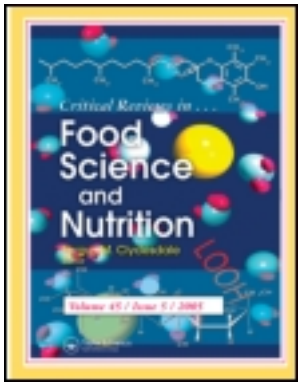

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Amy B. Howell [a]

[a] Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers, The State University of New Jersey

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Cranberry Proanthocyanidins and the Maintenance of Urinary Tract Health

Amy B. Howell

Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers, The State University of New Jersey

* Correspondence address: Marucci Center for Blueberry Cranberry Research and Extension at Rutgers, 125A Lake Oswego Rd., Chatsworth, NJ 08019. Tel: (609) 726-1590 ext 13; Fax: (609) 726-1593; Email: ahowell@aesop.rutgers.edu

ABSTRACT: One of the major health benefits attributed to the ingestion of cranberry juice is the maintenance of urinary tract health. Traditionally, the juice was thought to cause acidification of the urine resulting in a bacteriostatic effect. However, recent research has demonstrated that a bacterial antiadhesion mechanism is responsible. Proanthocyanidins with unique molecular structures have been isolated from cranberry fruit that exhibit potent bacterial antiadhesion activity. Little is known about the bioavailability and structure-activity relationships of cranberry proanthocyanidins. Data on how certain structural features of the molecules can influence bioactivity and bioavailability are reviewed.

KEY WORDS: *Escherichia coli*, tannins, bioavailability, antiadherence.

I. INTRODUCTION

Dietary consumption of cranberries (*Vaccinium macrocarpon*) has long been associated with the maintenance of urinary tract health. A number of clinical studies have demonstrated a positive link between cranberry consumption and prevention of urinary tract infections (UTIs).[1,2,3,4,5,6,7,8,9] There has been much speculation over the years as to how cranberries act to elicit the therapeutic effect and what might be the active ingredient(s) in the fruit. The first reports of a possible link between cranberries and UTIs appeared in the early 1900s.[10,11] For many years the theory was that hippuric acid excreted in the urine following cranberry consumption was responsible for the beneficial effects. Quinic acid in cranberries is the precursor of hippuric acid which is a strong antibacterial agent.[12] To achieve a bacteriostatic effect, urinary pH must be reduced to at least 5.0 with a minimum hippuric acid concentration of 0.02 *M*.[13] However, to achieve these levels, humans would need to consume at least 1500 ml of cranberry juice per day (a high intake for the average person).[14] While some studies have demonstrated a reduction in urinary pH levels following cranberry juice consumption with an associated reduction in UTIs,[1,12,15,16,17,18,19] other studies have found no reduction in urinary acidity using similar experimental conditions.[2,4,5,6,7,8,9,20] Due to a lack of scientific substantiation, it is generally accepted that urinary acidification is not the major factor responsible for cranberry's effect on UTIs.

The current theory on how cranberries maintain urinary tract health is based on the findings that certain compounds in the juice prevent the *Escherichia coli* bacteria that cause infection from adhering to the uroepithelium and multiplying.[21,22,23,24] Bacterial adherence

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to mucosal cells in the urinary tract is the initial step in the development of infection.[25] Once attachment has occurred, the bacteria are able to multiply and deliver toxins to susceptible host cells resulting in the tissue damage associated with UTIs. Bacterial adherence is facilitated by fimbriae that are proteinaceous fibers on the bacterial cell wall.[26] Fimbriae produce specific adhesins that adhere to corresponding carbohydrate receptors on the surface of uroepithelial cells.[25] The two common fimbrial types (type 1 and P-type) are morphologically identical, but they vary in their attachment sites on cell surfaces and have different antigenic specificities. Type 1 *E. coli* have fimbriae that bind to mannose-like receptors on uroepithelial cell surfaces.[27] Fructose in cranberry (and other fruits) is known to inhibit *in vitro* adherence of type 1 (mannose-sensitive) fimbriae to uroepithelial cells,[22] but there is no clinical evidence linking dietary fructose intake with maintenance of urinary tract health. P-type *E. coli* fimbriae attach to oligosaccharide (α-Gal(1$\rightarrow$4)β-Gal) receptor sequences on cell surfaces in the urinary tract.[28] P-fimbriated *E. coli* has been implicated in both cystitis and the more severe infection of the kidney known as pyelonephritis.[29] Cranberry juice is known to inhibit cellular adherence of uropathogenic strains of P-type (mannose-resistant) *E. coli*.[22,23]

Recently, a group of tannins called proanthocyanidins isolated from cranberry fruit by bioassay-directed fractionation were shown to inhibit the *in vitro* adherence of P-type fimbriae to uroepithelial cells.[24,30,31] Tannins are polyphenolic compounds found in leaves, fruits, seeds, bark, and roots of many higher plants.[32] The astringency of tannins makes them important in plant defense. Their bitter taste renders plant tissues unpalatable to animal and insect predators.[33] In addition to their bacterial antiadherence activity, tannin molecules exhibit other types of antimicrobial activity through the inhibition of enzyme production, substrate availability, and microbial metabolism.[34] They have been implicated in anti-cancer activity by inducing cancer-protective enzymes,[35] and by inhibiting cancer-causing superoxide radicals.[36] Tannins have demonstrated potential anti-Aids activity through the inhibition of HIV reverse transcriptase.[37,38]

Tannins are known to bind and precipitate proteins from solution,[39] and this is the reason for their use in the tanning of animal skins. The binding process between tannin and protein is similar to antigen-antibody interactions in that a binding agent and a ligand of similar size associate multivalently to form soluble and insoluble complexes.[40,41] The complexation can cause cross-linking of proteins aided by hydrogen bonding and hydrophobic interactions.[42] Interestingly, the binding of proteinaceous bacterial fimbriae to mucosal surfaces occurs as a specific receptor-ligand interaction[41] that is favored by hydrophobic interactions.[42] Thus, tannins from cranberry could be competitively inhibiting adherence of *E. coli* to mucosal cells through a similar binding mechanism.

The structure of tannins can influence certain biological activities. Two structurally distinct groups of tannins, hydrolyzable and condensed, have been defined. The bacterial anti-adhesion effect of cranberries is due to condensed tannins (proanthocyanidins)[24] that are polymers of flavans, such as flavan-3,4-diols. In these molecules, the B ring of the flavan monomer is substituted with two or three *ortho* hydroxyl groups. They are called proanthocyanidins because they are easily converted to anthocyanidins when heated in acid;[43] however, proanthocyanidins have no biosynthetic relationship to anthocyanidins. The interflavanoid bonds that link the proanthocyanidin units can be the more common B type (dimeric) or C-type (trimeric) single linkages or the less common double, A-type linkages. The single, B-types comprise the proanthocyanidin linkages found in certain tannin-containing fruits such as grapes.[44] Proanthocyanidins with the less com-

mon A-type linkages exhibited greater antiviral bioactivity when compared with those with B-type linkages.[45] Proanthocyanidins with at least one A-type linkage isolated from cranberry fruit demonstrated the ability to prevent P-fimbriated *E. coli* from attaching to uroepithelial cells.[30,31] The A-linked dimers from cranberry were more effective at inhibiting *in vitro* bacterial adherence than the B-linked dimers,[31] indicating that the doubly linked molecules in cranberry may play a role in eliciting the urinary tract benefits. However, *in vivo* studies need to be carried out to confirm this hypothesis.

The molecular weight of proanthocyanidins can influence various *in vitro* bioactivities. A-linked proanthocyanidin trimers from cranberry exhibited higher *in vitro* bacterial anti-adherence activity than A-linked dimers.[31] In another study, complement-modulating activity was greater for certain proanthocyanidin trimers than for dimers or monomeric flavonoids.[45] Bioactivity of proanthocyanidin polymers against cholera toxin-induced secretion in the colon increased as the molecular weight of the polymers increased.[46] The molecular weight of proanthocyanidins varies widely depending on the number of flavan-3-ol units that make up the structures. As plants age, proanthocyanidins can attain high-molecular-weights by forming large, insoluble polymers through condensation of B-ring oligomers with various substances.[47] High molecular weight proanthocyanidins have been isolated from unripe fruits, with average molecular weights of 63,000 (Dp 14.3) extracted from *Vaccinium corymbosum* fruits.[47] In some species of *Phormium*, values as high as 150,000 (Dp of 44) have been reported.[47] However, there is speculation as to the extent to which polymeric proanthocyanidins of high molecular weight are able to elicit *in vivo* biological effects due to their size. Proanthocyanidin fractions from cranberry with average Dp of 4 to 5 have been isolated that exhibited potent *in vitro* bacterial antiadherence activity,[30] but it remains to be seen whether intact oligomers of this size elicit a response *in vivo*.

Few pharmacokinetics studies have been carried out on proanthocyanidins due to the structural complexities of the molecules as well as the lack of commercial standards. Little data exist on the pharmacokinetics of cranberry proanthocyanidins; however, inferences can be made from research on grape (*Vitis vinifera*) proanthocyanidins. Comparisons must be viewed with caution, owing to the differences in molecular structures between the two groups of proanthocyanidins. Following oral delivery of ^{14}C-labeled grape proanthocyanidins to rats, 19% and 45% of the dose was excreted in urine and feces, respectively, and 6% was exhaled as CO_2.[48] In another study, gastrointestinal absorption of ^{14}C-labeled proanthocyanidins given orally to mice reached a peak at 45 min.[49]

The limited data from animal studies on bioavailability indicate that molecular weight influences metabolism and absorption of proanthocyanidins.[50] Larger proanthocyanidin polymers may actually undergo some degradation by colonic microflora and further biotransformation forming sulfate ester or glucoronide-conjugated metabolites.[50] ^{14}C-labeled hawthorn (*Crataegus* spp.) proanthocyanidins of different molecular weights were administered orally to mice.[51] In the first hour following administration, 65% of the trimers and 3% of the higher molecular weight oligomers were found in the blood. After 7 h, 81% of the trimers and 42% of the oligomers reached the organs, and 1.8% of both the trimers and oligomers were excreted in the urine. *In vivo* pharmacokinetics studies are needed utilizing distinct molecular weight fractions of cranberry proanthocyanidins to determine if similar absorption patterns are evident. The conformational rigidity that the A-linkage affords[32] to the cranberry proanthocyanidin molecules may also influence their bioavailability.

Owing to the lack of data on absorption of cranberry proanthocyanidins, the site(s) of action of the bacterial antiadherence activity have not been fully determined. *E. coli*

that cause UTIs are harbored in the colon and ascend up the urinary tract where they proliferate and can cause infection.[52] Urine of mice given cranberry juice in place of drinking water exhibited *in vitro* bacterial antiadherence activity.[21] In a recent study, mice fed isolated cranberry proanthocyanidins produced urine with similar antiadherence activity.[53] This indicates that a bioactive cranberry proanthocyanidin metabolite was present in the urine or that properties of the urine were altered by the proanthocyanidins such that adhesion was inhibited. There is speculation that the cranberry compounds could be active in the colon as well as the urinary tract.[22] The metabolic fate of proanthocyanidin metabolites from grape and hawthorn would support this theory. The relatively high percentage of oligomers that are eliminated in the colon[48] could potentially act on the colonic bacterial population, binding to the bacterial receptors. In the event that these colonic bacteria were to ascend up the urinary tract, they would be unable to bind to the uroepithelium and proliferate. Research utilizing cranberry proanthocyanidins is needed to substantiate site-of-action hypotheses and to determine dose response.

CONCLUSION

There is substantial *in vitro* and emerging *in vivo* evidence to suggest that cranberry proanthocyanidins effectively inhibit adherence of P-type *E. coli* bacteria to uroepithelial cells. A more substantial link needs to be established between the positive clinical effects of cranberry juice and the *in vitro* antiadherence activity of isolated proanthocyanidins. Research on the structures of cranberry proanthocyanidins has revealed some unique features that ultimately may be responsible for the *in vivo* effects of the compounds. Further studies are needed to determine bioavailability, sites of action, structure-activity relations, and dose-response of the compounds. In the meantime, ingestion of cranberry proanthocyanidins may offer women an effective means to maintain urinary tract health.

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