Cranberry prevents the adhesion of bacteria: overview of relevant health benefits

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INTRODUCTION

Overview of Bacterial Adhesion

The adherence of bacteria to cells or tissues in the body is the propagating step in infections. Bacterial surfaces contain several types of molecules that help them attach to cells, such as proteinaceous fimbriae or pili, flagella, lipopolysaccharides, and capsular polysaccharide molecules. When the bacterial structures find their complementary receptors on mammalian cells, the two bind tightly. In the case of urinary tract infections, fimbriae expressed by *Escherichia coli (E. coli)* must bind to receptors on uroepithelial cells. Compounds that prevent this adhesion represent an alternative therapy to the use of antibiotics, since the anti-adhesion molecules do not kill or impair the growth of the bacteria, yet they are able to prevent the infection from developing. A similar mechanism exists in gastric ulcers. In the case of a *Helicobacter pylori* infection, which can lead to the development of a gastroduodenal ulcer, bacteria must attach to human gastric mucosal cells for the infection to develop. A third bacterial infection that develops following adhesion of bacteria is related to periodontitis, an inflammatory disorder of tooth-supporting tissues. Gram-negative bacteria, such as *Porphyromonas gingivalis* can colonize teeth, gingival epithelial cells, and red blood cells, or interact with other oral bacteria and proteins in the mouth through receptors on their surfaces (1).
In each of these three systems, cranberry compounds have been implicated in preventing the bacterial adhesion process, thus presenting a complementary or alternative methodology to prevent urinary tract infections (2, 3), *H. pylori* infections (4, 5), and periodontitis (1). We present a brief overview of the current knowledge of how cranberry is beneficial for these three systems.

ABSTRACT

With growing antibiotic resistance, alternative therapies and preventions are needed to help control microbial infections in hospital and community environments. By altering the ability of bacteria to adhere to cells in the body, anti-adhesion therapies are a promising complementary method to control infections. Research suggests that ingestion of cranberry compounds can serve as an anti-adhesion treatment for bacteria, bringing benefits in several ways: urinary tract and kidney health, maintaining a healthy oral environment, and prevention of ulcers due to Helicobacter pylori infections. We review the mechanisms by which cranberries alter the ability of bacteria to adhere and lead to health benefits for these body systems. A focus is placed on new molecular level understandings of the role of cranberry on the interactions between Escherichia coli surfaces and uroepithelial cells.

Cranberries and UTIs

The American red cranberry (*Vaccinium macrocarpon* Ait., family Ericaceae) has long been recognized for benefits to maintenance of a healthy urinary tract. This is especially a concern for women, 1/3 of whom will have at least one UTI in their lifetime (6), leading to the infection of 11.3 million women per year in the U.S. alone (7). Elderly women are also extremely prone to UTIs, with some women over 65 experiencing at least one UTI per year (8, 9). UTIs are caused when bacteria attach to and colonize mucosa surfaces in the urinary system (10). The resulting infection can range from cystitis (bladder infection) to a more serious illness, acute pyelonephritis (kidney infection). The Gram-negative bacterium *E. coli* is implicated in 85-95 percent of cystitis and 90 percent of pyelonephritis infections in women (11). If untreated, UTIs can cause kidney failure, and in some cases death (10, 12, 13).

CRANBERRY EFFECTS ON *E. COLI- IN VIVO* STUDIES OF URINARY TRACT HEALTH

The pioneering clinical trial of Avorn et al. (3) was the first to conclusively demonstrate that consumption of cranberry juice helped prevent recurrent urinary tract infections in women. This study was conducted on female residents of a long-term care facility. The women drank 300 mL/day of artificially sweetened cranberry juice or a placebo with similar colour and taste for a period of 6 months. After one month, the prevalence of bacteria in the urine of the cranberry juice drinkers was significantly decreased.
Kontiokari et al. studied 150 university women (mean age of 30) who presented to the Finnish University of Oulu's student health center or occupational clinic, and had clinically documented *E. coli* UTIs (14). The three groups received either 1) cranberry-lingonberry juice concentrate (50 mL/day for 6 months), 2) 100 mL of a probiotic *Lactobaccillus GG* drink, five times per week, for one year, or 3) a control group who did not receive any intervention. The rate of recurrence of UTIs in the 12 months following the study was statistically different among these treatment groups. The overall absolute risk of recurrence of UTI was reduced by 20 percent for the cranberry group compared to the control group, but a benefit was not seen due to lingonberry (14).
Stothers et al. (15) studied 150 women (ages 21 to 72) who had prior histories of UTIs ($\geq$ 2 in previous year), and

provided them with either cranberry juice (250 mL at three times per day + placebo tablet), cranberry extract in pill form + placebo juice, or both juice and pills that were non-cranberry containing placebos, and followed the women for one year. The tablet group had the least recurrence of UTI in the following year (18 percent), with the cranberry juice group having a similar but significantly different recurrence rate of 20 percent. Both the tablet and juice groups had much lower recurrence than the non-cranberry placebo group, where 32 percent infection recurrence was observed.
In a pilot study of five women with culture-confirmed UTIs, participants who ate sweetened dried cranberries (SDC) in a single dose exhibited anti-adherence properties in their urine that were comparable to consuming a cranberry juice cocktail drink (16). More data from this and other clinical investigations will help demonstrate if SDCs can be used for prevention of UTIs in the same way as cranberry juice cocktail (CJC).

CRANBERRY EFFECTS ON *E. COLI- IN VITRO* STUDIES RELATED TO URINARY TRACT HEALTH

While the earliest studies suggested that acidification of urine was responsible for cranberry's benefits towards UT health (17), research since the 1980s has focused on the anti-adhesive properties of cranberry juice, and recent studies demonstrated that the pH of urine (after cranberry consumption) is only slightly decreased and that the effect is transient (18, 19), or showed no decrease in urine pH (2).
All uropathogenic *E. coli* (UPEC) isolates express protein molecules on their surfaces, known as fimbriae. These molecules include the nearly universally expressed type 1 fimbriae, which bind to a lectin on uroepithelial cells (20), and P fimbriae, which are associated with 23 percent of cystitis infections and nearly all pyelonephritis infections (21). Type 1 fimbriae are mannose sensitive, meaning that any mannose type sugar (i.e. fructose, common to all fruit juices) can block this protein from being able to attach to eukaryotic cells (22). P fimbriae are mannose resistant, but their binding to uroepithelial cells can be blocked by other compounds found in cranberries (23). The ground-breaking studies demonstrating an in vivo effect of cranberry juice on bacterial adhesion to epithelial cells were performed in the 1980s, although the bacterial surface fimbriae were not investigated in these initial studies (19, 22, 24). Next, researchers began to characterize how cranberry affected bacteria with specific types of fimbriae. Zafriri et al. were the first to postulate that different compounds in cranberry could affect P and type 1 fimbriae, with their studies showing that fructose inhibited the adhesion of bacteria with type 1 fimbriae only (22). In a follow up study, these researchers tried to characterize the material that was effective against type P-fimbriated bacteria, and they determined that a high molecular weight, non-dialyzable material (NDM) inhibited the adhesion of UPEC to epithelial cells (25). A breakthrough came in 1998, when Howell et al. identified through directed fractionation, specific proanthocyanidin compounds in cranberry that caused P fimbriated-*E. coli* to exhibit anti-adhesion properties (23). The chemical structure of these compounds was further elucidated (26, 27). The studies of these two independent groups suggest that perhaps multiple mechanisms of anti-adhesive properties can be demonstrated against bacteria, and different compounds could be responsible for the different effects.
Current laboratory research in this area approaches the problems from multiple perspectives, including: characterization of the types of proanthocyanidins in terms of their chemical structures (28); determination of whether the beneficial compounds in cranberries are degraded by the body and elucidating their ultimate form in urine (2), microbiological studies focusing on the genes responsible for the production of fimbriae, and the role of particular fimbrial proteins in determining adhesion of the *E. coli* to uroepithelial cells (29), physical characterizations of the conformation and morphology of bacterial fimbriae (30), and physical interaction force measurements between *E. coli* bacteria and uroepithelial cells (31).

Better Specification of Beneficial Compounds

In order to identify precisely which compounds have anti-adhesion activity, Howell et al. conducted in vitro studies of the anti-adhesive capability of A-type and B-type proanthocyanidins isolated from cranberry, and other foods, including grape and apple juices, green tea, and dark chocolate (28). The type-A proanthocyandins isolated from cranberry juice had anti-adherence activity at the lowest dose of 60 μg/mL. The B-type proanthocyandins from grape had some activity at a much higher dose (1200 μg/mL), while none of the other B-type proanthocyandins had any in vitro anti-adherence activity.

Are Beneficial Compounds Degraded in Urine?

Di Martino et al. studied 10 healthy men and 10 healthy women (21-25 years old) in a placebo-controlled double-blind investigation of cranberry consumption on in vitro bacterial adherence (2). Volunteers consumed a single dose of between 0 and 750 mL of cranberry juice, or an appropriate dilution with mineral water or placebo beverage. All of the participants eventually consumed all four of the tested doses in random order, with a "washout" period of >6 days in between. UPEC strains were grown in their urine and the ability of these *E. coli* to attach to bladder epithelial cells was then characterized. A statistically significant decrease in bacterial adherence was noted for the cranberry groups compared to the control groups, and this was dose dependent. These results suggest that growth in cranberry can condition bacteria and change their properties, making them less able to adhere to epithelial cells.

Physical and Morphological Effects of Cranberry on *E. coli* Bacteria

Some laboratory studies have concentrated on understanding how cranberry compounds affect fimbriae on *E. coli*, especially P fimbriae. For example, Ahuja et al. suggested that growth in media containing cranberry caused *E. coli* to be unable to express their P fimbriae (32). In another study where bacteria were not grown in cranberry juice but exposed to it after growth in normal media, Liu et al. used atomic force microscopy (AFM) to probe the physical conformation of P fimbriae on *E. coli* HB101pDC1 that were exposed to cranberry juice cocktail (CJC) in concentrations ranging from 0 to 20 percent CJC (30). They found that CJC caused the P fimbriae to collapse on the surface of the *E. coli* cells, decreasing the protein's height and ability to extend from the surface of the bacteria. Molecular adhesion forces between the *E. coli* cells with collapsed fimbriae were significantly decreased

compared to the molecular adhesion forces between the control (i.e. non-infective) strain of *E. coli*. This was the first study to quantify the molecular adhesion forces for *E. coli* treated with cranberry juice.

RECENT PROGRESS IN DEVELOPMENT OF MOLECULAR MECHANISMS OF CRANBERRY ACTION AGAINST *E. COLI*

Recently, we have investigated the molecular scale effects of cranberry compounds on *E. coli* bacteria (31). We examined the morphology and cellular membrane properties of *E. coli* HB101 cells grown in culture media (tryptic soy broth; TSB) supplemented with cranberry juice, compared to *E. coli* grown in only TSB. The cranberry juice was neutralized to pH 7.0 before the bacterial growth experiments. The growth rate of the bacteria changed in an unpredictable manner when their growth media was supplemented with 10 percent CJC. Initially the bacterial growth rate decreased, but then after some time of acclimation, they resumed normal growth rates. In addition, Gram staining of the bacterial membrane revealed that culture in media supplemented with CJC changed the cellular membrane of the *E. coli*.

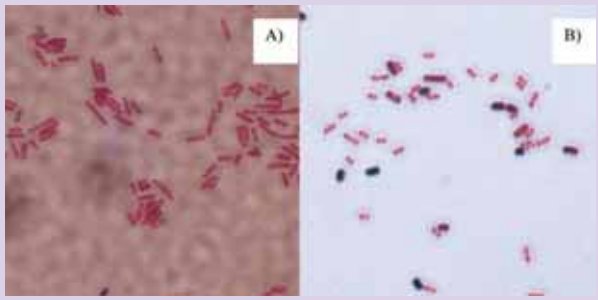

Figure 1. A) E. coli HB101 grown in TSB. B) E. coli HB101 grown in TSB supplemented with 10 percent neutralized CJC

For example, Figure 1A shows *E. coli* HB101 bacteria grown in only TSB, and stained with a Gram stain. The *E. coli* appear pink, which is characteristic for Gram-negative bacteria. For the *E. coli* bacteria that had been grown in media supplemented with CJC, some of the cells stained pink while some stained purple (Figure 1B). The purple appearance is an indication of Gram-positive bacteria and is an unusual finding for *E. coli*. While the mechanism of action is not yet clear, we speculate that some compounds from the cranberry juice are altering either the peptidoglycan layer or lipopolysaccharide layer of the *E. coli*, causing these apparent changes in the cell wall organization. In addition, we are using a nanotechnology-based tool, atomic force microscopy (AFM), to measure the nanoscale adhesion forces between *E. coli* bacteria and uroepithelial cells. By combining nanoscopic force measurements with calculations of the interaction energies surrounding bacteria and uroepithelial cells, we have found that cranberry juice affects the nature of the *E. coli*-uroepithelial cell in several ways: 1) cranberry juice causes P fimbriae on the *E. coli* to collapse, thus being unable to form attachments to uroepithelial cells (30), 2) cranberry juice causes an "energy barrier" to build up around the *E. coli* and the uroepithelial cells, thus making it unfavourable for the two to make contact with one another (31), and 3) cranberry juice decreases the forces of adhesion between P fimbriated *E. coli* and urinary tract cells from 9.64 nN (in buffer alone) to 0.50 nN (in buffer plus 10 percent cranberry juice; Figure 2) (31). Our nanoscale measurements can help researchers elucidate the mechanisms by which cranberry compounds can block the adhesion of *E. coli* bacteria to uroepithelial cells.

CRANBERRY AND *H. PYLORI*

A high molecular weight, non-dialyzable material (NDM) isolated from cranberry juice inhibited the adhesion of three different strains of *H. pylori* to human erythrocytes and human gastric mucous (33). These bacterial strains were found to have a sialic-acid specific adhesin on their surface. It is hypothesized that the compounds from cranberry blocked the ability of this adhesin to attach to receptors on the immobilized human mucus. A follow-up study examined the adhesion behaviour of 83 strains of *H. pylori*, and confirmed that 0.2 mg/mL of NDM was sufficient to inhibit adhesion of 53/83 of the strains (63.86 percent) to gastric cells (5). This research suggested that consumption of cranberry would make it more difficult for *H. pylori* to colonize the mucus and the epithelium of the gut, thus representing a possible preventive measure against peptic ulcers caused by *H. pylori*. It may be possible to use cranberry in combination with antibiotics to prevent infections from recurring. A randomized, double-blind, placebo-controlled clinical study investigated 189 adults infected with *H. pylori* (4). The cranberry juice group drank two boxes containing 150 mL cranberry juice per day for 90 days, while the control group received a placebo beverage at the same frequency and duration. At both 35 and 90 days after intervention, 14 of 97 participants (14.43 percent) from the cranberry group and 5 of 92 participants (5.43 percent) from the control group were free of *H. pylori*, as determined by a ^{13}C-urea breath test.

CRANBERRY'S ACTION AGAINST ORAL BACTERIA

Cranberry can also act against oral bacteria. For example, a high-molecular weight NDM of cranberry juice inhibited coaggregation of oral bacteria (25, 34) and reduced salivary counts of oral bacteria (34). Further, this NDM inhibited the ability of *P. gingivalis* to form biofilms, and prevented the microbes from attaching to surfaces coated with proteins, such as type I collagen, fibrinogen, and human serum, which represent periodontal sites (1). A pilot-type clinical study showed that six weeks of daily use of a mouthwash containing cranberry NDM reduced counts of mutans streptococci and total bacteria in saliva, compared to a control group receiving placebo mouthwash (35). Due to these encouraging results, it is likely that more clinical studies will follow.

FUTURE RESEARCH NEEDS

The use of cranberry as an anti-adhesive therapy for preventing a wide range of infections has great potential.

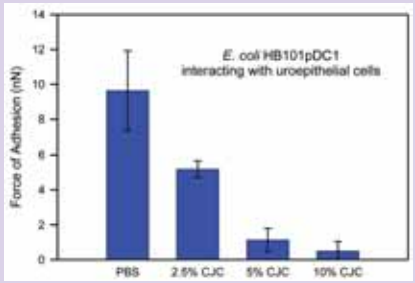

Figure 2. Adhesion force between individual bacterial cells of E. coli HB101pDC1 (expresses P fimbriae) and uroepithelial cells, probed in phosphate buffered saline (PBS), or in buffer supplemented with CJC at concentrations of 2.5, 5.0 or 10.0 percent

Clinical studies have focused mainly on urinary tract health (3, 14), and a few recent trials related to *H. pylori* (4) and oral bacteria (35), but future studies will likely be performed. Scientifically and clinically, more questions

need to be answered so that the appropriate dose, frequency, and duration of cranberry needed to bring about these benefits can be identified. Further, scientists can continue to search for other infections where cranberry can be of some benefit. A combination of molecular level characterization of bacterial interactions with genetic techniques to identify the genes responsible for the adhesion process can help in determining the mechanisms of bacterial adhesion for each system. These laboratory studies will be combined with clinical studies that seek to establish the optimal conditions for providing benefits.

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Health and Welfare (AHAW). These panels worked closely with the European Centre for Disease Prevention and Control (ECDC).
The two most commonly reported zoonotic diseases in 2004 were Salmonellosis and Campylobacteriosis.
The major sources of Salmonella are contaminated eggs and egg products and contaminated poultry meat. Contaminated poultry meat is also a major source of Campylobacter and EFSA recommends measures be taken along the poultry chain to reduce the prevalence of these bacteria.
Listeriosis is a cause of severe disease in humans and accounted for the highest number of reported human fatalities (107 deaths).
EFSA recommends that good manufacture, handling and hygienic practices - as well as the HACCP3 hazard identification approach - be applied effectively by food manufacturers.
Among other issues considered are Toxoplasmosis which can seriously affect the unborn child; and microbial resistance in zoonotic bacteria found in food producing animals; and the apparent higher incidence amongst young children of infection from Salmonella.
The EFSA Opinion stresses the need for further clarification on the role of contaminated water in causing zoonotic diseases, and identifies contaminated animal feed as an important route for introducing Salmonella into livestock.
According to the Opinion, rabies continues to pose a serious fatal human health risk in areas where rabies is present in wildlife.
EFSA also recommends that risk communication initiatives be targeted at food operators, vulnerable groups in the population, as well as the general public and makes several recommendations for improving the EU monitoring and reporting system for zoonoses.
The full text of the Opinion is available at **http://www.efsa.europa.eu/en/science/biohaz/biohaz_opinions/biohazahaw_ej403_zoonoses.html**

Two reports are available on zoonoses.
The zoonoses report based on the data of 2005, and the report providing comprehensive data on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the EU in the course of 2005.
Further information on the Zoonoses recommendations can be found at the following address:
http://www.efsa.europa.eu/en/press_room/press_release/pr_zoonoses_report2005.html

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