Protective effect of anthocyanin-rich extract from bilberry (*Vaccinium myrtillus* L.) against myelotoxicity induced by 5-fluorouracil

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Abstract. The toxicities associated with 5-fluorouracil (5-FU), a potent broad-spectrum chemotherapeutic agent, can not only affect the morbidity and the efficacy of chemotherapy but also limit its clinical use. The objective of this study is to investigate the effects of a commercial anthocyanin-rich extract from bilberry (AREB) against 5-FU-induced myelotoxicity in vivo, and against chemosensitivity to 5-FU in vitro. A single injection of 5-FU at 200 mg/kg induced severe peripheral erythrocytopenia, thrombocytopenia and leucopenia as well as hypocellularity of the spleen and bone marrow in C57BL/6 mice. Oral administration of 500 mg/kg of AREB for 10 days significantly increased the number of red blood cells, neutrophils, and monocytes in peripheral blood to 1.2-fold, 9-fold, and 6-fold, respectively, compared with those seen after treatment with 5-FU alone (\(p<0.05–0.001\)). The hypocellularity of the spleen and bone marrow caused by 5-FU was also distinctly alleviated in the AREB-treated group. Furthermore, AREB treatment with 50 and 100 µg/ml as a monomeric anthocyanin did not interfere with, but rather enhanced the chemotherapeutic efficacy of 5-FU in vitro. These results suggest that AREB may have protective potential against 5-FU-induced myelotoxicity and/or the ability to enhance the chemotherapeutic effectiveness of 5-FU.

Keywords: Anthocyanin, bilberry, 5-fluorouracil, myelotoxicity, chemotherapeutic efficacy

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1. Introduction

Many chemotherapeutic agents are cytotoxic to normal cells as well as to cancer cells, and consequently cause side effects such as immunosuppression, myelotoxicity, nausea, vomiting, diarrhea, and alopecia [30]. The consequences of toxicity have profound effects on patients affecting not only their therapy, but also their overall quality of life. 5-Fluorouracil (5-FU), a pyrimidine analogue, is an active anticancer drug used widely in the treatment of a variety of solid tumors such as colorectal, breast, and liver carcinomas [10]. However, 5-FU is known to induce gastrointestinal toxicity and myelotoxicity through its phosphorylation in the digestive tract [15] and bone marrow tissue [29]. These side effects restrict extensive clinical applications of 5-FU. More importantly, 5-FU-induced side effects sometimes prevent patients from receiving their next treatment cycle as scheduled [35]. As described by Jodrell et al. [16], 47% of patients receiving protracted venous infusion of 5-FU (300 mg/m²/day for 26 weeks) required delay and/or dose reduction due to the toxicity of 5-FU.

Natural products or their constituents have been reported to have beneficial effects against 5-FU-induced side effects. Lentinan, an anticancer polysaccharide, improved the burst forming units-erythroid reduced by 5-FU in mice [34], and catechin counteracted the 5-FU-induced reduction of myeloid colony formation [33]. In addition, antioxidants including vitamin C, vitamin E, and glutathione, reportedly enhanced the effectiveness of colon cancer treatment with 5-FU and/or reduced its side effects [3,4,32]. Although those results remain controversial [1], they indicate that natural products or their constituents with high antioxidative activity may enhance the effectiveness of 5-FU and/or reduce its side effects.

Recently, interest in anthocyanin-rich foods and extracts has intensified because of the possible health benefits that might arise from their potent antioxidative nature. Vaccinium species and various cherry and berry extracts, which are known for their high contents of anthocyanins, have demonstrated anticancer activity in experimental models. In particular, anthocyanin-containing extracts of chokeberry (Aronia meloncarpa E.), grape (Vitis vinifera) and bilberry (Vaccinium myrtillus L.) significantly inhibited growth of human colon cancer cells, HT-29, but not of normal colon cells [38]. Moreover, tart cherry extract inhibited intestinal tumor development in Apc (Min) mice [18], and freeze-dried black raspberries inhibited azoxymethane-induced colon tumors in rats [12].

Thus, we hypothesized that an anthocyanin-rich extract with high antioxidative and anticancer potential may reduce 5-FU-induced myelotoxicity and/or enhance the chemotherapeutic efficacy of 5-FU. To address this hypothesis, a commercially available anthocyanin-rich extract from bilberry (AREB) was examined for its protective potential against 5-FU-induced myelotoxicity in C57BL/6 mice, in parallel with analysis of the anthocyanin profile of AREB. Further, the effect of AREB on the cytotoxicity of 5-FU was also examined in vitro to evaluate the interference of AREB with the antitumor activity of 5-FU.

2. Materials and methods

2.1. Materials

A commercial anthocyanin-rich extract from bilberry (Vaccinium myrtillus L.) was supplied by Artemis International, Inc. (Fort Wayne, IN, USA). All HPLC reagents were purchased from Honeywell Berdick & Jackson (Muskegon, MI, USA). The anthocyanin standards were purchased from Polyphenols Laboratories AS (Sandnes, Norway) and their purities were > 97%. Dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), 5-FU, sulforhodamine B (SRB) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were of reagent grades.
2.2. Anthocyanin profile of AREB by HPLC

AREB was semi-purified by a solid-phase extraction (SPE) with a C18 cartridge (Waters Corp, Milford, MA, USA) and its anthocyanin profile was determined according to the method of Durst and Wrolstad [7]. For the analysis of aglycone forms, the anthocyanidins fraction was prepared by the acid hydrolysis of AREB with 2 N HCl for 30 min at 95°C. The hydrolysate was cooled at room temperature and then purified by SPE. The anthocyanin profile of AREB was determined by a Jasco HPLC instrument (Tokyo, Japan) equipped with a UV detector UV-975, autosampler AS-950-10 and column oven CO-965. Samples were filtered through 0.2 µm polypropylene filter (Whatman Inc., Clifton, NJ, USA). The anthocyanins were separated on an Allsphere ODS-2 column (4.6 × 150 mm i.d., 5 µm particle size; Alltech Inc., Deerfield, IL, USA) and the solvent system was composed of solvent A, 4% phosphoric acid-H2O (v/v) and solvent B, 100% acetonitrile. The gradient elution for anthocyanin analysis was 0–35 min, 90–87% A; 35–40 min, 87–90% A; and 40–50 min, 90% A. In anthocyanidin analysis, the gradient was 0–5 min, 88% A; 5–30 min, 88–85% A; 30–50 min, 85–80% A; and 50–55 min, 80–88% A. The column temperature was maintained at 35°C, and the injection volume was 20 µl, and the flow rate was 0.7 ml/min. The peaks were detected at 520 nm.

2.3. Effect of AREB on myelotoxicity induced by 5-FU in vivo

2.3.1. Animals
Male C57BL/6 mice, 6 weeks old, were purchased from Hanlim Animal Co., Ltd. (Hwaseong, South Korea). Mice were housed under standard laboratory conditions (12 h light: 12 h dark cycle and 22 ± 2°C) with free access to water and food. The mice were divided into four groups of 10 animals each for four treatment: Control (vehicle treated), 5-FU (200 mg/kg), 5-FU (200 mg/kg) + AREB 100 mg/kg and 5-FU + AREB 500 mg/kg. 5-FU was intraperitoneally injected at a single dose of 200 mg/kg body weight at day 5 to induce acute myelotoxicity in the mice. AREB was administered by gavage in a dose of 100 or 500 mg/kg body weight once a day for 10 days.

2.3.2. Hematological analysis
At the end of the experiments, peripheral blood was collected from the retro-orbital venous plexus with an EDTA-coated capillary tube. Red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), platelets, white blood cells (WBC), lymphocytes, neutrophils, and monocytes in each sample were measured by an autoanalyzer (ADVIA 120 Hematology system, Bayer Corporation, Tarrytown, NY, USA).

2.3.3. Histology
For histological evaluation of the spleen and bone marrow, the spleen and right femur from all mice were fixed in 10% buffered formalin, and the femur was decalcified with Calci-Clear Rapid (National Diagnostics, Atlanta, GA, USA). Spleen and femur were then embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin-eosin. The numbers of marrow and splenic nucleated cells were counted under a light microscope.

2.4. Effect of AREB on the cytotoxicity of 5-FU in vitro

2.4.1. Cell culture
HT-29, human colon adenocarcinoma cells, and MCF-7, human breast adenocarcinoma cells, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell culture
medium and supplements were purchased from GibcoBRL (Grand Island, NY, USA). Cells were cultured in complete Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37°C in a 5% CO₂ atmosphere. The medium was changed every 2 days.

2.4.2. Cytotoxicity assay

AREB semi-purified by SPE with a C18 cartridge as described by Guisti and Wrolstad [9] was used for this assay. The monomeric anthocyanin content of AREB was determined by pH-differential spectrophotometry and calculated as cyanidin-3-glucoside using an extinction coefficient [8]. The effect of AREB on the cytotoxicity of 5-FU was evaluated in vitro in HT-29 and MCF-7 cancer cells by SRB assay [31]. Briefly, sub-confluent cells were trypsinized and seeded onto 96-well plates at 0.5–1 × 10⁵ cells/ml. Cells were allowed to grow for 24 h prior to treatment. Thereafter cells were exposed to AREB at concentrations of 1–100 µg/ml as a monomeric anthocyanin and/or 5-FU for 48 h. Cultivation was stopped by the addition of 50% TCA solution. After 1 h at 4°C, the plates were washed five times with water and dried. The fixed cells were stained with 0.4% SRB solution in the dark for 30 min, and subsequently washed with 1% acetic acid to remove excess dye. The protein-bound dye was eluted with 10 mM Tris-buffer and quantified spectrophotometrically at 550 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). There were three or four replications for each tumor cell and cytotoxicity was determined as the percentage of cell survival compared with that of the control.

2.5. Statistical analysis

All experimental data were given as mean ± SEM. Statistical analysis was carried out by Student’s t-test. The level of significance was set at p < 0.05.

3. Results

3.1. Anthocyanin profile of AREB

The anthocyanin profile of AREB was analyzed by HPLC detected at 520 nm. The typical HPLC chromatogram of AREB showed 13 peaks (Fig. 1A). The anthocyanin peaks in the AREB contained five anthocyanidins glycosylated with three sugars, galactose, glucose and arabinose. Because of unavailability and incomplete separation of reference compounds, the HPLC quantitation of anthocyanin was difficult and inaccurate. To quantify the anthocyanin content of AREB, samples were acid hydrolyzed. Acid hydrolysis greatly simplified the anthocyanin profile, which comprised five aglycons, delphinidin, cyanidin, petunidin, peonidin, and malvidin (Fig. 1B). All anthocyanidins from AREB could be completely separated and all reference standards were commercially available. This made accurate quantitation of anthocyanin possible. According to the polarity, delphinidin, having the highest polarity, was eluted first, followed in order by cyanidin, petunidin, peonidin and malvidin. The anthocyanin profile and the anthocyanidin contents were given in Table 1. Delphinidin-3-glucoside was the major anthocyanin (15%) present in AREB. Delphinidin was the most abundant aglycone (18.7 mg/g extract) and the total anthocyanidin content of AREB was 65.9 mg/g extract.
Table 1

<table>
<thead>
<tr>
<th>Anthocyanins</th>
<th>Glycoside (%)</th>
<th>Aglycone Anthocyanidin contents (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galactoside</td>
<td>Glucoside</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>10.0</td>
<td>15.1</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>8.0</td>
<td>12.7</td>
</tr>
<tr>
<td>Petunidin</td>
<td>–</td>
<td>9.8</td>
</tr>
<tr>
<td>Peonidin</td>
<td>0.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Malvidin</td>
<td>3.8</td>
<td>9.9</td>
</tr>
</tbody>
</table>

a. The values were the percent area of the peak.

b. The contents of anthocyanidin were quantified with anthocyanidin standard in acid hydrolyzed AREB.

Fig. 1. HPLC chromatograms for AREB (A) and acid hydrolyzed AREB (B). (A) AREB: peaks 1, delphinidin-3-galactoside; 2, delphinidin-3-glucoside; 3, cyanidin-3-galactoside; 4, delphinidin-3-arabinoside; 5, cyanidin-3-glucoside; 6, cyanidin-3-arabinoside; 7, petunidin-3-glucoside; 8, peonidin-3-galactoside; 9, petunidin-3-arabinoside; 10, peonidin-3-glucoside; 11, malvidin-3-galactoside; 12, malvidin-3-glucoside; 13, malvidin-3-arabinoside. The number in parentheses is the percent area of the peak. (B) Acid hydrolyzed AREB: peaks 1, delphinidin; 2, cyanidin; 3, petunidin; 4, unknown; 5, peonidin; 6, malvidin.

3.2. The effect of AREB on myelotoxicity induced by 5-FU

The myelotoxicity induced by 5-FU was examined in the peripheral blood, spleen, and bone marrow in mice. As shown in Table 2, the intraperitoneal injection of 5-FU (200 mg/kg body weight) drastically reduced all hematological parameters in the peripheral blood. The RBC, Hb, and HCT values in mice receiving 5-FU alone were 22–23% lower than those of the control group. Circulating platelets, WBC,
The table below shows the effect of AREB on hematological parameters damaged by 5-FU treatment.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>RBC (10⁶/µl)</th>
<th>Hb (g/dl)</th>
<th>HCT (%)</th>
<th>Platelets (10⁵/µl)</th>
<th>WBC (10⁹/µl)</th>
<th>Lymphocytes (10⁵/µl)</th>
<th>Neutrophils (10⁵/µl)</th>
<th>Monocytes (10⁵/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.5 ± 0.1</td>
<td>13.9 ± 0.2</td>
<td>45.8 ± 0.9</td>
<td>708 ± 52</td>
<td>2.7 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>0.20 ± 0.07</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>5-FU only</td>
<td>6.6 ± 0.3</td>
<td>10.7 ± 0.4</td>
<td>35.3 ± 1.4</td>
<td>216 ± 31</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>5-FU + AREB 100</td>
<td>7.5 ± 0.3</td>
<td>11.5 ± 0.3</td>
<td>36.8 ± 1.1</td>
<td>348 ± 81</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.06 ± 0.03</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>5-FU + AREB 500</td>
<td>7.7 ± 0.2*</td>
<td>11.8 ± 0.3</td>
<td>36.9 ± 1.3</td>
<td>504 ± 133</td>
<td>1.2 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.27 ± 0.05**</td>
<td>0.06 ± 0.02</td>
</tr>
</tbody>
</table>

5-FU was injected intraperitoneally at a single dose of 200 mg/kg body weight at day 5. AREB was administered by gavage in a dose of 100 or 500 mg/kg body weight once a day for 10 days. *p < 0.05, **p < 0.001 compared with 5-FU treatment only. Values are expressed as mean ± SEM.

Fig. 2. Effect of AREB on splenic cells damaged by 5-FU treatment. Representative splenic sections for 5-FU and/or AREB treated mice (hematoxylin-eosin, original magnification × 400). (A) Control; normal splenocytes, (B) 5-FU (200 mg/kg body weight) only; hypocellularity, (C) 5-FU + AREB 100 mg/kg body weight; hypocellularity, (D) 5-FU + AREB 500 mg/kg body weight; hypocellularity significantly alleviated compared with (B). The splenic nucleated cells were counted under a microscope (100 × 100 µm²). *p < 0.05 compared with 5-FU treatment only. Values are expressed as mean ± SEM.

lymphocytes, neutrophils and monocytes were particularly seriously damaged, and their counts were decreased to 31, 30, 30, 15 and 5%, respectively, compared with those of the control group. The oral administration of 500 mg/kg of AREB for 10 days resulted in considerable improvement of these parameters. RBC, neutrophil, and monocyte counts were significantly higher than those of the 5-FU-alone group (p < 0.05–0.001). Platelet and WBC counts were increased by 133% and 50%, respectively, compared with those of 5-FU alone, but these were not significant. AREB at a dose of 100 mg/kg showed only slight positive effects on the parameters, although these were not statistically significant.

A single injection of 5-FU (200 mg/kg) caused a serious damage to the spleen and bone marrow (Figs 2 and 3). In 5-FU-alone group, the count of nucleated splenic cells was 63% and the count of femoral bone marrow cells was only 33% of those of the control group, and there was a marked histopathological hypocellularity. Treatment with AREB resulted in significantly higher numbers of splenic cells as well as of bone marrow nucleated cells, compared with 5-FU alone group, in a dose dependent manner (p < 0.05 and 0.01, respectively). The protection against bone marrow damage was particularly evident (Fig. 3) at a dose of 500 mg/kg AREB.
Effect of AREB on bone marrow cells damaged by 5-FU treatment. Representative bone marrow sections for 5-FU and/or AREB treated mice (hematoxylin-eosin, original magnification ×400). (A) Control; normal cells, (B) 5-FU (200 mg/kg body weight) only; severe hypocellularity, (C) 5-FU + AREB 100 mg/kg body weight; hypocellularity still severe but myelocytes slightly increased, (D) 5-FU + AREB 500 mg/kg body weight; hypocellularity significantly alleviated compared with (B). The marrow nucleated cells were counted under a microscope (100 × 100 µm²). **p < 0.01 compared with 5-FU treatment only. Values are expressed as mean ± SEM.

Effect of AREB on cytotoxicity, *in vitro* of 5-FU in (A) HT-29, colon adenocarcinoma and (B) MCF-7, breast adenocarcinoma cells. Cells were allowed to grow for 24 h and then exposed with 5-FU to different concentrations of AREB on the basis of their monomeric anthocyanin content, expressed as cyanidin-3-glucoside equivalents, for 48 h. Cell survival was evaluated by SRB assay. ***p < 0.001 compared with 5-FU treatment only. Values are expressed as mean ± SEM.

3.3. The effect of AREB on the cytotoxicity of 5-FU

To evaluate whether AREB could modify the chemotherapeutic efficacy of 5-FU, the effect of AREB on the 5-FU-induced cytotoxicity was investigated in HT-29, human colon cancer cells and MCF-7, human breast cancer cells by SRB assay. As shown in Fig. 4, cell survival was lowered by 5-FU alone, and co-administration for 48 h of 5-FU with AREB at 50 and 100 µg/ml as a monomeric anthocyanin significantly decreased cell survival in both cancer cell lines (p < 0.001). These results indicate that AREB did not interfere with the antitumor activity of 5-FU, but rather exhibited a synergistic effect.
4. Discussion

In the present study, oral administration of AREB demonstrated a protective effect against the myelo-toxicity caused by 5-FU in mice. Moreover, AREB treatment with 5-FU increased the antitumor activity of 5-FU synergistically in HT-29, human colon cancer cells and MCF-7, breast cancer cells *in vitro*. This study appears to be the first attempt to demonstrate the protective effect of the anthocyanin-rich extract from berries against 5-FU-induced myelotoxicity.

Although 5-FU has been widely used in the treatment of a variety of solid tumors such as colorectal, breast, and liver carcinomas, it induces severe side effects including myelotoxicity and gastrointestinal toxicity. It has been reported that peripheral thrombocytopenia and leucopenia, and also medullary erythrocytopenia and granulocytopenia were characteristic findings induced by 5-FU in mice [13]. The present study indicated that a single injection of 5-FU (200 mg/kg body weight) induced peripheral erythrocytopenia, thrombocytopenia and leucopenia, as well as hypocellularity in the hematopoietic organs, spleen and bone marrow. These myelotoxicities were considered to be due to the cytotoxic effects of 5-FU. Generally, 5-FU is subject to one of two alternative metabolic pathways: catabolism inactivates 80% of this compound in the liver, leading to the main metabolite 5-fluoro-5,6-dihydrouracil, whereas anabolism produces 5-fluorouridine-5′-monophosphate, 5-fluorouridine and 5-fluoro-2′-deoxyuridine [6]. These metabolites of 5-FU interfere with the synthesis of DNA, RNA and proteins. As a result, 5-FU can inhibit the proliferation of hematopoietic cells as well as cancer cells.

Oral administration of AREB at 500 mg/kg body weight significantly protected RBC, neutrophils, and monocytes in peripheral blood, and the spleen and bone marrow against the side effects of 5-FU. The AREB used in this study is a commercially available extract from bilberry, which is standardized as 6% anthocyanin; the total anthocyanin content was measured as 65.9 mg/g extract under our HPLC conditions after acid hydrolysis. Anthocyanins, major active components of AREB, have recently gained increasing attention because of their relatively high potential intake in humans and the broad spectrum of potentially positive health effects related to their potent antioxidative properties. Although the protective mechanism of AREB was not examined here, it is considered to be linked with two known mechanisms in relation to anthocyanin.

First, AREB could exhibit a protective effect against 5-FU-induced myelotoxicity in part as a result of its antioxidative activity. AREB can increase the stability of DNA and prevent DNA damage in hematopoietic cells. Lazzé et al. showed that anthocyanins were effective in reducing DNA single strand breakage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells [21]. Furthermore, it has been suggested that the cyanidin-DNA copigmentation may be a possible defense mechanism against the oxidative damage of DNA [28] and that anthocyanins have the ability to stabilize DNA triple-helical complexes [23]. Recently, it was reported that other antioxidants such as (+)-catechin and glutathione demonstrated a preventive effect against 5-FU-induced myelotoxicity [19,33].

Secondly, the anti-inflammatory properties of anthocyanin and/or berry extracts may play a role in protection by AREB. A high dose of 5-FU often induces cytotoxicity in intestinal tissue, resulting in ulceration, diarrhea, and bacterial translocation [5,36]. The tissue destruction and bacterial translocation induce an inflammatory response through the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) by epithelial cells and infiltrating leukocytes [14]. Kucuk et al. demonstrated that administration of 5-FU resulted in the elevation of acute inflammatory cytokine, TNF-α and interleukin-6 (IL-6) [20]. Some cytokines such as TNF-α, interferon-gamma (IFN-γ) and granulocyte macrophage-colony simulating factor (GM-CSF) have been reported to suppress or stimulate hematopoiesis [22,27]. Anthocyanins and/or berry extracts have been reported to modulate the levels of
TNF-\(\alpha\), prostaglandin-E2 (PGE2), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 [25,26]. Thus, AREB could affect hematopoiesis through cytokine modulation. However, there is no conclusive proof that the AREB and/or anthocyanins are directly related with the protection against myelotoxicity or the hematopoiesis. Therefore, further studies are necessary to clarify the mechanism of protection by AREB and/or anthocyanins.

Our data revealed that semi-purified AREB increased the chemotherapeutic efficacy of 5-FU in HT-29, human colon cancer cells and MCF-7, breast cancer cells, in vitro. In a preliminary trial, semipurified AREB alone at 50 and 100 \(\mu\)g/ml was cytotoxic to cancer cells (data not shown). These results corresponded to previous reports, which demonstrated that anthocyanins or anthocyanin-rich extracts suppressed tumor growth in vitro and in vivo: the tumor cells tested have included HT-29, HCT 15, AGS, and HCT 116 [17,18,38]. In an in vivo model, anthocyanins have inhibited chemically-induced colorectal carcinogenesis in F344/DuCrj rats and intestinal tumor development in Apc (Min) mice [11, 18]. Moreover, berry extracts have been reported to exert differential effects on normal or cancerous cells [38]. Grape seed proanthocyanin extract has been shown to protect human oral keratinocytes against tobacco-induced apoptotic cell death and human liver cells against drug-induced cytotoxicity [2,37].

Anthocyanins, as well as several other flavonoids, are largely found in the plant kingdom, in fruits and vegetables of nutritional interest. The consumption of this class of compounds has been shown to increase antioxidant defenses that might be effective in preventing diseases caused by reactive oxygen species. However, there is insufficient information on the bioavailability and eventual distribution and accumulation of anthocyanins in the organs, even though Milbury et al. [24] demonstrated that orally-consumed elderberry anthocyanins are found in low amounts in their intact glycosylated forms in the plasma of older women (maximum concentration reported for cyanidin 3-glucoside, \(\simeq 50\) nmol/l plasma). The present study indicates that the protective effect of AREB against 5-FU-induced myelotoxicity may be exploited at higher concentrations than those obtainable through the diet. Further studies are necessary, not only to better characterize the effects of dietary intake but also to evaluate its possible pharmacological use.

In conclusion, AREB significantly reduced myelotoxicity in peripheral blood and hypocellularity in hematopoietic organs caused by 5-FU in vivo. Furthermore AREB increased the effects of 5-FU on tumor cells in vitro. These findings suggest that AREB may lessen the side effects and enhance the beneficial activity of 5-FU in cancer chemotherapy. Confirmation of this result will require further investigation of the protective mechanisms of AREB against 5-FU-induced myelotoxicity as well as studies using tumor-bearing animal models in vivo.

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References


