The Effect of Cranberry Juice and Cranberry Derivatives on the Hemagglutination Activity of P-Fimbriated *Escherichia coli*

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ABSTRACT

Escherichia coli is the most common cause of urinary tract infections, especially those strains with hair-like fimbriae that aid in adhesion to the urinary tract. Binding to the epithelial cells lining the urinary tract is the first step in such an infection. A class of compounds found in cranberries called proanthocyanidins has been shown to inhibit P-fimbriated *E. coli* from binding to uroepithelial cells. P-fimbriated strains of *E. coli* have been shown to agglutinate human erythrocytes via P-fimbriae in the same manner they bind uroepithelial cells. Hemagglutination (HA) inhibition therefore correlates with a substance's ability to block P-fimbriae mediated uroepithelial cell binding. The goal of this project was to determine the ability of cranberry juice and various other cranberry products to inhibit P-fimbriated *E. coli* from binding to human erythrocytes as measured by HA. The results of this study demonstrate that cranberry juice can block P-fimbriae mediated binding of *E. coli* to human cells when incorporated into the bacterial growth media and also directly interferes with P-fimbriae binding to their ligand. Comparison of cranberry products revealed significant differences among individual products to block P-fimbriae mediated host cell binding. Preincubation of the bacteria with the cranberry products revealed further differences in their ability to inhibit HA in a time-dependent manner.

INTRODUCTION

Cranberry juice has been regarded for centuries as a potent defender of urinary tract health. It was once thought that the juice made the urinary tract a more acidic, bacteriostatic environment. While the juice is significantly acidic (pH=2.3), scientific studies have not reinforced this hypothesis.[3,7] *E. coli* is responsible for 80% of urinary tract infections (UTIs)[10]. Most uropathogenic strains of *E. coli* express hair-like projections called fimbriae that allow them to adhere to uroepithelium[2]. This adherence allows them to persist and multiply in the urinary tract, leading to a UTI. Research has shown that cranberry juice contains substances that inhibit the adhesion of *E. coli* to uroepithelial cells[10].

Cranberry juice contains two compounds that inhibit fimbrial adherence. Fructose, a sugar found in all fruit juices, inhibits the adhesion of *E. coli* with type-1 (mannose sensitive) fimbriae[9]. The second substance is a class of compounds known as proanthocyanidins. These

compounds inhibit the adhesion of P-fimbriated *E. coli* to uroepithelium[5]. The members of the genus *Vaccinum*, including blueberries and cranberries, are known sources of proanthocyanidins[8]. These compounds block the P-fimbriae adhesions from binding to the αGal(1→4)βGal oligosaccharide receptors on uroepithelium[2]. These receptors are common among several human cells, including erythrocytes. *E. coli* has been shown to agglutinate erythrocytes by binding via P-fimbriae. Therefore, hemagglutination assays are useful to measure *E. coli* adherence via P-fimbriae adhesion.

In this study, isogenic strains of *E. coli* expressing P-fimbriae and lacking P-fimbriae were exposed to cranberry juice and several cranberry derivatives. The ability of these *E. coli* to agglutinate erythrocytes was then tested to determine the ability of the cranberry derivatives to block adherence via P-fimbriae and thus predict their efficacy against UTIs. The first objective was to determine the effect of adding cranberry juice to growth media. Supplementing the growth media with cranberry juice had a concentration-dependent effect on HA. Secondly, it was demonstrated that cranberry juice could directly inhibit P-fimbriated *E. coli* from binding human cells. Finally, we have shown that cranberry products differ in their ability to block P-fimbriae mediated binding of *E. coli* to human cells.

MATERIALS AND METHODS

Bacterial Strains. Two isogenic strains of *E. coli* were used: DS17, which expresses P-fimbriae and DS17-8, which lacks P-fimbriae (generous gift of Dr. William Schwan, University of Wisconsin-La Crosse).

Cranberry Juice Agar Assay. Tryptic soy agar (Becton Dickinson Microbiology Systems, Sparks, MD) was prepared supplemented with 1%, 5%, 10%, 15%, 20% and 25% "first press" cranberry juice with no additives (Northland Cranberries, Wisconsin Rapids, WI). All cranberry products were adjusted to a pH of 7 before use in the assays. DS17 and DS17-8 were grown at 37°C on the cranberry juice agar, and tested after 24 and 48 hours of growth for their hemagglutination activity. ~2×10^9 bacteria were serially diluted in a U-bottom microtiter plate (Corning Incorporated, Corning, NY) and exposed to 0.5% human erythrocytes (final concentration) in a total volume of 50 µl phosphate buffered saline (Fisher Health Care, Baltimore, MD). The plates were incubated overnight at 4° C. The above assay was also repeated with *E. coli* DS17 and DS17-8 grown on the media supplemented with cranberry juice for 24 hours and then grown on tryptic soy agar (TSA) with no cranberry juice for an additional 24 hours.

Preparation and assay of cranberry products. Various cranberry products were solublized or emulsified in phosphate buffered saline to a final concentration of 50 mg/ml. These products included commercially available cranberry pills (Nature's Way, GNC, Spring Valley), as well as juice (Northland Cranberries, Wisconsin Rapids, WI). These products were compared to dried cranberry stick, seed, powder and oil products prepared from a manufacturer who identity could not be disclosed due to a disclosure agreement.

DS17 and DS17-8 were grown up on TSA at 37°C, and ~2×10^9 bacteria were serially diluted in a microtiter plate. These bacteria were then exposed to 0.5% human erythrocytes and 5 mg/ml concentrations of the various cranberry products prepared above, as well as 10% concentration of cranberry juice for a final well volume of 50 µl. The plates were incubated overnight at 4° C.

Preincubation Assay. The cranberry products were serially diluted in a microtiter plate. ~5×10^8 bacteria were added to each well. Human red blood cells were added at fifteen-minute intervals to a final concentration of 0.5%. The final well volume was 50 µl. The

plates were incubated overnight at 4° C. The results were expressed as the reciprocal of the lowest dilution of cranberry product that inhibited HA.

RESULTS

Percent Cranberry Juice in Media	Number of Bacteria Required for HA at 24 Hours	Number of Bacteria Required for HA at 48 Hours
None	6.25×10^7	6.25×10^7
1%	6.25×10^7	6.25×10^7
5%	1.25×10^8	2.50×10^8
10%	2.50×10^8	5.00×10^8
15%	5.00×10^8	1.00×10^9
20%	$>1.00 \times 10^9$	$>1.00 \times 10^9$
25%	$>1.00 \times 10^9$	$>1.00 \times 10^9$

TABLE 1. The effect of cranberry juice in the growth media on the ability of *E. coli* DS17 to agglutinate red blood cells. Note: at concentrations of 20% and 25%, HA required greater than 1×10^9 bacteria. Each assay was performed in duplicate and repeated on three independent days with no difference in observed titers.

Cranberry Juice Agar Assay. *E. coli* DS17 grown on cranberry-supplemented media displayed a concentration-dependent reduction in hemagglutination activity (see Table 1). At concentrations of 5% and over, the cranberry juice impaired the ability of DS17 to adhere to red blood cells. At concentrations of over 20%, the bacteria were unable to elicit levels of hemagglutination detectable by this assay (> 1 ? 10^9 bacteria required for HA). This data indicates that cranberry juice inhibits the function of P-fimbriae of uropathogenic *E. coli*.

Bacteria that had been grown on cranberry-supplemented media for 24 hours were then plated on TSA and allowed to grow for an additional 24 hours. The HA ability of these bacteria was then retested. No changes were observed, indicating that the effects of cranberry juice persist in the bacteria for at least 24 hours post-exposure (data not shown). The isogenic strain *E. coli* DS17-8 that lacks P-fimbriae was also tested in all of the above assays and displayed no HA under any conditions. The bacteria grown on the cranberry juice media also displayed cellular elongation as observed by Gram staining (data not shown). The direct cause of this phenomenon was not established and could be an area for future studies.

Cranberry Product	Number of Bacteria Required for HA
None	6.25×10^7
Cranberry Juice	$>1.00 \times 10^9$
Nature's Way Pills	1.00×10^9
GNC Pills	2.50×10^8
Spring Valley Pills	1.25×10^8
Cranberry Stick	6.25×10^7
Cranberry Seed	6.25×10^7
Cranberry Powder	6.25×10^7
Cranberry Oil	6.25×10^7

TABLE 2. Comparative efficacy of various cranberry products against E. coli DS17 hemagglutination activity. Cranberry juice was at a final concentration of 10%, and all other cranberry products were at a final concentration of 5 mg/ml. Note: When the bacteria were exposed to cranberry juice, HA was not observed, and presumably requires greater than 1×10^9 bacteria. Each assay was performed in duplicate and repeated on three independent days with no difference in observed titers.

Assay of Various Cranberry Products. The efficacy of the cranberry products' ability to directly inhibit *E. coli* DS17 adherence varied widely (see Table 2). Cranberry juice was by far the most effective at disrupting bacterial adherence. The commercially available cranberry pills displayed varying degrees of efficacy. Nature's Way pills appeared to be the most effective, followed by GNC pills and Spring Valley pills. Cranberry stick, seed, powder and oil showed no ability to inhibit DS17 adherence as detected by this assay. Strain DS17-8 was also tested in this assay and showed no HA under any conditions.

Cranberry Product	Reciprocal of Lowest Dilution of Cranberry Product that Inhibited HA
Cranberry Juice	64
Nature's Way Pills	32
GNC Pills	16
Spring Valley Pills	8
Cranberry Stick	<2
Cranberry Seed	<2

TABLE 3. The hemagglutination activity of *E. coli* DS17 after thirty minutes of preincubation with cranberry products. Each assay was performed in duplicate and repeated on three independent days with no difference in observed titers.

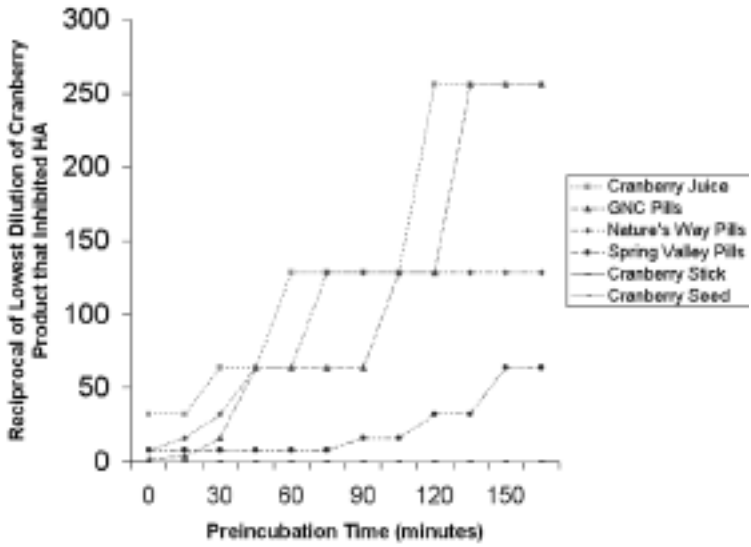

FIGURE 1. Time course assay of *E. coli* DS17 hemagglutination inhibition when preincubated with various cranberry products. Each assay was performed in duplicate and repeated on three independent days with no difference in observed titers.

Time Course Preincubation Assay. Preincubation of the cranberry products with *E. coli* DS17 improved their efficacy in a time-dependent fashion. The ability of cranberry juice to inhibit hemagglutination rose steadily through the duration of the assay (see Fig. 1). Interestingly, in relation to GNC pills, the Nature's Way pills were more effective during brief preincubation periods (see Table 3). The HA inhibition potential of Nature's Way pills was reduced in relation to GNC pills during longer preincubation periods. The GNC pills had effectiveness that was equal to cranberry juice during longer preincubation times. The Spring Valley pills showed significantly less efficacy than the other pills over time. The cranberry seed and stick showed no ability to inhibit hemagglutination throughout the assay. Strain DS17-8 was also tested in this assay and showed no HA under any conditions.

DISCUSSION

Cranberry juice is able to inhibit the ability of P-fimbriated *E. coli* to bind human cells through both indirect and a direct means. The indirect mechanism is demonstrated by the cranberry juice agar assay results in Table 1. The ability of *E. coli* DS17 to agglutinate red blood cells was inversely proportional to cranberry juice concentrations used to supplement the bacterial growth media. The observed decrease in HA ability remained even when the bacteria were transferred to growth media lacking cranberry juice for a period of 24 hours. This long-lasting indirect inhibition is presumably due to an induced alteration of bacterial physiology. This physiological change also results in the unusual elongation of the *E. coli* DS17 observed by Gram staining, which had also been recognized in previous studies[1]. Previous studies have also found an absence of P-fimbriae on the surface of *E. coli* DS17 after prolonged exposure to cranberry juice, as observed by electron microscopy[1]. The disruption of the P-fimbriae structure as well as the cellular elongation indicate that the cranberry juice may be altering the gene expression of *E. coli* DS17, which is an area for future research.

The second mechanism of cranberry juice protection is direct inhibition of the interaction between P-fimbriae and the αGal(1$\rightarrow$4)βGal receptors on human cells. This action has been demonstrated by the results in Tables 2 and 3. The juice significantly reduced the ability of *E. coli* DS17 to agglutinate red blood cells when it was added directly to the assay well. While cranberry juice has demonstrated utility in reducing DS17 adherence, this activity is not uniformly provided among commercially available cranberry extract products (see Table 2). This indicates that the active protective ingredient in cranberry juice, namely proanthocyanidins, is not present in equal amounts in the various commercial cranberry products.

Time course preincubation studies may provide the best evidence for predicting a cranberry product's ability to protect against bacterial adherence. Preincubation of the bacterial cells with cranberry products increased the sensitivity of the direct assay for inhibitory activity. The time course preincubation results suggest that proanthocyanidins may have altered structure or stability properties dependent upon the method by which they were extracted and prepared. This suggests that simple chemical measurement of proanthocyanidins in a product alone may not correlate with the biologic activity of the product to prevent bacterial adherence. These studies also reinforce the precautions one must consider when interpreting the results of a single time point during a binding assay such as this (compare Table 3 and Figure 1). Further studies are necessary to answer the questions raised here.

These *in vitro* studies cannot predict the *in vivo* stability or distribution of proanthocyanidins in the body. However, the *in vitro* differences observed here suggest that

commercial cranberry products differ widely in their ability to protect from urinary tract infections. The varying efficacy of these products indicates that the preparation methods used in the manufacturing of these products is a key component in their ability to protect against urinary tract infections.

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