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ORIGINAL ARTICLE

Urine post equivalent daily cranberry juice consumption may opsonize uropathogenicity of *Escherichia coli*

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Abstract Basic studies have proven that cranberries may prevent urinary tract infections through changing the adhesiveness of *Escherichia coli* (*E. coli*) to urothelial cells. Various cranberry preparations, including extract powder, capsules, and juice, have been shown to be effective in clinical and epidemiological research. Because cranberries are most commonly consumed as juice in a diluted concentration, the aim of this study was to investigate whether the equivalent daily dose of cranberry juice is sufficient to modify host urine to change the uropathogenicity of *E. coli*. Urine from rats taking an equivalent daily dose of cranberry juice has been shown to decrease the capability of *E. coli* in hemagglutination, urothelium adhesion, nematode killing, and biofilm formation. All these changes occurred after *E. coli* was incubated in cranberry metabolite-containing urine, defined as urine opsonization. Urine opsonization of *E. coli* resulted in 40.9 % ($p = 0.0038$) decrease in hemagglutination ability, 66.7 % ($p = 0.0181$) decrease in urothelium adhesiveness, 16.7 % ($p = 0.0004$) increase in the 50 % lethal time in killing nematodes, and 53.9 % ($p = 5.9 \times 10^{-4}$) decrease in biofilm formation. Thus, an equivalent daily dose of cranberry juice should be considered sufficiently potent to demonstrate urine opsonization in *E. coli*.

Keywords Urinary tract infection · Cranberry juice · Opsonization · Equivalent daily dose

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Introduction

Cranberries (*Vaccinium macrocarpon*) have been used for many years to prevent urinary tract infections (UTI) in women [1]. The effect seems to be more pronounced in women with recurrent UTI, and a protective effect has also been shown in pregnant women [2] and UTI in children [3] with or without neurogenic bladder [4]. This clinical evidence has led to further investigations in the role that cranberries play in UTI.

Type A proanthocyanidins from cranberries have been proven to inhibit the adherence of bacterial P fimbriae in a dose-dependent manner in vitro [5]. It has also been found that macroscale commercial extracts may inhibit up to 55 % of uropathogenic *E. coli* in adhesion during the exponential phase [6]. Both macroscale and nanoscale adhesion forces between *E. coli* and human uroepithelial cells have been studied with or without cranberry juice treatment. It has been confirmed that if *E. coli* expressing P fimbriae, which is often more uropathogenic, is incubated in cranberry juice solution, it will lose the adhesion forces originating from the P fimbriae [7].

Different cranberry preparations have been shown to have similar efficacy, and consumption of either cranberry juice or extracts may inhibit *E. coli* adhesion. Taking cranberry extracts has been shown to produce urine that may result in *E. coli* with decreased abilities of hemagglutination, adhesiveness to urothelial cells, and toxicity to

Caenorhabditis elegans [8]. It has also been shown that taking cranberry juice may result in urine that causes *E. coli* to lose its adherence activity in a dose-dependent manner [9] and ability in biofilm formation on microtiter plates. This finding indicates that even when cranberries are processed into different forms, the metabolites in urine are still active against uropathogenic *E. coli* (UPEC).

Consumption of cranberry juice may therefore change the character of urine, and individuals may produce urine with an inhibitory effect on UPEC adhesiveness. It has also been shown that the more cranberry juice consumed, the greater the inhibition on adhesiveness [9]. This inhibition on adhesiveness is independent from phenotypes with antibiotic resistance or genes encoding type P pili.

Escherichia coli (*E. coli*) morphology and surface structure are modified after incubation in cranberry juice or extract [10], and urine containing cranberry metabolites seems to have a similar effect on the surface characteristics of *E. coli* [8, 11]. Gene expressions of flagellar basal body rods and motor proteins may also be modified. In this study, we describe this condition as the "urine opsonization" of *E. coli*, as we believe it is similar to the actions of antibodies in that antibodies may act as binding enhancers for phagocytosis during opsonization. We investigated whether urine containing cranberry metabolites at daily equivalent doses may change UPEC surface characteristics.

Materials and methods

Animals and urine collection

A total of 12 adult female Sprague–Dawley rats (6–8 weeks old, 250–350 g) purchased from BioLASCO Taiwan were used following 1 week of acclimation. All experiments were performed in compliance with the National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals and the guidelines of the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital. The rats were divided into three groups: the control group, and the 25 % Juice-EC and 100 % Juice-EC groups, which were tube-fed with 1 ml H_2O and 25 % cranberry juice or 1 ml 100 % cranberry juice three times per day, respectively. Cranberry juice concentrate was kindly provided by Ocean Spray Cranberries (Ocean Spray International Services, Taipei, Taiwan). Urine was collected to evaluate host modification after 3 days of juice consumption. Urine samples were sequentially collected in metabolic cages with 50-ml conical tubes containing a thin layer of paraffin oil to prevent evaporation. Collections were continued during the night until at least 8 h and recorded for volume (about 10 ml). Before a urine sample was frozen at −20 °C, it was centrifuged at 2,000 rpm and filtered with Minisart syringe filters (Sartorius-Stedim Biotech, Taipei, Taiwan), then held until analysis.

Hemagglutination assay

Uropathogenic *E. coli* (UPEC0061) with P-fimbriae *papG* and type-1 pili was used, which had previously been isolated from a patient with recurrent cystitis. To detect PapG-mediated agglutination, the bacteria were incubated overnight on a Luria–Bertani broth (LB broth; Merck Millipore, Taipei, Taiwan) agar plate at 37 °C. Agglutination was performed with human red blood cells [HRBC, A1, Rh+, 3 % vol/vol in phosphate-buffered saline (PBS)]. Equivalent daily cranberry juice consumption may modify host urine and change its antihemagglutination activity; this was tested by measuring the ability of urine dilutions to suppress agglutination of HRBC, following incubation with UPEC.

The bacteria were treated with urine from the different groups of rats for 6 h and then precipitated to remove the urine. They were then resuspended in PBS solution at pH 7.2. The initial bacterial suspension (50 µl, with an initial concentration of 5×10^8 bacteria/ml) was placed on a 96-well polystyrene plate and diluted twofold serially. Each dilution was incubated with HRBC 3 % 50 µl for 60 min at room temperature on a rotary shaker. The final dilution concentration at which hemagglutination was suppressed by the diluted urine was recorded. Wells containing HRBC plus PBS served as negative controls for hemagglutination, and wells containing nontreated bacteria plus HRBC served as positive controls for hemagglutination. All assays were performed in triplicate.

Bacterial adhesive assay

A uropathogenic *E. coli* strain purchased from Bioresource Collection and Research Center (BCRC No. 15479, originated from ATCC 23499), in which serotypes O1a and 1b and P fimbriae were confirmed, was used for this test. To allow for the direct observation of adherent bacteria under fluorescence microscopy, BCRC15479 was genetically modified to express a green fluorescent protein (GFP) using a pCOM-derived non-mobilizable plasmid carrying a GFP expression sequence, subsequently referred to as GFP-UPEC [12]. The bacteria were incubated in trypticase soy broth (bioMerieux, Marnes La Coquette, France) for 12 h at 37 °C to enhance the expression of P fimbriae [11].

This ex vivo bacterial adhesion assay was evaluated with the use of a human urinary bladder carcinoma T24 epithelial cell line (ATCC HTB-4). Monolayers of T24 cells were grown in McCoy's 5a medium containing 10 % (v/v) fetal calf serum, 1.5 mM glutamine, and antibiotics (50 mg/ml streptomycin, 50 U/ml penicillin) on coverslips in 24-well

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