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Cranberry Juice to Reduce Bladder Biofilms and Infection in Geriatric and Spinal Cord Injured Patients with Dysfunctional Bladders

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Abstract

There is evidence to suggest that cranberry juice supplements improve the health of the urinary tract by inhibiting the binding of fimbriated uropathogenic *E. coli* to the bladder mucosa. In patients with neurogenic bladders, urinary tract infections (UTI) are particularly common and often poorly managed by antibiotic treatment. A double-blind, randomized, placebo-controlled trial was undertaken on 29 geriatric and spinal cord injured patients with dysfunctional bladders. They received three times daily at mealtimes a 4 oz bottle of cranberry juice (Ocean Spray Cranberries, USA) or a specially prepared synthetic placebo drink. Two episodes of UTI arose in week one of cranberry intake and none thereafter, compared to four episodes of UTI in 4 placebo patients in weeks four, six and 10. Mean bacterial adhesion counts on bladder cells of the patients rose during the first month of treatment in 71% of the placebo patients compared to only 31% of cranberry patients ($p < 0.001$). The difference persisted to some extent for the second and third months. Bacterial adhesion levels correlated with culture findings (higher adhesion and higher viable counts in urine) ($p < 0.001$), positive leukocyte nitrite tests (136 ± 131 bacteria per cell versus 52 ± 86 in negative tests) ($p < 0.001$), and higher white blood cell counts (>10) per high power field (126 ± 125 versus 48 ± 85 bacteria per cell) ($p < 0.001$). *E. coli* was the most frequently isolated organism (40% samples) followed by *K. pneumoniae* (17%) and a number of other uropathogens. Group B *Streptococci*, and coagulase negative *Staphylococcus* were recovered from urine in 4 samples but were not associated with any red blood cell presence. The daily intake of cranberry juice, in amounts which are not detrimental to long term compliance, appeared to have a role in reducing the risk of bladder colonization and infection in a highly susceptible patient population.

Key words: cranberry, spinal cord injury, biofilms, randomized controlled trial

INTRODUCTION

There is still some controversy over the extent to which intake of cranberry juice influences uropathogen colonization and infection of the urinary bladder (1-4). Studies suggest that cranberry juice supplements improve the health of the urinary tract by inhibiting the binding of fimbriated uropathogenic *E. coli* to the bladder mucosa (5,6). Three days intake of cranberry supplement has been shown to produce urine which is more acidic and less able to support the binding of uropathogens to surfaces (7). A more demanding challenge for a functional food to enhance patient care is in spinal cord injured (SCI) or other patients with neurogenic bladder where urinary tract infection (UTI) is common, life-threatening and complicated by dense biofilms in the bladder that are recalcitrant to antibiotic eradication (8,9). A pilot study suggested that daily intake of cranberry juice could reduce the biofilm load in these patients, perhaps by discouraging growth and colonization of the bladder by uropathogenic bacteria, thereby maintaining an infection free state without the need for antibiotic therapy (10). Given the high prevalence of multidrug resistant pathogens amongst hospitalized patients, and the potential for cranberry juice to impact these organisms, further studies on neurogenic bladder patients using cranberry juice are warranted.

A clinical study was undertaken on SCI and geriatric and spinal cord injured patients with neurogenic or dysfunctional bladder to determine if daily intake of cranberry juice reduced urinary biofilms and the risk of UTI.

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MATERIALS AND METHODS

Patients and study protocol

In a double blind, randomized, placebo-controlled trial, 7 geriatric and 22 spinal cord injured patients with neurogenic or dysfunctional bladders received three times daily at mealtimes a 4 oz bottle of cranberry juice (Ocean Spray Cranberries, USA) or a specially prepared synthetic placebo drink that was indistinguishable in taste, appearance, and vitamin C content but lacked cranberry content (1) for three months. Urine samples were collected monthly and tested for bacteria by standard culture, pH, white and red blood cells by microscopy, leukocyte-nitrite test by commercial strips. Uroepithelial cells were harvested, washed and examined microscopically for adherent bacteria. The study was approved by the Ethics Review Board at the University of Western Ontario and all patients signed consent forms after reading the Letter of Information.

Statistics

Various analytical tools were used to analyse the data, including an untransformed t-test, square root t-test and Wilcoxon 2 sample test.

RESULTS

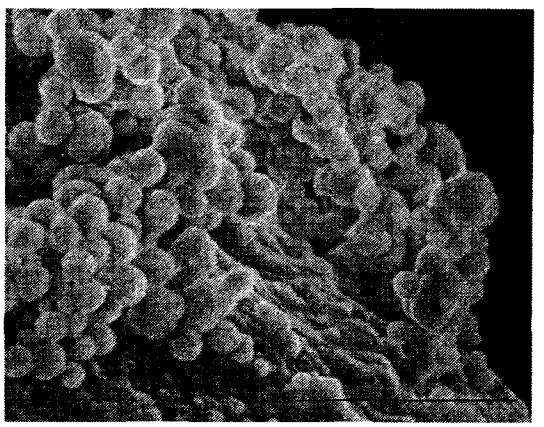
Thirty eight male and female (equally distributed amongst the two test groups) patients were recruited of which 29 (14 on cranberry and 15 on placebo) provided sufficient data for analysis. The main reason for non-compliance was the sour taste of the juice.

No side effects arose from cranberry juice intake in the 29 subjects, although some patients complained of its sour taste. In four patients in each group, they commented on having cloudy urine and increased urinary output. Two episodes of UTI arose in week one of cranberry intake and none thereafter, compared to four episodes of UTI in 4 placebo patients in weeks four, six and ten. Urinary pH ranged from 5 to 8 (mean 6.3) and did not correlate with cranberry juice consumption or bacterial adhesion counts, urine culture counts or UTI, in agreement with previous findings (11).

Bacterial biofilms were noted adherent to uroepithelial cells, especially when adhesion counts were >100 per cell (Fig. 1). Mean biofilm adhesion counts were lower or unchanged in patients receiving cranberry juice compared to placebo at one and three month timepoints ($p<0.001$). (Fig. 2).

The lower the culture result ($<10^5$ cfu/mL), the lower the mean bacterial adhesion (23 ± 48 per cell) compared to 134 ± 123 per cell for $\geq10^5$ cfu/mL ($p<0.001$, Table 1). One placebo patient had only 5 bacteria adherent per cell at the time of infection. High counts (>100 bacteria per cell) were found in 12 placebo subjects (mean=208 ± 98 bacteria per cell) and 12 cranberry subjects (mean=252 ± 125 bacteria per cell) without symptoms or signs of infection. Higher adhesion counts were found in positive leukocyte nitrite tests (136 ± 131 versus 52 ± 86 in negative tests) ($p<0.001$, Table 2). Furthermore, higher adhesion counts were associated with higher white blood cell counts (>10) per high power field (126 ± 125 versus 48 ± 85 bacteria per cell) ($p<0.001$, Table 3).

E. coli was the most frequently isolated organism (40% samples) followed by *K. pneumoniae* (17%) and a number of other uropathogens. Interestingly, *Serratia marcescens*, found in only 5% of samples, was highly adhesive ($>125\sim550$ bacteria per cell) and was associated with red and white blood cells in urine of 3/4 patients. Group B *Streptococci*, and coagulase negative *Staphylococcus* were recovered from urine in 4 samples but were not associated with any red blood cell presence.



Bar = 2.31 μm

Fig. 1. Bacterial biofilm adherent to the surface of a uroepithelial cell harvested from the urine of a patient with spinal cord injury and asymptomatic bacteriuria.

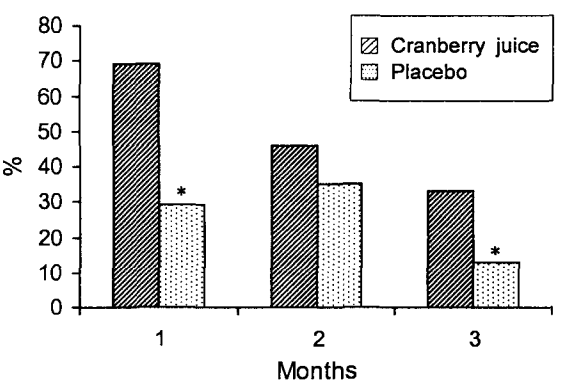


Fig. 2. Percentage patients whose biofilm levels improved or stayed the same during cranberry juice intake (Series 1 - bold) or placebo (Series 2 - open) compared to Day 0. *$p<0.05$.

Table 1. Adhesion counts from uroepithelial cells obtained when patients had low ($<10^5$ bacteria per mL) and high ($\geq 10^5$/mL) urine culture counts

$<10^5$ bacteria per mL		$\geq 10^5$ bacteria per cell	
Range of adhesion counts	# Values in this range	Range of adhesion counts	# Values in this range
0	18	0	0
1~20	26	1~20	6
21~50	4	21~50	7
51~100	3	51~100	16
>100	4	>100	22
Total mean counts	23±48	Total mean counts	134±123***

***$p<0.001$.

Table 2. Adhesion counts from uroepithelial cells obtained when patients had positive leukocyte nitrite strip test results compared to negative findings

Positive leukocyte nitrite test		Negative leukocyte nitrite test	
Range of adhesion counts	# Values in this range (%)	Range of adhesion counts	# Values in this range (%)
0	1 (3%)	0	17 (22%)
1~20	4 (12%)	1~20	30 (39%)
21~50	3 (9%)	21~50	7 (9%)
51~100	11 (33%)	51~100	8 (10%)
>100	14 (43%)	>100	15 (20%)
Total mean counts	136±131	Total mean counts	52±86***

***$p<0.001$.

Table 3. Adhesion counts from uroepithelial cells obtained when patients had low (≤10 cells) and high (>10 cells) white blood cell (WBC) counts per field of microscope

≤10 WBC		>10 WBC	
Range of adhesion counts	# Values in this range	Range of adhesion counts	# Values in this range
0	16	0	2
1~20	28	1~20	6
21~50	6	21~50	4
51~100	7	51~100	12
>100	11	>100	17
Total mean counts	48±85	Total mean counts	126±125***

***$p<0.001$.

The study was not designed to compare geriatric and spinal cord injured subjects per se, but no obvious consistent differences appeared between the subjects.

DISCUSSION

This study provided more evidence that daily cranberry juice intake can confer health benefits in patients with dysfunctional and neurogenic bladder, in terms of fewer episodes of symptomatic UTI and reduced presence of uropathogens within the bladder.

Previous studies (6) indicate that cranberry juice has proanthocyanidins that inhibit *E. coli* binding to cells. In the present study, when *E. coli* was detected in urine, the adhesion counts were the same in the cranberry and placebo groups (67 per cell) and one case of UTI occurred in each group. This implies no effect of cranberry on biofilms in dysfunctional and neurogenic bladder patients. However, in the 5 patients who had *E. coli* in urine at recruitment, bacterial adhesion counts dropped 40% following one month on cranberry compared to no change in 3 placebo cases. Thus, further studies are needed to show conclusively that cranberry juice has an anti-adhesive effect over *E. coli in vivo*. Mean adhesion counts fell or remained unchanged in more cranberry juice treated patients than controls, especially for the first month of treatment, suggesting that cranberry intake did indeed lower the risk of bladder colonization and infection in some patients. The study sample size was not set up to achieve large numbers of symptomatic UTI episodes, but after the

first week of intake of juice, there were four cases of UTI in the placebo group and none in the cranberry group.

The study showed a good correlation between lower culture findings ($< 10^5$ cfu/mL), and lower mean bacterial adhesion counts (23 ± 48 per cell), as well as higher culture findings ($\geq 10^5$ cfu/mL) and higher mean bacterial counts (134 ± 123 per cell). This suggests that assessment of bacterial adhesion counts, using microscopy, could provide caregivers with more information when deciding whether or not to administer antibiotics, such as fluoroquinolones which might reduce the biofilm burden and the risk of sepsis (9). The higher levels of bacterial adhesion to bladder cells correlated with positive leukocyte nitrite test results and higher white blood cell counts (> 10) per high power field. This suggests that dense biofilms with large numbers of adherent organisms are more likely to induce a host immune response.

However, there are exceptions. In one patient, UTI arose with only a mean of 5 bacteria attached per cell. Similar findings have been reported previously in acute, uncomplicated UTI patients (12), thus contradicting the belief that high counts are always associated with symptomatic UTI (13). Therefore, while high bacterial levels were found in the urine (adherent to cells and in planktonic form suspended in urine), these invariably were not associated with UTI, and therefore further studies are required to determine the key components which induce symptoms and signs of an infectious state. The trigger for perception of pain, spasms, urgency etc may involve neurotransmitter signaling by pathogens. Afferent nerves lie suburothelially, where they form a plexus immediately beneath the epithelial lining. Extracellular adenosine triphosphate (ATP) can mediate excitation of small-diameter sensory neurons via P2X3 receptors and evoke a neural discharge (14). Urinary functions are dependent on the activity of smooth and striated muscles in the urinary bladder, urethra, and external urethral sphincter, which is controlled by neural circuits in the brain, spinal cord, and peripheral ganglia. Clearly in SCI and other patients with dysfunctional or neurogenic bladder, the action of neurotransmitters such as acetylcholine, norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids, adenosine triphosphate, nitric oxide, and neuropeptides, may be compromised (15), but the method by which some pathogens (even in low numbers) in some patients cause symptomatic UTI is uncertain and worthy of closer investigation. Similarly, the lack of any symptomatic effects when very large numbers of bacteria are attached remains somewhat of a mystery. One possible explanation is that such strains are not able to invade uroepithelial cells, a step which may be critical for some virulent *E. coli* (16).

In summary, chronic bacterial colonization and biofilm formation is prevalent in patients with dysfunctional or neurogenic bladder, such as geriatric and spinal cord injured patients. Failure of antibiotics to eradicate these biofilms, and fear that long term use of these agents will lead to multi-drug resistant pathogens infecting the host, make it imperative that other management approaches be evaluated. The continued presence of pathogens in the bladder can not only lead to production of toxic compounds including carcinogens, such as N,N-dimethylnitrosamine produced by *E. coli* (17), but a continued inflamed state detrimental to the host's health and prognosis. In these patients, the daily intake of cranberry juice, in amounts which are conducive to long term compliance, has a role to play in reducing the risk of UTI and affiliated complications.

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