Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

Research Article

Cancer Prevention Research

Effects of Black Raspberry on Dibenzo[*a,l*]Pyrene Diol Epoxide Induced DNA Adducts, Mutagenesis, and Tumorigenesis in the Mouse Oral Cavity

Kun-Ming Chen[1], Joseph B. Guttenplan[2,3], Yuan-Wan Sun[1], Timothy Cooper[4], Nora A.E. Shalaby[2,3], Wieslawa Kosinska[2,3], Gabrielle Benitez[1], Cesar Aliaga[1], Junjia Zhu[5], Jason Liao[5], Krishne Gowda[6], Shantu Amin[6], Gary Stoner[7], and Karam El-Bayoumy[1]

Abstract

We previously showed that metabolic activation of the environmental and tobacco smoke constituent dibenzo[*a,l*]pyrene (DB[*a,l*]P) to its active fjord region diol epoxide (DB[*a,l*]PDE) is required to induce DNA damage, mutagenesis, and squamous cell carcinoma (SCC) in the mouse oral cavity. In contrast to procarcinogens, which were employed previously to induce SCC, DB[*a,l*]PDE does not require metabolic activation to exert its biological effects, and thus, this study was initiated to examine, for the first time, whether black raspberry powder (BRB) inhibits postmetabolic processes, such as DNA damage, mutagenesis, and tumorigenesis. Prior to long-term chemoprevention studies, we initially examined the effect of BRB (5% added to AIN-93M diet) on DNA damage in B6C3F1 mice using LC/MS-MS and on mutagenesis in the *lacI* gene in the mouse oral cavity. We showed that BRB inhibited DB[*a,l*]PDE-induced DNA damage ($P < 0.05$) and mutagenesis ($P = 0.053$) in the oral cavity. Tumor incidence in the oral cavity (oral mucosa and tongue) of mice fed diet containing 5% BRB was significantly ($P < 0.05$) reduced from 93% to 66%. Specifically, the incidence of benign tumor was significantly ($P < 0.001$) reduced from 90% to 31% (62% to 28% in the oral cavity and 28% to 2% in the tongue), a nonsignificant reduction of malignant tumors from 52% to 45%. Our preclinical findings demonstrate for the first time that the chemopreventive efficacy of BRB can be extended to direct-acting carcinogens that do not require phase I enzymes and is not just limited to procarcinogens. *Cancer Prev Res; 11(3); 157–64. ©2017 AACR.*

Introduction

Worldwide, head and neck cancer is the sixth most common human cancer, and oral cancer is the most common type of this disease (1, 2). Cancer of the oral cavity is a deadly disease and can strip away the patient's voice and certain basic needs in life such as eating and drinking; there are currently over 300,000 cases, and more than 145,500 deaths occurred in 2012 worldwide, and in the United States, over 30,000 cases and over 6,000 deaths from the disease occur annually (2–4). In 2017, the number of new cases of oropharyngeal carcinomas in the United States is estimated to be 49,670 and the estimated deaths from the disease 9,700 (5). The most common histologic type of oral cancer is oral squamous cell carcinoma (OSCC); it accounts for more than 90% of oral cancers (1, 2). Early diagnosis for oral cancer has not improved over time; up to 77% of oral cancer cases are diagnosed at advanced stages (6). The conventional treatments include surgery, radiotherapy, and chemotherapy (7). However, approximately one third of treated patients will experience local or regional recurrence and/or distant metastasis (8). The survival rate is stagnant at about 50%; it varies greatly with the stage of disease detection (6).

[1]Department of Biochemistry and Molecular Biology, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania. [2]Department of Basic Science, New York University College of Dentistry, New York, New York. [3]Department of Environmental Medicine, New York University School of Medicine, New York, New York. [4]Charles River Laboratories-Contractor Supporting: National Institute of Allergy and Infectious Diseases (NIAID), Frederick, Maryland. [5]Department of Public Health Sciences, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania. [6]Department of Pharmacology, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania. [7]Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Corresponding Author: Karam El-Bayoumy, Department of Biochemistry and Molecular Biology, Pennsylvania State College of Medicine, Hershey, PA 17033. Phone: 717-531-1079; Fax: 717-531-0002; E-mail: kee2@psu.edu

doi: 10.1158/1940-6207.CAPR-17-0278

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

The main risk factors for oral cancer are exposure to exogenous carcinogens, such as tobacco smoke, smokeless tobacco, excess alcohol, and human papillomavirus (HPV). These factors are estimated to account for 90% of oral cancers (1, 2). In general, avoidance of risk factors has only been partially successful, largely because of the addictive power of tobacco smoking and alcohol consumption. Although it is not clear which chemical compounds in tobacco smoke contribute to the development of human oral cancer, certain classes of chemical carcinogens, such as tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons (PAH), are recognized as potential etiologic agents for oral cancer (9–11).

Our laboratory has focused on developing an animal model that mimics human exposure to environmental carcinogens and reflects tumor heterogeneity; in 2012, we introduced a new mouse model to study oral carcinogenesis (12). We chose to focus on the tobacco smoke constituent and the environmental pollutant, the fjord-region PAH, dibenzo[*a,l*]pyrene (DB[*a,l*]P; ref. 12) and its ultimate diolepoxide metabolite DB[*a,l*]PDE (13). Both compounds were potent carcinogens, and DB[*a,l*]PDE greatly enhanced carcinogenicity relative to the parent compound in the mouse oral cavity (12–14).

Treating cancers including OSCC at late stages, even with recent advances in targeting therapies continues to be a major challenge, and thus, prevention remains a desirable approach. Numerous sources of phytochemicals have been proposed (15), and one that has shown promise in inhibiting carcinogenesis including head and neck cancers is freeze-dried black raspberry (BRB; refs. 14–19). In a previous study, BRB has been shown to inhibit dimethylbenz[a]anthracene (DMBA)-induced oral cancer in the hamster cheek pouch (20). In that study, inhibition could have resulted from inhibiting initiation or postinitiation processes. For initiation, metabolic activation of DMBA (and also other oral procarcinogens, such as 4-nitroquinoline-N-oxide) is essential to induce DNA damage, mutagenesis and carcinogenesis. Therefore, the chemopreventive effect of BRB on DBMA-induced oral cancer could be due to factors affecting initiation, such as inhibition of phase I, induction of phase II enzymes, and enhancement of DNA repair capacity of bulky lesions derived from DMBA. In addition, postinitiation processes, such as promotion and progression, could have been affected by BRB. In recent *in vitro* studies carried out in our laboratory (21), we showed that BRB extract enhanced repair of DB[*a,l*]P-induced bulky DNA lesions. We also showed that metabolic activation of DB[*a,l*]P to its active fjord-region diol epoxide DB[*a,l*]PDE is required to induce DNA damage, mutagenesis, and squamous cell carcinoma in the mouse oral cavity (13, 22). In contrast, to DMBA and 4-NQO, DB[*a,l*]PDE does not require metabolic activation to exert its biological effects, and thus, this study was initiated to examine, for the first time, whether BRB inhibits postmetabolic processes such as DNA damage, mutagenesis, and tumorigenesis.

Materials and Methods

Chemicals

(±)-Anti-DB[*a,l*]PDE was prepared according to a published method by our group (23). Structural characterization of this carcinogen was based on NMR and high-resolution mass spectral data, and its purity (≥99%) was determined by high-performance liquid chromatography (HPLC).

Animals

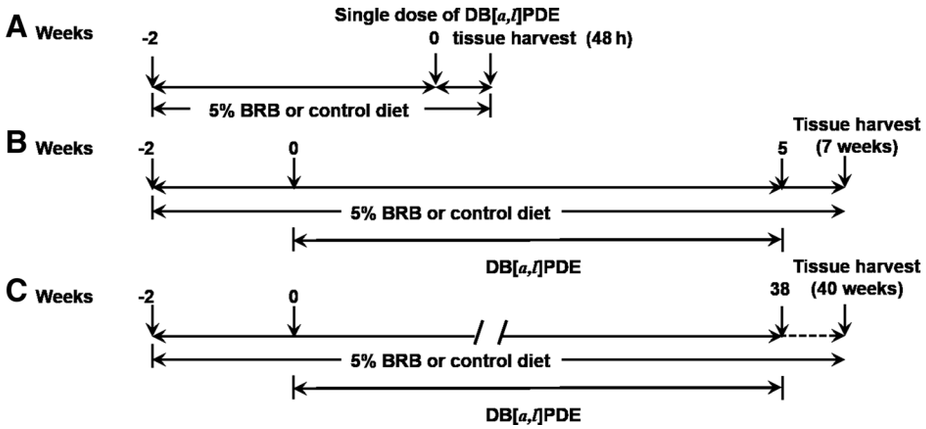
The selection of species, strain, and sex of the animals used in the current study is based on our previous report (13). Thus, female B6C3F$_1$ mice (6–8 weeks of age, The Jackson Laboratory) were used to examine the effects of BRB on DNA adducts formation and tumorigenesis induced by DB[*a,l*]PDE in the oral cavity; Big Blue C57BL/6 (*lacI*) mice (BioReliance) were used to examine the effect of BRB on mutagenesis induced by DB[*a,l*]PDE. Mice were quarantined for 1 week; then, they were transferred to the bioassay laboratory. All mice were kept on a 12-hour light/dark cycle, maintained at 50% relative humidity and 21°C ± 2°C. Water and food were provided *ad libitum*. The bioassay was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by Institutional Animal Care and Use Committee.

Effect of BRB on DNA adducts induced by DB[*a,l*]PDE in oral tissues of mice

Two groups of B6C3F1 mice (6 mice/group) were fed AIN-93M diet (5% corn oil) containing BRB (5%) or control diet (AIN-93M) starting 2 weeks prior to the administration of a single dose of DB[*a,l*]PDE (6 nmol/mouse) by topical application into the oral cavity of mice (Scheme IA). On the basis of previous preclinical studies (reviewed in ref. 14), 5% BRB was the most protective, and thus, such level was used in the current investigation. The carcinogen dose was selected on the basis of our previous studies (13, 24, 25). Mice were sacrificed 48 hours after DB[*a,l*]PDE treatment, and tissues were removed for DNA isolation.

We employed our previously published method for the analysis of the major deoxyadenosine adducts by LC/MS-MS (21, 24, 25). Briefly, we isolated DNA from oral tissues using the Qiagen genomic DNA isolation procedure. The level of DNA was quantified by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Prior to enzymatic digestion, 150 pg of the internal standard [$^{15}N_5$]-anti-*trans*-DB[*a,l*]PDE-dA adduct was added to approximately 60 μg DNA. Subsequently, in the presence of 10 μL of 1 mol/L $MgCl_2$/mg DNA and DNAse I (0.2 mg/mg DNA) DNA was hydrolyzed at 37°C for 1.5 hours, followed by snake

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278



Scheme 1.
Experimental protocol to determine the effects of BRB on DB[*a,l*]PDE-induced DNA adducts (**A**) mutagenesis (**B**) and tumorigenesis (**C**) in oral tissues of mice.

venom phosphodiesterase (0.08 U/mg DNA) and alkaline phosphatase (2 U/mg DNA). An aliquot of the DNA hydrolysate was taken for analysis of dA by HPLC. The remaining supernatant was partially purified on an Oasis HLB column (1 cm^3, 30 mg, Waters Ltd.). Then, the analysis was carried out on an API 3200 LC/MS-MS triple quadrupole mass spectrometer interfaced with an Agilent 1200 series HPLC using an Agilent extend-C18 5 μm 4.6 × 150 mm column. Adducts were monitored in multiple reaction monitoring mode, and the MS/MS transitions of *m/z* 604→ *m/z* 335, and *m/z* 609→ *m/z* 335 were monitored for targeted adducts and the internal standard (Fig. 1), respectively.

The effect of BRB on DB[*a,l*]PDE-induced mutagenesis in oral tissues of mice

Two groups of Big Blue C57BL/6 (*lacI*) mice (10/group) received 3 nmol (3 times weekly for 5 weeks) of (±)-anti-DB[*a,l*]PDE in DMSO by topical application as depicted in Scheme IB for 5 weeks. The dose was selected on the basis of our previous report (13). One group of mice were fed AIN-93M control diet, and the other group were fed AIN-93M diet containing 5% BRB starting 2 weeks prior to carcinogen treatment and continued until termination. Mice were sacrificed 2 weeks after the cessation of carcinogen administration. The oral tissues were excised and stored at −80°C until isolation of DNA. The palate and pharynx were pooled and homogenized together to give a mixture designated oral tissue. Each tissue was gently homogenized by hand in a microcentrifuge tube using a Teflon pestle in three volumes of 10 mmol/L Tris-HCl (pH, 8.0), 10 mmol/L EDTA, and 150 mmol/L NaCl/g tissue weight (w/v). SDS and protease K were added to this homogenate to obtain final concentrations of 10 and 1 mg/mL, respectively. The mixture was incubated for 3 hours at 50°C, and

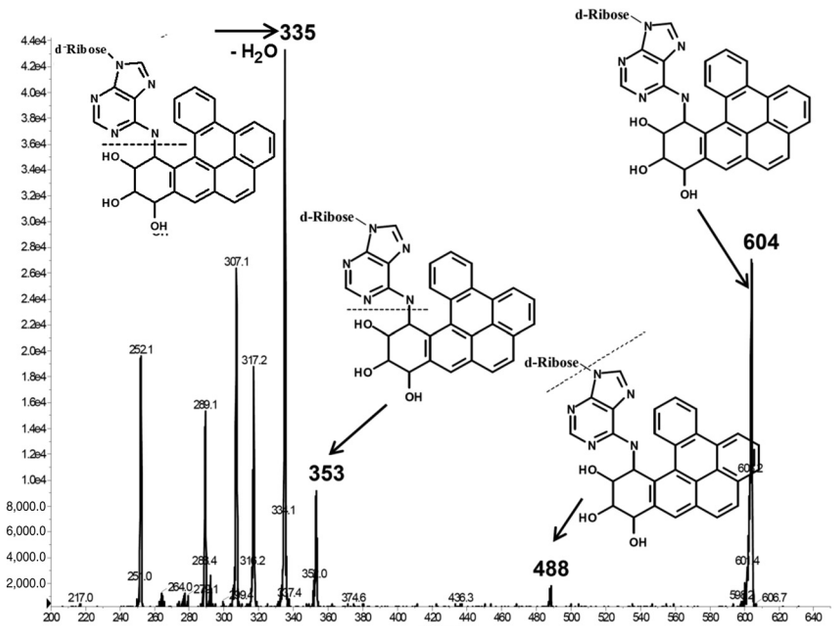


Figure 1.
A representative mass spectra of (±)-anti-DB[*a,l*]PDE-dA adducts.

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

then for 30 minutes at 37°C with 0.1 mg/mL RNase A. After incubation, one third volume of 6 mol/L ammonium acetate (pH 7.4) was added, and the mixture was gently mixed and then centrifuged at 14,000 rpm in an Eppendorf microfuge. The supernatant was carefully removed, leaving a small volume behind, to avoid transfer of any of the precipitate. To the supernatant, an equal volume of isopropyl alcohol was added at room temperature to precipitate DNA. The supernatant was removed, the DNA was washed once with 70% ethanol, suspended in 10 mmol/L Tris-HCl (pH, 8.0), 1 mmol/L EDTA, and left overnight at room temperature to dissolve.

Phage packaging was carried out using a homemade packaging extract prepared from bacterial strains supplied by Dr. Peter Glazer (Yale University School of Medicine, New Haven, CT), and the positive selection *cII* mutation assay was performed as described previously (26). At least three packaging reactions were carried out for each DNA sample. The mean mutant fractions of each group were compared with those from the control (vehicle alone) group using a one-tailed *t* test.

The effect of BRB on DB[*a,l*]PDE-induced tumorigenesis in oral tissues of mice

Two groups of B6C3F$_1$ mice (30/group) at the age of 8 weeks received 3 nmol of (±)-anti-DB[*a,l*]PDE in DMSO, 3 times a week for 38 weeks as depicted in Scheme IC. The dose was selected on the basis of our previous study (13). One group of mice was fed AIN-93M control diet, and the other group was fed AIN-93M diet containing 5% BRB starting 2 weeks prior to the initiation of carcinogen treatment. Treatments continued until termination. A third group of mice ($n = 20$) treated only with the vehicle (DSMO) and fed 5% BRB diet was used as a negative control. Mice were weighed weekly in the first month and then biweekly until termination. During the progress of the bioassay, mice were culled from the group and sacrificed if we observed a sudden weight loss of more than 20% or a tumor size exceeding 0.5 cm in diameter. At termination, mice were sacrificed by CO_2 asphyxiation, and soft tissues of the oral cavity, including tongue, pharynx, and other oral tissues (hard palate, buccal mucosa, and floor of mouth), were collected and fixed in 10% neutral-buffered formalin. Tissues were processed in an automated Tissue-Tek VIP processor and paraffin-embedded with a Tissue-Tek TEC embedding station. Sections were cut at 6 μm for routine hematoxylin and eosin (H&E) staining. All tissues were examined by an ACVP diplomate pathologist blinded to treatment according to established criteria (27). Benign tumors were not invasive into the basement membrane and subjacent stroma. Microinvasive malignant tumors had focal limited invasion, less than a single 400× high-power field deep to the basement membrane. Invasive malignant tumors were more extensively invasive.

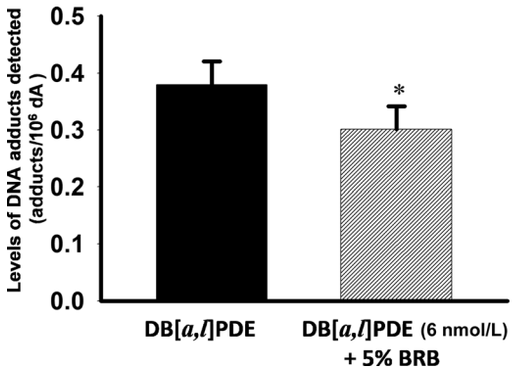


Figure 2.
Inhibition of DB[*a,l*]PDE-dA adduct levels by BRB in oral tissues of mice. *, $P < 0.05$.

Results

Effect of BRB on DB[*a,l*]PDE-induced DNA adducts in oral tissues of mice

Prior to the examination of the chemopreventive effects of BRB on DB[*a,l*]PDE-induced tumorigenesis, we initially examined the effect of BRB on DB[*a,l*]PDE-induced DNA adducts in oral tissues of mice. We showed that 5% BRB in the diet significantly ($P < 0.05$) reduced the levels of (-)-anti–*trans*-DB[*a,l*]PDE-dA in the oral cavity of mice 48 hours after a single dose of 6 nmol DB[*a,l*]PDE (Fig. 2). BRB in the diet reduced the levels of (-)-anti-*trans*-DB[*a,l*]PDE-dA adduct from 3.8 ± 0.4 to 3.0 ± 0.4 adducts/10^7 dA (20.7% inhibition).

The effect of BRB on DB[*a,l*]PDE-induced mutagenesis in oral tissues of the laci mice

We found that 5% BRB in the diet reduced the mutation fraction induced by DB[*a,l*]PDE in the oral cavity of mice by about 20% ($P = 0.053$; Fig. 3). A background of 2.4 mutants/10^5 has been subtracted from the mutant fractions in Fig. 3. That represents the mutant fraction in the untreated mice from our earlier study (13).

The effect of BRB on DB[*a,l*]PDE-induced tumorigenesis in oral tissues of mice

Figure 4 shows the cumulative mortality of mice during the progress of the bioassay. The probability of survival was displayed using Kaplan–Meier plot with death as an endpoint. Log-rank test was used to evaluate the difference between this pair of groups. No significant difference was observed in survival analysis in both groups of mice. All mice treated with DMSO and fed 5% BRB survived the duration of the bioassay. Body weights (mean ± SE) of mice treated with DB[*a,l*]PD + 5% BRB and those treated with DB[*a,l*]PDE alone are provided in Supplementary Table S1 and were not significantly different with the exception at week 4 and 20; however, the difference at these two time points was about 5%.

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

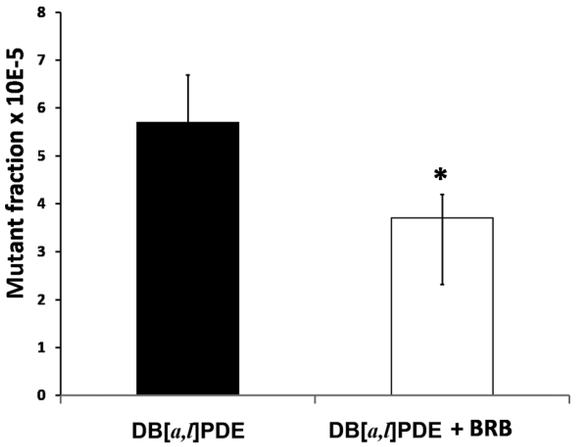


Figure 3.
Inhibition of DB[*a,l*]PDE-induced mutagenesis by BRB in oral tissues of mice. *, $P = 0.053$.

We found that the tumor incidence in the oral cavity (oral mucosa and tongue) of mice fed diet containing 5% BRB was reduced significantly from 93% to 66% (Table 1). Benign tumors induced by DB[*a,l*]PD consist of papilloma and keratoacanthoma, papilloma accounted for 94.7%. Specifically, overall benign tumor incidence was significantly reduced from 90% to 31% (62% to 28% in the oral cavity and 28% to 2% in the tongue). Malignant tumors induced by DB[*a,l*]PDE consist of squamous cell carcinoma, invasive anaplastic cell carcinoma, and fibrosarcoma; squamous cell carcinoma accounted for 88.8%. BRB reduced malignant tumors from 52% to 45%. No tumors were found in mice treated with DMSO and fed 5% BRB diet.

Discussion

The results of the current study clearly demonstrate the protective effects of BRB against the development of oral carcinogenesis induced by a powerful carcinogenic metabolite derived from the environmental pollutant and tobacco smoke constituent, DB[*a,l*]P. Numerous compounds as well as complex mixtures have been used to induce oral cancer in animal models (28). Preclinical animal models that used carcinogens found in the environment and/or tobacco smoke can mimic human exposure to such carcinogens and can recapitulate the tumor heterogeneity and can also provide a realistic platform to evaluate chemopreventive agents (29). In this study, we selected DB[*a,l*]PDE because of (i) its remarkable carcinogenicity in the mouse oral cavity (14) and (ii) to demonstrate for the first time that the efficacy of BRB can be extended to direct-acting carcinogens, which do not require metabolic activation by phase I enzymes, and is not just limited to procarcinogens.

Prior to the initiation of the long-term chemopreventive bioassay, it was essential to initially determine in a short-term animal bioassay whether BRB was capable of inhibiting DNA damage and consequently mutation induction by DB[*a,l*]PDE. Because of the positive outcomes demonstrating *in vivo* the protective effects of BRB on these endpoints, we then initiated the long-term bioassay to examine the efficacy of BRB against the development of oral carcinogenesis.

It was previously shown that the diet containing 5% BRB resulted in a 45% reduction in tumor incidence induced by the procarcinogen DMBA in hamster cheek pouch model, and this effect was associated with reduced formation of DNA adducts in epithelial cells of the oral mucosa (20). However, in this previous report, ^{32}P-postlabelling technique was used, which may not provide accurate adduct quantification, and adduct distribution remains to be assessed (20). In the current study, our LC/MS-MS method (25) was used to provide an accurate quantification and structural information on the nature of DNA adducts derived from DB[*a,l*]PDE. Clearly, the protective effect of BRB on DNA

Table 1. Inhibition of oral tumorigenesis by BRB in DB[*a,l*]PDE-treated mice

	Treatments	
	DB[*a,l*]PDE	DB[*a,l*]PDE+BRB
Number of mice[a]	29	29
Total (M + B)	27 (93)[b]	19 (66)[c]
Benign tumors (B)	26 (90)[d]	9 (31)[e]
Malignant tumors (M)	15 (52)[f]	13 (45)

[a]Mice that died before the first tumor appeared in the study or did not reach histology due to cannibalism were not counted.
[b]Number in parentheses, percentage of mice developed tumors.
[c]Tumor incidence was significantly reduced compared with DBPDE treatment, $P < 0.05$.
[d]Benign tumors induced by DB[*a,l*]PD consist of papilloma and keratoacanthoma; papilloma accounted for 94.7%.
[e]Tumor incidence was significantly reduced compared with DBPDE treatment, $P < 0.001$.
[f]Malignant tumors induced by DB[*a,l*]PDE consist of squamous cell carcinoma, invasive anaplastic cell carcinoma, and fibrosarcoma; squamous cell carcinoma accounted for 88.8%.

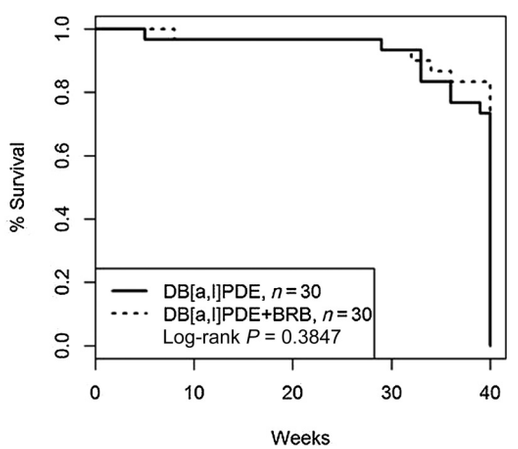


Figure 4.
Percentage survival of B6C3F1 mice treated by topical application of DB[*a,l*]PDE at 3 nmol three times a week for 38 weeks during the progress of the bioassay.

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

damage is not due to inhibition of phase I enzymes but its effects on phase II and/or DNA repair enzymes could be relevant. Although we did not examine the levels of phase II or DNA repair enzymes in the current study, our results are in line with our recent *in vitro* cell culture study that demonstrated that BRB extract resulted in reduced DNA adducts derived from DB[*a*,*l*]PDE via enhancing DNA repair capacity, which represents the integrated effects of proteins involved in the DNA repair pathways. (21, 30).

This observed inhibition of DNA damage by BRB stimulated a follow-up investigation aimed at determining the effect of BRB on mutation induction by DB[*a*,*l*]PDE *in vivo* using Big Blue C57BL/6 (*lacI*) mice. Presumably, a reduction in DNA damage by BRB would be expected to reduce mutation induction. Using *lacI* and *lacZ* rodents, we have reported that all of the known oral carcinogens are also mutagens in the mouse and/or rat oral cavity (31). As mutagenesis is considered an important step in carcinogenesis, it can represent an early biomarker in the multistep carcinogenesis process. We previously assayed mutagenesis induced by DB[*a*,*l*]P (12) and DB[*a*,*l*]PDE (13) in the oral cavity of BGC3F1 *lacI* mice and showed that both are powerful mutagens in the oral cavity of mice.

In the current study, we showed that BRB is capable of inhibiting mutagenesis induced by DB[*a*,*l*]PDE in the mouse oral cavity. In our previous *in vitro* studies, using rat oral fibroblast, we showed that BRB extract was also effective at reducing mutagenesis induced by DB[*a*,*l*]PDE (21). Presumably, a reduction in DNA damage by BRB, as discussed above, is responsible for the reduction in mutagenesis. We also showed that BRB extract reduced oxidative stress *in vitro* (21). Taken together, these results suggest that BRB is potentially an inhibitor of several steps in the multistep carcinogenesis, including tumor initiation by DB[*a*,*l*]PDE, and thus, we performed the following long-term chemoprevention bioassay.

Our results on survival and body weights demonstrated that mice tolerated 5% BRB in the diet. Clearly, the ability of BRB to inhibit tumorigenesis is not limited to synthetic procarcinogens, such as DMBA (20). A significant protective effect of BRB was observed against benign tumors, but the inhibition of malignant formation did not reach significance. A longer duration of the bioassay might have allowed some or all of the benign tumors to progress to malignant tumors, resulting in significant inhibition of malignancy, but this could not be possible because animals needed to be sacrificed because tumor size reached >0.5 cm in the oral cavity. In the current study, dietary BRB powder was given 2 weeks before carcinogen administration and continued until termination of the bioassay. Thus, our design cannot dissociate the effects of berry on tumor initiation versus tumor progression. However, based on our previous studies (21, 30), we showed that BRB enhanced DNA repair of the damage induced by DB[*a*,*l*]P, which contributes to the initiation phase of carcinogenesis.

In addition to the protective effects of BRB in preclinical animal models, the results of several clinical trials demonstrate its safety and that it is easy to administer and can modulate critical markers of cancer in a manner consistent with prevention/treatment (14). In the oral cavity of healthy volunteers, a 10% mucoadhesive freeze-dried BRB gel, when treated topically on dysplastic lesions four times per day for 6 weeks, was found to result in a histologic regression of about 60% of the lesions (32, 33). It was also found in oral cancer patients that proinflammatory and prosurvival markers were suppressed by BRB phytochemical-rich troche (34). The active components in BRB, which can account for its observed effects in our oral carcinogenesis model, remain to be determined. However, we recently reported on the effects of select constituents of BRB on carcinogen-induced DNA damage in cell cultures; our preliminary results indicate that the structure of the constituent is an important determining factor in inhibiting DNA damage (30). Our results provide a basis to translate our findings into future studies designed, for instance, to examine the effect of BRB on the initiation stage of carcinogenesis by assessing levels of DNA adducts derived from DB[*a*,*l*]P and benzo[*a*]pyrene in smokers who are considered at high risk of developing this disease.

Disclosure of Potential Conflicts of Interest

Gary Stoner reports receiving a commercial research grant from Oregon Raspberry and Blackberry Commission and has ownership interest (including patents) in Berristone, LLC. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: K.-M. Chen, J.B. Guttenplan, Y.-W. Sun, G. Stoner, K. El-Bayoumy

Development of methodology: K.-M. Chen, J.B. Guttenplan, C. Aliaga, S. Amin, K. El-Bayoumy

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.-M. Chen, J.B. Guttenplan, Y.-W. Sun, N.A.E. Shalaby, W. Kosinska, C. Aliaga, K. Gowda, S. Amin, K. El-Bayoumy

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.-M. Chen, J.B. Guttenplan, T. Cooper, N.A.E. Shalaby, J. Zhu, J. Liao, K. El-Bayoumy

Writing, review, and/or revision of the manuscript: K.-M. Chen, J.B. Guttenplan, Y.-W. Sun, J. Liao, S. Amin, G. Stoner, K. El-Bayoumy

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.-M. Chen, N.A.E. Shalaby, W. Kosinska, C. Aliaga, K. El-Bayoumy

Study supervision: J.B. Guttenplan, S. Amin, K. El-Bayoumy

Tissues collection, process, and analysis: G. Benitez

Acknowledgments

This work was supported by the NIH NCI grant R01-CA173465.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 1, 2017; revised October 16, 2017; accepted November 15, 2017; published OnlineFirst November 20, 2017.

References

1. Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. J Oncol 2011;2011:603740.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65: 87–108.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7–30.
6. Groome PA, Rohland SL, Hall SF, Irish J, Mackillop WJ, O'Sullivan B. A population-based study of factors associated with early versus late stage oral cavity cancer diagnoses. Oral Oncol 2011; 47:642–7.
7. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet 2008;371:1695–709.
8. da Silva SD, Hier M, Mlynarek A, Kowalski LP, Alaoui-Jamali MA. Recurrent oral cancer: current and emerging therapeutic approaches. Front Pharmacol 2012;3:149.
9. El-Bayoumy K, Chung FL, Richie J Jr, Reddy BS, Cohen L, Weisburger J, et al. Dietary control of cancer. Proc Soc Exp Biol Med 1997;216:211–23.
10. Hecht SS, Hoffmann D. The relevance of tobacco-specific nitrosamines to human cancer. Cancer Surv 1989;8:273–94.
11. Ng SK, Kabat GC, Wynder EL. Oral cavity cancer in non-users of tobacco. J Natl Cancer Inst 1993;85:743–5.
12. Guttenplan JB, Kosinska W, Zhao ZL, Chen KM, Aliaga C, Del-Tondo J, et al. Mutagenesis and carcinogenesis induced by dibenzo[a,l]pyrene in the mouse oral cavity: a potential new model for oral cancer. Int J Cancer 2012;130:2783–90.
13. Chen KM, Guttenplan JB, Zhang SM, Aliaga C, Cooper TK, Sun YW, et al. Mechanisms of oral carcinogenesis induced by dibenzo[a,l]pyrene: an environmental pollutant and a tobacco smoke constituent. Int J Cancer 2013;133:1300–9.
14. El-Bayoumy K, Chen KM, Zhang SM, Sun YW, Amin S, Stoner G, et al. Carcinogenesis of the oral cavity: environmental causes and potential prevention by black raspberry. Chem Res Toxicol 2017;30:126–44.
15. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3:768–80.
16. Mallery SR, Tong M, Shumway BS, Curran AE, Larsen PE, Ness GM, et al. Topical application of a mucoadhesive freeze-dried black raspberry gel induces clinical and histologic regression and reduces loss of heterozygosity events in premalignant oral intraepithelial lesions: results from a multicentered, placebo-controlled clinical trial. Clin Cancer Res 2014;20:1910–24.
17. Rao AV, Snyder DM. Raspberries and human health: a review. J Agric Food Chem 2010;58:3871–83.
18. Stoner GD, Wang LS, Zikri N, Chen T, Hecht SS, Huang C, et al. Cancer prevention with freeze-dried berries and berry components. Semin Cancer Biol 2007;17:403–10.
19. Stoner GD. Foodstuffs for preventing cancer: the preclinical and clinical development of berries. Cancer Prev Res (Phila) 2009;2:187–94.
20. Casto BC, Kresty LA, Kraly CL, Pearl DK, Knobloch TJ, Schut HA, et al. Chemoprevention of oral cancer by black raspberries. Anticancer Res 2002;22:4005–15.
21. Guttenplan JB, Chen KM, Sun YW, Kosinska W, Zhou Y, Kim SA, et al. Effects of black raspberry extract and protocatechuic acid on carcinogen-DNA adducts and mutagenesis, and oxidative stress in rat and human oral cells. Cancer Prev Res 2016;9:704–12.
22. Sun YW, El-Bayoumy K, Aliaga C, Awad AS, Gowda K, Amin S, et al. Tissue distribution, excretion and pharmacokinetics of the environmental pollutant Dibenzo[def,p]chrysene in mice. Chem Res Toxicol 2015;28:1427–33.
23. Krzeminski J, Lin JM, Amin S, Hecht SS. Synthesis of Fjord region diol epoxides as potential ultimate carcinogens of dibenzo[a, l]pyrene. Chem Res Toxicol 1994;7:125–9.
24. Zhang SM, Chen KM, Aliaga C, Sun YW, Lin JM, Sharma AK, et al. Identification and quantification of DNA adducts in the oral tissues of mice treated with the environmental carcinogen dibenzo[a,l]pyrene by HPLC-MS/MS. Chem Res Toxicol 2011; 24:1297–303.
25. Zhang SM, Chen KM, Sun YW, Aliaga C, Lin JM, Sharma AK, et al. Simultaneous detection of deoxyadenosine and deoxyguanosine adducts in the tongue and other oral tissues of mice treated with Dibenzo[a,l]pyrene. Chem Res Toxicol 2014;27:1199–206.
26. Guttenplan JB, Zhao ZL, Kosinska W, Norman RG, Krzeminski J, Sun YW, et al. Comparative mutational profiles of the environmental mammary carcinogen, 6-nitrochrysene and its metabolites in a lacI mammary epithelial cell line. Carcinogenesis 2007;28:2391–7.
27. Leininger JR, Jokinen MP. Tumours of the oral cavity, pharynx, oesophagus and stomach. IARC Sci Publ 1994:167–93.
28. Mognetti B, Di Carlo F, Berta GN. Animal models in oral cancer research. Oral Oncol 2006;42:448–60.
29. Vitale-Cross L, Czerninski R, Amornphimoltham P, Patel V, Molinolo AA, Gutkind JS. Chemical carcinogenesis models for evaluating molecular-targeted prevention and treatment of oral cancer. Cancer Prev Res (Phila) 2009;2:419–22.
30. Guttenplan JB, Chen K-M, Sun Y-W, Lajara B, Shalaby N, Kosinska W, et al. Effects of black raspberry extract and berry compounds on repair of DNA damage and mutagenesis induced by chemical and physical agents in human oral leukoplakia and rat oral fibroblasts. Chem Res Toxicol 2017 Nov 15. [Epub ahead of print].
31. von Pressentin MM, Kosinska W, Guttenplan JB. Mutagenesis induced by oral carcinogens in lacZ mouse (MutaMouse) tongue and other oral tissues. Carcinogenesis 1999;20:2167–70.
32. Shumway BS, Kresty LA, Larsen PE, Zwick JC, Lu B, Fields HW, et al. Effects of a topically applied bioadhesive berry gel on loss of heterozygosity indices in premalignant oral lesions. Clin Cancer Res 2008;14:2421–30.
33. Mallery SR, Zwick JC, Pei P, Tong M, Larsen PE, Shumway BS, et al. Topical application of a bioadhesive black raspberry gel modulates gene expression and reduces cyclooxygenase 2 protein in human premalignant oral lesions. Cancer Res 2008;68:4945–57.
34. Knobloch TJ, Uhrig LK, Pearl DK, Casto BC, Warner BM, Clinton SK, et al. Suppression of proinflammatory and prosurvival biomarkers in oral cancer patients consuming a black raspberry phytochemical-rich troche. Cancer Prev Res 2016;9:159–71.

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

Published OnlineFirst November 20, 2017; DOI: 10.1158/1940-6207.CAPR-17-0278

Cancer Prevention Research

Effects of Black Raspberry on Dibenzo[*a,l*]Pyrene Diol Epoxide Induced DNA Adducts, Mutagenesis, and Tumorigenesis in the Mouse Oral Cavity

Kun-Ming Chen, Joseph B. Guttenplan, Yuan-Wan Sun, et al.

Cancer Prev Res 2018;11:157-164. Published OnlineFirst November 20, 2017.