antioxidative, anti-inflammatory, anti-thrombotic, anti-atherogenic, anti-allergenic, anticoagulant, analgesic, immune-modulating, vasodilatory, and anti-mutagenic properties (7–24). They play a role in the prevention and treatment of many chronic diseases in humans, such as metabolic syndrome, cancer, eye disease, and cardiovascular disease (24, 25).

Structure

The flavonoid chemical structure consists of 2 phenyl rings and a heterocyclic ring (**Figure 1**). The anthocyanin structure is a slightly modified version, resulting in a flavilium (2-phenylchromenylium) structure, and including a sugar group (**Figure 2**). The most common anthocyanidins differ by the presence or absence of -H, -OH, or -OCH3 groups at R1 and R2 positions. The sugar molecule moiety (G in **Figure 2**) of the individual anthocyanidin can vary. Glucose is the most common, but others such as arabinose or galactose occur. Glucose gives an anthocyanidin the highest solubility and a more significant antioxidant action than disaccharide moieties (26). In a study of cranberry juice, a glucose moiety created a more bioavailable anthocyanidin than a galactose or arabinose moiety (4). The flavilium characteristic made them more reactive and among the least thermally stable of flavonoid groups (27).

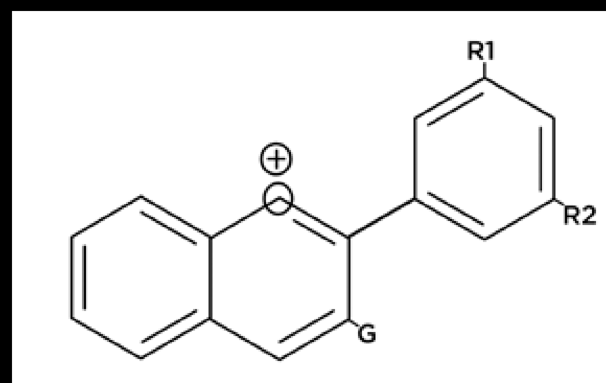
Over 635 anthocyanidins have been identified. More than 90% of anthocyanins in edible plants are comprised of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (5). Non-glycosylated anthocyanidins have poor chemical stability, so glycosylated forms predominate. The chromophore of 8 conjugated double bonds carrying a positive charge on the heterocyclic oxygen ring is responsible for the intense red-orange to blue-violet color produced by anthocyanins under acidic conditions (26).

Figure 1



Structural body of a flavonoid

Figure 2



Structural body of an anthocyanidin, also called a flavilium body, resulting from slight modification of the basic structural body of flavonoids. G indicates site of sugar moiety attachment. R1 and R2 indicate site of -H, -OH, or -OCH3 groups in the 6 anthocyanidins most commonly found in foods.

Bioavailability

Anthocyanins are among the few plant polyphenols that can be detected in plasma in their native intact forms as glycosides. Initially they were thought to have very low bioavailability, with less than 1% of the intact forms detectible in human plasma after absorption by the upper small intestine (2). The anthocyanins absorbed by the proximal small intestine subsequently go through phase II transformation in the liver (28). Later studies showed that the primary protective effects of anthocyanins occur after conversion by an intact microbiome in the colon (29). Bioavailability of anthocyanin metabolites is 42 times higher than that of parent anthocyanins (30). Metabolites are found in much higher concentrations in the blood than are intact anthocyanins (2). Thus, the bioavailability of the most active components of anthocyanins should be judged by the absorption of metabolites, not of whole anthocyanidins.

Studies in rats showed that anthocyanins are rapidly absorbed in the stomach and intestine (31). In one study, uptake of black raspberry anthocyanins in gastric and small intestinal tissue samples of rats reached 7.5% of the administered dose, which is much higher than the reported bioavailability of these compounds based on plasma and urine concentrations. Thus, intact anthocyanins may be taken up into the stomach and small intestine tissues efficiently but not transported into the circulation (4). Dietary anthocyanins have been shown to accumulate in the tissues of pigs during long-term feeding, with longer residence time in tissues than in the bloodstream (32).

A study of human ileostomy patients showed that 90% to 95% of anthocyanins reach the large intestine chemically intact (29). Within 4 hours, 69% of the original anthocyanidins disappeared from the colon, transformed by intestinal flora into many circulating and excreted anthocyanin metabolites and catabolic products (27, 32–35).

Anthocyanins can alter bacterial metabolism and species numbers within the intestines. Diets rich in anthocyanins protect against intestinal inflammation and increased gut permeability and improve colon health (6). Anthocyanins can modulate the inflammatory process associated with host-microbiome interactions and inhibit activation of the signaling pathway mediated by transcription factor Nuclear Factor-κB (NF-κB), resulting in increased beneficial bacteria in the gut, especially bifidobacteria (36).

Several in vivo studies suggest that the type of berry has a significant effect on the absorption and metabolism of anthocyanins. For example, one study found that 5% of anthocyanins in cranberry juice were recovered from the urine of humans after drinking the juice. In contrast, other studies recovered only 1.8% to 2% of anthocyanins from humans drinking strawberry juice (37, 38). This could reflect either higher excretion of anthocyanins in cranberry juice or greater metabolism of anthocyanins in strawberry juice and illustrates that one cannot generalize about availability or metabolism of anthocyanins from a study of a single anthocyanin source.

Effects in humans

Anthocyanin metabolites contribute to the improvement of human health (39). In vitro and in vivo studies of both lab animals and humans have shown that diets high in anthocyanidins or anthocyanidin-containing foods can improve or reverse oxidative stress, lung inflammation, type 2 diabetes, Alzheimer disease, cognitive impairment resulting from neuroinflammation, other CNS-related aging processes, obesity, cardiovascular disease, congestive heart failure, and inflammatory bowel disease (1, 4–6, 32, 40-45).

Anthocyanins alleviate oxidative stress through the downregulation of inflammatory cytokines and suppression of cellular signaling pathways of inflammatory processes (40). Several mechanisms are utilized, including capturing free radicals and anions, inhibiting xanthine oxidase, chelating metal ions, targeting arachidonic acid, and increasing adhesion of molecules (26).

Individual anthocyanidins block NF-κB via their antioxidant capacity, decreasing oxidative stress (46). Through the nuclear factor erythroid 2-related factor 2 pathway, they induce antioxidant defense (27). Anthocyanidins inhibit cyclooxygenase-2 activity and inhibit the xanthine oxidase pathway via increased uric acid concentration (47, 48). They reverse the activation of c-Jun N-terminal kinase and lower the levels of inflammatory markers, including p-NF-κB, tumor necrosis factor-α, and interleukin-1β (49). The antioxidant potential of anthocyanins and degree of inhibition depends on the number of free hydroxyl groups or conjugation groups, the degree of glycosylation, and the presence of donor electrons in the ring structure (26, 47).

Anthocyanins have a prebiotic action, demonstrated to both protect against and improve the symptoms of inflammatory bowel disease (40, 52). They promote the growth of beneficial bacteria, inhibit harmful gut bacteria associated with inflammatory bowel disease such as *Clostridia* species, and protect against inflammation-associated colorectal cancer in humans. There is a synergistic effect between mixed types of anthocyanins, so the benefits are increased when more than one kind of anthocyanidin or anthocyanin-rich food is used (42).

Human studies of the effects of foods high in anthocyanins have utilized pomegranate juice, blueberries, cranberries, chokecherries, strawberries, elderberries, blackberries, blackcurrants, mulberries, cherries, black elderberries, lingonberries, red cabbage microgreens, black soybeans, and jaboticabas (1). These studies have demonstrated benefits such as increases in high-density lipoproteins and decreases in cholesterol, low-density lipoproteins, triglycerides, blood pressure, and platelet aggregation (26). They protect the hippocampus in a mouse model of Alzheimer disease, have a protective effect against inflammation, and improve health in states of chronic low-grade inflammation (1, 32, 41, 44, 45). In contrast, studies of individual anthocyanidins have shown mixed success in improving chronic disease, similar to studies using other single antioxidants such as vitamin E, beta carotene, SOD, or glutathione (41). Individual antioxidants are more or less oxidant-specific. For example, vitamin E targets lipid peroxyl radicals, SOD regulates free oxygen to hydrogen peroxide conversion, and glutathione removes reactive aldehydes through conjugation. No single antioxidant can target all oxidants produced by the body (50). Therefore, one would expect that mixtures of anthocyanidins would produce superior results by having a broader range of antioxidant activity.

The few studies comparing the use of single versus multiple anthocyanidins or high-anthocyanin-containing foods showed better effects with combinations. Multiple types of anthocyanidins reduce inflammatory markers in hypercholesterolemic human subjects better than any single anthocyanidin (50). Likewise, the use of a combination of blackberries and raspberries showed a synergistic, not merely additive, total antioxidant capacity when compared to the effects of either berry alone (51). This would suggest that future studies to identify the health benefits of anthocyanins should use combinations of anthocyanidins or anthocyanin-containing foods. Likewise, anthocyanin supplements should be most effective when multiple anthocyanidins are used, and anthocyanin-containing foods in a healthy diet should be most beneficial when including different species of plants.

Effects in dogs

There is a single published study of the effects of supplementing a canine diet with nutraceuticals, with results similar to those in humans. Anthocyanidins were among the 4 nutraceuticals studied. A commercial diet was supplemented for 60 days in 13 dogs with 0.2 mg/kg of anthocyanins extracted from *Vaccinium myrtillus*. Blood was drawn at the beginning and end of the 60-day feeding trial. Tumor necrosis factor, interleukin 8, NF-κB1, and PG S2 were significantly down-regulated, and plasma ceruloplasmin was significantly decreased (53).

Anthocyanins are non-toxic in humans. Blood profiles of healthy humans given high doses of anthocyanins purified

from bilberries, blackberries, and elderberries showed no indication of harming kidney or liver functions (22). The same was found for postmenopausal women taking an anthocyanin extract from elderberries for 12 weeks (54). Anthocyanins are likely as benign in dogs as they are in humans. This author could find no published articles relating to anthocyanin toxicosis in dogs or humans. The ASPCA Animal Poison Control Center has seen only mild gastrointestinal signs in dogs that have ingested large amounts of commercially available concentrated anthocyanin products, and no renal issues have been reported (a).

Loss in food processing

Anthocyanin concentration in berries was reduced by 46% during the high temperature and pressures of canning and 85% by pasteurization, even though juice from several types of berries processed in this way maintained its flavor and color (55, 56). Anthocyanins in berries are also degraded by cooking, while antioxidant activity remains the same. The degradation from average cooking temperatures appears to be similar to metabolic transformation in the colon. The degree of degradation differs among the types of berries tested (57).

High anthocyanin-containing foods that were encapsulated with pectins or wheat flour (but not a combination of starch and wheat gluten) were protected against anthocyanin breakdown. They were also protected by fermentation, using the specific yeast strains used to stabilize the red color in wines (44). Encapsulation in liposomes and alginate/chitosan microcapsules increased bioefficiency for anthocyanins (58, 59). These processing methods could increase the value of berries added to commercial dog food.

Discussion

Berries, dried berries, berry extracts, or berry powders have the potential to aid in the control of chronic inflammatory diseases which are not responsive to conventional treatment. Evidence for their use in dogs is empirical, based on in vitro and in vivo studies in mice, humans, pigs, and a small study in dogs. Currently, no dosages have been identified or recommended. Further studies to point the way for their use should include the pharmacokinetics, metabolism, excretion, and bioavailability of various anthocyanidins and their metabolic breakdown products using combinations of berries as the source. Clinical studies should be done to determine their efficacy and recommended dose.

Berries are present in some commercial dog foods. Because of degradation of anthocyanins from average cooking temperatures and even more so from canning and pasteurization, it is likely that there is not enough present to cause a positive effect in the health of dogs consuming these diets. However, it would be easy for owners to add berries to home-prepared or commercial dog food. Due to their potential anti-inflammatory effect with no reported toxicosis, berries could be substituted for high calorie treats for obese dogs to decrease total calories in the diet and possibly decrease inflammation associated with obesity.

Endnote

a. Personal communication via email from Tina Wismer, DVM, MS, of the American Society for the Prevention of Cruelty to Animals Animal Poison Control Center, March 8, 2020.

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