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The effect of cranberry juice and cranberry proanthocyanidins on the infectivity of human enteric viral surrogates

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ABSTRACT

The effect of cranberry juice (CJ) and cranberry proanthocyanidins (PAC) on the infectivity of human enteric virus surrogates, murine norovirus (MNV-1), feline calicivirus (FCV-F9), MS2(ssRNA) bacteriophage, and phiX-174(ssDNA) bacteriophage was studied. Viruses at high (~7 $\log_{10}$ PFU/ml) or low (~5 $\log_{10}$ PFU/ml) titers were mixed with equal volumes of CJ, 0.30, 0.60, and 1.20 mg/ml final PAC concentration, or water and incubated for 1 h at room temperature. Viral infectivity after treatments was evaluated using standardized plaque assays. At low viral titers, FCV-F9 was undetectable after exposure to CJ or the three tested PAC solutions. MNV-1 was reduced by 2.06 $\log_{10}$ PFU/ml with CJ, and 2.63, 2.75, and 2.95 $\log_{10}$ PFU/ml with 0.15, 0.30, and 0.60 mg/ml PAC, respectively. MS2 titers were reduced by 1.14 $\log_{10}$ PFU/ml with CJ, and 0.55, 0.80, and 0.96 $\log_{10}$ PFU/ml with 0.15, 0.30, and 0.60 mg/ml PAC, respectively. ϕ-X174 titers were reduced by 1.79 $\log_{10}$ PFU/ml with CJ, and 1.95, 3.67, and 4.98 $\log_{10}$ PFU/ml with PAC at 0.15, 0.30, and 0.60 mg/ml, respectively. Experiments using high titers showed similar trends but with decreased effects. CJ and PAC show promise as natural anti-virals that could potentially be exploited for foodborne viral illness treatment and prevention.

1. Introduction

Cranberries originated in North America and historically have been used by Native Americans for treating bacterial infections (Brinckmann, 2003). Cranberry juice (CJ) and its extracts are known to exhibit a range of antibacterial, anti-viral, and pharmacological activities (Greenberg et al., 2005; Kerr, 1999; Lipson et al., 2007a,b; Lynch, 2004; Matsushima et al., 2008; Nogueira et al., 2003; Weiss et al., 2005; Wilson et al., 2008; Wu et al., 2008, 2009). Studies show that CJ or cranberry products can help prevent and reduce the recurrence of urinary tract infections by decreasing bacterial adhesion (Avorn et al., 1994; Greenberg et al., 2005; Kerr, 1999; Kontiokari et al., 2001; Liu et al., 2008; Lynch, 2004; Pinzon-Arango et al., 2009; Stothers and Brown, 2007). Similar effects have been identified in preventing *Helicobacter pylori* infections in the gastric lumen (Gotteland et al., 2008; Matsushima et al., 2008, 2005, 2006; Zhang et al., 2005; Shmuely et al., 2007). CJ has also been reported to inactivate other foodborne bacterial pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. (Nogueira et al., 2003; Pedigo et al., 2007; Wu et al., 2008).

Proanthocyanidins (PAC) have been determined to be the active compounds in cranberry that inhibit the adhesion of pathogenic strains of *E. coli* to human uroepithelial cells (Howell, 2007; Howell et al., 2005; Vorsa, 2003). Cranberry PAC are compositionally different from other tannin-rich foods (Foo et al., 2000a) in that they have unusual A-type linkages (Foo et al., 2000a) compared to B-type linkages in PAC from other foods (Howell et al., 2005). Howell et al. (2005) found that after consumption of CJ cocktail, human subjects exhibited anti-adhesion activity in their urine, but not after consumption of food containing B-type PAC.

Some work on the anti-viral effects of CJ and cranberry PAC has also been reported (Lipson et al., 2007a,b; Schlesinger et al., 2003; Weiss et al., 2005). Weiss et al. (2005) showed that high molecular weight constituents from cranberry inhibit influenza virus adhesion and reduced viral infectivity. Lipson et al. (2007b) found that CJ and PAC have marked anti-viral activity on bacteriophages T2 and T4, the waterborne rotavirus SA-11 and bovine reovirus.

The anti-viral effects of CJ and its constituents on foodborne viruses have not yet been extensively studied. Human noroviruses are the leading cause of non-bacterial gastroenteritis outbreaks worldwide (Turcios et al., 2006; Widdowson et al., 2004). They are highly transmittable, have low infectious doses and are resistant to environmental degradation (Cheesbrough et al., 2000; Kuusi et al., 2002). The symptoms associated with norovirus infections are nausea, vomiting, diarrhea, abdominal pain and low grade fever,

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with the newly emergent strains becoming highly virulent and capable of causing death (Siebenga et al., 2009). The epidemiologically significant foodborne viruses other than noroviruses include the hepatitis A virus, rotavirus, reoviruses, astrovirus, adenovirus, to name a few (Sair et al., 2002). Natural alternative remedies are needed to lessen the symptoms and prevent infections associated with foodborne viral infections, especially in the elderly and immune-compromised individuals.

The inability of human noroviruses to be propagated in cell culture has hampered the development of reliable infectivity assays (Duizer et al., 2004). However, surrogates such as feline calicivirus (FCV) (Steinmann, 2004), bacteriophage MS2 (Dawson et al., 2005), bacteriophage ϕ-X174 (Charles et al., 2009) and murine norovirus (MNV-1) (Wobus et al., 2006) that can be assayed for infectivity are used instead. In addition, these bacteriophages are also routinely used as indicators of fecal contamination (Finch and Fairbairn, 1991; Guan et al., 2006; Shirasaki et al., 2009). Among the described surrogates, murine norovirus (MNV-1), a recently cultivable norovirus (a genogroup V norovirus of the Caliciviridae family), is being increasingly used as alternate to feline calicivirus (Bae and Schwab, 2008; Baert et al., 2008; Belliot et al., 2008; Cannon et al., 2006; Kingsley et al., 2007; Lee et al., 2008; Sosnovtsev et al., 2006; Steinmann et al., 2008; Wobus et al., 2004, 2006). MNV-1 was reported to have greater biochemical, pathological, and molecular similarity to human norovirus than the other surrogates (Sosnovtsev et al., 2006; Wobus et al., 2006) and is currently considered to be the most appropriate surrogate for human noroviruses.

The objective of this research was to investigate the effectiveness of CJ and cranberry PAC against human norovirus surrogates as alternate natural therapies for the treatment and prevention of foodborne viral illness. The anti-viral effects of CJ and cranberry PAC on four human enteric virus surrogates were evaluated using standardized plaque infectivity assays. For comparative purposes, the anti-viral effects of orange and grape juices were also studied.

2. Methods

2.1. Viruses, bacterial hosts, and cell lines

Bacteriophage MS2 and host *E. coli* B-15597; FCV-F9 and Crandell Reese Feline Kidney (CRFK) cells were obtained from ATCC (Manassas, VA). Bacteriophage ϕ-X174 and its host *E. coli* CN-13 were gifts from Dr. Suresh Pillai (Texas A and M University, College Station, TX). Murine norovirus, MNV-1 was provided as a gift by Dr. Skip Virgin (Washington Univ., St Louis, MO) and RAW 264.7 cells were obtained from the University of Tennessee at Knoxville.

2.2. Propagation of viruses

CRFK and RAW 264.7 cell lines were grown at 37 °C in an atmosphere containing 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin. Viral stocks of FCV and MNV-1 were prepared by inoculation of FCV and MNV-1 stock onto monolayers of CRFK and RAW 264.7 cells, respectively until >90% cell lysis. Briefly, the viral suspensions were harvested by freeze-thawing and centrifuging for 10 min at 5000 × g. The resulted supernatants were filtered through 0.2 μm membrane filters, stored at −80 °C until use, assayed and used as viral stocks for the study. Bacteriophage MS2 and ϕ-X174 were propagated in *E. coli* B-15597 and *E. coli* CN-13 host, respectively in 3% Trypticase Soy Broth containing 0.1% glucose, 2 mM $CaCl_2$, and 10 μg/ml Thiamine at 37 °C for 18 h. The two bacteriophages were harvested by centrifugation and filtration using the procedure described above for FCV and MNV-1.

2.3. Anti-viral effects of juices and PAC

CJ (Cranberry Juice Cocktail, Sam's Choice, Wal-Mart Store, Inc., Bentonville, AR), orange juice (Tropicana 100% Orange Juice, Tropicana Manufacturing Company, Inc., Bradenton, FL), and grape juice (Welch's 100% Grape Juice, Welch's, Concord, MA) were sterilized by filtration through 0.22 micron filters. Cranberry PAC obtained from Dr. Amy Howell, Rutgers University, was dissolved in sterile deionized distilled water at concentrations of 1.2 mg/ml, filter sterilized (0.2 micron filter), and further diluted aseptically to 0.6 mg/ml and 0.3 mg/ml in sterile deionized distilled water. Equal volume of juice or PAC solutions was mixed with equal volume of each virus to reach titers of ~7 $\log_{10}$ PFU/ml or ~5 $\log_{10}$ PFU/ml and incubated at room temperature for 1 h. For time-dependence study, CJ or PAC were mixed with equal volume of MNV-1 and incubated for 0, 10, 20, 30, 40, 50 and 60 min, respectively. Individual viruses mixed with sterile deionized distilled water were used as the untreated controls. After incubation, treatments were neutralized by 10-fold serial dilution of virus in DMEM containing 10% FBS. All treatments were run in duplicates. Cytotoxicity assays were run using one-log serial dilution of the filter sterilized juice or PAC samples on CRFK and RAW 264.7 cell lines. Cytopathic effects were determined by visual inspection under optical microscope after incubation for 1–3 h and 3–5 d.

2.4. Infectious plaque assays

Infectivity of each treated virus was evaluated using standardized plaque assays in comparison to untreated virus controls. For MNV-1, the plaque assay previously described by Wobus et al. (2004) was followed with minor changes. Briefly, RAW 264.7 cells were seeded into 6-well plates at a density of 2×10^6/well and incubated until ~90% confluency. Ten-fold serial dilutions of viral samples were prepared in DMEM containing 10% fetal bovine serum and 0.5 ml of the dilution was inoculated into each well after aspiration of media. Viruses were adsorbed for 2.5 h at 37 °C in a CO_2 incubator. After adsorption, inocula were removed and cells were overlaid with 2 ml of DMEM containing 0.75% agarose, 10% FBS and 1% penicillin-streptomycin. Plates were incubated for 72 h and overlaid with a second overlay media containing 0.02% neutral red. Plaques were counted after incubation at 37 °C for 5 h.

FCV plaque assays were conducted using confluent CRFK cells in 6-well plates as described earlier (D'Souza et al., 2006). Ten-fold serial dilutions of treated and untreated samples in DMEM containing 2% fetal bovine serum were prepared and 0.5 ml of the dilution was inoculated into each well. Viruses were adsorbed for 2 h at 37 °C in a CO_2 incubator. Cells were then overlaid with 2 ml of DMEM containing 0.75% agarose, 2% FBS and 1% penicillin-streptomycin. Plates were incubated for 48 h and overlaid with a second overlay media containing 0.01% neutral red (Sigma). Plaques were counted after incubation at 37 °C for 20–24 h.

The method of Bae and Schwab (Bae and Schwab, 2008) was followed for the infectivity of MS2 and ϕ-X174 except that 0.7 ml serially diluted phage (treated or untreated) and 0.3 ml of respective 6 h culture host, *E. coli* B-15597 or *E. coli* CN-13 were mixed with 0.6% top agar and poured on tryptic soy agar (TSA) bottom agar plates. Plates were incubated at 37 °C overnight and plaques were counted. All assays were run in duplicates and repeated twice.

2.5. Statistical analysis

Results from the treatments and controls were statistically analyzed using ANOVA with SAS software (version 9.2, SAS Institute, Cary, NC, USA) and student's t-distribution on a completely randomized design with two or more replications.