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Review Article

ANTHOCYANINS: A LEAD FOR ANTICANCER DRUGS

Yogesh Joshi[1]* and Bhawana Goyal[2]

Department of Pharmacy, Himalayan Institute of Pharmacy & Research, Atak Farm, P.O.-Rajawala, Via Premnagar, Dehradun, Uttarakhand, India.

***Corresponding Author: yogeshjoshi1583@rediffmail.com**

ABSTRACT

This article presents a review about anthocyanins and shows they have anticancer property. Mechanism that may be responsible for the inhibitory effect of anthocyanins in carcinogenesis and tumor growth is either cellular redox status modification or any interference with basic cellular functions like cell cycle, apoptosis, inflammation, angiogenesis, invasion and metastasis. New anticancer agents can be developed taking reference of anthocyanins or by using them as basic structure. Further studies have to be done to explore the anthocyanins as anticancer lead.

Keywords: anthocyanin, anticancer agents, antioxidants, cell cycle, cancer cells.

INTRODUCTION

Anthocyanins are water soluble, flavonoid pigments, responsible for the attractive colors in fruits and vegetables, widely distributed in plants[1]. Flavonoids are most abundant polyphenols, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled as A, B and C. Flavonoid are further divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. The structural variation in each group is due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation[2].

Chemically anthocyanins are mostly 3-glucosides of the anthocyanidins. The most common anthocyanidins are pelargonidin, delphinidin, peonidin, petunidin, malvidin and cyanidin. They are water-soluble glycosides or acylglycosides of polyhydroxy and polymethoxy derivates of 2-phenylbenzopyrylium or flavylium salts. The ability to form flavylium cations distinguishes anthocyanins from other flavonoids as a distinct class. Plants rich in anthocyanins are Vaccinium species, such as blueberry, cranberry and bilberry, Rubus berries including black raspberry, red raspberry and blackberry, cherry eggplant peel, black rice, red cabbage etc[3].

ANTHOCYANINS AND CANCER

Cancer development involves initiation, promotion, progression, invasion and metastasis. Tumor initiation begins when DNA, in a cell or population of cells, is damaged by exposure to carcinogens, which are derived from three major sources: cigarette smoking, infection / inflammation, and

nutrition/diet[4]. In addition to the antioxidant activity shown by anthocyanins, they also display a wide variety of biological functions which are mainly related to modulation of carcinogenesis. Cancer occurs due to alteration in cancer regulating genes[5], such as oncogenes, tumor suppressor genes, resulting in altered cellular processes namely, decreased apoptosis, increased proliferation, cell maturation and differentiation. The inhibitory effect of anthocyanins in carcinogenesis and tumor growth may be through two main mechanisms: 1) redox status modification and 2) interference with basic cellular functions (cell cycle, apoptosis, inflammation, angiogenesis, invasion and metastasis)[6].

Cellular redox status modification by anthocyanins

Oxidative damage contributes to carcinogenesis and evolution of cancer. Reactive Oxygen Species, particularly hydrogen peroxide, are potent regulators of cell replication and play an important role in signal transduction[7]. These are constantly produced during normal cellular metabolism, by the metabolism of environmental toxins or carcinogens, by ionizing radiation and also by phagocytic cells involved in the inflammatory response. Anthocyanins can exert a major chemopreventive activity due to their antioxidant property[6]. They can inhibit carcinogen/toxin-induced cellular oxidative damage. The antioxidant properties of anthocyanins involves (1) scavenging radical species such as ROS/RNS (Reactive Oxygen Species/ Reactive Nitrogen Species); (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production[8].

Free radical scavenging activity of anthocyanins

Phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical and extended conjugated aromatic system to delocalize an unpaired electron are some structural properties that makes phenolic compounds ideal for free radical scavenging activities. Anthocyanins are particularly reactive towards ROS/RNS because of electron deficient nature of ROS/RNS. Relationship between structure and reduction potential shows that 3-hydroxyl group (C ring) is important; glycosylation of this reduces their activity as antioxidant. Presence of ortho-dihydroxy groups on B ring makes radical form more stable and participates in electron delocalization because of their electron donating properties[9, 10].

Anthocyanins as metal chelators

Optimum metal-binding and antioxidant activity is associated with the structures which contain a large number of catechol/gallol groups[11, 12]. Phenolic compounds with catecholate and gallate groups can inhibit metal-induced oxygen radical formation either by coordination with Fe^{2+} and enhancing autoxidation of Fe^{2+}, or the formation of inactive complex with Cu^{2+}, Fe^{2+}, or Cu^{+} with relatively weaker interaction[13, 14]. The attachment of metal ions to the anthocyanins molecule can be 3',4'-*o*-diphenolic groups in the B ring[11, 15]. Based on these potential capacities, extensive studies have demonstrated the antioxidant activities of anthocyanins[16]. Studies shows, combination of phenolic or other non-phenolic antioxidants exert better antioxidant effect than pure individual compound[17]. Anthocyanins and proanthocyanidin were found to inhibit the UV-radiation induced oxidative stress and cell damage in human keratinocytes[18, 19]. Phenolic antioxidants behave like prooxidants under the conditions that favor their autoxidation, for example, at high pH with high concentrations of transition metal ions and oxygen[20]. Easily oxidizable small phenolics such as quercetin, gallic acid, possess prooxidant activity; while high molecular weight phenolics, such as condensed and hydrolysable tannins, have little or no prooxidant activity[21]. It has been reported that delphinidin, resveratrol, curcumin etc can cause oxidative strand breakage in DNA *in vitro*[22, 23]. This study suggested that the antiproliferative effects of some polyphenol antioxidants on cancer cells are partially due to their prooxidant actions. However, this prooxidant property depends on the amount of dissolved oxygen in the test

medium[24]. It is not clear whether a similar mechanism could also occur *in vitro*.

Interference of basic cellular functions by anthocyanins

Anthocyanins can affect basic cell functions related to cancer development. They may inhibit the formation and growth of tumors by induction of cell cycle arrest and apoptosis. The regulation of cell cycle is altered in cancerous cells. Thus, any perturbation of cell cycle specific proteins can potentially affect and/or block the continuous proliferation of these tumorigenic cells. Anthocyanidins are more effective in inhibition of cell proliferation than anthocyanins[25]. Anthocyanin-rich extract from chokeberry was found to induce cell cycle block at G1/G0 and G2/M phases in colon cancer HT-29 cells but not in NCW460 normal colonic cells[26].

Apoptosis eliminate seriously damaged cells or tumor cells[27, 28]. Therefore, apoptosis-inducing agents are expected to be ideal anticancer drugs. The induction of apoptosis and/or inhibition of proliferation/survival by polyphenols has been reported to result from a number of mechanisms including inducing cell cycle arrest; blocking the extracellular regulated kinase (ERK), c-Jun *N*-terminal kinase (JNK), and P38 mitogen-activated protein kinase (MAPK) pathway; inhibition of the activation of transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP1); suppression of protein kinase C (PKC); suppression of growth factor-mediated pathways[29, 30].

A study showed that pomegranate fruit extract, rich in anthocyanins and hydrolysable tannins, protected against the adverse effect of both UVB-radiation in normal human epidermal keratinocytes *in vitro* and 12-*O*-tetradecanoylphorbol-13- acetate (TPA) in CD-1 mouse skin *in vitro*, by inhibiting the activation of NF-κB and MAPK pathway[31, 32].

Chronic inflammation predisposes to carcinogenesis. The over-expression of inducible cyclooxygenases (COX-2) is believed to be associated with colon, lung, breast and prostate carcinogenesis. A study examined the inhibitory effects of five kinds of green tea proanthocyanidins on cyclooxygenase-2 (COX-2) expression and PGE-2 release in LPS-activated murine macrophage RAW-264 cells[33]. It was revealed that the galloyl moiety of proanthocyanidins appeared important to their inhibitory actions. Natural phenolics such as grape seeds proanthocyanidins, anthocyanins etc were found to suppress malignant cell migration, invasion and metastasis *in vitro* and *in vitro*. The inhibition effect has been shown to be related to their ability to down-regulate the matrix metalloproteases (MMPs), namely, MMP-2 and MMP-9, as well as urokinase-plasminogen activator (uPA) and uPA receptor (uPAR) expression[34, 35, 36, 37, 38, 39].

In addition, phenolic compounds possess antiangiogenesis effects, which is an important aspect in the inhibition of tumor growth, invasion and metastasis[40]. It has been reported that anthocyanin-rich berry extracts inhibit tumor angiogenesis through down-regulation of vascular endothelial growth factor (VEGF), VEGF receptor-2 (VEGFR-2), platelet-derived growth factor (PDGF), PDGF receptor (PDGFR), hypoxia-inducible factor 1α (HIF-1α) and MMPs, as well as inhibition of phosphorylation of EGFR, VEGFR and PDGFR[30].

Various *in vitro* and *in vitro* experiments on cancer cell lines have also been performed to verify the antitumor efficacy of plant anthocyanins. Studies investigated the chemoprotective activity of anthocyanin-rich extracts (AREs) from bilberry, chokeberry, and grape and concluded this extract reduces colonic aberrant crypt foci formation in male rats treated with a colon carcinogen azoxymethane[41, 42, 43, 44].

The isolated polyphenols from strawberry including anthocyanins, kaempferol, quercetin, esters of coumaric acid and ellagic acid, were shown to inhibit the growth of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT-116), and prostate (LNCaP, DU-145) tumor cell line[45, 46].

A study investigated, cyanidin-3-glucoside (C3G), the major anthocyanin in blackberry, for the ability to inhibit 7,12-dimethylbenz[a]anthracene (DMBA)-12-*O* tetradecanolyphorbol-13-acetate (TPA)-

induced skin papillomas in animal skin model. The results showed that treatment of the animals with C3G decreased the number of tumors. C3G was also tested on human lung carcinoma xenograft growth and metastasis in athymic male nude mice. The results showed that reduction in size of tumor xenograft growth and significantly inhibited metastasis in nude mice. The authors concluded that C3G exhibits chemoprevention and chemotherapeutic activities by inhibiting tumor promoter-induced carcinogenesis and tumor metastasis *in vitro*[47].

A group of researchers investigated a novel mucoadhesive gel formulation for local delivery of freeze-dried black raspberries (FBRs) to human oral mucosal tissues. The results indicated that a gel formulation was well-suited for absorption and penetration of anthocyanins into the target oral mucosal tissue site. The greater penetration of anthocyanins into tissue explants was observed in berry gels with a final pH of 6.5 versus pH 3.5[48]. The effects of FBR gel formulation was examined clinically on oral intraepithelial neoplasia (IEN), a precursor to oral squamous cell carcinoma. Results showed that FBR gel topical application significantly reduced loss of heterozygosity (LOH) indices at chromosomal loci associated with tumor suppressor genes, uniformly suppressed gene associated with RNA processing, growth factor recycling and inhibition of apoptosis and significantly reduced epithelial COX-2 levels in human oral IEN lesions. It was found gel application also reduced microvascular density in the superficial connective tissues and induced genes associated with keratinocyte terminal differentiation in a subset of patients[49, 50].

A study shows reduction in oxidative damage in hemodialysis patients during anthocyanin/polyphenolic-rich fruit juice uptake. There is significant decrease of DNA oxidation damage, protein and lipid peroxidation, and NF-Kb binding activity, and an increase of glutathione level and status during juice uptake[51]. Anthocyanin fraction from potato extracts is found to be cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways[52].

CONCLUSION

Anthocyanins, natural compounds being powerful antioxidants, may be very useful in the prevention of oxidative stress induced diseases. It is further concluded that anthocyanins appears to be a promising preventive measures to reduce chronic diseases such as cancer. From various studies, it is found that some plants or their parts containing anthocyanins have anticancer property and their analogues may be helpful in synthesizing newer effective anticancer agents in future. Such advancement may play important role to aggravate the anthocyanins applications as a lead in cancer treatment.

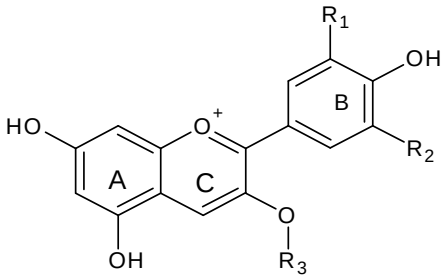

Fig. 1: Structure of Anthocyanidins

Anthocyanidins	R_1	R_2	R_3
Cyanidin	H	OH	H
Delphinidin	OH	OH	H
Pelargonidin	H	H	H
Peonidin	H	OMe	H
Petunidin	OH	OMe	H
Malvidin	OMe	OMe	H

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