Use of Freeze-dried Watercress for Detoxification of Carcinogens and Toxicants in Smokers: Implications of the Findings and Potential Opportunities

Karam El-Bayoumy[1] and Gary Stoner[2]

ABSTRACT

Bonorden and colleagues designed a clinical trial to test the hypothesis that daily consumption of freeze-dried watercress, a rich source of the chemopreventive agent phenethyl isothiocyanate, can enhance the detoxification of well-known tobacco and environmental carcinogens and toxicants. Initial results have validated subject compliance and a positive outcome of this study would further support the use of watercress as a whole food-based approach to cancer chemoprevention. On the basis of the design of the clinical trial and the various biological samples to be collected, we discuss potential opportunities to test future hypotheses.

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Introduction

The National Cancer Act that was signed into law in 1971 documents the commitment of the United States to a war on cancer as stated by the late president Richard Nixon. The past 50 years of fiscal support from the NIH has resulted in a better understanding of the multi-step carcinogenesis process and improved strategies for cancer screening, prevention, and treatment which in turn reduced cancer mortality by 30% since 1971. Although a cancer-free society remains a desirable goal, it constitutes major challenges to researchers in the United States and around the globe. There is no doubt that much of the decline in cancer mortality is due, in part, to lifestyle changes including reduction in smoking because of effective tobacco control policies. In fact, cigarette smoking has declined from 21% of adults in 2005 to 14% in 2019 (1). Unfortunately, there are still more than 30 million adult cigarette smokers in the United States and more than 1 billion tobacco smokers worldwide (2, 3).

Tobacco smoke contains over 7,000 chemicals of which more than 70 have been classified by the International Agency for Research on Cancer (IARC) as human carcinogens. Several agents in tobacco have also been shown to act as tumor promotors or co-carcinogens. Among the established tumor initiators include the polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines (TSNA). Tobacco smoke also contains high levels of reactive oxidants, such as free radicals and aldehydes known to impact various pathways involved in the multi-step carcinogenesis process. Smoking continues to be a major source of human exposure to carcinogens and toxicants and remains a significant risk factor for increased incidences of numerous diseases including cancer at multiple organ sites. In addition, cigarette smoking causes one-third of cancer deaths in the United States (3).

Current Challenges to Cancer Prevention

Treating cancers at late stages even with targeted therapies remains a major challenge and thus prevention represents the most effective strategy to manage and control tobacco smoking–related cancers. Effective control and prevention of tobacco consumption and the reduction of environmental exposure to known carcinogens remain major goals for cancer prevention. An important approach to prevention involves an improved understanding of the molecular mechanisms responsible for cancer induction by carcinogens in tobacco smoke and in the environment as a whole. In fact, in 2005 we (El-Bayoumy and Sinha) introduced the term "molecular chemoprevention" as a guiding principle in the design of clinical chemoprevention trials (4). Chemoprevention studies focused on molecular mechanisms of carcinogenesis can result in the development of biomarkers of potential use for the early detection of cancer as well as for monitoring the impact of chemopreventive agents.

It has been known for several decades that the consumption of diets rich in fruits and vegetables can lower the risk of cancer development at multiple organ sites. For example, a meta-analysis of more than 8,000 lung cancer cases and 684,000 non-cancer subjects showed an overall 20% lower risk of lung cancer associated with cruciferous vegetable (broccoli, cabbage, brussel sprouts, garden cress, and watercress) intake with the strongest risk reduction in subjects who were *glutathione S-transferase (GST) M1* or *GSTT1* null, and particularly in

[1]Department of Biochemistry & Molecular Biology, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania. [2]Department of Medicine, College of Medicine, Ohio State University, Columbus, Ohio.

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

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the double nulls (5). The identification of naturally occurring agents in routinely consumed foods that exhibit cancer preventative effects has been and continues to be a significant area of research activity worldwide. Many of these agents have been identified in animal models of specific cancers and mechanistically, they exhibit a broad range of effects on cellular and molecular events involved in both tumor initiation and tumor promotion/progression. However, in human clinical trials, most of these agents have either been ineffective or they have exhibited side effects that severely limit their use for routine chemoprevention. For example, the negative impact of beta-carotene and retinol on lung cancer occurrence in active tobacco smokers. Furthermore, the results of the Selenium and Vitamin E prostate cancer prevention trial (SELECT) were disappointing. The outcome of these results led Potter to conclude that chemoprevention is not a viable approach to cancer prevention (6).

An alternative approach to chemoprevention utilizing whole foods was proposed by one of us (Stoner) based upon chemoprevention studies with freeze-dried black raspberries (7). The berries were found to be efficacious for chemoprevention in both animals and humans because: (i) they elicited little or no toxicity at relatively high doses; and, (ii) they exhibited a broad spectrum of chemopreventive effects on both cellular and molecular levels probably due to the fact that they contain many individual compounds with demonstrated chemopreventive activity. Currently, we (El-Bayoumy) have an ongoing clinical trial (NCT04372914) funded by the NCI to determine the inhibitory effect of a black raspberry lozenge on tobacco-induced DNA damage in buccal cells of smokers. Similarly, Kensler and his colleagues pioneered the use of broccoli sprouts as a whole food approach to the chemoprevention of aflatoxin-induced liver cancer in humans (8). The most active chemopreventive compounds in black raspberries appear to be the anthocyanins and in broccoli, sulforaphane, an inducer of phase II detoxification enzymes. Recently, Bonorden and colleagues discuss the rationale and the benefits of freeze-dried watercress as a whole-food approach to enhance the detoxification of tobacco carcinogens in smokers (9). The rationale for the use of watercress is described below.

Why watercress?

Extensive studies have shown that naturally occurring isothiocyanate compounds have potential cancer protective and therapeutic effects. Our interest in the role of phenethyl isothiocyanate (PEITC) began in 1985 when Chung, and colleagues at the Institute of Cancer Prevention (formally the American Health Foundation) in Valhalla, New York showed that PEITC inhibited the metabolic activation of the powerful tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) (NNK; ref. 10). NNK is classified by IARC as carcinogenic to humans, Group 1. Subsequent studies by these investigators demonstrated the preventative effects of PEITC against NNK-induced lung carcinogenesis in rodents and as an inhibitor of the metabolic activation of NNK in a clinical trial involving cigarette smokers (11). The results of this trial showed a modest but significant 7.7% inhibition of NNK metabolic activation when cigarette smokers were dosed with PEITC versus olive oil alone. However, this trial revealed more encouraging results demonstrating significant increases in the level of urinary mercapturic acid detoxification of several compounds (acrolein, crotonaldehyde, benzene, and 1,3-butadiene) which were observed particularly in subjects who were null for either *GSTM1* or *GSTT1* genes or both. On the basis of these encouraging results, Bonorden and colleagues hypothesize that daily consumption of a drink containing freeze-dried watercress, a rich source of PEITC, would have a similar effect in humans particularly in those who are null in certain *GST* genes (9).

Tobacco smoke is a major source of acrolein exposure in the general population; however, acrolein is also found in other environmental sources (12). Acrolein was recently classified by IARC as probably carcinogenic to humans (Group 2A) on the basis of sufficient evidence of carcinogenicity in experiential animals and strong mechanistic evidence (12). In addition to other sources, tobacco smoke is a major source of crotonaldehyde which was classified as possibly carcinogenic to humans (Group 2B) on the basis of strong mechanistic evidence (12). The IARC previously classified benzene as Group 1 and 1,3-butadiene as Group 2B. Clearly, the hypothesis stated by these authors is based on compelling evidence resulting from extensive studies on PEITC and watercress; furthermore, the specific carcinogens and toxicants being examined in this article are highly relevant (9).

From the Laboratory to the Food Industry Setting to the Clinic

Bonorden and colleagues describe a partnership between scientists in academia and the food industry to prepare a beverage containing freeze-dried watercress for use in a clinical chemoprevention trial to determine the impact of watercress on detoxification of carcinogens and toxicants (9). However, the authors were faced with a major challenge to scale up from the laboratory setting to a clinical trial involving 400 subjects (smokers and non-smokers in their relative proportion in Minnesota) who are null in both *GSTM1* and *GSTT1* and who would consume the beverage for 2 weeks. Although this is a huge task, the authors successfully prepared approximately 100 lbs. of fully validated freeze-dried watercress in compliance with all food safety requirements for the trial. It is encouraging that the initial results validate subject compliance in the proposed trial. Importantly, a choice of a drink formulated from freeze-dried watercress was based on a number of well-defined practical considerations. The trial started on March 1, 2021, and 19 subjects have completed the study protocol. At each clinic visit, first morning void urine samples are collected and participants are asked to submit saliva and oral cells. The primary endpoints are urinary levels of the mercapturic acids of the carcinogens and toxicants as described above which will test the hypothesis that watercress consumption will increase the

detoxification of these volatile toxicants and carcinogens *via* their conjugations with glutathione, and that such effects will most clearly be observed in subjects who are null in both *GST* genes.

Implications of the study findings and potential opportunities

Bonorden and colleagues remind us that the design of a clinical trial with a large number of participants, requiring the preparation of an ample amount of freeze-dried watercress in order to test a well-defined hypothesis, would not be possible without a partnership between scientists in academia and the food industry. Initial results clearly document the tolerability of freeze-dried watercress and subject compliance. A positive outcome of this trial is anticipated on the basis of previous results generated from extensive studies employing PEITC and watercress in preclinical and clinical studies. On the basis of the trial design and the various biological samples to be collected, we discuss additional potential research opportunities. The authors stated that in addition to urine collection, participants will be asked to submit saliva and oral cells (9). The oral cavity is the first site of exposure to cigarette smoke and these cells can be easily collected and used in studies aimed at assessing DNA damage, a reliable marker for cancer risk, not just for head and neck squamous cell carcinoma (HNSCC) but possibly in lung cancer (13). Early studies quantified the levels of DNA damage derived from TSNA N′-nitrosonornicotine (NNN) in buccal cells of smokers and such damage was shown to be an independent risk factor for HNSCC (14). More recent studies by these authors reported the detection of acrolein-DNA adducts and apurinic/apyrimidinic sites in oral cells from cigarette smokers and nonsmokers (15, 16). Collectively, these biomarkers could provide reliable targets not just to evaluate cancer risk following exposures to tobacco smoke and environmental pollutants but also to monitor potential effects of chemopreventive agents, such as watercress. After the oral cavity, the second largest microbial community in humans is the gut (17) which contains a diverse community of about 700 bacterial species, many of which contribute to carcinogenesis. Thus, another potential opportunity is the utilization of saliva and oral cells to be obtained in the trial to assess the impact of cigarette smoking on microbiome profile and to further assess the impact of freeze-dried watercress on the oral microbiome. Successful achievement of the proposed clinical trial could provide the basis for a phase III chemoprevention trials using freeze-dried watercress in smokers.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

K. El-Bayoumy: Conceptualization, methodology, writing–original draft, writing–review and editing. **G. Stoner:** Conceptualization, methodology, writing–original draft, writing–review and editing.

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