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Anthocyans from fruits and vegetables – Does bright colour signal cancer chemopreventive activity?

Darren Cooke, William P. Steward, Andreas J. Gescher *, Tim Marczylo

Cancer Biomarkers and Prevention Group, Department of Cancer Studies, University of Leicester, Leicester Royal Infirmary, 5th Floor, RKCSB, Leicester LE2 7LX, UK

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Abstract

Consumption of fruits and berries has been associated with decreased risk of developing cancer. The most abundant flavonoid constituents of fruits and berries are anthocyans (*i.e.* anthocyanins, glycosides, and their aglycons, anthocyanidins) that cause intense colouration. In this review, we describe epidemiological evidence hinting at the cancer preventive activity of anthocyan-containing foods in humans, results of chemoprevention studies in rodent models with anthocyans or anthocyan-containing fruit/vegetable extracts, and pharmacological properties of anthocyans. Anthocyanidins have been shown to inhibit malignant cell survival and confound many oncogenic signalling events in the 10^{-6}–10^{-4} M concentration range. Studies of the pharmacokinetics of anthocyanins after their consumption as single agents, anthocyanin mixtures or berry extracts suggest that anthocyanins reach levels of 10^{-8}–10^{-7} M in human blood. It is unclear whether such concentrations are sufficient to explain anticarcinogenic effects, and whether anthocyanins exert chemopreventive efficacy themselves, or if they need to undergo hydrolysis to their aglyconic counterparts. The currently available literature provides tantalising hints of the potential usefulness of anthocyans or anthocyan mixtures as cancer chemopreventive interventions. Nevertheless further studies are necessary to help adjudge the propitiousness of their clinical development.

Keywords: Chemoprevention; Flavonoids; Anthocyanins; Anthocyanidins

1. Introduction

Some media reports on health issues imply that a diet rich in colourful fruits and vegetables is the nutritional panacea in a society plagued by problems such as obesity, atherosclerosis and cancer. There is increasing interest in the role of nutrition and specific dietary constituents in the causation and prevention of cancer [1]. Prominent among dietary constituents, that are the focus of such interest, are the flavonoids. Considerable pre-clinical evidence suggests that some flavonoids can delay, or abrogate, carcinogenesis in rodents. Examples include the isoflavone genistein occurring in soya, the flavonol quercetin from onions and the flavone apigenin from leafy vegetables. Anthocyans are flavanols, which occur ubiquitously in the plant kingdom and confer bright red or blue colouration on berries and other fruits and vegetables. What renders anthocyans especially interesting *vis-à-vis* other flavonoids is that they occur in the diet at relatively high concentrations. The daily intake of anthocyans in the US diet has been suggested to be 180–215 mg/day [2], whilst in contrast, the daily intake of most other dietary flavonoids, including genistein, quercetin and apigenin, is estimated to be only 20–25 mg/day. The term ''anthocyan'' encompasses ''anthocyanin'' for the glycoside and ''anthocyanidin''

* Corresponding author.
E-mail address: ag15@leicester.ac.uk (A.J. Gescher).

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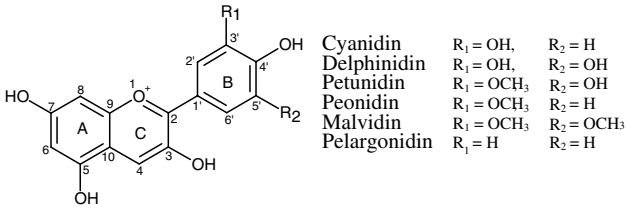

Fig. 1. Structures of anthocyanidins.

for the aglycon. The anthocyanidins found in higher plants are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin (for structures, see Fig. 1), with a distribution in nature of 50%, 12%, 12%, 12%, 7% and 7%, respectively, and they occur almost exclusively as anthocyanins [3]. The aim of this review is to summarise the evidence suggesting that anthocyans possess cancer chemopreventive properties, and to discuss whether they are worthy of further investigation leading to their clinical development.

2. Epidemiological evidence

What is the evidence to support the notion that coloured fruits and vegetables, i.e. foods containing high levels of anthocyans, possess cancer delaying or preventive properties? Whilst robust epidemiological data is not abundant, there are reports hinting at their potential anti-carcinogenicity. In a cohort of elderly individuals, who consumed large amounts of strawberries, the odds ratio for developing cancer at any site was 0.3, compared to subjects who refrained from high berry consumption [4]. Consumption of coloured fruits and vegetables has also been associated with a reduced risk of human breast cancer [5] and colorectal polyp recurrence [6]. Anthocyan-containing foodstuffs have been linked with a decreased risk of coronary heart disease. They have been shown to possess beneficial effects in several parts of the organism [7], including the central nervous system and the eye, and have been suspected to account, at least in part, for the "French paradox", *i.e.* the decreased risk of cardiovascular disease despite a high-fat diet in individuals living in France.

3. Chemical properties

The most common sugar components of anthocyanins are glucose, galactose and arabinose, which are usually conjugated to the anthocyanidin molecule *via* the C3 hydroxyl group in ring C. Some anthocyanins comprise multiple sugar moieties involving hydroxyl functionalities of the aglycon molecule other than that at C-3. Anthocyans are extremely water-soluble and occur in different pH-dependent conformations with varying colours or colour intensities (Fig. 2). At pH < 3,

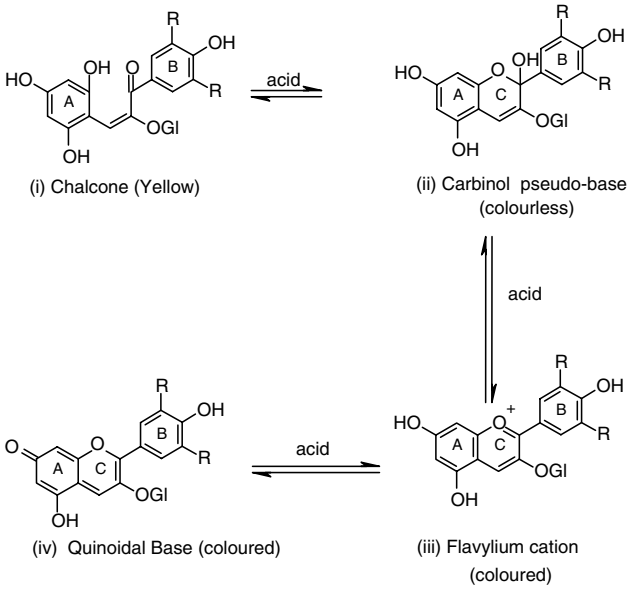

Fig. 2. pH-dependent conformational rearrangement of the anthocyan molecule, shown here for anthocyanins bearing a sugar ("Gl") on C3. Which conformer predominates depends upon pH. At neutral pH anthocyans occur as chalcones with an open C ring (i). Under mildly acidic conditions the ring is closed to form a carbinol pseudo base (ii). In strong acid (pH 2), ring C acquires aromaticity involving a flavylium cation, which imparts intense colour on the molecule (iii). In alkali, oxidation of ring A generates a quinoid structure with elimination of the positive charge, this species is also coloured (iv). The ring-opened chalcone can be reformed at neutral pH.

the middle ring (ring C) of the predominant anthocyan conformer harbours a flavylium cation which confers intense colour onto the molecule. Although the stability of the flavylium cation, as judged by UV–Vis spectrophotometry, is compromised by increasing pH [8,9], the implications of the different conformational manifestations of anthocyans for pharmacological activity are unclear.

4. Cancer chemopreventive properties in animals

There are, to our knowledge, eight reports in which the ability of anthocyans or anthocyan-containing mixtures to interfere with carcinogenesis in animals has been described. In a comparative investigation in the Apc^{Min} mouse, animals received either a mixture of anthocyanins at 800 mg/l or pure cyanidin at 200 mg/l with the drinking water or tart cherries added to the diet (200 g/kg diet). These amounts correspond to doses of approximately 2.4 and 0.6 mg anthocyans/animal/day or 600 mg of tart cherries/animal/day, respectively based on average daily intake of diet and water. In the mice that received these interventions, the number of caecal adenomas was reduced compared with animals on control diet or those that received the non-steroidal anti-inflammatory drug sulindac [10]. Numbers of intestinal adenomas were not significantly influenced by

anthocyans. Apc^{Min} mice carry an *APC* gene mutation and develop adenomas in the small intestinal tract, and they are considered to reflect in many respects human familial adenomatous polyposis coli (FAP) [11]. A recent study conducted in our laboratory suggests a moderate, but significant reduction in small intestinal adenoma number in Apc^{Min} mice that received either an anthocyanin-containing blueberry extract or pure cyanidin-3-glucoside at 0.1% (w/w) in their diet. This concentration translates into a dose of approximately 3.0 mg anthocyanin/mouse/day (Cooke et al., unpublished).

In a xenograft model in which Balb/C mice were inoculated intraperitoneally with syngeneic Meth/A lymphoma cells, animals receiving a diet of anthocyanin-containing red glutinous rice showed evidence of antitumour efficacy in terms of increased survival time compared to animals on normal rice or control diet [12].

Commercially available anthocyanin-rich extracts of chokeberry, bilberry or grape were compared in terms of their effects on azoxymethane-induced colonic aberrant crypt foci in rats [13]. In animals that received these extracts at 4 g/kg diet (translating into a dose of approximately 35 mg/animal/day) one week before the carcinogen, the number of aberrant crypt foci was significantly reduced compared to control rats. Aberrant crypt foci reduction was accompanied by inhibition of cyclooxygenase-2 gene expression in malignant tissue.

Female rats, in which mammary tumour formation had been induced by exposure to 7,12-dimethylbenz(a)anthracene, received Concord grape juice containing 15 different anthocyanins (constituting ~12% of grape juice phenols) in the drinking water at doses of approximately 10, 15 or 20 mg anthocyanins/rat/day [14]. In the animals which consumed juice, the incidence, multiplicity and final weight of mammary tumours were reduced compared to rats on drinking water omitting grape juice [14]. This study was designed with suitable control animals such as to eliminate any potential preventive effect of sugars or organic acids contained in the juice, so that the observed efficacy was probably mediated by the grape anthocyanins.

Dyes derived from purple sweet potato, red cabbage and purple corn, which are rich in anthocyanins, have also been shown to inhibit chemical-induced carcinogenesis in a rat model [15,16]. Colonic adenomas and adenocarcinomas were induced by treatment with 1,2-dimethyl hydrazine (DMH), and tumour incidence was increased by supplementing the diet with the heterocyclic amine carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Rats ingested purple sweet potato, purple corn or red cabbage colourings at doses of approximately 490, 233 or 620 mg anthocyanins/rat/day, respectively, together with PhIP (0.02%) in the diet. In the dye-treated rats, incidence and multiplicity of adenomas and adenocarcinomas were significantly decreased compared to control rats. Furthermore, dyes from purple corn or red cabbage also inhibited PhIP-induced aberrant crypt formation in rats that did not receive DMH.

Consumption of lyophilized black raspberries by rats at approximately 0.38, 0.75 or 1.5 g/animal/day decreased the multiplicity of azoxymethane-induced aberrant crypt foci by 21–36% and that of adenocarcinomas by 28–80% [17]. This protective effect was paralleled by a significant reduction in urinary levels of 8-hydroxy-2′-deoxyguanosine, a marker of oxidative DNA damage.

Anthocyans have also been shown to prevent skin cancer in rodents [18]. Skin carcinogenesis was initiated in female CD-1 mice by 7,12-dimethylbenz(a)anthracene and promoted by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). Topical application of anthocyanin-containing pomegranate extract elicited a delay in onset and decrease in incidence and burden of skin tumours [18]. Tumour attenuation was accompanied by inhibition of phorbol ester-induced biochemical events including phosphorylation of ERK1/2, p38 and JNK1/2, activation of NFkB and IKK and degradation of IkB.

5. Pharmacological effects *in vitro*

Studies, in which effects of anthocyans on cell growth or on cellular events germane to tumour promotion or progression have been investigated, are summarised in Tables 1 and 2. In terms of inhibition of neoplastic cell survival, anthocyanidins have shown greater potency than their glycosylated counterparts. Anthocyanidins compromised cell survival when present in the 10^{-5}–10^{-4} M concentration range, whilst anthocyanins were hardly growth-inhibitory at concentrations below 10^{-4} M. Anthocyanidins have been shown to inhibit the growth of embryonic fibroblasts and of cancer cells derived from malignant human tissues (see Table 1) from a variety of origins including lung, breast, uterus, vulva and colon [3,19–23]. Among anthocyanidins, delphinidin possessed the highest growth-inhibitory activity. This finding hints at a structural determinant in anti-proliferative activity suggesting dependence of potency on the presence of hydroxyl groups on ring B of the anthocyanidin molecule. Inconsistent with this inference is the finding that in some cell types the potency of malvidin was equivalent to, or greater than, that of delphinidin [20–23].

Anthocyanin-containing extracts of grapes, bilberries or chokeberries at 25–75 μg/ml inhibited the growth of human malignant HT29 colon cancer cells, but not that of non-malignant colon-derived NCM460 cells [24].

Anthocyans can interfere with biochemical activities germane to promotion or progression of malignancies, such as those mediated by cyclooxygenase (COX) enzymes, tyrosine kinases and phosphodiesterases

Table 1
Summary of growth inhibitory effects of anthocyans and anthocyanin-rich extracts

Agent	Cell line	Effect	Reference
Anthocyanidin			
Delphinidin	CaCo-2	Growth inhibition (20%), 200 μM	[23]
	HeLa S3	IC_{50} 200 μM	[23]
	Human embryonic fibroblasts	Growth inhibition (10%), 200 μM	[23]
	LXFL529L	IC_{50} 33 μM	[19]
	A431	IC_{50} 18 μM	[19]
	HT29	IC_{50} 35 μM	[32]
	MCF-7	Growth inhibition (66%), 662 μM	[3]
	HL60	Growth inhibition (88%), 100 μM	[21]
		↑Apoptosis, 200 μM	[21]
	HCT116	Growth inhibition (64%), 100 μM	[21]
Cyanidin	U937[a]	IC_{50} 210 μM	[22]
	HT29	IC_{50} 63 μM	[10]
		IC_{50} 57 μM	[32]
	HCT-116	IC_{50} 85 μM	[10]
		Growth inhibition (82%), 200 μM	[21]
	LXFL529L	IC_{50} 73 μM	[19]
	A431	IC_{50} 42 μM	[19]
	MCF-7	Growth inhibition (47%), 699 μM	[3]
	HL60	Growth inhibition (85%), 200 μM	[21]
		↑Apoptosis, 200 μM	[21]
Malvidin	U937[a]	IC_{50} 121 μM	[22]
	LXFL529L	IC_{50} >100 μM	[19]
	A431	IC_{50} 61 μM	[19]
	HT29	IC_{50} 35 μM	[32]
	HCT-116	IC_{50} 218 μM	[3]
	SF-268	IC_{50} 433 μM	[3]
	AGS	IC_{50} 258 μM	[3]
	NCI H460	IC_{50} 267 μM	[3]
	MCF-7	IC_{50} 97 μM	[3]
	HL60	Growth inhibition (97%), 200 μM	[21]
		↑Apoptosis, 200 μM	[21]
	HCT116	Growth inhibition (22%), 200 μM	[21]
Peonidin	HT29	IC_{50} 90 μM	[32]
	HL60	Growth inhibition (80%), 400 μM	[21]
Pelargonidin	HT29	IC_{50} > 100 μM	[32]
	HCT-116	Growth inhibition (63%), 645 μM	[3]
	SF-268	Growth inhibition (34%), 645 μM	[3]
	AGS	Growth inhibition (64%), 645 μM	[3]
	NCI H460	Growth inhibition (62%), 645 μM	[3]
	MCF-7	Growth inhibition (63%), 645 μM	[3]
Petunidin	MCF-7	Growth inhibition (53%), 633 μM	[3]
Anthocyanin			
Delphinidin-3-galactoside	HL60	Growth inhibition (~80%), 431 μM	[21]
Delphinidin-3-glucoside	HCT116	Growth inhibition (~85%), 863 μM	[21]
	HT29	Growth inhibition (87%), 431 μM	[39]
	MCF-7	Growth inhibition (82%), 431 μM	[39]
	HL60	Growth inhibition (~75%), 216 μM	[21]
	HCT116	Growth inhibition (~80%), 431 μM	[21]
Cyanidin-3-galactoside	LXFL529L	IC_{50} > 100 μM	[19]
	A431	IC_{50} > 100 μM	[19]
Cyanidin-3-glucoside	HT29	Growth inhibition (88%), 446 μM	[39]
	MCF-7	Growth inhibition (85%), 446 μM	[39]
	Jurkat	IC_{50} 391 μM	[36]
	HL60	Growth inhibition (37%), 446 μM	[36]

Table 1 (*continued*)

Agent	Cell line	Effect	Reference
Malvidin-3-glucoside	LXFL529L	$IC_{50} > 100$ μM	[19]
	A431	$IC_{50} > 100$ μM	[19]
	HT29	Growth inhibition (90%), 407 μM	[39]
	MCF-7	Growth inhibition (84%), 407 μM	[39]
Anthocyanin-rich extract			
Chokeberry extract	NCM 460	IC_{50} 25 μg/ml[b]	[24]
	HT29	IC_{50} 10 μg/ml[b]	[24]
		Growth inhibition (37%), 5 mg/ml	[39]
		Growth inhibition (69%), 10 mg/ml	[13]
Grape extract	MCF-7	Growth inhibition (19%), 5 mg/ml	[39]
	HT29	IC_{50} 25 μg/ml[b]	[24]
	NCM 460	IC_{50} 75 μg/ml[b]	[24]
	RBA	IC_{50} ~14 μg/ml	[14]
Cherry extracts	HT29	IC_{50} 360 μg/ml	[10]
	HCT116	IC_{50} 130 μg/ml	[10]
Bilberry extract	NCM 460	IC_{50} 25 μg/ml	[24]
	HT29	IC_{50} 25 μg/ml[b]	[24]
		Growth inhibition (69%), 5 mg/ml	[13]
	MCF-7	Growth inhibition (25%), 5 mg/ml	[39]
	HL60	Growth inhibition (84%), 4 mg/ml	[21]
	HCT116	Growth inhibition (97%), 4 mg/ml	[21]
Black currant	MCF-7	Growth inhibition (45%), 5 mg/ml	[39]
Cranberry anthocyanins	CAL27	Growth inhibition (~20%)[c]	[44]
	KB	Growth inhibition (~20%)[c]	[44]
	HCT116	Growth inhibition (~15%)[c]	[44]
	SW620	Growth inhibition (~15%)[c]	[44]
	RWPE-1	Growth inhibition (~55%)[c]	[44]
	RWPE-2	Growth inhibition (~60%)[c]	[44]
	22Rv1	Growth inhibition (~70%)[c]	[44]

HT29, HCT116, SW620 and CaCo-2, human colon carcinoma cells; NCM460, normal colon cell; LXFL529L and NCI H460, human lung carcinoma cells; A431, human vulva carcinoma cell; HeLa S3, human uterine carcinoma cell; U937, human monocytic leukaemia cell; RBA, rat mammary adenocarcinoma cell; MCF-7, a human mammary cancer cell; KB and CAL27, human oral cancer cells; RWPE-1 (normal), RWPE-2 (k-ras transfected) and 22Rv1, human prostate cancer cells; SF-268, human glioblastoma cell; AGS, human stomach carcinoma cell; HL60, human promyelocytic leukaemia cell.

Growth inhibition is expressed as the percentage decrease in cell number compared to cells unexposed to anthocyans.

[a] Aglycon formed by alcoholic acid hydrolysis of pigmented rice.

[b] IC_{50} estimated after 72 h exposure to extracts.

[c] Concentrations equivalent to content of anthocyanins in 200 μg/ml total cranberry extract.

(Table 2). All three systems play key roles in tumourigenesis [25–27]. Several reports suggest that anthocyanidins, anthocyanins and certain berry extracts can inhibit COX activity [28–30]. For example, 40 μM anthocyanidins inhibited the activities of purified COX enzyme preparations in a cell-free system, with cyanidin, the most potent cogener, able to decrease COX-1 and -2 activities by 52% and 74%, respectively [30]. Delphinidin and bilberry extract inhibited lipopolysaccharide-induced expression of COX-2 mRNA and protein in mouse macrophage RAW264 cells [31]. The ability of delphinidin to downregulate COX-2 expression may have been, at least in part, the consequence of its suppression of lipopolysaccharide- or phorbol ester-induced degradation of IκB and activation of IKK and NFκB [18,31].

Delphinidin, cyanidin and malvidin inhibited the activity of epidermal growth factor receptor (EGFR) tyrosine kinase obtained from A431 cells with IC_{50} values of 18, 42 and 61 μM, respectively [19]. In another study, the abilities of anthocyanidins to inhibit EGFR tyrosine kinase decreased in the order delphinidin = cyanidin > pelargonidin > peonidin > malvidin [32], suggesting that potency might be positively correlated with the presence of hydroxyl functions in positions 3′ and 5′ of ring B of the anthocyanidin molecule, and inversely with the presence of methoxy groups in these positions. Similarly, anthocyanidins bearing hydroxyl moieties in ring B were better inhibitors of the activity of activator protein 1 (AP1), a transcription factor with an integral role in carcinogenesis [33], and of ERK and JNK phosphorylation than their methyoxylated

Table 2
Summary of mechanisms of anthocyans potentially contributing to chemopreventive efficacy

Agent	Cell line/fraction	Effect	Reference
Anthocyanidin			
Delphinidin	JB6	↓TPA-induced AP-1 activation	[34]
		↓JNK/ERK phosphorylation	[34]
	A431	↓EGFR tyrosine kinase activity	[19]
		↓Elk-1 activation	[19]
	HT29	↓EGFR tyrosine kinase activity	[32]
		↑PDE4 inhibition	[32]
	CaCo-2	G_2/M phase arrest	[23]
		↑Apoptosis	[23]
	HeLa S3	G_2/M phase arrest	[23]
		↑Apoptosis	[23]
	Human embryonic fibroblasts	↑Apoptosis	[23]
		S-Phase arrest	[23]
	HL60	↑Apoptosis	[20]
		Caspase 3 activation	[20]
		↑JNK phosphorylation	[20]
	RAW264	↓LPS-induced IkB degradation	[31]
		↓LPS-induced NFkB activation	[31]
		↓LPS-induced Cox 2 expression	[31]
	Rat seminal vesicle	↓Cox-1 activity	[30]
	Insect cell lysate	↓Cox-2 activity	[30]
Cyanidin	JB6	↓TPA-induced AP-1 activation	[34]
	HL60	↑Apoptosis	[20]
	Normal human fibroblasts	G1 phase arrest	[23]
	A431	↓EGFR tyrosine kinase activity	[19]
		↓Elk-1 activation	[19]
	HT29	↓EGFR tyrosine kinase activity	[32]
		↑PDE4 inhibition	[32]
	U937[a]	Arrest of G_2/M Phase	[22]
		Apoptosis	[22]
	Rat seminal vesicle	↓Cox-1 activity	[29]
	Rat seminal vesicle	↓Cox-1 activity	[30]
	Insect cell lysate	↓Cox-1 activity	[29]
	Insect cell lysate	↓Cox-1 activity	[30]
	Rat liver microsomes	Antioxidant	[46]
	Liposomes	Antioxidant	[46]
	Rabbit erythrocyte membranes	Antioxidant	[46]
Malvidin	U937[a]	Arrest of G_2/M phase	[22]
		↑Apoptosis	[22]
	A431	↓Elk-1 activation	[19]
	JB6	Weak inhibition of AP-1 activity	[34]
	HT29	↓EGFR tyrosine kinase activity	[32]
		PDE4 inhibition	[32]
	Rat seminal vesicle	↓Cox-1 activity	[30]
	Insect cell lysate	↓Cox-2 activity	[30]
Petunidin	JB6	↓TPA-induced AP-1 activation	[34]
	HL60	↑Apoptosis	[20]
Peonidin	HT29	↓EGFR tyrosine kinase activity	[32]
		↑PDE4 inhibition	[32]
	Rat seminal vesicle	↓Cox-1 activity	[30]
	Insect cell lysate	↓Cox-2 activity	[30]
Pelargonidin	JB6	Weak inhibition of AP-1 activity	[34]
	HT29	↓EGFR tyrosine kinase activity	[32]
		PDE4 inhibition	[32]
	Rat seminal vesicle	↓Cox-1 activity	[30]
	Insect cell lysate	↓Cox-2 activity	[30]
Anthocyanin			
Cyanidin-3-glucoside	Lymphocytes	↑Apoptosis	[35]
	Jurkat cells	↑Apoptosis	[36]
		↑p53	[36]
		↑bax levels	[36]

Table 2 (*continued*)

Agent	Cell line/fraction	Effect	Reference
HL-60	↑Apoptosis		[36]
	↓c-myc		[36]
	↓bcl-2		[36]
Rat liver microsomes	Antioxidation		[46]
Liposomes	Antioxidation		[46]
Rabbit erythrocyte membranes	Antioxidation		[46]
Bovine artery endothelial cells	↑eNOS activity		[57]
	↑Akt phosphorylation		[57]
Anthocyan-rich extract			
Cherry extract	Rat seminal vesicle	↓Cox-1 activity	[29]
	Insect cell lysate	↓Cox-2 activity	[29]
Raspberry extract	Rat seminal vesicle	↓Cox-1 activity	[29]
	Insect cell lysate	↓Cox-2 activity	[29]
Grape colour extract	RBA	↓DNA synthesis	[14]
		G_1 phase arrest	[14]
Bilberry extract	RAW264	↓LPS-induced COX-2 expression	[31]
	HL60	↑Apoptosis	[21]
	HCT116	↑Apoptosis	[21]
	Plasma	Antioxidation	[40]
Black currant juice	Plasma	Antioxidation	[37]

JB6, mouse skin epidermal cell; U937, human monocytic leukaemia cell; A431, human vulva carcinoma cell; Jurkat, human T-lymphoblastoid cell; HL-60, human promyelocytic leukaemia cell; RBA, rat mammary adenocarcinoma cell; HT-29 and CaCo-2, human colon adenocarcinoma cells; HeLa S3, human uterine carcinoma cell; and Raw264, mouse macrophage cell.

Abbreviations. TPA, tetradecanoylphorbol acetate; EGFR, epidermal growth factor receptor; PDE, phosphodiesterases; LPS, lipopolysaccharide; COX-2, cyclooxygenase-2 and eNOS, endothelial nitric oxide synthase.

[a] Aglycon formed by alcoholic acid hydrolysis of pigmented rice.

counterparts [34]. In contrast, inhibition by anthocyanidins of phosphodiesterase activity in HT29 cells displayed the inverse molecular structure–activity relationship, as borne out by the decreasing rank order of inhibitory potency of malvidin > peonidin > pelargonidin = cyanidin > delphinidin [32]. This observation suggests that phosphodiesterase-inhibitory potency is positively correlated with number of methoxy moieties and inversely with number of hydroxyl groups in positions 3′ and 5′ in ring B. For comparison, the rank order of potency of anthocyanidins with respect to growth-inhibitory properties in HT-29 cells was delphinidin ≈malvidin > cyanidin > peonidin > pelargonidin, with IC_{50} values of 35, 35, 57, 90 and 213 μM, respectively [32].

In terms of their effect on cell-cycle regulation, anthocyanidins have been shown to interrupt the cell cycle at G_1 or G_2/M, which might contribute to induction of apoptosis and antiproliferation [14,20,22,35,36]. In terms of structure–activity relationship, hydroxyl groups in ring B conferred higher pro-apoptotic activity measured in human leukaemia cells onto the anthocyanidin molecule than methoxyl groups [20].

Last but no means least, berry extracts demonstrated potent antioxidant activity that correlated with anthocyanin content [37–39]. Anthocyans have been shown to be powerful antioxidants and may interfere with carcinogenicity by engaging this property [37,40–42]. Using the "ferric reducing ability of plasma" (FRAP) method to reflect antioxidant activity, the 3-glucosides of delphinidin, petunidin and malvidin were found to be 2–2.5 times more potent antioxidants than ascorbic acid [43]. Similarly, in the "trolox equivalent antioxidant capacity" (TEAC) or "oxygen radical absorbing capacity" (ORAC) tests, the antioxidant capacity of anthocyans was 3–6-fold that of the standard antioxidant trolox. The structure–antioxidant activity pattern emerging from these and other studies [42,44,45] suggests that potent antioxidant activity is associated with the presence of hydroxyl groups in the anthocyan B-ring. The antioxidant capacities of cyanidin and cyanidin-3-*O*-glucoside were similar to that of α-tocopherol in assay systems employing linoleic acid, liposomes, rabbit erythrocyte membranes or rat liver microsomes [46]. Antioxidant capacity has also been demonstrated *in vivo* in vitamin E-depleted rats [47]. In such rats that received a diet supplemented with an anthocyanin-rich extract, plasma antioxidant capacity was superior and hepatic 8-hydroxy-2′-deoxyguanosine levels were inferior compared to those in rats on diet without anthocyanins [47].

6. Pre-clinical and clinical pharmacology

The evidence summarised above intimates that anthocyanidins are more potent as confounders of certain activities regulating neoplastic promotion/progression than their glycosidic counterparts. Therefore, a crucial question concerning the pharmacokinetic properties of anthocyanins is whether they undergo metabolic de-glycosylation in the mammalian organism. It has been suggested that gut villi can catalyse anthocyanin hydrolysis to generate the aglycon [48–52], but until very recently convincing evidence for this hypothesis has been lacking. Two recent studies in rats support the notion that hydrolysis might occur *in vivo*. In one study, animals received cyanidin-3-glucoside either as an oral bolus or intravenously. Only rats that received the oral dose showed evidence of cyanidin-3-glucuronide in the plasma [52]. The presence of the glucuronide implies the intermediate formation of the aglycon, albeit possibly only fleetingly. The absence of the cyanidin-3-glucuronide from the plasma after intravenous dosing suggests that formation of the aglycon is a consequence of metabolism either by the gut microflora or the intestinal mucosa. In another recent report, cyanidin was unambiguously identified by HPLC-mass spectrometric analysis in the plasma and jejenum of rats which had received blackberry extract containing predominantly cyanidin-3-glucoside [53]. Metabolic conjugates of anthocyanins other than the 3-glucuronide has also been reported, including *O*-methylated species [48–50,52,53]. Glucuronides have also been found in rats that received delphinidin-3-glucoside [54] and in pigs on a marionberry diet [51].

The metabolism of anthocyanins when administered in the form of berry extracts to humans has received considerable attention. Serum and urine of healthy volunteers who ingested chokeberry extract containing four cyanidin-3-glycosides were analysed for parent phytochemicals and metabolic conjugates [50]. The maximal total concentration of anthocyanins in serum reached 0.59 μM within 2 h of consumption. In urine the peak concentration of total anthocyans was 18 μM within 5 h, and high levels persisted for 24 h. The metabolites were identified as glucuronide conjugates as well as methylated and oxidised derivatives of cyanidin-3-galactoside and cyanidin-3-glucoside. In a study of adults who received a mixture of four cyanidin-3-glycosides in concentrated elderberry juice, the majority of excreted anthocyanin species consisted of parent compounds. Maximal urinary excretion was observed after one hour Within five hours, 0.053% of the administered dose was excreted, of which 6.2% consisted of glucuronide metabolites [55]. The excretion profile in this study suggests first-order excretion kinetics. The results lead to the suggestion that small doses of anthocyans undergo metabolism in the intestinal mucosa, whilst at larger doses hepatic metabolism gains importance, a difference that may affect the anthocyan excretion profile.

Other human studies of the bioavailability and pharmacokinetics of anthocyanins have been conducted in elderly women. In one study, blueberry and elderberry extracts were compared [48]. The urinary concentration of anthocyanins after consumption of blueberries was much lower than that after ingestion of elderberry extract and anthocyanins were not detected in the plasma. In male volunteers, who ingested freeze-dried wild blueberry powder, anthocyanin pharmacokinetics was correlated with serum anti-oxidant capacity [56]. A maximum amount of 13 ng/ml of total anthocyanins was detected in the serum after 4 h. Results obtained using the ORAC test, which reflects antioxidant properties, suggest a positive correlation between postprandial serum anthocyanin content and antioxidant status [40], hinting at the potential usefulness of antioxidant capacity as a pharmacodynamic marker of anthocyan efficacy. In another study, volunteers received different beverages rich in malvidin-3-glucoside [57]. Plasma levels of malvidin-3-glucoside peaked after 50, 90 or 120 min for ingestion of red wine, de-alcoholized red wine or red grape juice, respectively. In women who received elderberry extract, peak plasma concentrations of cyanidin-3-sambubioside and cyanidin-3-glucoside were 39 and 43 nM, respectively [58].

7. Conclusion

Anthocyans, especially the anthocyanidins, possess a variety of pharmacological properties rendering them interesting as potential cancer chemopreventive agents. They occur in fruits and vegetables in relatively high amounts. The pharmacokinetic studies summarised above suggest that anthocyanins are easily ingested with their natural diet sources at amounts that can furnish blood levels in humans in the 10^{-8}–10^{-7} M range. Issues that remain unresolved are whether such levels are sufficient for exertion of robust anticarcinogenic effects, and whether anthocyanins exert chemopreventive efficacy themselves or if they need to undergo hydrolysis to their aglyconic counterparts. Anthocyanidins have been shown to inhibit malignant cell survival and oncogenic signalling with reasonable potency. Whilst the survey of the currently available literature provides tantalising hints to the potential usefulness of anthocyans as cancer chemopreventive interventions, further studies are necessary to help adjudge the propitiousness of their clinical development. Two types of investigations seem especially pertinent. Firstly, pharmacological potency differences between anthocyan derivatives need to be further defined, in order to allow prioritisation to be made as to which anthocyanidin(s) and/or anthocyanin(s) may be especially worthy of development. Secondly, studies

should focus on the tissue types that might be particularly susceptible to cancer prevention by anthocyans. Results from such studies together with those presented above will provide the robust scientific basis to support the notion that ingestion of five portions of colourful fruits per day is really good for you, because it may well prevent cancer.

Conflict of interest statement

None declared.

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