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Research Article

Cranberry cocktail juice, cranberry concentrates, and proanthocyanidins reduce reovirus infectivity titers in African green monkey kidney epithelial cell cultures

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Studies were performed to investigate the effect of several cranberry and grape juice extracts on the inhibition of reovirus infectivity following cell culture inoculation. Infectivity testing was performed utilizing cranberry juice extracts NutriCran-100™ and NutriCran-90™. At 5% extract concentrations, titers were reduced by *ca.* 50%. Cranberry cocktail juice caused an infectivity loss of *ca.* 10%. We ascribe these data to higher concentrations of proanthocyanidins (PACs) in the cranberry extracts. Further testing was performed utilizing purified high and low molecular weight cranberry PAC fractions (CB HMW and CB LMW, respectively), a cranberry flavonol glycoside (CB EToAc), cranberry anthocyanins (CB CA), and a grape PAC extract. Reovirus titers were reduced to undetectable levels at PAC concentrations ≤0.2%. CB CA had no effect on the inhibition of infectivity titers. Loss of infectivity titers was in the order: GP PAC>CB HMW>CB LMW>CB EToAc. Probe homogenization of CB HMW enhanced the extract to efficacy levels equal to that of grape PAC. Reovirus dsRNA segments were undetectable 96-h postcranberry cocktail juice pretreatment of MA-104 cell cultures. This study indicates an inhibition of reovirus infectivity titers by cranberry or grape juices or their purified PAC extracts. Viral inhibition probably occurs at the host cell surface.

Keywords: Cell culture / Cranberry / Infectivity titer / Proanthocyanidins / Reovirus

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1 Introduction

The presence of naturally occurring antimicrobial agents in foods and food products remains an area of continual investigation [1]. The effects of naturally occurring antimicrobial/antiviral agents in comestible beverages have been studied both *in vitro* and *in vivo* [2–4].

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Abbreviations: CB CA, cranberry anthocyanins; **CB EToAc,** cranberry flavonol glycoside; **CB HMW,** cranberry high molecular weight extract; **CB LMW,** cranberry low molecular weights extract; **CPE,** cytopathic effect; **dsRNA,** double-stranded RNA; **GP PAC,** grape proanthocyanidins; **JAM-A,** junction adhesion molecule; **NC-90,** NutriCran™-90; **NC-100,** NutriCran™-100; **MA-104,** African green monkey kidney epithelial cells; **PAC,** proanthocyanidins

Cranberries (*Vaccinium macrocarpon*) and their products have been recognized by the general population and most medical experts as a preventative of urinary tract infections (UTIs). Recent clinical studies have confirmed the efficacy of cranberry juice consumption to a reduction in the recurrence of UTIs, especially among sexually active women [5, 6].

Progress has been made in understanding the mechanism of cranberry juice as an antimicrobial agent. Proanthocyanidins (PACs) of the cranberry have been shown to be potent inhibitors in the adhesion of pathogenic strains of *Escherichia coli* to human uroepithelial cells. This effect has been ascribed to the blockage of P-fimbriated *E. coli* binding to uroepithelial cell receptors [7, 8]. Howell and colleagues reported an enhanced antiadhesion activity by A-type PACs in the blockage of P-type *E. coli* to cellular receptor sites. A-type PACs are unique to *V. macrocarpon* and several other species (*e.g.*, *Prunus armenica*, *Carallia brachiata*) [9–11].

Relatively little work has been performed on the antiviral benefits of cranberries and other juices. Early studies by Konowalchuk and Speirs [12] using attenuated poliovirus as a model system showed that selected juices reduce significantly poliovirus infectivity *in vitro*. In the mouse model, intranasal and intraperitoneal administration of a plant flavonoid inhibited replication of the influenza virus [13]. More recently, *in vitro* studies using the influenza virus ascribed reduction of viral titers to a blockage of hemagglutinin determinants by PACs and another ill-defined *V. macrocarpon* chemical constituents [14].

Investigations by Lipson *et al.* [15] have extended this research to bacteriophage and enteric virus model systems. Lipson and coworkers have shown marked antiviral activity to bacterial viruses (*i.e.*, bacteriophages T4 and T2) and a mammalian enteric virus (the simian rotavirus, SA-11) by cranberry cocktail juice. Exposure or treatment of these infectious agents at different temperatures and pH values had no significant effect on loss of infectivity compared with that of cranberry juice. Inhibition of rotavirus-induced hemagglutination by cranberry cocktail juice and failure of the virus to yield morphologically intact particles (determined by electron microscopy) was also observed [15]. Additional studies using the reovirus as an enteric virus model system showed a cranberry cocktail juice-mediated adverse effect on the integrity of the host cell junction adhesion molecule A (JAM-A) [16]. Importantly, the JAM-A serves as a reovirus receptor molecule, suggesting, in part, a cranberry cocktail juice-induced modification of the JAM-A host cell receptor, thereby compromising virus adsorption and, in turn, penetration into the host cell [17, 18].

There still remains a paucity of data assessing the mechanism of infectivity inhibition/reduction of virus titer by the American cranberry. A need exists furthermore to investigate the effects of *V. macrocarpon* and its products on mammalian enteric viruses. The purpose of this study was to investigate the effects and to determine a mechanism(s) of cranberry cocktail juice, cranberry concentrates/extracts, and selected PACs on the inhibition/infectivity reduction of a mammalian enteric virus in a cell culture assay system. Bovine reovirus type 3 was used as a model enteric virus.

2 Materials and methods

2.1 Animal viruses

The bovine reovirus (Genus: *Reovirus*, Family: Reoviridae) used consisted of a type 3 strain (American BioResearch Laboratories (ABL), Sevierville, TN). The host cell system for the virus consisted of African green monkey kidney epithelial cells (MA-104) grown in monolayer culture (see below). Upon the appearance of a +3 to +4 cytopathic effect (CPE), cells and medium were frozen-thawed twice, centrifuged, followed by concentration of the virus from the supernatant using polyacrylamide absorbent gel (Sigma, Prod. No. P-7651). After titration, aliquots were stored at –60°C.

2.2 Cells and cell culture

MA-104 were obtained from Viromed Laboratories (Minnetonka, MN) as monolayer cultures grown on 12 mm circular cover slips in 40×13 mm^2 capped vials. Cell culture monolayers were prepared from commercial vials and maintained in T25 cm^2 plastic flasks (Corning). Monolayers were subpassaged not more than five times prior to the receipt of additional commercially prepared cell cultures. Growth medium (GM) consisted of Earle's minimal essential medium (E-MEM) supplemented with L-glutamine, penicillin/streptomycin, amphotericin B, and 10% fetal bovine serum (FBS). Maintenance medium was identical to that of GM but consisted of 2% FBS. Titrations of the virus were performed using the commercially prepared MA-104 monolayer cultures [19].

2.3 Reovirus infectivity titer assay

Reovirus infectivity titers were determined by the antigen detection-cell culture amplification technique. Experimental and control monolayers were washed twice with PBS and fixed in cold acetone. Immunostaining of monolayers was performed by a direct immunofluorescence assay. A fluorescein isothyocyanate (FITC)-conjugated caprine polyclonal antibody to reovirus types 1–3 was used throughout these experiments (REO FA conjugate, ABL). The tagged immunoreagent was added to monolayers, incubated for 30 min at 37°C, washed in PBS, fixed in acetone, mounted, and viewed with a Nikon Eclipse FA microscope (model no. E400). The microscope was equipped with a 100 W halogen bulb. Slides were read with a 40× objective. Cells displaying cytoplasmic immunofluorescence, characteristic of the genus reovirus, were deemed positive. Positive cells were counted and expressed as fluorescent focus units (FFUs)/mL of virus inoculum. Where appropriate, data were normalized to percent of control.

2.4 Juices and juice concentrates/extracts

NutriCran-90™ (NC-90) and NutriCran-100™ (NC-100) were prepared from a "spray-dried cranberry concentrate powder" and from a proprietary "cranberry extract derived from whole cranberry solids," respectively (Decas Botanical Synergies, Wareham, MA). The processing resulted in increased ellagic acid (200–415 μg/g), PACs (0.80–1.5% (for the NC-90), 1.0 – 1.5% (for the NC-100)), and phenolic content (2.0–3.8%). Extracts of cranberry cocktail juice (Ocean Spray Cranberry Juice Cocktail Drink) and grape (Welch's Purple 100% Grape Juice (made with Concord grapes)) were kindly supplied by Dr. Amy B. Howell (Marucci Center for Blueberry Cranberry Research, Rutgers

University, Chatsworth, NJ). The extracts included (i) grape proanthocyanidins (GP PAC), (ii) cranberry juice high and low molecular weight PAC fractions (CB HMW and CB LMW, respectively), (iii) a cranberry flavonol glycoside (CB EToAC), and (iv) cranberry juice anthocyanins (CB CA). Chemical extraction/preparatory procedures for the cranberry (*V. macrocarpon*) and the Concord grape (*V. labrusca* cultivar) components have been described [9]. Cranberry cocktail juice and Concord grape juice (Welch's Purple 100% Grape Juice) were commercially purchased at a local food store.

2.5 Treatment of cell culture monolayers

2.5.1 NC-90 and NC-100

Medium from the MA-104 cell culture monolayers was removed by aspiration, followed by a 3–5 min pretreatment of the monolayer with 0.2 mL of NC-90 ranging from 0.1 to 40% w/v in PBS without Ca^{2+} or Mg^{2+} (PBS). After the pretreatment step at 23°C the concentrate was removed, the monolayers were washed with PBS, and inoculated with 0.2 mL of reovirus. Monolayers were incubated at 36.5°C for 40 min, followed by the addition of 1 mL of fresh maintenance medium. The positive control consisted of monolayers treated with PBS and the reovirus. The negative control consisted of cell culture monolayers treated with only PBS. Antiviral activity of the NC-100 was tested as described for the NC-90 concentrate.

2.5.2 Proanthocyanidins, CB EToAC, CB CA extracts, and GP PAC

Antiviral testing of *V. macrocarpon* and *V. labrusca* cultivar extracts was performed by pretreatment of MA-104 monolayers using the CB HMW PAC, CB LMW PAC, CB EToAC, and CB-CA and GP-PAC extracts ranging in concentration from 25 (0.0025%) to 2000 (0.2%) μg/mL. Briefly, monolayers were pretreated with 0.2 mL of each extract for 3–5 min, followed by washing with PBS, and then inoculation with 0.2 mL of reovirus suspension. The immiscible CB HMW PAC fraction was brought into solution as follows: a 3 mL suspension of the CB HMW extract (400 μg/mL) was prepared in PBS; the suspension was homogenized at 20 000 rpm for 5 min using an Omni TH Homogenizer equipped with a generator probe (Omni-Tips™ plastic Disposable Rotor Stator Generator Probes) and the homogenate was filtered through a 0.45 μm polyethersulfone membrane enclosed in a polypropylene housing unit (PURADIS™ 25 AS; Whatman, Clifton, NJ). Both nontreated and treated (homogenized) CB HMW PAC filtrates were used in the pretreatment of monolayers.

2.5.3 Cell viability testing by trypan blue exclusion cell growth determinations

Cell viability was determined by trypan blue exclusion according to standard procedures (http://www.bio.com/protocolstools/protocol.jhtml?id=p2151). Briefly, MA-104 monolayer cultures were grown in T25 cm^2 plastic flasks. GM was removed followed by the addition of a 200 μg/mL concentration of the "homogenized"/filtered (described above) CB HMC PAC and a filtered 25% solution of cranberry juice cocktail drink. Sufficient volumes (1.5 mL) of each HMW PAC and cranberry juice were added to flasks to cover the monolayer cultures. After a 3–5 min incubation at room temperature (23°C), the PAC and cranberry juice additives were removed by aspiration, the monolayers were washed with PBS without Ca^{2+} or Mg^{2+}, followed by trypsinization. The digested monolayers were washed in the PBS, raised in the same solution to 1 mL, and gently agitated on a vortex mixer to attain a homogeneous suspension. Zero point 5 mL of each cell suspension was added to an equal volume of 0.4% trypan blue (Sigma-Aldrich, St. Louis, MO). The suspensions were incubated for 5 min at room temperature, followed by determination of percent viable cells. Cells were examined by light microscopy (400×) upon placement in a hemocytometer. The trypsinized MA-104 cells were also examined for any overt morphological aberrancy (*e.g.*, cytoplasmic streaming/projections from the plasma membrane) following the PAC or cranberry juice treatment. The control consisted of monolayers treated with the PBS alone. Additional cell viability testing was performed by the measurement of MA-104 cell growth over time employing monolayer subpassage. Cell growth determinations in this latter phase of viability testing utilized a grape juice (Welch's) monolayer pretreatment. Briefly, monolayers were pretreated for a period of 3–5 min at room temperature with a 30% (in PBS) grape juice preparation. Monolayers were washed with PBS, subpassaged, and placed into a 37°C incubator. After 9 days in culture (with appropriate medium changes), monolayers were trypsinized and cell quantitative determinations were performed. The control was treated exactly as the pretreatment monolayers but PBS was utilized in place of the grape juice. Experiments were performed in triplicate or quadruplicate.

2.5.4 Detection of the reovirus dsRNA by the polyacrylamide gel electrophoresis technique

T25 cm^2 cell culture flasks containing 4–5-day-old MA-104 monolayers were inoculated with 1 mL of an 80% suspension (in PBS) of a commercially purchased cranberry cocktail juice (Ocean Spray, 27% cranberry juice [9]). After a 5-min incubation at room temperature (23°C), the monolayers were washed twice with PBS, followed by the inoculation of a 1-mL suspension of reovirus stock (reovirus stock titer: 1×10^7 FFU/mL). Flasks were incubated at 36.5°C for 40 min followed by the removal of the viral inocula by aspiration, washing with PBS, and the addition of 7 mL of maintenance medium. The positive control was treated identically as the experimental, except that the pretreatment inoculum consisted of only PBS. The negative

control consisted of pretreatment of the monolayers with PBS but in the absence of subsequent reovirus inoculation. Monolayers were also pretreated with cranberry cocktail juice alone, and tested in parallel with the above experimental, positive, and negative control systems. All monolayers were monitored daily for the presence of characteristic reovirus-induced CPE (cellular rounding). With the appearance of a CPE in the positive control (96-h p.i.), all monolayers were removed by scrapping, washed in PBS, and then processed for PAGE [20]. RNA extraction was performed using the AquaPure RNA isolation kit (cat. no. 732-6370; BioRad Laboratories, Hercules, CA). The RNA was suspended in Laemmli sample buffer (cat. no. 161-0737, Bio-Rad) for loading onto polyacrylamide gels (Precast PAGEr (4–12% T-G) for electrophoresis (cat. no. 58520, Cambrix Bio Science Rockland, Rockland, ME). The gels were run for 50 min at 70 V. RNA segments were visualized by silver staining (Silver Stain Plus™ kit, BioRad), as described previously [21–23].

2.6 Statistics

The arithmetic mean ± SEM of control and experimental results were calculated using the Student's *t*-test. A p value <0.05 was considered statistically significant.

3 Results

Pretreatment of MA-104 cell culture monolayers with a concentration of 5% NC-90 and NC-100 extracts reduced reovirus infectivity titers to approximately 50% of control. At a similar concentration, a commercially purchased cranberry cocktail juice caused a reduction in infectivity titer of only 10% (Fig. 1).

Utilizing purified cranberry and GP PACs infectivity titers were reduced from 75% of control to levels below the detectable limit attainable by cell culture assay. Notably, these reductions in viral infectivity titer occurred with concentrations of PACs ranging from 0.003 to 0.2%. At the same concentrations, cranberry anthocyanins (CB CA) had no significant effects on the reduction of reovirus infectivity titers (Fig. 2). The CB EToAc caused a reduction in infectivity titers, but at efficiencies below that of the cranberry or grape PACs. Reductions in reovirus infectivity titers among the PAC fractions and the EToAc extract prior to probe homogenization of the CB HMW PAC were in the order: GP PAC>CB LMW>CB HMW>CB EToAc (Fig. 2). Homogenization of the CB HMW PAC extract which contained a visibly immiscible particulate fraction resulted in a greater reduction of viral infectivity titers. Utilizing a 400 μg/mL (0.04%) suspension, the antiviral effect (or reduction of infectivity titer) caused by homogenized CB HMW PAC resulted in reductions in infectivity viral titers

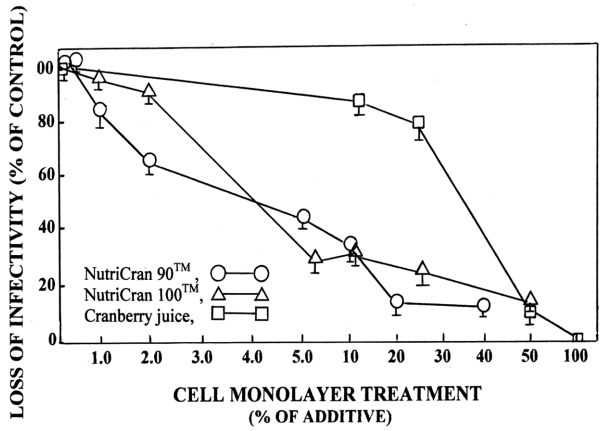

Figure 1. Reovirus infectivity titers following pretreatment with NutriCran-90™ (NC-90) and NutriCran-100™ (NC-100) of cell culture monolayers. MA-104 cell culture monolayers were pretreated for 3–5 min with NC-90 and NC-100 extracts at concentrations ranging from 0.1 to 50%. After pretreatment, the monolayers were washed with PBS and inoculated with reovirus. The positive control consisted of monolayers treated with PBS and inoculated with reovirus. The negative control consisted of cell culture monolayers treated with only PBS. Stock virus titer: 1.5×10^6 fluorescent focus units (FFU)/mL.

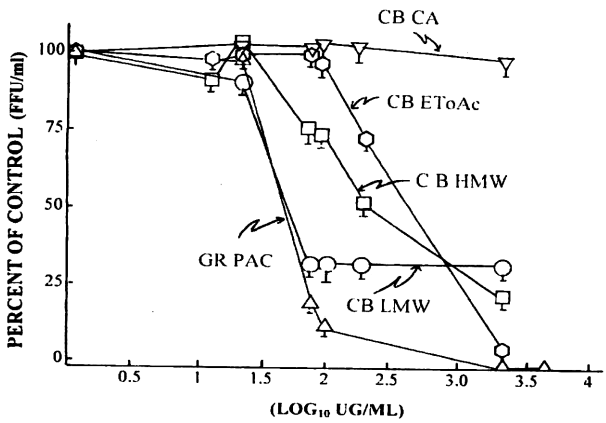

Figure 2. Effect of proanthocyanidins, CB EToAC, CB CA extracts, and Concord grape juice on the reduction of reovirus infectivity titers in host cell cultures. Antiviral testing of cranberry extracts and grape juice PAC was performed by pretreatment of MA-104 monolayers using CB HMW PAC (non-homogenized suspension), CB LMW PAC, CB EToAC, and CB-CA and GP-PAC extracts ranging in concentration from 25 to 2000 μg/mL (0.0025–0.2%). Monolayers were pretreated for 3–5 min with each extract, following by washing of the monolayer with PBS and then inoculation of stock reovirus. Stock virus titer: 1.5×10^6 FFU/mL.

slightly lower but not significantly different from that achieved with GP PAC (*viz.* 41 *vs.* 60%; $p = 0.17$; Fig. 3).

Reovirus double-stranded RNA (dsRNA) genomic segments were undetectable 48- or 96-h postcranberry cocktail juice pretreatment of host monkey kidney epithelial (MA-

104) cell culture monolayers. The characteristic reovirus (ten segmented) dsRNA genomic patterns were clearly identified in positive control cell cultures by PAGE (Fig. 4). Either PBS or cranberry cocktail juice pretreated monolayers did not show any reovirus genomic RNA segments (not shown). An early CPE (*viz.* 15–20% cellular rounding) was observed in positive control monolayers 48- and 96-h postreovirus inoculation but not in monolayers pretreated with only PBS or cranberry cocktail juice.

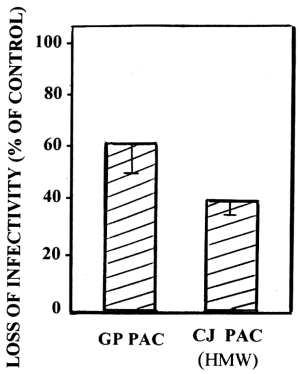

Figure 3. Comparison of homogenized CB HMW and GP PACs on the inhibition of reovirus infectivity titers. A 3-mL suspension of the CB HMW extract (400 μg/mL) was prepared in PBS followed by probe homogenization at 20 000 rpm for 5 min (Omni TH Homogenizer). Cell culture monolayers were pretreated in parallel with GP PAC and filtered CB HMW homogenate. No significant differences were identified in reductions of reovirus infectivity titer between the two PAC preparations ($p = 0.17$). Reovirus stock titer: 1.5×10^6 FFU/mL.

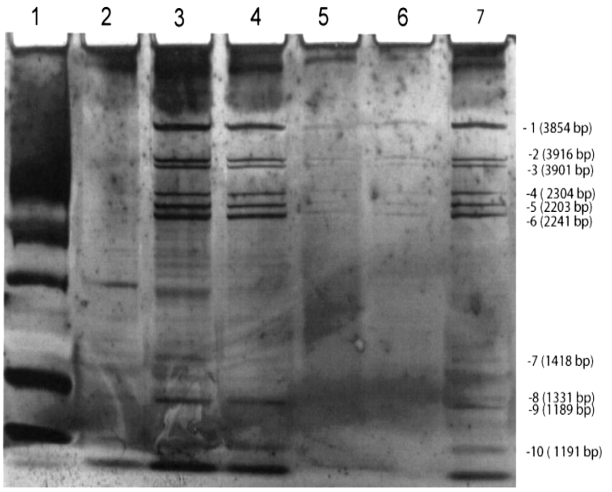

Figure 4. Effect of cranberry cocktail juice on the integrity of reovirus dsRNA segments. Lane 1, ProSieve^R 11–190 kDa color protein marker; lane 2, cranberry cocktail juice (80%) pretreated monolayer. Note the absence of viral dsRNA bands; lanes 3, 4, and 7, positive control displaying characteristic dsRNA electrophoretic bands; lanes 5 and 6, stock virus diluted 10^{-3}. The gel shows dsRNA segment patterns 96-h p.i. Reovirus stock titer: 1×10^7 FFU/mL.

No significant differences in cell viability were observed between PAC ($p = 0.42$) and cranberry juice-treated ($p = 0.79$) monolayers to the PBS-treated monolayer controls. Viability greater than *ca.* 90% was observed in each monolayer-treated system. Individual morphologically intact spherical cells were observed by light microscopy following trypsinization and during the reading of cells after the addition of the trypan blue stain. No significant differences in growth rates occurred between grape juice treated (8.2×10^5 cells/mL) and control (9.3×10^5 cells/mL) monolayer cultures ($p = 0.14$).

4 Discussion

Pretreatment of MA-104 monolayers with CB HMW PAC, CB LMW PAC, GP PAC, CB EToAc, and, to a relatively lesser extent, NC-90, NC 100, and a commercially purchased cranberry cocktail juice reduced reovirus infectivity titers in a dose-dependent manner. An anthocyanin (CB CA) extract, structurally different from the PACs tested, had no effect on the viral infectivity titers. Importantly, both cranberry cocktail juice and each juice concentrate/extract (except CB CA) displayed an efficacy in the reduction of reovirus infectivity titers following only a brief (several minutes) pretreatment of the MA-104 host cell culture monolayers. Neither cranberry juice cocktail drink nor the CB HMW PAC displayed any significant detrimental effect upon the viability of the MA-104 cells in monolayer culture. Similarly, no significant differences were identified in cell (monolayer) growth rates between grape juice and PBS pretreated cell monolayers. Based upon our testing, it is unlikely that the reported reduced infectivity titers in this study reflect the presence of dead cells.

The data from the use of *V. macrocarpon* (cranberry) extracts/concentrates and the *V. labrusca* cultivar (grape) PAC extract suggest an effect at the virus–cell interface, specifically mediated at the host cell surface. This suggestion is supported by a reduction of viral infectivity titers following cell culture monolayer pretreatment by several *V. macrocarpon* and *V. labrusca* cultivar concentrates/extracts. Prior studies additionally showed a reduction of monolayer tight junction protein integrity (specifically the JAM-A and claudin-1), after pretreatment of MA-104 cell monolayers to cranberry juice [16, 17; Lipson *et al.*, submitted]. Importantly, the JAM-A serves as a reovirus cellular receptor molecule [24]. It is not unreasonable to suggest, therefore, that reduced rates or even the total elimination of reovirus infectivity titers might be ascribed to an alteration in reovirus receptor site integrity on the MA-104 cell surface by cranberry cocktail juice or the cranberry/grape juice concentrates/extracts.

Among the numerous concentrates and extracts tested, our data indicate a marked effect by CB HMW and GP PAC in the reduction/inhibition of viral infectivity titers. Reduc-

tions of reovirus infectivity titers were reduced following pretreatment of MA-04 cell cultures with PAC extract concentrations of <0.2%. At identical concentrations, CB CA caused no reductions in viral infectivity titers. These data may be the result of the structural differences between the two molecules. Anthocyanins are positively charged monomeric flavonoids consisting of an unstable flavylium cation unit as its basic unit. PACs, on the other hand, are oligomeric and polymeric compounds consisting of different flavan-3-ol subunits, primarily (+)-catechin and (–)-epicatechin stereoisomers [25, 26]. Chemical structural differences (*viz.* stereochemistry) between the above phenolic groups may be associated with differences in binding avidity at the epithelial (MA-104) host cell surface. The differences in infectivity titers by pretreatment of the monolayers with the PACs and CB CA probably reflect the inherent nature (molecule structure) of each flavonoid subgroup. Pretreatment of cell culture monolayers with the cranberry cocktail juice flavonol glycoside (CB EToAc) extract also caused an inhibitory effect on reovirus infectivity. This effect, however, was less than that observed with the CB or GP PAC extracts. Little information exists on the bioactivity of CB EToAc [27]. Utilizing the human immunodeficiency virus type-1 (HIV-1) as a model system, for example, an inhibitory effect on infectivity titer was identified using the flavonol glycoside of the tropical plant, *Hiraea reclinata* [28]. A mechanism ascribed for this effect was not proposed. Other workers suggested an *Acer okamotoanum* (Chinese maple) flavonol glycoside-associated loss of the HIV-1 integrase [29]. In the current study, loss of reovirus infectivity titers may be ascribed to an event at the cell surface following flavonol pretreatment of the culture monolayer.

These data emphasize the efficacy of cranberry and grape PAC extract in the reduction or inhibition of viral infectivity titers in cell culture. The CB HMW and GP PAC extracts caused the greatest viral titer reductions among the tested juice concentrates/extracts. Although the CB HMW extract initially had reduced efficacy in the inhibition of viral infectivity titer, the extract's activity was markedly increased following treatment of the molecule's immiscible particulate matter by probe homogenization. The initially reduced viral inhibitory effect by the CB HMW was the result of the extract's inability of its particulate component to enter into solution.

PAGE was used (i) to identify the presence of reovirus dsRNA segments in infected cell cultures, and (ii) determine whether pretreatment of host cells with cranberry cocktail juice resulted in the selection of viral particles with any atypical RNA profiles (*e.g.*, an absence or comigration of segments), which would suggest the presence of antigenically distinct particles. Atypical dsRNA electropherotypes (or migration profiles) in human and animal rotavirus strains have been reported [22, 30]. In the current study, PAGE showed an absence 48–96-h postreovirus inoculation of dsRNA segments in cell culture monolayers pretreated with cranberry juice. Characteristic virus dsRNA migration patterns were clearly identified in positive control (*i.e.*, not pretreated with cranberry cocktail juice) virus infected cell culture monolayers (Fig. 4). Several faint bands in lane 2 might be interpreted as dsRNA segments. However, an inability to visualize the characteristic dsRNA banding pattern [31] suggests an absence of infectious viral particles. In a related study using the simian rotavirus strain SA-11, electron microscopy failed to reveal any morphologically intact viral particles in MA-104 cultures pretreated with cranberry cocktail juice [15]. These ultramicroscopic findings (albeit utilizing a different but related virus) coupled with PAGE assay and infectivity titer determinations suggest an inhibition of adsorption or viral penetration following cranberry juice pretreatment of the host cell cultures.

In summary, cranberry (*V. macrocarpon*) high molecular weight (CB HMW) PAC and grape (*V. labrusca* cultivar) PAC extracts markedly reduced reovirus infectivity titers in cell culture. The cranberry HMW PAC and GP PAC extracts affected greater reductions in viral infectivity titers than identified using cranberry cocktail juice alone or other cranberry juice extracts. The reductions in viral infectivity titers might be explained by an effect on the host cell surface, possibly through an alteration of viral receptor sites. The inability to detect reovirus dsRNA segments in infected MA-104 host cell culture monolayers pretreated with cranberry juice confirms the absence of infectious virus in the assay system. These data were supported by infectivity titration (viral isolation) testing.

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