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Blackberry extracts inhibit UV-induced mutagenesis in *Salmonella typhimurium* TA100

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Abstract

The development of metastatic cancer involves a series of mutagenic events. Compounds in blackberries effectively block mutagenesis by metabolically activated carcinogens and can serve as effective anticancer agents. We have compared the effects of 8 different varieties of blackberry on UV-C–induced mutagenesis in *Salmonella typhimurium* TA100. The 8 varieties were cultivated under identical conditions and harvested as ripe berries. Two varieties had no effect on UV-C–induced mutagenesis. Two showed significant suppression of mutagenesis. The other 4 had intermediate effects. Berry extracts were added only after irradiation of the cells was complete. The results imply that some varieties of blackberries contain substances that might inhibit error-prone DNA repair.

Keywords: Blackberry; Fruit extracts; Mutagenesis; UV radiation; Anticancer compounds; Ames assay; *Salmonella typhimurium*

1. Introduction

Diets high in fruits and vegetables are known to correlate with decreased probability of developing cancer. Green tea [1-4], broccoli (reviewed in Ref. [5]), berries [6-8], and other fruits [9,10] have been found to exhibit anticancer activity in animal studies, and in vitro studies have demonstrated that several plant extracts block or inhibit specific steps in carcinogenic process. In particular, some plant extracts block cancer cell metabolism or kill cancer cells in culture [11,12], inactivate free radicals and active oxygen species [13,14], exhibit antiestrogenic activity [15], and inhibit mutagenesis [16]. Several chemical species have been isolated and found to possess activity. The most intensively studied of these are the ellagitannins, especially ellagic acid [17-20]. However, it is almost certain that any particular fruit contains several compounds that can affect carcinogenesis and that these can interact either synergistically or antagonistically. In evaluating dietary components for anticancer potential, it is necessary to take these interactions into account by studying whole fruits or fruit juices. The complexity of the system is enhanced by the fact that different varieties of the same fruit could vary in activity and fruits of the same variety could vary with the culture conditions.

We have shown previously that berry extracts exhibit anticancer activity in that they can inhibit the metabolism of cells cultured from breast and cervical tumors [21]. Blackberry extracts also inhibit chemically induced mutagenesis as measured by the Ames assay for mutagenesis [22]. Cancer is initiated by an unrepaired lesion in an oncogene or tumor suppressor gene of a somatic cell. Accumulation of additional mutations in genes controlling cell division, cell-cell interactions, apoptosis, and cell surface markers results in an invasive, autonomous phenotype free of the usual restrictions on cell replication and capable of evading immune destruction. Hence, mutation is critical to the

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initiation and evolution of metastatic cancer. In the study reported here, we compared 8 varieties of blackberry for their effects on mutagenesis induced by UV radiation. The berries were all ripe, grown in the same location, under the same conditions, and harvested at the same time. Ultraviolet radiation in the C range (UV-C) was used as the mutagen because damage to the cell is localized primarily in the nucleic acids and results from direct absorption of photons by the target molecules. The types of lesions produced in DNA have been well characterized, and repair of these has been studied intensively. In addition, dosage and kinetics are better defined than for chemical carcinogens. Complications involving diffusion into the cell, reaction with other molecular species, transport to the nucleus, and the time required for these to occur are avoided.

2. Methods and materials

2.1. Berry extracts

Ripe blackberries were obtained from Callaham Orchards, Belton, SC. The 8 varieties (Arapaho, Chester, Chickasaw, Choctaw, Hull, Kiowa, Navajo, Triple Crown) had been cultivated and harvested under the same conditions. The berries were washed thoroughly and homogenized in a blender. For each variety, 8.0 mL of water was added to 3.0 g of homogenate to facilitate subsequent filtration. These solutions were clarified by centrifugation at 7000 *g*, and the supernatant was sterilized by filtration through a 0.2-μm filter (Nalgene; Nunc International, Rochester, NY).

2.2. Bacteria

Salmonella typhimurium strain TA100 originally generated by Ames et al [23] was purchased from EBP (Ontario, Canada). This is a His^- base substitution mutant in which the wild-type CTC codon has been replaced by CCC in the hisG46 allele. This mutant also has a deletion of *uvrB* function and carries the pKM101 plasmid coding for the SOS repair and mutagenesis [24]. Upon arrival, the bacteria were cultured. Single-colony isolates were tested for genetic characteristics, and stock cultures were prepared from these and stored at -80°C. These were used only to prepare master plates.

2.3. Mutagenesis assay

The protocol of Maron and Ames [25] was followed. A colony selected from one of the master plates was used to generate an overnight culture. This culture was diluted 1:10 and incubated for 0.5 hour at 37°C. After the incubation, 1 mL of culture was removed and irradiated with a germicidal lamp (G 1578) having a sharp intensity maximum at 254 nm. A dose rate of 0.86 J/m^2 was used to produce a final dose of 15 J/m^2. After irradiation, aliquots of 100 μL were placed into separate tubes. To the appropriate tubes, either 200 μL of berry extract or 200 μL of double-distilled water was added. Similar 100 μL aliquots of unirradiated cells were also placed in separate tubes, and either 200 μL of berry extract or double-distilled water was added. Four sets of samples were generated as follows: (1) 100 μL unirradiated cells plus 200 μL water, (2) 100 μL unirradiated cells plus 200 μL berry extract, (3) 100 μL irradiated cells plus 200 μL water, and (4) 100 μL irradiated cells plus 200 μL berry extract. To each of these samples, 2 mL of agar was added. The samples were then plated on selective medium and incubated for 48 hours, and the number of his^+ revertants was counted. Experiments were done under yellow light to prevent photoreactivation [26].

2.4. Data analysis

In each experiment, culture plates were done in triplicate. For each condition, the 3 plate counts were averaged. Because the number of revertant colonies on the control plates varied slightly from one experiment to another, the data for each experiment were normalized to the average number of revertants on control plates for that experiment. For each variety of blackberry except Arapaho and Chester, 3 independent experiments were done on different days. The normalized data from the 3 independent experiments were averaged. Because they represent the extreme cases, 5 experiments were averaged for Arapaho and Chester. For each extract, the levels of mutagenesis measured in the presence and the absence of berry extract were compared by Student *t* test, with a probability of .05 or less being considered significant [27].

3. Results

To determine the effect of a berry extract on mutagenesis, the following parameters were calculated for each experiment.

Mutagenesis induced by UV-C exposure alone is represented by

$$M_{\mathrm{UV}} = \frac{\text{no. of colonies from UV exposed cells}}{\text{no. of colonies from unirradiated cells}}.$$

Effect of berry extract alone is represented by

$$M_{\mathrm{EX}} = \frac{\text{no. of colonies from unirradiated cells + extract}}{\text{no. of colonies from unirradiated cells}}.$$

Mutagenesis induced by UV-C exposure followed by addition of extract is represented by

$$M_{\mathrm{UV+EX}} = \frac{\text{no. of colonies from UV exposed cells + extract}}{\text{no. of colonies from unirradiated cells + extract}}.$$

The effect of the berry extract on UV-C mutagenesis is given by comparing the amount of mutagenesis observed when the extract was added to the irradiated cells to the amount of mutagenesis observed in the absence of extract:

$$R = M_{\mathrm{UV+EX}}/M_{\mathrm{UV}}.$$