Whole Food Approach to Cancer Prevention: Berries as an Example

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Abstract Our laboratories have been evaluating a "food-based" approach to cancer prevention using black raspberries (BRBs) for the past 20^{+} years. Black raspberries contain multiple compounds with chemopreventive potential including vitamins A, C and E, selenium and calcium, numerous complex and simple polyphenols including anthocyanins, ellagitannins, quercetin, ferulic and coumaric acids, various carotenoids, and phytohormones such as β-sitosterol. Because BRBs are about 90% water, freeze-drying them concentrates these putative chemopreventive agents about 10-fold. Preclinical studies have shown that freeze-dried BRB powder inhibits the development of oral, esophageal, colon and breast tumors in animals. In humans, BRB powder exhibits chemopreventive effects on premalignant lesions in the oral cavity, esophagus and colon at dose levels that elicit little or no toxicity. BRBs function by reducing cell proliferation, inflammation and angiogenesis, and enhancing apoptosis, cell adhesion and differentiation. Molecular studies have identified multiple genes associated with these cellular functions that are protectively modulated by BRBs. Bio-fractionation studies suggest that most of the chemopreventive effects of BRBs are due to their content of polyphenols and fiber. It is likely that many other foodstuffs would exhibit protective effects if formulated in a manner similar to that described for BRBs.

Key words chemoprevention; berries; oral cavity; esophagus; colon; animal; human

1 Introduction

For thousands of years, humankind has relied on plant derivatives for the prevention and treatment of a wide variety of ailments, including cancer. Anecdotal evidence from traditional medicine has led to numerous epidemiological studies to confirm whether or not the consumption of certain plant derivatives elicits preventative effects on disease occurrence. Perhaps the most convincing results from these studies is the protective effect of vegetable and fruit consumption on the occurrence of multiple human cancers [1, 2]. Experimental studies have now identified more than 1 000 individual compounds in vegetables and fruits that exhibit chemopreventive effects in vitro and in animal model systems. Indeed, most chemoprevention studies have been conducted with individual compounds, including various nutrients and non-nutrient phytochemicals. The most effective chemoprevention agents are those that elicit protective effects on a broad range of cellular functions such as proliferation, apoptosis, inflammation, angiogenesis, adhesion and differentiation, and on multiple signaling pathways that influence these cellular functions. Even with broadly based chemoprevention agents however, the extent of inhibition of spontaneous or carcinogen-induced tumorigenesis in animals rarely exceeds 50%—60%. The rational development of combinatorial approaches to chemoprevention therefore remains an important goal for cancer prevention.

Beginning in the early 1980's, we devoted considerable efforts toward developing individual compounds for cancer prevention, especially the non-nutrient phytochemicals, ellagic acid and phenylethyl isothiocyanate [3—5]. Both agents elicit chemopreventive effects in vitro and in multiple organ sites in animals.

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More recently, however, we have devoted most of our efforts to developing and applying a "food-based" approach to cancer prevention using freeze-dried, edible berries. Interest in berries stemmed from our early studies with ellagic acid, which is found in the pulp and seeds but not the juice of berries [6]. Because water accounts for about 80%—90% of the wet weight of berries, we reasoned that the removal of water from berries would result in an approximate 10-fold concentration of the ellagic acid and any other potential chemoprevention agents the berries might contain. Black raspberries (BRBs) were found to have higher levels of ellagic acid than the other berry types analyzed [6], therefore, we decided to conduct studies with BRBs (Fig. 1).

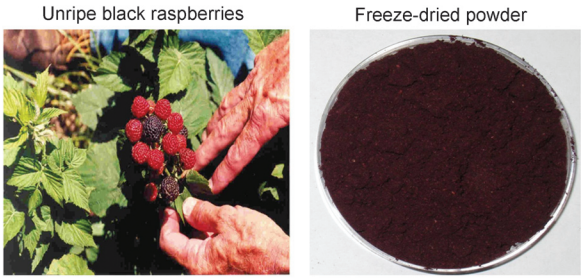

Fig. 1 On the left are unripe black raspberries about 1 week before harvest. At harvest, the berries will all be black. On the right is black raspberry powder made by freeze drying the berries.

The present chapter describes the approach we have taken to evaluate the chemoprevention potential of BRBs. It is largely an update of a Commentary written on this topic and published in *Cancer Prevention Research* in 2009 [7].

2 Scheme for Evaluating the Chemopreventive Potential of Berry Powder

We have proposed a stepwise approach for evaluating the chemopreventive potential of berry powders (Fig. 2)[7]: (1) develop "standardized" powders using nutrient, non-nutrient, chemical and microbial analyses; (2) evaluate toxicity in rodents; (3) determine anti-tumorigenic effects and the mechanism(s) for these effects in rodents; (4) conduct phase Ⅰ clinical trials of toxicity and pharmacokinetics in humans; (5) conduct "pilot" trials of different berry powder formulations for effects on precancerous lesions and biomarkers in humans; (6) conduct randomized, placebo-controlled phase Ⅱ biomarker trials; and (7) conduct phase Ⅲ trials to determine cancer prevention efficacy. Our proposed approach is similar to that described by Kelloff et al. [8] for the preclinical and clinical development of individual compounds. The scheme of Kelloff et al. differs from ours principally in their proposed initial step, which is to either synthesize an individual compound or isolate one from naturally occurring sources; in contrast, a standardized berry powder in our approach contains multiple compounds. This approach could easily be applied to the assessment of powders from other foodstuffs. Indeed, we were encouraged to test berry powder by early reports on the chemoprevention potential of other foodstuffs such

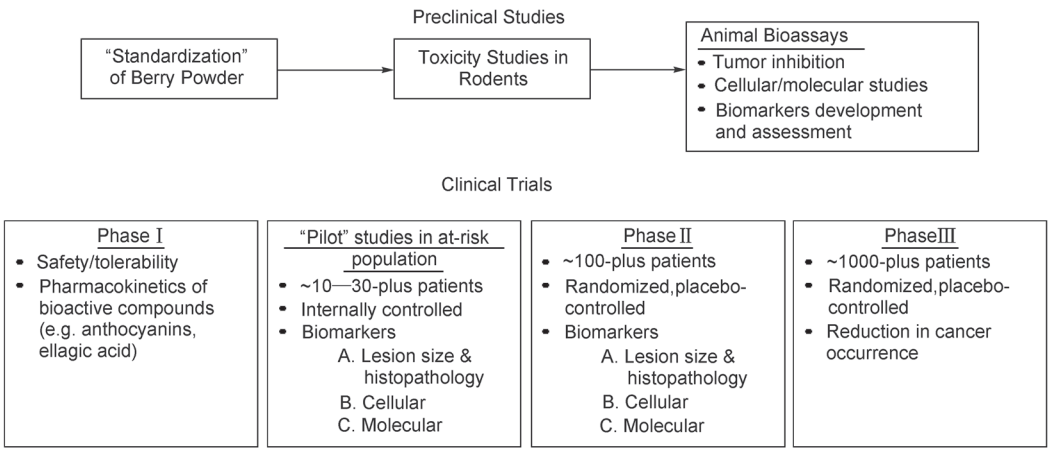

Fig. 2 Stepwise approach to evaluate berries for cancer prevention.

as tea [9, 10], broccoli [11], tomato juice [12], soybeans [13], garlic [14], and red beetroot [15]. The specific steps of our approach for developing berries and berry components for cancer prevention are summarized in the following sections.

2.1 "Standardizing" berry powders

Early studies revealed that the ellagic acid and anthocyanin contents in BRBs obtained from different farms in Ohio varied as much as 2 to 4 fold [6, 16]. To minimize this variability, we have obtained all berries either from a single farm in Southern Ohio or, more recently, from another farm in Central Oregon. Most studies have been conducted with BRBs (Rubus occidentalis) of two varieties (Jewel or Bristol) because BRBs have among the highest levels of anthocyanins and ellagitannins [16] and exhibit higher antioxidant activity [17] compared with most other commercially available berry types. BRBs are picked mechanically when ripe, washed thoroughly with water, and frozen at −20° C on the farm within 2—3 hours of picking. The berries are then shipped frozen to facilities in either Illinois (Ohio berries) or Oregon where they are freeze-dried under anoxic conditions to protect the integrity of berry components. After freeze-drying, whole BRBs can be ground into powder and used as such for experimental studies. Alternatively, the seeds can be removed by forcing the freeze-dried berries through a sieve, and the dried pulp ground into powder. Berry powder prepared from pulp only is preferable for human studies because some humans develop gastrointestinal disturbances from ground seed. We have observed that powders prepared either from whole BRBs or from BRB pulp are equally capable of inhibiting chemically-induced tumorigenesis in the rat esophagus (data not published). The berry powder is shipped frozen from the freeze-drying facilities to our laboratories where it is stored at −20° C until used for experimental studies. For standardization purposes, each batch of powder undergoes a quantitative chemical analysis of 20+ randomly selected nutrients and non-nutrient components, including some agents with chemopreventive potential [18]. Recently, we have found that levels of the four anthocyanins in BRBs remain within 20%—25% of the initial analyses for at least ten years in powder stored in sealed plastic bags at −20° C (unpublished data).

Recent events have re-emphasized the importance of evaluating the safety of food products for human consumption. Therefore, it is recommended that each batch of freeze-dried berry powder be analyzed for contamination with microbes (i.e., *Listeria, E. coli, Salmonella* and fungi), and potentially harmful chemicals (pesticides, herbicides and fungicides). These analyses are routinely performed by commercial firms that require only about 100 grams of powder

Table 1 Some potential chemopreventive agents in powder made from black raspberries harvested in 1997, 2001, 2006 and 2010

	Crop year*			
Component	1997	2001	2006	2010
Minerals				
calcium	215.00	175.00	188.00	234.00
selenium*	< 5.00	< 5.00	< 5.00	< 5.00
zinc	2.69	2.34	2.16	2.00
Vitamins				
α-carotene	< 0.02	< 0.02	< 0.03	< 0.02
β-carotene	< 0.02	0.06	< 0.07	< 0.02
α-tocopherol	n.d.	n.d.	10.40	n.d.
γ-tocopherol	n.d.	n.d.	11.20	n.d.
folate	0.06	0.08	0.14	0.12
Sterols				
β-sitosterol	80.10	88.80	110.00	84.20
campesterol	3.40	5.90	5.50	4.60
Simple phenols				
ellagic acid	166.30	185.00	225.00	320.00
ferulic acid	17.60	< 5.00	47.10	90.80
ρ-coumaric acid	9.23	6.82	6.92	n.d.
chlorogenic acid	n.d.	n.d.	0.14	0.11
quercetin	n.d.	43.60	36.50	143.00
Anthocyanins				
cyanidin-3-*O*-glucoside	n.d.	250.00	278.50	277.80
cyanidin-3-*O*- sambubioside	n.d.	220.00	56.00	76.49
cyanidin-3-*O*-rutinoside	n.d.	2002.00	1790.00	1981.43
cyanidin-3-*O*-xylosylrutinoside	n.d.	510.00	853.50	373.21

Abbreviation: n.d., not determined.

*All measures in the crop-year columns are mg/100 g dry weight, except for that of selenium which is μg/100g dry weight. Berries in crop years 1997, 2001, and 2006 were obtained from a farm in Ohio. Those from year 2010 were obtained from a farm in Oregon.

to test for microbial and chemical contamination as well as nutrient and non-nutrient content. In addition, to prevent degradation of nutrients and non-nutritive constituents, as well as the growth of microbes, berry powders should be stored frozen at -20°C or colder in sealed bags before and during use in animal studies and in human clinical trials. Recently, we found that the total content of the four anthocyanins in BRBs was reduced only about 20% when BRB powder was stored at -20°C in sealed bags for 10 years (data unpublished).

Table 1 shows some of the potential chemopreventive agent content of powders that were prepared from BRBs obtained from an Ohio farm in 1997, 2001 and 2006 and from an Oregon farm in 2010. Note that the content of these components in the Ohio berries is similar to that of the Oregon berries. Further studies are required however, to provide an adequate comparison of the nutrient and non-nutrient content of berries from different geographical areas of the U.S. BRBs contain relatively high levels of calcium, ß-sitosterol, ellagic acid, quercetin and the four anthocyanins. The amounts of calcium, zinc, ß-sitosterol, α-carotene, ellagic acid, ρ-coumaric acid, cyanidin-3-O-glucoside, and cyanidin-3-O-rutinoside in the yearly powders varied from 10%–40%, whereas the amounts of other constituents (β-carotene, folate, ferulic acid, quercetin, cyanidin-3-O-sambubioside and cyanidin-3-O-xylosylrutinoside) varied from 60%–90%. The relatively high variability in levels of ß-carotene and folate is likely due to difficulties in accurately measuring the low levels of these agents in the powder. Selenium is present in microgram quantities in BRBs; therefore, values for selenium are reported as <5.00 μg/100 g dry weight. Because we analyze only a small percentage of the overall number of compounds in BRBs, it is likely that BRBs contain chemopreventive agents in addition to those listed in Table 1. Therefore, berries, like other foods, represent combinations of agents that may exhibit chemopreventive potential, particularly when concentrated several fold by freeze-drying.

2.2 Toxicity studies in rodents

One of the properties of an "ideal" chemopreventive agent is to exhibit chemopreventive efficacy at concentrations that cause little or no toxicity. We have evaluated the toxicity of BRBs in rats fed a synthetic diet (AIN-76A) plus either 2.5, 5.0 or 10% BRB powder by weight (w/w) for up-to nine months [19]. These percentages of BRB powder in a rat diet would be equivalent to approximately 0.9 to 1.8 oz of BRB powder in the daily human diet, as calculated on a body surface area basis [20]. Since one ounce of berry powder is equivalent in content to about 10 ounces of fresh berries, 0.9 to 1.8 oz of powder averages out to about 0.8 lb of fresh whole BRBs per day.

Histopathologic studies indicated that the BRB diets did not produce toxic effects in any major organs of the animals, and there were no significant differences in body weights, food consumption or cell blood counts between rats on either of the BRB-supplemented diets versus control rats on the AIN-76A-alone diet during the nine month treatment. An unexpected benefit of the berry diets in rats was a 10% reduction in total blood cholesterol [19].

Table 2 Cancer prevention in animal models with dietary black raspberries

Species	Site	Carcinogen	Berries	% Tumor Reduction	ref
Hamster	cheek pouch	[a]DMBA	5 & 10% in diet	8 – 56 [b] (m)	21
F344 rat	esophagus	[a]NMBA	5 & 10% in diet	43 – 68 (m)	18
F344 rat	colon	[a]AOM	2.5, 5.0 & 10% in diet	42 – 71 (m)	19
ACI rat	breast	estrogen	2.5% in diet	37 (m)	24
Apc1638$^{+/-}$ MIN mouse	intestine	spontaneous	10% in diet	57 (m)	25
Muc2$^{-/-}$	intest., colon	spontaneous	10% in diet	50 – 55 (m)	25
SKH–1 mouse	skin	[a]UVB	500 μg extract	77 (m)	26

[a]DMBA, 7, 12-dimethylbenz[*a*]anthracene; NMBA, *N*-nitrosomethylbenzylamine; AOM, azoxymethane; UVB, ultraviolet light;

[b] (m) = tumor multiplicity; (i) = tumor incidence

2.3 Inhibition of carcinogen-induced and spontaneous tumors and mechanistic studies in vivo

Table 2 summarizes the results of numerous studies to evaluate the ability of BRB diets to prevent the development of carcinogen-induced and spontaneously-occurring tumors in animal models. AIN-76A diets containing either 2.5, 5.0 or 10% BRB powder have been shown to reduce carcinogen-induced tumors in the Syrian golden hamster cheek pouch [21], in the F344 rat esophagus and colon [18, 19, 22, 23] and in the ACI rat mammary gland [24]. A 10% BRB diet inhibited the development of spontaneous intestinal tumors in Apc1638+/– Min mice and both intestinal and colon tumors in $Muc2^{-/-}$ mice [25]. Finally, an anthocyanin-rich extract of BRBs was shown to inhibit UVB-induced skin tumors in SKH-1 mice [26]. The most reliable measure of tumor inhibition in these studies is tumor multiplicity; in general and depending on the temporal sequence of administration of the carcinogen and the berry diet, the extent of inhibition of tumor multiplicity ranges from about 30%–75%. Optimal tumor inhibition occurs when the BRBs are added to the diet before, during and after treatment with carcinogens, suggesting that consumption of berries throughout life may maximize their chemopreventive effectiveness in humans. That berry diets do not inhibit 100% of tumorigenesis suggests that the inhibitory components of BRBs and/or their metabolites are not completely absorbed, which has been shown to be the case for the anthocyanins and ellagitannins [27]. In addition, berry compounds may not sufficiently affect all of the critical signaling pathways of carcinogenesis. It should be mentioned that diets containing 5% and 10% strawberry and blackberry powders [28, 29], or 5% red raspberry, blueberry, noni, goji or acai powder [30] were nearly as effective as BRB powder in inhibiting tumors induced in the rat esophagus by the carcinogen N-nitrosomethylbenzylamine (NMBA). These data suggest that multiple berry types have the capability for cancer prevention in vivo as has been shown in numerous studies with extracts of different berry types in vitro [31].

The cellular and molecular mechanisms of chemoprevention by berries have been studied most often in vivo with BRBs in the NMBA model of esophageal carcinogenesis in F344 rats. BRBs influence cellular events including proliferation, apoptosis, inflammation, angiogenesis and differentiation (Fig. 3). Using real-time PCR, western blot and quantitative immunohistochemistry techniques, we have identified multiple NMBA-dysregulated genes associated with these cellular events that are protectively modulated by BRBs [22, 23, 32, 33] (Fig. 3). In addition, an early study involving DNA microarray identified 462 of 2261 NMBA-dysregulated genes in the initiation stage of rat esophageal carcinogenesis that were restored to near normal levels of expression by BRBs [34]. These restored genes were associated with multiple cellular functions including carcinogen metabolism indicating that the active components of BRBs elicit a genome-wide effect in modulating genes involved in the early events of esophageal carcinogenesis. A more recent study involving DNA microarray identified genes in the late stages of rat esophageal tumorigenesis that were protectively modulated by BRBs [33]. Six hundred and twenty-six of 4807 NMBA-dysregulated genes in preneoplastic rat esophagus (PE) and 625 of 17 846 genes in esophageal papillomas were restored to normal levels of expression by BRBs. In both PE and in papillomas, BRBs modulated the mRNA expression of genes associated with carbohydrate and lipid metabolism, cell proliferation, apoptosis and inflammation as well as both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. Interestingly, matrix metalloproteinases involved in tissue invasion and metastasis were also modulated by BRBs. Therefore, as for initiation events in carcinogenesis, BRBs elicit

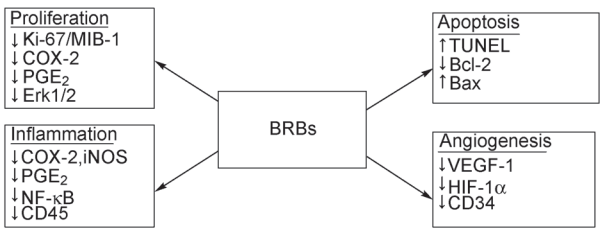

Fig. 3 Black raspberries (BRBs) reduce proliferation, inflammation and angiogenesis and stimulate apoptosis by influencing expression of relevant genes.

a genome-wide effect in modulating genes involved in the late stages of esophageal carcinogenesis.

The effects of BRBs on the expression levels of genes in the esophagus of control rats not treated with NMBA have also been studied using microarray [35]. Three weeks treatment of rats with 5% BRBs altered the expression levels of only 36 genes in control esophagus; 24 were upregulated and 12 were downregulated. Among the upregulated genes were genes associated with cellular matrix, signaling cascades, transcription regulation, apoptosis, metabolism and, intriguingly, contraction. The downregulated genes are involved in cell regulation, signal transduction and metabolism. Histopathological analysis indicated that the berries have little or no effect on esophageal morphology. Therefore, BRBs alone produce only modest effects on normal rat esophagus.

Two mouse models of colorectal cancer were used to evaluate the effects of BRBs on colorectal tumor development and to investigate the underlying mechanisms[25]. A 12-week feeding of BRBs significantly inhibited intestinal tumor formation in both models; reducing tumor incidence by 45% and tumor multiplicity by 60% in *Apc*1638+/- mice and tumor incidence and multiplicity by 50% in *Muc*-/- mice. Mechanistic studies showed that BRBs inhibit tumor development in *Apc*1638+/- mice by suppressing β-catenin signaling and in *Muc*-/- mice by reducing chronic inflammation. Intestinal cell proliferation was reduced in both mouse models by BRBs, however, mucus differentiation was not affected in either model. In another study, the effect of a 10% BRB diet on inflammation events in an experimental mouse model of ulcerative colitis (UC) using 3% dextran sodium sulfate (DSS) was investigated [36]. The berries markedly reduced DSS-induced acute injury to the colonic epithelium and they suppressed tissue levels of several pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β). Colonic cyclooxygenase-2 (COX-2) levels were also suppressed by BRB treatment, with a concomitant decrease in plasma prostaglandin E_2. These results demonstrate a potent anti-inflammatory effect of BRBs during DSS-induced colonic injury and suggest that BRBs should be evaluated for potential effects on UC in humans.

2.4 Phase Ⅰ human clinical trial

Based upon promising results in preclinical studies, clinical trials with BRBs were initiated and several have been completed. An initial phase Ⅰ trial evaluated the safety and tolerability of BRB powder (45 g as a slurry in water daily for 7 days) and measured anthocyanins and ellagic acid in the plasma and urine of 11 healthy participants [27]. This dose of BRB powder is equivalent to the human consumption of about 16 ounces (1 lb) by weight of fresh whole BRBs daily. All participants were on a "phenol-free" diet (no tea, coffee, alcoholic beverages or vegetables and fruit) during the 7 day treatment with BRBs. BRBs were administered in powder form rather than fresh for two reasons: (1) 1 lb of fresh BRBs is a substantial, problematic quantity to consume on a daily basis, particularly for individuals who cannot tolerate berry seed; (2) where available, fresh BRBs can be purchased in stores only 1−2 months of each year, whereas high-quality BRB powder is available during the entire year. Therefore, berry powder is more feasible for routine chemoprevention. Results of the phase Ⅰ trial indicated that BRB powder is well tolerated, with a low incidence of mild or moderate constipation in 4 of the 11 subjects. Maximum concentrations of anthocyanins and ellagic acid occurred at 1—2 hours in plasma and at 1/2—4 hours in urine. As is the case in rats [37], the overall uptake of anthocyanins and ellagic acid in humans was <1% of the administered dose as determined by measurement of free anthocyanins and ellagic acid in plasma. It is probable, however, that the uptake of these compounds was underestimated since their metabolites and protein-bound forms were not measured in plasma [27]. In a subsequent study of oral BRB powder (32 or 45 g/day for 6 months) in Barrett's esophagus patients [38], about 15% of patients reported symptoms of occasional diarrhea, constipation or epigastric pain, but the symptoms were not severe and all patients continued BRB consumption throughout the study. Similar gastrointestinal effects were observed in a phase Ⅰb trial of BRB powder in patients with

familial adenomatous polyposis (FAP) who were treated with a total oral dose of 60 g/day (20g/3x/day) for nine months (data not published). The collective human data suggest that BRB powder is well tolerated in humans at doses of at least 45—60 g/day for at least 9 months.

2.5 "Pilot" clinical trials with berry formulations

A series of "pilot" clinical trials have been conducted in individuals at higher-than-normal risk for cancer to determine if BRBs have potential for chemoprevention in humans (Table 3). These trials are internally controlled (i.e., each patient serves as his/her own control), involve relatively few patients (14 to 20), and determine the effects of BRBs on dysplastic lesions and relevant biomarkers after relatively short-term (1 to 9 months) treatment. Exceptions are the FAP trial in which one-half [7] of the patients were treated orally with a placebo powder (i.e., a placebo control), and an esophageal dysplasia trial in China in which 75 patients were treated orally with strawberry (STRW) powder rather than BRB powder. The reasons for treatment with strawberries were the following: (1) they are the major berry type grown in China; (2) the reluctance by the Chinese government to permit the introduction of black raspberry powder into China for fear that some of the seed in the powder might be viable; and (3) strawberry powder is less expensive than BRB powder. We view "pilot" trials as a time- and cost-effective means of assessing whether berries exhibit effects in specific cohorts with desirable characteristics for further examination in randomized, placebo-controlled, phase Ⅱ and Ⅲ clinical trials. Results from these pilot studies were as follows.

2.5.1 Barrett's esophagus

A chemoprevention trial was conducted in 20 patients with Barrett's esophagus [38]. Patients were treated with either 32g/d (female) or 45g/d (male) of BRB powder orally in a slurry of water for six months. Barrett's lesions were biopsied before and after treatment with berries. Results indicated that the berries had little effect on biomarkers of proliferation and apoptosis in the Barrett's lesion itself, however, they caused reductions in two urinary biomarkers of oxidative stress, 8-epi-prostaglandin F2α (8-Iso-PGF2) and to a lesser extent, 8-hydroxy-2'-deoxyguanosine (8-OHdG). It is possible that the transit time of the BRB powder across the Barrett's lesions may have been too rapid to permit localized absorption of berry bioactives into the tissue.

2.5.2 Esophageal dysplasia

A randomized (noncomparative) phase Ⅱ trial was conducted in China to investigate the effects of freeze-dried strawberries (STRW) in patients with esophageal dysplasia in a high-risk area for esophageal squamous cell carcinoma [39]. Seventy-five patients were randomized to receive either 30 g/d (37 patients) or 60 g/d (38 patients) of STRW powder for six months; the powder was mixed in water and patients were encouraged to drink it slowly over a period of one hour each use. Changes in histologic grade of the dysplastic lesions was the primary endpoint of the trial. The dose of 30 g/d did not significantly affect histology or any other measured parameter. The dose of 60 g/d,

Table 3 Pilot clinical trials with berry formulations

Berry Type	Cohort	No. Patients	Berry Dose	Treatment Mode	Treatment time	Ref
BRB	Barrett's Esophagus	20	32 g/d,♀; 45 g/d, ♂	Oral, in water	6 mos.	38
STRW	Esophageal dysplasia	75	30 g/d (37) 60 g/d (38)	Oral, in water	6 mos.	39
BRB	Colorectal cancer	20	60 d/g	Oral, in water	1—9 wks.	41
BRB	Rectal polyps	14	60 g BRB/d + suppository (7) 60 g placebo/d + suppository (7)	Oral, in water, intra-rectal	9 mos.	Unpublished
BRB gel	Oral dysplasia (leukoplakia)	10 normal 17 dysplasia	0.5 g/application	topical, 4x/d	6 wks.	45 & 45

however, reduced the histologic grade of about 80% of mildly dysplastic lesions ($p < 0.0001$), but there were too few moderately dysplastic lesions evaluated to draw any conclusions. The STRWs were well tolerated, with no toxic effects or adverse events. The high dose of STRWs also reduced protein expression levels of COX-2 by about 62.9% ($p < 0.001$), inducible nitric oxide synthase (iNOS) by 79.5% ($p < 0.001$), phospho-nuclear factor kappa B (NFκB)-p65 (NFκB-p65) by 62.6% ($p < 0.001$), and phospho-S6 (pS6) by 73.2% ($p < 0.001$). The STRWs (60 g/d) also significantly inhibited the Ki-67 labeling index by 37.9% ($p = 0.023$). These results are encouraging in view of the fact that several agents tested previously in China for their ability to affect dysplastic lesions in the esophagus have been ineffective [40].

2.5.3 Colorectal cancer

A study was undertaken in 20 colorectal cancer patients to determine whether the oral administration of BRB powder might have any effect on biomarkers of cell proliferation (Ki-67), apoptosis (TUNEL), angiogenesis (CD105), expression of Wnt signaling pathway genes (c-Myc, β-catenin, E-cadherin), and methylation of tumor suppressor genes (*p*16, *PAX*6a, *SFRP*2, *SFRP*5, *WIF*1) in colorectal tumor specimens when compared to adjacent "normal" tissues [41]. Patients consumed a total of 60g/d (20g/3x/d) BRB powder orally in water for periods of 1-to-9 weeks; biopsies were taken immediately before BRB treatment and at surgery for removal of the tumors. Quantitative immunohistochemistry indicated that BRB treatment for at least 4 weeks resulted in modulating the expression of genes associated with the Wnt pathway, proliferation, apoptosis and angiogenesis in a protective direction however, only the reduction in Ki-67 cell proliferation rates was significant ($p < 0.05$). The methylation of the three Wnt inhibitors, *SFRP*2, *SFRP*5, and *WIF*1, all upstream genes in the Wnt pathway, and *PAX*6a, a developmental regulator, was modulated in a protective direction by BRBs. This was associated with decreased expression of DNA methyltransferase-1 (DNMT1), an enzyme that functions as the maintenance DNA methyltransferase in mammalian cells. These results suggest that BRBs might be useful in the treatment of colorectal cancer, preferably in conjunction with routine chemo/radiotherapy and surgery.

2.5.4 Rectal polyps in familial adenomatous polyposis (FAP) patients

Familial adenomatous polyposis (FAP) is an inherited colorectal cancer syndome characterized by colonic polyposis and a lifetime risk of subsequent colon cancer of nearly 100%. Total abdominal colectomy with ileorectal anastomosis or total proctocolectomy with ileal pouch anal anastomosis are the traditional management strategies for colonic polyposis. Lifelong endoscopic surveillance of the rectum is required for the management of recurrent polyposis and does not obviate the development of uncontrolled rectal polyposis or rectal cancer which may require proctectomy.

Non-steroidal anti-inflammatory drugs (NSAIDs) were first reported to cause regression of colonic polyps in patients with FAP over two decades ago. However, the gastrointestinal toxicity of non-selective NSAIDs such as sulindac led to the development of selective COX-2 inhibitors. Celecoxib® and rofecoxib® have been shown in randomized controlled trials to induce regression of colonic adenomas in FAP [42], and celecoxib is FDA approved for this purpose as an adjunct to standard endoscopic management. Unfortunately, the increased risk of cardiovascular, thromboembolic and cerebrovascular events [43] led to the withdrawal of rofecoxib from the market and remains a concern for celecoxib.

Because of the lack of observable toxicity in clinical trials, we decided to evaluate BRBs for their ability to regress rectal polyps in FAP patients. Subjects were randomly assigned to two treatment groups (9 subjects per group): Group 1: 20 g of placebo powder administered as an oral slurry 3 times per day, plus 2 berry suppositories administered at bedtime. Group 2: 20 g of BRB powder administered orally 3 times per day, plus 2 berry suppositories administered at bedtime (Fig. 4). Each rectal suppository contained 730 mg BRB. The treatment period was nine months and the size and number of rectal polyps were counted at each

visit. No more than 2 rectal polyps were biopsied at baseline for biomarker evaluation and all polyps were counted and removed at nine months.

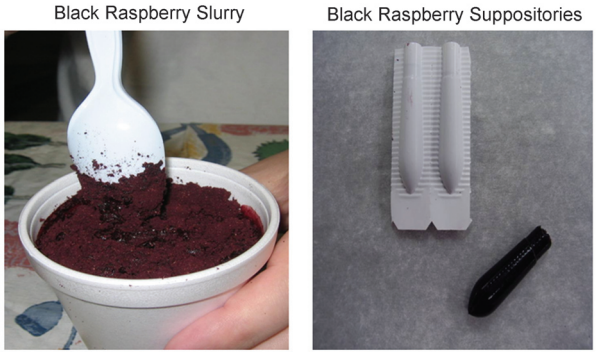

Fig. 4 Black raspberry powder and suppositories for FAP trial.

Fig. 5 illustrates the results from this study (data not published). Two patients in each group dropped out of the study due to rectal fissure associated with insertion of the suppositories. This was due to the fact that, in the beginning, the suppositories were made from whole

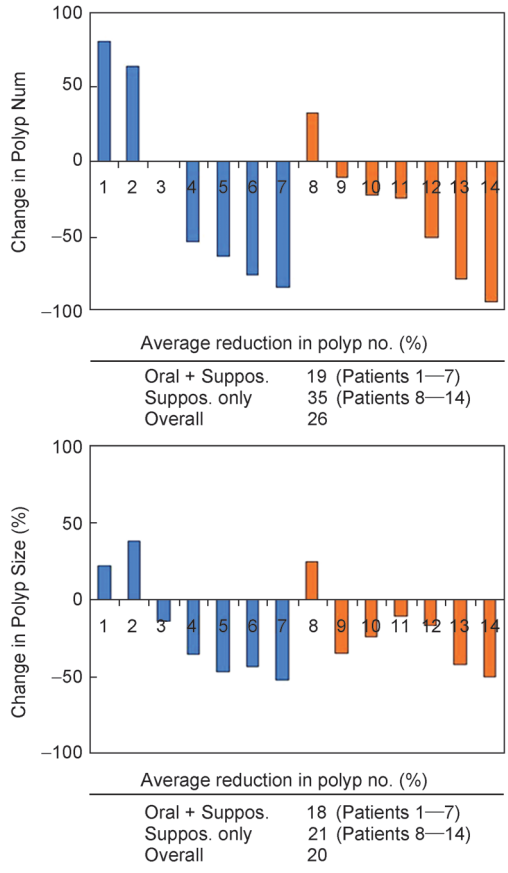

Fig. 5 Effects of BRBs on polyp number and size in FAP patients.

berry powder including the ground seed. Unfortunately, the seed was not sufficiently ground which led to injury of the anal opening upon insertion of the suppositories. This was rectified by removing the seed by pushing the dried berries through a sieve and grinding the berry pulp into powder. Interestingly, of the seven patients who received oral BRB plus suppositories, only four had reductions in polyp number, one had no change in polyp number and two had more polyps at the end of the nine-month study. The overall reduction in polyp number was 19%. In contrast, in the seven patients treated with the oral placebo plus the rectal suppositories, the overall reduction in polyp number was 35%. These results suggest that treatment with the suppositories only is more effective than treatment with oral berries plus suppositories. However, more patients in each arm are required in order to draw conclusions regarding the effect of berries on polyp number. There is a good correlation between the effects of the berries on polyp number and polyp size (Fig. 5). The reduction in polyp size after nine months of treatment correlated with reduced cell proliferation (Ki-67 nuclear staining) and increased apoptosis (TUNEL) in berry treated polyps. Additional biomarker studies are currently underway. Overall, our results suggest that berry suppositories might be an alternative to celecoxib® for the treatment of patients with FAP.

2.5.5 Oral dysplasia

The aim of this trial was to assess the effects of topical application of a 10% (w/w) black raspberry gel on oral dysplasia variables that included histologic diagnoses and loss of heterozygosity (LOH) indices [44]. Ten patients with normal oral mucosa and 17 patients with oral dysplasia were treated with a 10% black raspberry gel applied topically (0.5 g per application) to the tissues four times daily for six weeks. Before treatment, all dysplastic lesions and normal tissues were photographed, and lesional tissue was hemisected to obtain a pretreatment diagnosis and baseline biochemical and molecular variables. Gel dosing was begun one week after the initial biopsy. Genomic DNA was isolated from laser captured basilar and suprabasilar surface epithelium followed by PCR amplification

using primer sets that targeted known and presumed tumor suppressor gene loci associated with INK4a, /ARF, p53 and FHIT. None of the 27 participants developed BRB gel associated toxicities during the study. Histologic regression of dysplastic lesions occurred in about 50% of treated patients, as well as a similar reduction in LOH at the tumor suppressor gene-associated loci. The majority of participants showed post-treatment decreases in epithelial iNOS and COX-2 proteins, but only the reductions in COX-2 were significant [45]. Array analysis showed that the berry gel uniformly suppressed genes associated with RNA processing, growth factor recycling, and inhibition of apoptosis. In a patient subset, berry gel application also reduced vascular densities in the superficial connective tissues and induced genes associated with keratinocyte terminal differentiation. Currently, an NCI-supported Phase Ⅱ randomized, placebo controlled trial of the BRB gel in 70 patients with oral dysplasia is underway to confirm the results of the pilot study in 27 patients. In the Phase Ⅱ trial, the gel is being applied topically for a period of 12 weeks. Preliminary results from this trial appear to confirm the published data from the pilot study in 27 patients (Susan Mallery, personal communication).

Collectively, results from these pilot studies suggest that BRBs in different formulations may be very promising for the treatment of preneoplastic lesions in the oral cavity, esophagus and colon. Further studies are needed to confirm these results. Berries may become a non-toxic and relatively inexpensive alternative for the prevention of cancer, especially in sites where berry compounds can be delivered locally in relatively high concentrations.

2.6 Phase Ⅱ and Ⅲ clinical trials

To date, only two phase Ⅱ clinical trials of BRBs for cancer prevention have been initiated. One is the trial mentioned above to confirm the ability of the 10% BRB gel to cause histologic regression of oral dysplasia as well as modulate cellular and molecular biomarkers. In another study at the Ohio State University, BRB lozenges are being evaluated for their ability to prevent the recurrence of oral cancer in patients who been treated for the disease (Christopher Weghorst, personal communication). To date, there have been no publications of the results of this trial. There have been no Phase Ⅲ chemoprevention trials with berries.

3 Conclusions

A major focus of effort in the fields of cancer therapy and prevention is to develop drugs that target specific genes in signaling pathways to either kill cancer cells or prevent precancerous cells from progressing to cancer, while causing minimal effects on their normal counterparts. In contrast, berry powders contain a mixture of compounds that affect the expression levels of a wide range of cancer-related genes (to lesser extents than therapeutic agents; ref. 34), thus either preventing or slowing the conversion of premalignant cells to malignancy at doses that cause minimal or no cytotoxicity. In this regard, berries seem to fulfill the requirement of an "ideal" chemopreventive agent [46]. The same is undoubtedly true of many other foodstuffs; e.g., a freeze-dried aqueous extract of broccoli sprouts was effective at dietary levels in inhibiting chemically induced bladder cancer with no observable toxicity in rats [47].

One of the major concerns with the use of food-based approaches to cancer prevention is that of "standardizing" the foodstuff under investigation. There is no question that our approach to obtaining berries from only one or two sources is not the "real world" and one might expect considerable variation in the content of black raspberries, for example, obtained from different geographical areas of the U.S. or other countries. One wonders how important this is however, because we have repeatedly demonstrated chemopreventive effects of BRB powder in our rat model of esophageal carcinogenesis using more than 10 batches of berries obtained in different years from two different states (Ohio and Oregon) and varying considerably in nutrient and non-nutrient content. Perhaps the "common denominator" is the fiber in berries because we have shown that fiber from different berry types (which varied markedly in content of anthocyanins and ellagitannins) were all about

equally chemopreventive [48]. Thus, the concern about standardization may be misplaced to some degree,even though it is clearly desirable.

From a practical standpoint, we have found that high-risk individuals are usually willing to participate in clinical trials of berry formulations, and compliance in these trials is excellent. Moreover, the general public is intrigued with food-based approaches for the prevention of diseases including cancer. With potentially lower toxicity and costs, effective food-based approaches not only would be attractive for developed countries but would offer greater portability (versus highly synthesized agents) to underdeveloped countries as well. Therefore, in my opinion, food-based approaches with rational developmental schemes such as the one outlined in this commentary should be an integral part of the overall strategies for the prevention of cancer and other diseases.

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癌症预防的完全食物途径：以浆果为例

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摘 要 在过去的二十多年中，Gary D. Stoner 实验室一直在评估利用黑树莓进行基于食物的癌症预防途径。黑树莓含有多种具有潜在化学预防作用的化合物，包括维生素 A、C、E，硒，钙，以及大量或复杂或简单的多酚类物质，包括花青素，鞣花，槲皮素，阿魏酸和香豆酸，各种类胡萝卜素，β- 谷甾醇等激素。因为黑树莓含有大约 90% 的水分，对其冷冻干燥可使其中假定的化学预防成分浓缩约十倍。临床前研究表明，黑树莓冻干粉末可以抑制动物的口腔，食道，结肠和乳腺中肿瘤发生发展。在人体实验中，黑树莓冻干粉末对口腔，食道和结肠的癌前病变具有预防作用，且其剂量水平仅引起少量或不引起毒性。黑树莓预防癌症的机理可能包括抑制细胞增殖、炎症和血管生成，并且促进细胞调亡、黏附和分化。分子生物学研究表明，黑树莓保护性地调控了多个和上述细胞功能相关的基因表达。对黑树莓不同组分的生物活性分析显示其化学预防作用主要来自其中的多酚类物质和纤维素。这些结果提示，很多具有黑树莓相似组成的食物可能也有类似的保护作用。

关键词 化学预防 浆果 口腔 食道 结肠 动物 人类

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