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REVIEW ARTICLE

Effects of anthocyanins and anthocyanin-rich extracts on the risk for cancers of the gastrointestinal tract

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Summary

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. Anthocyanins are responsible for most of the red, blue, and purple colors of fruits, vegetables, flowers, and other plant tissues or products. In recent years, numerous studies have shown that anthocyanins display a wide range of biological activities. This review summarises recent literature evidence on the association of anthocyanins and anthocyanin-rich extracts consumption with the risk for gastrointestinal tract cancer, concentrating on the results from in vivo animal model tumor systems, as well as data from human epidemiological studies. Potential cancer chemopreventive activities of anthocyanins were revealed from in vitro studies. In vivo animal model tumor systems showed that dietary anthocyanins inhibit cancers of the gastrointestinal tract. Some epidemiological studies have revealed protective effects of anthocyanins consumption on gastrointestinal cancer risk in humans. Pharmacokinetic data indicate that absorption of anthocyanins into the bloodstream of rodents and humans is minimal, suggesting that they may have little efficacy in tissues other than the gastrointestinal tract and skin. Future studies should be undertaken to determine if the anticancer effects of anthocyanins are due to the parent compounds and/or to their metabolites.

Key words: anthocyanins, gastrointestinal cancer, in vitro, in vivo, prevention

Introduction

Anthocyanins (Greek *anthos*, flower and Greek *kyanos*, blue) originally used to describe the blue pigment of the cornflower *Centaurea cyanus*, are an important group of water-soluble plant pigments. They belong to the most common class of phenolic compounds, known collectively as flavonoids with more than 8000 flavonoid and more than 500 anthocyanin structures [1]. Anthocyanins are widespread in food plants, occurring in 27 families. The worldwide annual consumption has been estimated as 10 000 tons from black grapes alone. During the past two decades an increasing number of studies have investigated the diverse protective effects elicited by polyphenolics present in various fruits and vegetables. These effects include antioxidant, antiallergic, antiinflammatory, antiviral, antiproliferative, antimutagenic, antimicrobial, anticarcinogenic, protection from cardiovascular damage and allergy, microcirculation improvement, peripheral capillary fragility prevention, diabetes prevention, and vision improvement [2-13]. Polyphenolic research has recently intensified because of this increasing understanding and awareness of the potential beneficial effects on human health.

This review summarises recent literature evidence on the association of anthocyanins and anthocyanin-rich extracts consumption with the risk for gastrointestinal tract cancer, concentrating on the results from *in vivo* animal model tumor systems, as well as data from human epidemiological studies.

Chemical structure

Anthocyanins are water-soluble glycosides of polyhydroxyl and polymethoxyl derivatives of 2-phenylbenzopyrylium or flavylium salts. The anthocyanins found most commonly in plants are the glycosides of 6 anthocyanidins: cyanidin (cy), delphinidin (dp), malvidin (mv), peonidin (pn), pelargonidin (pg) and petunidin

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(pt). These 6 anthocyanidins commonly found in plants are classified according to the number and position of hydroxyl groups on the flavan nucleus. The most common sugar components of anthocyanins are glucose, galactose and arabinose, usually conjugated to the C3 hydroxyl group in the anthocyanidin C ring. The differences between individual anthocyanins come from the number and the position of hydroxyl groups, the degree of methylation of these hydroxyl groups, the nature, number and location of sugars attached to the molecule, and the aliphatic or aromatic acids attached to the sugars in the molecule [14]. Glycosylation confers increased structural stability and water solubility to the parent anthocyanidin [15]. Acylation of the sugar residues with cinnamic (*p*-coumaric, caffeic, ferulic) or aliphatic (acetic, malonic, succinic) acids [14] further improves anthocyanin stability. Generally, di-, tri-, or polyacylated anthocyanins have increased stability over simple and monoacylated anthocyanins [16].

It is generally accepted that anthocyanins are the most important group of water-soluble pigments in plants. In the plant tissues the anthocyanins produce blue, purple, red and intermediate hues and appear black in some products. Their hues and structure are dependent on pH and the presence of co-pigments [17,18]. At pH 1-3 the flavylium cation is red colored, at pH 5 the resultant carbinol pseudo base is colorless, and at pH 7-8 the blue purple quinoidal base is formed.

Distribution and consumption

The glycosides of the 3 nonmethylated anthocyanidins (cyanidin, delphinidin and pelargonidin) are the most widespread in nature, being present in 80% of the pigmented leaves, 69% of fruits and 50% of flowers. The most abundant anthocyanins in edible parts of plants are the glycosides of cyanidin (50%), followed by pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%), and malvidin (7%) [15,19,20].

Anthocyanins form the colors of many fruits and vegetables and are probably the most widespread food colors occurring as red colors in fruit juices, wines and jams. These pigments have been identified in edible plant materials as diverse as apple, berries (blackcurrant, boysenberry, blueberry, bilberry, strawberry, blackberry, raspberry, cranberry, elderberry, lingonberry, chokeberry etc.), black carrot, cabbage, cherry, grape, radish, red onions and sweet potato, to mention only a few of the vast array known [21].

The discovery of more stable bis- and polyacylated anthocyanins has attracted the attention of the food industry for use as safe and effective food additives.

The average intake of dietary flavonoids is estimated at about 23 mg/day in the Netherlands [22] and 650 mg/day in USA [23,24]. The daily intake of anthocyanins in USA has been estimated at 180-215 mg/day [23]. This value is considerably higher than the intake of other flavonoids such as flavones and flavonols in Dutch diet (23 mg/day, measured as aglycones) [22]. Servings of 100 g of berries can provide up to 500 mg of anthocyanins [14].

In vitro studies

Potential cancer chemopreventive activities of anthocyanins revealed from *in vitro* studies include radical scavenging activity [25], stimulation of phase II detoxifying enzymes [26], reduced cell proliferation [27-32], inflammation [27,30], angiogenesis [33-35], invasiveness [36-38], induction of apoptosis [39-41] and differentiation [27,42,43]. Anthocyanins modulate the expression and activation of multiple genes associated with these cellular functions including genes involved in the phosphoinositide 3-kinase (PI3K)/Akt [34], extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and mitogen-activated protein kinase (MAPK) [44] pathways.

In vivo studies in animals

In a model of squamous cell carcinoma of the oesophagus, Fischer-344 rats were treated repeatedly with the carcinogen N-nitrosomethylbenzylamine (NMBA), after which esophageal tumors appear in all treated animals within 20-25 weeks [45]. Using this model, the laboratory of Stoner et al. has demonstrated in 2007 the ability of multiple chemopreventive agents, including lyophilized black raspberries (BRB), to prevent the development of NMBA-induced esophageal tumors and determined their mechanism of action [46]. In a recent study, they compared the ability of diets containing either 5% black raspberry powder, an anthocyanin-rich fraction isolated from black raspberries, or an ethanol: H_2O extract from black raspberries, to inhibit esophageal tumorigenesis in NMBA-treated rats [45]. All three diets contained approximately the same quantity of anthocyanins (3.5 μmol/g diet). The results of this study indicated that all three diets were equally effective in preventing the development of esophageal tumors, reducing the number of tumors by 42-47%, thus suggesting that the anthocyanins of black raspberries are important for their chemopreventive activity [46]. The mechanism by which the anthocyanin-rich diet pre-

vented esophageal tumorigenesis is under investigation, however results from another study by the same investigators have shown that whole 5% black raspberry diets inhibit the mRNA and protein expression levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), c-Jun, vascular endothelial growth factors (VEGF) and other genes associated with cell proliferation, inflammation and angiogenesis [46].

In summary, results from the study of Wang et al. published in 2009 [47] indicate that the anthocyanins in BRB are important for their chemopreventive potential. In addition, the residue of BRB seems to be equally as effective as the anthocyanin fraction in preventing esophageal cancer in rats, and studies are ongoing to identify the active constituents in the residue. Finally, these data suggest that berry fractions may have therapeutic value for esophageal tumorigenesis and, perhaps, consideration should be given to the use of these in conjunction with radiotherapy and/or chemotherapy.

In the APC (Min) mouse model of intestinal cancer, animals fed with an anthocyanin-rich tart cherry extract (375-3000 mg/kg diet) had 74% fewer cecal tumors ($p<0.05$) than untreated mice, but the percent changes in colon tumors (17%) and small intestinal tumors (30%) in treated vs. untreated mice were not significant [20]. In a subsequent study using a similar protocol, Min mice fed with the anthocyanin-rich tart cherry extract (375-3000 mg/kg diet) plus the non-steroidal anti-inflammatory drug (NSAID) sulindac (100 mg/kg diet), had significantly ($p<0.05$) fewer tumors in the proximal and medial thirds of the small intestine, but not in the distal third, than mice fed with sulindac alone [48]. In the same model, APC (Min) mice ingested isolated cyanidin-3-glucoside (C3G), the most abundant anthocyanin in diet or mirtoselect, an anthocyanin mixture from bilberry, at 0.03, 0.1 or 0.3% in the diet for 12 weeks, and intestinal adenomas were counted. Ingestion of either C3G or mirtoselect reduced adenoma load dose-dependently. At the highest doses of C3G and mirtoselect (0.3%) the adenoma numbers decreased by 45% ($p<0.001$) or 30% ($p<0.05$), respectively, compared to controls. [49]. In this study, anthocyanins were detected in plasma, and both glucuronide and methyl metabolites of anthocyanins were detectable in the intestinal mucosa and urine. In the azoxymethane (AOM)-induced model of colon cancer in F344 rats, diets containing 2.5, 5 and 10% lyophilized black raspberries significantly decreased the total tumors (adenomas and adenocarcinomas) by 42, 45 and 71% and urinary 8-hydroxy-2´-deoxyguanosine (8-OHdG) levels by 73, 81 and 83%, respectively [50]. The reduction in urinary 8-OHdG levels indicated that berries reduce reactive oxygen species (ROS)-induced DNA damage in animals. Lala et al. [51], using the AOM-induced rat colon cancer model, reported that an anthocyanin-rich extract from bilberry, chokeberry and grape (containing 3.85 g anthocyanins per kg diet) significantly reduced AOM-induced aberrant crypt foci by 26-29%. This reduction was associated with decreased cell proliferation and COX-2 gene expression, however, the levels of urinary 8-OHdG were similar among rats fed with the different diets. Anthocyanins from purple sweet potatoes, red cabbage and purple corn (at 5% in the diet), significantly reduced colorectal carcinogenesis by 48, 63 and 89%, respectively, in rats treated with 1,2-dimethylhydrazine, but the mechanisms of tumor inhibition were not investigated [52,53].

Human studies

Anthocyanin-rich fruit or berry preparations have been investigated in healthy volunteers, in individuals at high risk of developing cancer or in cancer patients.

A 10% freeze-dried black raspberry (FBR) gel was topically applied 4 times per day to 17 patients with oral intraepithelial neoplasia (IEN) [54]. After 6 weeks pre- and post-treatment biopsies were evaluated for change in histopathology grade. Seven patients showed histopathological improvement, 6 exhibited stable disease and 4 evidence of progression. A reduction in loss of heterozygosity at tumor suppressor gene-associated loci was also observed. There was an association, although relatively weak, between reduction in loss of heterozygosity and improvement in histopathology grade [54].

Supplementation of anthocyanins in the diet of cancer patients receiving chemotherapy did not result in increased inhibition of tumor development when compared to chemotherapy alone [55].

However, some epidemiological studies suggest that anthocyanin intake may reduce certain parameters of oxidative damage.

A study from Germany showed that individuals who consumed an anthocyanin/polyphenolic-rich fruit juice had reduced oxidative DNA damage and a significant increase in reduced glutathione when compared to controls [56].

In an investigation of patients with Barrett's esophagus, the daily oral administration of 45 or 32 g (males and females, respectively) of lyophilized black raspberry powder (which contains about 5-7% anthocyanins) in a slurry of water for 6 months reduced the levels of 8-epi-prostaglandin F2α (8-Iso-PGF2) and 8-OHdG in urine [57].

A study conducted in the United Kingdom indicated that dietary anthocyanins from cranberry juice had no effect on basal or induced oxidative DNA damage

or cellular antioxidant status in leukocytes taken from treated individuals [58].

In a presurgical model, 25 colon cancer patients having not received prior therapy consumed 60 g/day (20 g/×3/day) of an anthocyanin-rich black raspberry powder daily for 2-4 weeks. Biopsies of normal-appearing and tumor tissues were taken before and after berry treatment. The berries reduced the proliferation rates and increased apoptosis in colon tumors but not in normal-appearing crypts. The number CD 105 stained blood vessels was also reduced in berry-treated colon tumors suggesting an antiangiogenic effect of short-term berry treatment [47].

Twenty-five colorectal cancer patients scheduled to undergo resection of the primary tumor or of liver metastases received mirtocyan, an anthocyanin-rich standardized bilberry extract, 1.4, 2.8. or 5.6 g (containing 0.5-2.0 g anthocyanins) daily for 7 days before surgery [59]. Mirtocyan anthocyanins and methyl and glucuronide metabolites were identified in plasma, colorectal tissue, and urine, but not in liver. Anthocyanin concentrations in plasma and urine were roughly dose-dependent, reaching approximately 179 ng/g in tumor tissue at the highest dose. In tumor tissue from all patients on mirtocyan, proliferation was decreased by 7% compared with preintervention values. Thus, a repeated administration of bilberry anthocyanins in humans resembles what is seen in APC (Min) mice [49]. Studies of doses containing $p<0.5$ g bilberry anthocyanins are necessary to conclude whether they may be appropriate for development as colorectal cancer chemopreventive agents.

Pharmacokinetics and metabolism of anthocyanins

In general, both in animals and humans, the anthocyanins are absorbed as intact glycosides, and their absorption and elimination is rapid. However, the efficiency of their absorption is relatively poor [60,61]. In 2005 Stoner et al. investigated the absorption and metabolism of black raspberry anthocyanins in humans when administered orally at high doses (2.69±0.085 g/day) [62]. Peak plasma levels of the 4 anthocyanins in black raspberries were observed within 2 hours of oral berry intake and their elimination from plasma followed first-order kinetics. They were excreted both as intact anthocyanins and as methylated derivatives in the urine within 4-8 hours of berry ingestion. Overall, less than 1% of the administered dose of the berry anthocyanins was absorbed and excreted in urine. Similar results have been obtained in studies of the absorption and metabolism of anthocyanins in rodents [61].

Anthocyanins have been shown to inhibit malignant cell growth, stimulate apoptosis and modulate oncogenic signaling events *in vitro* in the 10^{-6} to 10^{-4} M concentration range. Studies on the uptake of anthocyanins in humans after their consumption as mixtures suggest that they reach levels of 10^{-8} to 10^{-7} M in human blood, or far below the levels required to exhibit anticarcinogenic effects *in vitro*. Thus, it is unclear whether the *in vivo* concentration is sufficient to elicit anticarcinogenic effects in humans, and whether they exert chemopreventive activity by themselves or if they need to undergo hydrolysis to their aglyconic counterparts to be effective [63].

The anthocyanin enigma

An enigma is defined as anything that perplexes because it is inexplicable, hidden, or obscure; something that serves as a puzzle to be solved. The whole realm of anthocyanin consumption and human health fits into this definition, because several aspects of anthocyanins' pharmacological roles have remained elusive to the scientist. Generally, in most of the interventions of the anthocyanins in human health, details on the mechanisms of action for bioactivity, uptake, absorption, bioavailability, whole body distribution, and tissue localization are still not fully elucidated.

There are at least 4 primary obstacles that have impeded the formulation, by medical professionals, of a robust dietary or prescriptive guidelines on the consumption of anthocyanins [64].

Probably the most complicated piece of the puzzle is that, in terms of biological activity in the human body, an anthocyanin pigment is (almost) never acting independently. Typically, anthocyanins and other flavonoid components or anthocyanins and other nonflavonoid phytochemicals, are interacting together in order to provide full potency. So, traditional bioprospecting approaches, which search for single purified plant-derived compounds as a means of drug discovery, will not capture the full potency of a plant extract when multiple potentiating interactions are responsible for bioactivity.

Another common well-recognized handicap to the scientists exploring the bioactive properties of flavonoids, and the second part of the anthocyanin puzzle, is the fact that these phytochemicals can be of an evanescent nature. The susceptibility of anthocyanins to oxidation and degradation is one of the concerns of food processors who wish to maximize the shelf life of products enhanced with natural pigment colors. In particular, many of the classic phytochemical methods used to extract from plant tissues and fractionate components

out of crude extract, can degrade anthocyanins and/or inactivate them during the purification steps. As a result, research that aims to identify bioactive entities and gauge potencies of an extract can easily fail to assess the actual sources of biological activity *in situ*.

A third piece of the puzzle is the inducible nature of many of the bioactive flavonoids, including anthocyanins. As is true for a plethora of secondary plant products, the initial production and accumulation of phytochemicals is triggered by physical or chemical microenvironmental triggers, usually a stress factor. The genes responsible for flavonoid synthesis are highly inducible. As such, a researcher's intent on maximizing the production of anthocyanin pigments must recognize the induction factors and deliberately elicit production of bioactive flavonoids by providing these stimuli to the plant material of interest.

The final puzzle piece in the "Anthocyanin Enigma" is the inability of the scientist or medical practitioner to track the metabolic progress of anthocyanins after ingestion, due to the plethora of metabolic breakdown products that are rapidly produced *in situ*. There is substantial current interest in the quest follow the transport of bioflavonoids through the body, to determine absorption and bioavailability, and to see where breakdown products accumulate and for how long.

Conclusion

Experimental studies have clearly demonstrated the anticancer activity of anthocyanins. Some epidemiological studies have revealed protective effects of anthocyanins consumption on gastrointestinal cancer risk in humans. Pharmacokinetics data indicate that the absorption of anthocyanins into the bloodstream of rodents and humans is minimal, suggesting that they may have little efficacy in tissues other than the gastrointestinal tract (and skin), where they can be absorbed locally. Future studies aimed at enhancing the absorption of anthocyanins and/or their metabolites, may be necessary for their optimal use in the chemoprevention of human cancer.

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