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Invited article

Cancer prevention with freeze-dried berries and berry components

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Abstract

Our laboratory is developing a food-based approach to the prevention of esophageal and colon cancer utilizing freeze-dried berries and berry extracts. Dietary freeze-dried berries were shown to inhibit chemically induced cancer of the rodent esophagus by 30–60% and of the colon by up to 80%. The berries are effective at both the initiation and promotion/progression stages of tumor development. Berries inhibit tumor initiation events by influencing carcinogen metabolism, resulting in reduced levels of carcinogen-induced DNA damage. They inhibit promotion/progression events by reducing the growth rate of pre-malignant cells, promoting apoptosis, reducing parameters of tissue inflammation and inhibiting angiogenesis. On a molecular level, berries modulate the expression of genes involved with proliferation, apoptosis, inflammation and angiogenesis. We have recently initiated clinical trials; results from a toxicity study indicated that freeze-dried black raspberries are well tolerated in humans when administered orally for 7 days at a dose of 45 g per day. Several Phase IIa clinical trials are underway in patients at high risk for esophagus and colon cancer; i.e., Barrett's esophagus, esophageal dysplasia and colonic polyps, to determine if berries will modulate various histological and molecular biomarkers of development of these diseases.

Keywords: Black raspberries; Colon; Esophagus; Cancer; Prevention

Contents

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1. Cancers studied

1.1. Esophageal cancer

Adenocarcinoma and squamous cell carcinoma are the principal types. The prognosis of both is poor (<10%, 5-year survival) [1]. Adenocarcinoma is more prevalent in the United States and develops primarily in patients with Barrett's esophagus [2]. Barrett's is defined by columnar-lined metaplastic mucosal cells replacing the normal squamous epithelium in the distal esophagus. Barrett's in turn is caused by chronic gastroesophageal reflux disease. Barrett's patients are offered endoscopic surveillance involving biopsies to aid in early cancer detection [3]. Chemoprevention may represent an effective means to prevent or slow the development of cancer in these individuals.

Squamous cell carcinoma (SCC) accounts for >90% of esophageal cancers worldwide [1,4]. This disease probably develops through a progressive sequence from mild to severe dysplasia, carcinoma *in situ* and, finally, invasive carcinoma [1,5,6]. This cancer shows marked variation in geographic distribution, with high frequencies in parts of China, South Africa, Iran, Uruguay, France, Italy and Puerto Rico [7–9]. The highest incidences are in the Henan, Shansi and Hopei provinces in China where the age-adjusted mortality rates are 151/100,000 for males and 115/100,000 for females [7–10]. Risk factors are tobacco smoking, alcohol consumption, ingestion of salt-pickled, salt-cured and moldy foods, deficits in certain vitamins and minerals, and human papilloma virus (HPV) infection [11–15]. Thus, life-style changes, avoidance of tobacco and reduced alcohol use, (potentially) vaccination against oncogenic HPV, and enhanced consumption of chemopreventive foods may reduce the incidence of the disease.

1.2. Colon cancer

Colorectal cancer is the most prevalent digestive tract cancer in the Western world. It is third most common cancer in both men and women, and there will be 149,000 new cases in the U.S. in 2007. Colorectal cancer accounts for 10% of all annual cancer deaths in the U.S. [16]. Age is the primary risk factor; >90% of cases are diagnosed in individuals older than 50. Risk is also increased by certain inherited syndromes {familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC)}, a personal or family history of colorectal cancer and/or polyps, or a personal history of inflammatory bowel disease. Other factors include obesity, physical inactivity, smoking, alcohol consumption, a diet high in red meat, and inadequate intake of vegetables and fruit. The sentinel preventative is endoscopic screening after age 50 to detect and remove precursor lesions (adenomatous polyps), and detection of early stage carcinomas [16]. Sadly, screening compliance is poor; however, encouraging pre-clinical chemoprevention studies suggest that regular use of aspirin, estrogen and progestin hormone therapy, and HMG Co-A reductase inhibitors (statins) may reduce colon cancer risk.

2. Rationale for freeze-dried berries as chemopreventive agents

In the mid-1980s we showed that the polyphenol ellagic acid inhibited carcinogen-induced esophagus tumors in rats when administered in their diet [17]. Analysis of 28 fruits showed that all contained some ellagic acid, but the highest concentrations (630–1500 μg/g dry weight) were in blackberries, raspberries, strawberries and cranberries [18]. Ellagic acid is abundant in the pulp and seeds, with little in the juice. As berries are 85–90% water we concentrated the ellagic acid 9–10-fold by freeze-drying. The freeze-dried preparation was found to have active ellagic acid along with many known chemopreventive agents including vitamins A, C and E and folic acid; calcium and selenium; β-carotene, α-carotene, lutein, gallic acid, ferulic acid, *p*-coumaric acid, quercetin, several anthocyanins, β-sitosterol, stigmasterol, and kaempferol [19]. Regarding toxicity, extensive histopathological studies of rats fed freeze-dried black raspberries (*Rubus occidentalis,* BRBs) or strawberries (*Fragaria ananasia*, STRWs) as 5 and 10% of a synthetic diet for a period of 9 months showed no evidence of changes in any organ or tissue. In addition, liver-specific enzymes were not affected, and there was about a 10% reduction in blood cholesterol. Thus, freeze-dried berries are well tolerated.

3. "Standardized" berry powders

The content of two major anthocyanins in BRBs grown on different farms in Ohio varies two- to three-fold (unpublished data). Thus, to obtain a "standardized" preparation, we purchase berries (*Jewel* variety) from a single farm on a yearly basis. These berries are grown in the same part of the field, and are picked mechanically at the same degree of ripeness. After picking, they are washed and stored frozen at −20 °C. They are then shipped frozen to VanDrunen Farms (Momence, IL) to be freeze-dried and ground into a powder. The powder is stored frozen until

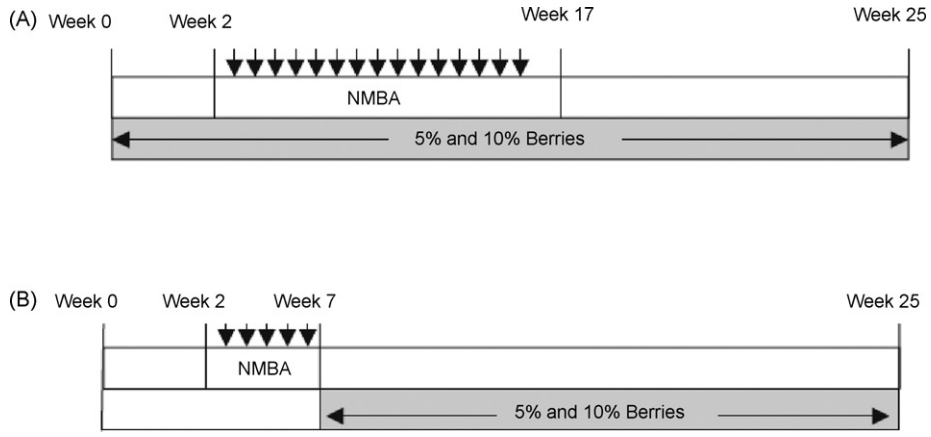

Fig. 1. Experimental protocols for the complete carcinogenesis (A) and the post-initiation (B) studies. Rats were treated with NMBA once per week for 15 weeks (A) or 3 times per week for 5 weeks (B). Freeze-dried berry diets were administered 2 weeks prior to initiation of NMBA treatment and for the duration of the study (A) or only following NMBA treatment (B) [23,24].

use [19]. We have found that powders have relatively constant concentrations of the above-mentioned components on a crop-to-crop basis. Also, these components remain stable (i.e., <20% degradation) for at least 2 years. The vitamins, especially vitamin C, are exceptions; they undergo considerable degradation in frozen berries before the berries are freeze-dried.

4. Prevention of esophagus cancer in rodents

4.1. Rodent esophagus model system

Esophageal tumors are induced in the Fischer 344 (F344) rat by repeated subcutaneous (s.c.) injection of the *N*-nitrosamine carcinogen, *N*-nitrosomethylbenzylamine (NMBA). We either inject NMBA at 0.25–0.5 mg/kg body weight 3 times a week for 5 weeks or once per week for 15 weeks; both protocols yield 100% tumor incidence by 25 weeks [20–23]. Squamous papilloma is the predominant pathology; the incidence of squamous cell carcinomas (SCC) is low because the large papillomas occlude the esophagi before carcinomas can develop. The quantifiable pre-neoplastic lesions include simple hyperplasia, leukoplakia, and epithelial dysplasia.

4.2. Bioassays with berries

Freeze-dried BRBs and STRWs were administered as 5 and 10% of the diet, before, during and after NMBA treatment [19,23,24]. The data are summarized in Fig. 1A and B, and Table 1. In experiments in which BRBs were continuously administered, the tumor incidence was reduced from 8 to 22% (not significant) and the tumor multiplicity by 40–50% ($p < 0.05$). In the same protocol, STRWs reduced the tumor incidence by 20% (not significant) and the tumor multiplicity by 24–56% ($p < 0.05$). These reductions in tumor response were correlated with similar reductions in the frequency of pre-neoplastic lesions, and with the ability of dietary BRBs and STRWs to reduce the formation of O^6-methylguanine (O^6-MeGua) adducts in esophageal DNA [23–26]. Inhibition of DNA adduct formation might be expected to inhibit tumor initiation, in part, because one of the principal initiation events in rat esophageal carcinogenesis is mutational activation of the H-ras oncogene [27,28]. Activation of this gene by NMBA is associated with GC → AT transition mutations in the second base of codon 12; these mutations are consistent with the formation of O^6-MeGua adducts in DNA.

BRBs and STRWs were also evaluated for their post-initiation activity (Fig. 1B) by providing the berries after NMBA treatment stopped [23,24]. At this time point, about 50–60% of the esophageal epithelium in NMBA-treated rats is hyperplastic, and also contains occasional foci of mild to moderate dysplasia. Five and 10% dietary BRBs reduced the ultimate tumor incidence from 40 to 47% ($p < 0.05$) and tumor multiplicity by 40–60% ($p < 0.05$) (Table 1). In contrast, 5 and 10% STRWs had no effect on tumor incidence; however, they reduced tumor multiplicity by 31–38% ($p < 0.05$). Dietary BRBs were shown to reduce the proliferation rate of pre-neoplastic cells in NMBA-treated esophagus almost to levels seen in vehicle-treated esophagus and in the esophagus of rats admin-

Table 1
Freeze-dried berries as effective agents against NMBA-induced esophageal tumorigenesis in the F344 rat

Berry type	Experimental protocols	Inhibition of tumor incidence (%)	Inhibition of tumor multiplicity (%)	References
BRBs[a]	Complete	8–22	40–50	[24]
STRWs[b]	Complete	20	24–56	[19,23]
BRBs	Post-initiation	40–47	40–60	[24]
STRWs	Post-initiation	0	31–38	[23]

[a] BRBs, freeze-dried black raspberries.
[b] STRWs, freeze-dried strawberries.

istered a 10% BRB diet [24]. These observations suggest that berries might be expected to be preventive in humans who have esophageal dysplasia.

4.3. Effects of berries on gene expression in the esophagus

A number of genes are overly expressed in the preneoplastic stages of rat esophagus [29–31]. Among these are cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and vascular endothelial growth factor (VEGF). COX-2 expression and enzyme activity increases progressively with tumor development resulting in elevated levels of prostaglandin E_2 (PGE_2) [29]. Elevated levels of PGE_2 may contribute to increased cell proliferation and inflammatory events. Dietary 5% BRBs reduced the level of expression of COX-2 mRNA in NMBA-treated esophagus almost to that in control esophagus, with a corresponding 50% reduction in PGE_2 levels. Since up-regulation of COX-2 has been detected in human esophageal SCCs [32], dietary freeze-dried berries might produce inhibitory effects on the development of esophageal SCC in humans, in part, by down-regulation of COX-2.

The iNOS enzyme converts L-arginine to citrulline with the production of nitric oxide (NO) [33]. NO is a free radical that can donate or accept an electron to become a nitrosonium cation (NO^+) or a nitroxyl anion (NO^-), which leads to nitrosative stress or oxidative stress, respectively. Nitrosative stress can lead to inactivation of DNA repair proteins, deamination of DNA bases and the formation of nitrosamine carcinogens. Oxidative stress contributes to the formation of peroxynitrite, which can damage DNA, and can be catalyzed by tissue peroxidases to products that induce COX-2. iNOS is overly expressed in many human cancers including esophageal SCC [34]. Dietary 5% BRBs significantly down-regulated iNOS mRNA with a reduction in nitrite levels in esophageal tissues [30]. By inhibiting iNOS and NO production, berries could reduce nitrosative and oxidative stress, inflammation, and COX-2 activity.

Angiogenesis is essential for tumor growth and expansion [35]. Several growth factors exhibit angiogenic activity including VEGF, basic fibroblast growth factor and platelet-derived growth factors [36]. VEGF-C, which induces proliferation and migration of endothelial cells [37], is upregulated in esophageal SCC [38], and administration of 5% BRBs in the diet of NMBA-treated rats resulted in a significant reduction in VEGF-C mRNA [31]. There was also a marked reduction in microvessel density (MVD), indicating that modulation of angiogenesis by BRBs is associated with down-regulation of VEGF, COX-2 and iNOS in NMBA-treated rat esophagus.

cDNA microarray analysis of 30,000 genes has been conducted to depict the molecular functions berries affect (unpublished data). Rats received three subcutaneous injections of NMBA during a period of 1 week. One-half of the animals were fed control diet and the other half were fed control diet containing 5% freeze-dried BRBs. All esophagi were harvested 24 h after the last NMBA injection. More than 3100 genes showed expression changes in the NMBA alone animals. The berry diet reversed the expressions of 578 of these genes to levels seen in control esophagus, regardless of whether the genes were overexpressed or underexpressed.

5. The active chemopreventive constituents

We have extracted freeze-dried berries with organic solvents and water to identify the chemopreventative components [39] and assessed fractions using mouse epidermal JB6 Cl 41 cells that are stably transfected with either a nuclear factor kappa B (NFκB) or an activator protein 1 (AP-1)-luciferase reporter. The carcinogen *anti*-benzo[a]pyrene-7,8-diol-9,10 epoxide (BPDE) induces both NFκB and AP-1 in these cells, and both ethanol and water extracts inhibited up-regulation of these genes [40]. These extracts were then fractionated by high-performance liquid chromatography (HPLC) to yield several bioactive subfractions. Interestingly, the major constituents of the most active subfractions were the anthocyanins: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and cyanidin 3-*O*-(2^G-xylosylrutinoside) [39]. Thus, the cyanidin glucosides account for some of the chemopreventive activity of BRBs. In a follow-up study, rats were fed different fractions before, during and after treatment with NMBA (unpublished data). The anthocyanin fraction and the 80% ethanol/20% water (80/20) extracts contain the same amount of anthocyanins (~3.5 μmol/g diet) that are present in a diet containing 5% freeze-dried BRBs. The 80/20 residue contained <1.0 μmol anthocyanins/g of diet, and the hexane extract and a sugar fraction contained no anthocyanins. Fig. 2 illustrates the results. As expected, the hexane extract and the sugar fraction (principally fructose) were inactive, whereas the anthocyanin fraction and the 80/20 extract were equally effective as the 5% BRB diet, suggesting that anthocyanins are chemopreventive. Interestingly, however, the 80/20 residue, with minute anthocyanin levels, was as effective as the 5% BRB diet. Studies are currently underway to determine if the flavan-3-ols (ellagitannin) and the flavonols (quercetin) content might be responsible for the chemopreventive activity of the 80/20 residue.

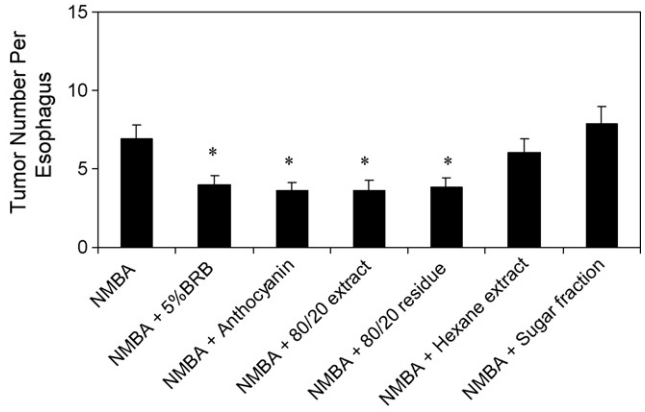

Fig. 2. Effects of BRB extracts on tumor development in NMBA-treated rat esophagus at 30 weeks. Rats treated with NMBA + 5%BRBs, NMBA + anthocyanins, NMBA + 80/20 extract, and NMBA + 80/20 residue had fewer tumors than rats treated with NMBA only. Bars represent mean ± S.E., $n = 15$. The symbol (*) shows significantly lower ($p < 0.05$) than rats treated with NMBA only.

Table 2
Effects of freeze-dried BRBs on azoxymethane (AOM)-induced colon cancer in the F344 rat [41]

Treatment	Tumor multiplicity[a,b,c] (% inhibition)	Tumor volume[b,d] (mm^3)	Tumor burden[b,e] (% inhibition)
Control diet	0	NA	NA
5.0% BRBs	0	NA	NA
AOM only	2.3 ± 0.5	33.8 ± 7.7	120.9 ± 25
2.5% BRBs + AOM	1.4 ± 0.3[c] (42)	35.9 ± 17.8	87.5 ± 41.5 (28)
5.0% BRBs + AOM	1.3 ± 0.2[c] (45)	42.4 ± 15.0	69.8 ± 22.5 (42)
10.0% BRBs + AOM	0.7 ± 0.2[c] (71)	15.7 ± 5.0	30.2 ± 9.8 (75)

[a] Mean number of tumors per animal within a given treatment group ($n = 18$).
[b] Values are means ± S.E.
[c] Significantly different from AOM only ($p < 0.05$).
[d] Calculated as follows: tumor length × width × height × $\pi/6$. Tumor volume means are calculated by summing tumor volumes within a group and dividing by the number of tumor-bearing animals in that treatment group. NA, not applicable.
[e] Sum of tumor volumes for a tumor-bearing animal. Tumor burden means are calculated by summing tumor burdens within a group and dividing by the number of tumor-bearing animals in that treatment group.

6. Prevention of colon cancer in rodents

A BRB diet was tested to prevent colon cancer development in F344 rats induced by the chemical carcinogen, azoxymethane (AOM) [41] (this model is used extensively to evaluate the effects of putative chemopreventatives, in part because the histopathology of the resulting cancers resembles that of human colon cancer). AOM was administered at 15 mg/kg body weight intraperitoneally once per week for 2 weeks. At 24 h after the final AOM injections, animals were switched to treatment diets of 0, 2.5, 5 or 10% BRBs. The data are summarized in Table 2. No control animals developed tumors. Tumor multiplicity was reduced significantly by 42, 45 and 71% in the 2.5, 5 and 10% BRB + AOM groups, respectively, when compared to animals treated with AOM alone. No significant reductions in tumor volume were observed. Mean tumor burden decreased by 28, 42 and 75% in the 2.5, 5 and 10% BRB-treated groups, but none of these decreases were significant. This trend toward reduction in mean tumor burden indicates that a lower quantity of tumor tissue was present in the BRB + AOM-treated animals than in animals given AOM alone. Tumors were distributed throughout the colon, but most were in the distal region. No mechanistic studies have followed these initial observations.

7. *In vitro* studies with berry extracts

There have been multiple cell studies with berry extracts produced as described by Xue, et al. [42] to investigate the mechanisms of action of BRBs and to identify bioactive components. Only the alcohol fraction showed a dose-dependent decrease in ability of benzo[a]pyrene (B[a]P)-to induce transformation of Syrian hamster embryo (SHE) cells [42]. Similarly, Han, et al. [43] found that the dichloromethane and alcohol fractions, and two known components; namely, β-sitosterol and ferulic acid, selectively inhibited the growth of pre-malignant and malignant human oral cavity cell lines but not normal oral cells. They further found reduced levels of cyclin A and cell division cycle gene 2 (cdc2) in the pre-malignant cells, and reduced levels of cyclin B1, D1 and cdc2 in the malignant cells. The alcohol fraction also increased the level of $p21^{waf1/cip1}$ but did not influence cell cycle distribution. In contrast, treatment of pre-malignant and malignant oral cells with ferulic acid resulted in accumulation of the cells in the G_2/M phase of the cell cycle. Ferulic acid also induced cyclin B1 and cdc2 in both cell lines and $p21^{waf1/cip1}$ in the malignant cell line. β-sitosterol treatment led to accumulation of both pre-malignant and malignant cell lines in the G_2/M and G_0/G_1 phases of the cell cycle, increased levels of $p21^{waf1/cip1}$ and decreased levels of cyclin B1 and cdc2 genes in both cell lines. Rodrigo, et al. [44] also evaluated the preventative effects of the alcohol fraction on human oral cancer cell lines and found inhibition of cell proliferation, VEGF production, nitric oxide synthase activity, apoptosis, and terminal cell differentiation. Lastly, preliminary data from our laboratory indicates that treatment of tumorigenic and non-tumorigenic rat esophageal epithelial cells with the alcohol fraction, or individual anthocyanins (cyanidin-3-*O*-glucoside or cyanidin-3-*O*-rutinoside) inhibits proliferation, induces apoptosis and modulates the expression of COX-2 and iNOS in tumorigenic cells as compared to non-tumorigenic cells [45].

In initial studies with the JB-6 Cl 41 mouse epidermal cell model [40] with the alcohol fraction showed inhibition of BPDE-induced AP-1 and NFκB transactivation. The inhibitory effects appeared to be mediated via inhibition of mitogen activated protein kinase activation and inhibitory subunit κB phosphorylation, respectively. Pretreatment of cells with this fraction did not result in an inhibition of BPDE binding to DNA; thus, this was not a mechanism of reduced AP-1 and NFκB activation. Interestingly, none of the tested fractions was found to affect p53-dependent transcription activity [40]. The ability of BRBs to inhibit tumor development may be mediated by impairing signal transduction pathways leading to activation of AP-1 and NFκB. This supposition is supported by the demonstration that the alcohol fraction markedly inhibited activation of PI-3K, Akt, and p70 S6 kinase, suggesting that another mechanism for the chemopreventive activity may be through inhibition of the PI-3K/Akt/AP-1/VEGF pathway (Fig. 3) [46].

Our [39] fractionation studies point to cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-xylosyl-rutinoside as the active components of BRBs. However, another laboratory [47] found that the ellagitannins in red raspberries were the most effective inhibitors of the growth of human cer-

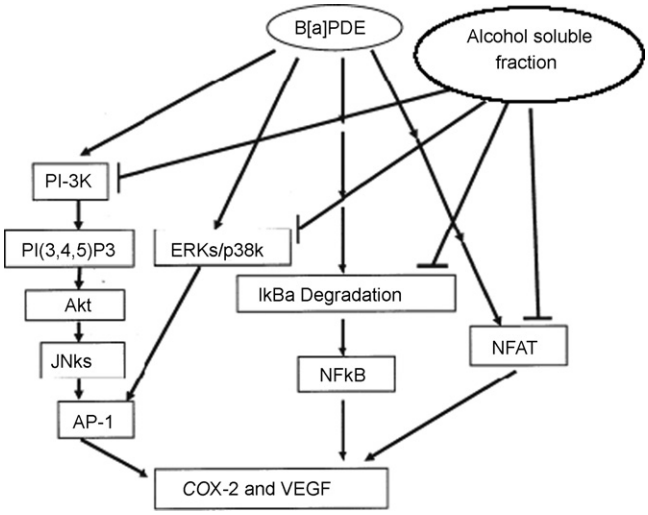

Fig. 3. Schematic illustration of known molecular mechanisms that may be involved in the chemopreventive activities of alcohol-soluble black raspberry fraction [57].

vical cancer (HeLa) cells *in vitro*. Also, extracts of other berry types (bilberry, black currant, cloudberry, lingonberry, raspberry and strawberry) reduced the growth of HT-29 colon cancer cells [48]. The bilberry extract was the most potent and the strawberry extract the least. Interestingly, even though having a low content of anthocyanins, the cloudberry extract caused a 14-fold induction of $p21^{waf1}$ and induced BCL-2 associated X protein (Bax) leading to the overall conclusion that, in addition to the anthocyanins, other berry components are responsible for their chemopreventive effects.

8. Human clinical trials with berries

8.1. Toxicity

A trial with eleven subjects was undertaken to determine the safety/tolerability of freeze-dried BRBs and to measure anthocyanins and ellagic acid in the plasma and urine [49] in subjects fed 45 g (equivalent to a 5% BRB diet in animals) of freeze-dried BRB powder as a slurry in water daily for 7 days. Blood samples were collected pre-dose on days 1 and 7 and at 10 time points over a period of 12 h post dose. Urine was collected for 12 h pre-dose on days 1 and 7 and at three 4-h intervals post dose. There was a low incidence of mild or moderate constipation in four of the eleven subjects. Maximum concentrations of anthocyanins and ellagic acid in plasma occurred at 1–2 h, and maximum quantities in urine appeared from 0 to 4 h. Absorption of both anthocyanins and ellagic acid was less than 1% of the administered dose.

8.2. Esophagus trials

8.2.1. Barrett's esophagus

Twenty BE Patients were provided 32 or 45 g (female and male, respectively) of BRB powder daily as a slurry in water for 6 months. Biopsies were taken before and after BRB treatment for biomarker analysis. Each subject collected urine for a 3-h period in the morning, at study baseline (pre-berry treatment), at week 12 of study, and at the final time point of 26 weeks. Urine was evaluated for the oxidative damage biomarkers 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-epi-prostaglandin F2α (8-Iso-PGF2) [51–53]. To date, only limited data from 10 of the 20 patients has been reported [50]. The length of the Barrett's lesion was unchanged in these 10 subjects following the 26-week dietary intervention. However, levels of urinary 8-Iso-PGF2 were significantly reduced, but there was no significant change in mean levels of urinary 8-OHdG (although, at the individual level, five patients experienced significant declines in 8-OHdG).

8.3. Colon trials

8.3.1. Colon cancer patients

One trial (pilot) will study 50 subjects with colorectal cancer and/or polyps. Upon enrollment, baseline biopsies of normal and tumor/polyp tissues are collected, as well as blood and urine specimens. Patients then consume 20 g of freeze-dried BRB powder as a slurry in water, three times per day (60 g total) until their scheduled surgery date, usually within two to 4 weeks. Post-treatment biopsy specimens are collected during the surgery. The pre-and post-treatment specimens will be analyzed for cell proliferation, inflammation, apoptosis and angiogenesis biomarkers. In a second colon trial (randomized design) we are evaluating FAP patients with an ileorectal anastamosis. Group 1 is consuming 20 g of BRB powder orally as a slurry in water, three times per day, for a total of 60 g daily. This group is also placing two suppositories composed of BRB powder into their rectum once a day before bedtime. Group 2 is being treated orally with 20 g of a placebo slurry, three times daily, plus two BRB suppositories once daily before bed. The treatment period is 9 months, with 3 time points for endoscopic evaluation of size and number of polyps (baseline, 18 and 36 weeks). Preliminary results for patients 18 weeks on protocol suggest that BRB powder causes ∼50% regression rate of rectal polyps (unpublished data). Biomarkers of cell proliferation, apoptosis, inflammation and angiogenesis are being measured.

9. Summary

Data from 15 years of investigations endorse the use of freeze-dried berries for prevention trials of digestive tract cancers. Berries contain a number of known chemopreventive agents; however, most if not all of these agents is too low to be protective unless concentrated 9–10-fold by the freeze-drying. Studies with extracts of freeze-dried berries suggest that the anthocyanins are important for their cancer preventive effects, particularly in view of their relative abundance in black raspberries. It is likely, however, that other compounds contribute significantly to the overall protective effect and studies to identify and rank these compounds are underway. Ultimately it may be possible to achieve equal or better cancer preventive effects with a single berry component or several components in combination which can be produced synthetically. One important factor is localized

absorption of the berry components into target tissues [54]. For example, dietary administration of 10% strawberry powder to strain A mice failed to protect against chemically induced cancer in the lung, suggesting that preventive components did not reach the lung in sufficient concentrations to be effective [55].

Mechanistic studies indicate that berries affect both the initiation and progression stages of tumor development [56]. Berry components influence carcinogen metabolism with lowered levels of DNA adducts. They also reduce the growth rate of pre-malignant cells, stimulate apoptosis and cellular differentiation, and reduce inflammation and angiogenesis. It is clear that berry components influence multiple signal transduction pathways through modulation of key regulatory genes such as NFκB, AP-1, P1-3K/Akt, p38/Erk1/2 leading to effects on downstream genes such as COX-2, VEGF and iNOS [57]. Although it is known that oxidative radicals can induce expression of regulatory genes in signal transduction pathways, it is unlikely that the effects of berry components on gene expression reside solely in their antioxidant activity; i.e., their ability to quench oxidative radicals. This is evidenced by the fact that berry extracts down-regulate luciferase reporter genes such as AP-1 and NFκB in cultured mouse epidermal cells for periods of at least 1–2 h after treatment of the cells with agents that induce expression of these genes. In view of the very short half-life of oxidative radicals, it is unlikely that this down-regulation can be attributed to the quenching of oxygen radicals.

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