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Antiviral effects on bacteriophages and rotavirus by cranberry juice

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Abstract

Studies were undertaken to investigate the antiviral effects of comestible juices, especially cranberry juice, on non-related viral species. After exposure of bacteriophage T2 to a commercially available cranberry (*Vaccinium macrocarpon*) juice cocktail (CJ), virus infectivity titer was no longer detectible. After a 60-min exposure to orange (OJ) and grapefruit juices (GJ), phage infectivity was reduced to 25–35% of control, respectively. Similar data were observed for the bacteriophage T4. CJ inactivation of phage T4 was rapid, dose-dependent, and occurred at either 4 or 23 °C. Neither pH nor differences in sugar/carbohydrate levels among the juices may be ascribed to the recognized antiviral effects. Further studies were performed to identify the occurrence of antiviral activity by CJ to a mammalian enteric virus. The treatment of the simian rotavirus SA-11 with a 20% CJ suspension was sufficient to inhibit hemagglutination. Under scanning and transmission electron microscopy, CJ was observed to inhibit the adsorption of phage T4 to its bacterial host cells and prevented the replication of rotavirus in its monkey kidney (MA-104) host cells, respectively. The data suggest, for the first time, a non-specific antiviral effect towards unrelated viral species (viz., bacteriophages T2 and T4 and the simian rotavirus SA-11) by a commercially available cranberry fruit juice drink.

Keywords: Cranberry juice; Antiviral activity; Bacteriophages; Rotavirus

Introduction

The existence of anti-microbial agents in comestible plants and their products (e.g., fruit juices) has been of interest to biomedical and nutrition researchers for decades. Studies investigating the effects of naturally occurring anti-microbial and, to a lesser extent, antiviral agents in food and food products, have been performed in both in vivo and in vivo settings (Jassim and Naji, 2003).

Kontiokari et al. (2003), for example, reported a reduction in urinary tract infections (UTIs) among women following the addition of cranberry juice (CJ) to their diets. Cinatl et al. (2003) reported that glycyrrhizin, a component of licorice roots, inhibited the in vivo replication of the severe acute respiratory syndrome (SARS)-associated corona virus and alluded to the use of glycyrrhizin as a possible therapeutic agent. These and similar findings have broad implications, as consumption of defined foods containing antiviral/anti-microbial agents may benefit the health and well-being of diverse

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populations including, but not limited to infants, geriatric, and the immunocompromised.

Public interest in nutrition and food science has surged within the last two decades. A plethora of professional organizations now address the interests of researchers and lay persons in this growing field. Numerous organizations on both the national and international levels support divisions dedicated to the field of food science and nutrition (Food and Nutritional Information Center, 2005).

Scientific interest in naturally occurring antiviral activity in foods was bolstered during the late 1970s by the studies of Konawalchuk and Spears (1978). Using poliovirus as a model system, selected juices were found to reduce in vivo poliovirus infectivity significantly. More recent studies showed in vivo and/or in vivo antiviral activity against human immunodeficiency virus type-I and Freund's leukemia virus by the plant extracts hypericin and pseudohypericin, from *Hypericum perforatum* (St. John's wort) (Degar et al., 1992; Meruelo et al., 1988). New classes of antiviral compounds in the edible gypsy mushroom *Rozities caperata*, were reported recently. Specifically, in vivo antiviral activity by extract RC-183 from this mushroom was deemed effective in inhibiting the replication of herpes simplex virus types 1 and 2, varicella-zoster virus (chicken pox, zoster), influenza, and respiratory syncytial virus (Piraino and Brandt, 1999). In the mouse model, intranasal and intraperitoneal administration of flavonoids (water-soluble plant pigments) inhibited the replication of influenza virus types A and B (Nagai et al., 1995).

It was proposed that flavonoids may be responsible for the in vivo and in vivo (mouse model) antiviral effects of various herbs and comestible plant products (Middleton et al., 2000).

Most studies investigating the effects of naturally occurring antimicrobial agents have been performed on bacterial species using plant extracts or isolated/synthesized plant components. Relatively little work has been reported on the antiviral effects of commercially available juices. Moreover, few if any studies have addressed the antiviral effects of juices on enteric virus infectivity. The purpose of this study was to identify the occurrence and extent of antiviral activity by comestible juices (viz., orange, grapefruit, and especially CJs) on diverse viral species. Studies were performed using the bacteriophages T2 and T4 of *Escherichia coli* C and B, respectively, with subsequent testing using the simian enteric virus, rotavirus SA-11.

Materials and methods

Bacteriophages and plaque assay

Bacteriophages T2 and T4 of *E. coli* strains C and B, respectively, were obtained from Ward's Natural Science (Rochester, NY) and Carolina Biological Supply Company (Burlington, NC). Bacteriophage titrations were performed by the double agar layer technique (Lipson and Alsmadi, 1989). Briefly, 0.1 ml virus was inoculated into 10 ml overlay medium (0.75% tryptic soy agar containing log growth phase *E. coli* strains B or C). Underlay consisted of the same medium but contained 1.5% agar. Plates were incubated at 37 °C for 24–48 h. Plaques were counted on a bacteriologic colony counter and recorded as plaque-forming units (PFU)/ml. The bacteriophage strains were temporarily stored at 4 °C.

Effect of juices on bacteriophage T2 and T4 infectivity titers

Equal volumes of undiluted orange juice (OJ; Tropicana Pure Premium Original Orange Juice, Bradenton, FL), grapefruit juice (GJ; Florida Natural Premium Grapefruit Juice, Florida Nature Flavors-Beverage Manufacturer, Casselberry, FL) and CJ (Cranberry Juice Cocktail, Ocean Spray Cranberry, Inc., Lakeville-Middleboro, MA), were added to equal volumes of bacteriophage T2 suspensions. The suspensions were titered within several minutes of preparation (this time period is designated T_0), and then after 30 (T_{30}) and 60 (T_{60}) min. The controls, performed at each time period, were identical to experimentals except that PBS was used in place of each juice. Identical experiments were performed using bacteriophage T4, except that infectivity titers were performed only at T_0. All experiments were performed in triplicate or quadruplicate. Data are graphically expressed as percent of control at each titration period.

Dose response

A 0.1-ml quantity of stock suspensions of bacteriophage T4 was added in triplicate to 0.9 ml of CJ. The CJ was diluted in PBS to 50%, 30%, 10%, 0.01%, 0.005%, 0.001%, and 0.0005% of the commercial product. The suspensions were incubated for 30 min at 37 °C followed by titration by plaque assay. The control was identical to experimentals, except that PBS was substituted in place of either juice.

Effect of temperature

The effects of 4 and 23 °C were tested to determine any effect of these temperatures on the antiviral activity of the bacteriophage T4 by CJ. A total of 0.1 ml bacteriophage T4 was added in triplicate to 0.9 ml of non-diluted CJ, followed by incubation for a period of 60-min at 4 and 23 °C. The control was treated as the experimentals, except PBS was used in place of the CJ.