Oral Consumption of Cranberry Juice Cocktail Inhibits Molecular-Scale Adhesion of Clinical Uropathogenic *Escherichia coli*

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ABSTRACT Cranberry juice cocktail (CJC) has been shown to inhibit the formation of biofilm by uropathogenic *Escherichia coli*. In order to investigate whether the anti-adhesive components could reach the urinary tract after oral consumption of CJC, a volunteer was given 16 oz of either water or CJC. Urine samples were collected at 0, 2, 4, 6, and 8 hours after consumption of a single dose. The ability of compounds in the urine to influence bacterial adhesion was tested for six clinical uropathogenic *E. coli* strains, including four P-fimbriated strains (B37, CFT073, BF1023, and J96) and two strains not expressing P-fimbriae but exhibiting mannose-resistant hemagglutination (B73 and B78). A non-fimbriated strain, HB101, was used as a control. Atomic force microscopy (AFM) was used to measure the adhesion force between a silicon nitride probe and bacteria treated with urine samples. Within 2 hours after CJC consumption, bacteria of the clinical strains treated with the corresponding urine sample demonstrated lower adhesion forces than those treated with urine collected before CJC consumption. The adhesion forces continued decreasing with time after CJC consumption over the 8-hour measurement period. The adhesion forces of bacteria after exposure to urine collected following water consumption did not change. HB101 showed low adhesion forces following both water and CJC consumption, and these did not change over time. The AFM adhesion force measurements were consistent with the results of a hemagglutination assay, confirming that oral consumption of CJC could act against adhesion of uropathogenic *E. coli*.

KEY WORDS: • anti-adhesion • urinary tract infections • *Vaccinium macrocarpon*

INTRODUCTION

URINARY TRACT INFECTIONS (UTIs) are the most common community-required infections affecting the human body,¹ with yearly costs estimated to be \$1.6 billion in the United States.^{1,2} The main UTI pathogen is *Escherichia coli*, which causes 90% of community-acquired UTIs and 30% of nosocomial UTIs.^{3,4} Infections are initiated when pathogenic bacteria attach to uroepithelial cells via the binding of bacterial adhesins to the receptors on the host cell membrane.⁵ Typical adhesins of uropathogenic *E. coli* include type 1 fimbriae, P fimbriae, and X adhesins.⁵ Type 1 fimbriae-mediated adherence can be blocked by D-mannose, α -methylmannoside, and many other mannose analogs, and therefore this mechanism is termed mannose-sensitive adhesion.⁶ The binding of P fimbriae and X adhesins to uroepithelial cells cannot be blocked by mannose or its analogs, and thus this mechanism is termed mannose-resistant adhesion.^{7,8}

UTIs are conventionally treated with antibiotics, but there are concerns due to the development of antibiotic resistance and the problem of recurrent UTIs that affect some patients. Therefore, alternative therapies are drawing increasing interest among researchers. The American cranberry (*Vaccinium macrocarpon*) was shown to prevent UTIs in some studies,^{9–11} but the underlying mechanisms have not been fully explored. A family of phytochemicals in cranberry, A-type proanthocyanidins (A-PACs), were identified as the anti-adhesive components.¹² The nondialyzable material in cranberries, containing mainly A-PACs and some unidentified molecules, has been used in some studies and had anti-adhesive activity.¹³ Incubation of P-fimbriated *E. coli* with 60 μ g/mL A-PACs significantly decreased mannose-resistant human red blood cell (HRBC) hemagglutination, and similar effects were observed when A-PAC solution was replaced with urine that was collected from volunteers who drank cranberry juice cocktail (CJC).¹⁴ In our previous study using atomic force microscopy (AFM) to measure adhesion forces between P-fimbriated *E. coli* and a silicon nitride probe, the average adhesion forces decreased after 12 cultures in the presence of light CJC (from 1.60 ± 0.71 nN to 0.56 ± 0.3 nN) or PACs (from 1.60 ± 0.71 nN to 0.42 ± 0.2 nN), and the frequency distribution of adhesion forces

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shifted to lower values as the concentration of cranberry juice in the culture medium increased.^{15,16}

Previous studies have not addressed whether cranberry juice compounds have molecular-scale anti-adhesive activity after passing through the digestive system. Therefore, we investigated CJC's effects on adhesion forces of clinical *E. coli* strains, including antibiotic-resistant strains that were isolated from patients with cystitis or acute pyelonephritis. Using direct adhesion force measurements with AFM, the effects of cranberry components or metabolites remaining in urine on the adhesion of *E. coli* were investigated. The adhesion forces measured with AFM were correlated to the results of a macroscopic assay, namely, the agglutination of red blood cells (RBCs) exposed to specific *E. coli* agglutinins. To our knowledge, this is the first molecular-scale study to confirm the existence of anti-adhesive molecules in urine after oral consumption of CJC.

MATERIALS AND METHODS

Urine samples

Urine samples had been collected for other Institutional Review Board–approved research studies at IRB through Rutgers University, Cook College, New Brunswick, NJ, USA. The samples sent to Worcester Polytechnic Institute (Worcester, MA, USA) were de-identified, and no personal information was provided. A random, crossover design was applied, with a single volunteer. The volunteer was a healthy white male, 42 years old. He drank 16 oz (473 mL) of commercial CJC or water. After he consumed the single dose, urine was collected at intervals of 0, 2, 4, 6, and 8 hours. Samples were immediately frozen and shipped to Worcester Polytechnic Institute, where they were stored at -20°C . Urine samples were later thawed and filtered through polyethersulfone syringe filters with $0.8\text{-}\mu\text{m}$ (Pall Corp., East Hills, NY, USA) and $0.2\text{-}\mu\text{m}$ (VWR International™, West Chester, PA, USA) pores, sequentially.

Bacterial cultures

Six *E. coli* clinical strains that cause acute pyelonephritis or cystitis were selected, and a laboratory strain with no

fimbriae was used as a control. The clinical strains possess various surface properties, adhesin types, and antibiotic sensitivities (Table 1). To study different types of adhesins we chose two strains from each of the most common subgroups: (1) strains that demonstrate mannose-resistant hemagglutination (MRHA) but no P-fimbriae (B73 and B78); (2) strains that express P-fimbriae from class II (B37 and CFT073); and (3) strains that express P-fimbriae from class III (BF1023 and J96).

Bacteria were cultured at 37°C in colonizing factor antigen medium, composed of 1% (wt/vol) casamino acids (Bacto™, Sparks, MD, USA), 0.078% (wt/vol) yeast extract (Bacto), 0.4 mM MgSO_4 (Sigma-Aldrich, St. Louis, MO, USA), and 0.04 mM MnCl_2 (Sigma-Aldrich) in ultrapure water, and the pH was adjusted to 7.4 using sodium hydroxide (Sigma-Aldrich). For culture plates, 2% agar (Bacto) was added to the medium. Bacteria were grown overnight and harvested at late exponential phase, when the absorbance of the culture was 0.9–1.1 at a wavelength of 600 nm, measured with a spectrophotometer (Thermo Spectronic, Rochester, NY, USA). Bacteria were washed three times with ultrapure water by centrifuging the solution at 2,000 g and removing the supernatant.

AFM adhesion force measurements

Bacteria were attached to glass slides before AFM experiments.^{21,22} Glass slides were treated with 3:1 (vol/vol) HCl/HNO_3 solution (Fisher Chemical, Fair Lawn, NJ, USA) for 45 minutes and rinsed with ultrapure water. Slides were soaked with 7:3 (vol/vol) $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ solution (Fisher Chemical) and rinsed with ultrapure water. The acid-cleaned glass slides were stored at 4°C in ultrapure water until use. Glass slides were functionalized with an amine group to facilitate bacterial attachment. Glass slides were treated with ethanol (Sigma-Aldrich) and methanol (Sigma-Aldrich) and then immersed in a solution of 35% 3-aminopropyltrimethoxysilane (Sigma-Aldrich) in methanol for 15 minutes, followed by rinsing with ultrapure water. A 300- μL solution of 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Pierce, Rockford, IL, USA) was added to the

TABLE 1. SUMMARY OF PROPERTIES AND SOURCES OF SEVEN *E. COLI* STRAINS STUDIED

Strain	Fimbriae/description	Source
B73	No P-fimbriae but exhibits MRHA (may be Dr adhesins); trimethoprin/sulfamethoxazole resistant	Dr. James Johnson (VA Medical Center, Minneapolis, MN, USA); isolated from a woman with cystitis ¹⁷
B78	No P-fimbriae but exhibits MRHA (may be Dr adhesins); ampicillin/sulfamethoxazole intermediate resistance	Dr. James Johnson; isolated from a woman with cystitis ¹⁷
B37	P-fimbriae from class II, ampicillin/sulfamethoxazole intermediate resistance	Dr. James Johnson; isolated from a woman with cystitis ¹⁷
CFT073[WAM 2267]	Type P-fimbriae from class II	ATCC 700928; isolated from blood and urine of a woman with acute pyelonephritis
BF1023	P-fimbriae from class I and class III	ATCC 700414; isolated from a female patient with cystitis ¹⁸
J96	P-fimbriae from class I and class III	ATCC 700336; isolated from a patient with pyelonephritis ^{19,20}
HB101	Non-fimbriated, laboratory strain (nonpathogenic control)	ATCC 33694

MRHA, mannose-resistant hemagglutination.

washed *E. coli* cells and incubated at 37°C for 10 minutes with rotation at 18 rpm, followed by addition of a 600- μ L solution of 40 mM *N*-hydroxysulfosuccinimide (Pierce) solution and incubation at 37°C for 10 minutes with rotation at 18 rpm. Bacterial solution incubated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysulfosuccinimide was added to the 3-aminopropyltrimethoxysilane-treated glass slides and agitated at 70 rpm for 4 hours to allow bacteria to bind onto the slides. Viability and morphology of bacteria cells were maintained during the binding process.²¹

AFM adhesion force measurements were performed using a Dimension 3100 instrument with a Nanoscope IIIa controller (Veeco Metrology, Santa Barbara, CA, USA). Silicon nitride cantilevers with spring constants of 0.12 ± 0.02 N/m (DNPS, Veeco Metrology) were used to acquire images and force data. Before experiments, the cantilevers were cleaned by immersion in ethanol and exposure to ultraviolet light. All the measurements were done with the slides and cantilevers immersed in the same urine sample with which the slides were treated, in order to mimic the physiological environment. From each slide, five bacteria were probed, and 10 force measurements were made in the center of each bacterium. Force data were exported in ASCII format and analyzed as described previously²³ to acquire adhesion force–separation distance data. During the portion of the force profile where the AFM tip retracts from the bacterial surface, adhesion peaks are often observed. On each retraction curve the peaks represent the moment when the AFM probe pulls off from the cell surface, and the values of the forces at the peaks are defined as adhesion forces between the probe and the cell surface.

Statistical analysis

SigmaStat[®] version 2.03 (Systat Software, San Jose, CA, USA) was used to analyze force data. A two-way analysis of variance test was used to compare the two groups that were treated with urine samples collected after water or CJC consumption. Background (bacteria treated with urine samples that were collected before drinking water or CJC) adhesion forces were also compared with adhesion forces measured for the bacteria treated with urine samples that were collected after consumption of water or CJC.

HRBC agglutination assay

Whole blood (type O positive) acquired from a volunteer was stored with EDTA at 4°C. Before the agglutination experiment, the RBCs were separated from whole blood by centrifugation at 200 g for 10 minutes, in order to remove the platelets and plasma. The resulting RBCs were suspended in phosphate-buffered saline and washed three times. After each wash the supernatant was removed, and the remaining cells were resuspended in phosphate-buffered saline. The concentration of RBCs in the suspension was measured using a hemacytometer (Hausser Scientific, Horsham, PA, USA). The suspension was diluted with urine to a concentration of 10^7 RBCs/mL. The urine samples used in

the HRBC agglutination assay were those collected at intervals of 0–2 hours or 6–8 hours after water or CJC consumption, as described above.

E. coli bacteria harvested at late exponential phase were diluted in urine to 10^9 cells/mL. RBCs and bacteria were incubated separately in urine at 37°C for 3 hours. Then, the two were co-incubated for 90 minutes to allow agglutination. The number of bacteria that attached to RBCs was counted under a light microscope (Eclipse E400, Nikon, Tokyo, Japan). For each urine sample, the number of attached *E. coli* bacteria was counted on 20 RBCs.

RESULTS

Adhesion force measurements and analysis

When the clinical *E. coli* strains were incubated with the background urine sample (urine collected just before water or CJC consumption), this resulted typically in adhesion forces above 1 nN, which varied somewhat depending on the strain (Table 2). *E. coli* BF1023 had the highest background adhesion force of 1.68 ± 1.01 nN, whereas B73, B37, B78, CFT073, and J96 had background adhesion forces between 1.00 and 1.30 nN. HB101, the laboratory strain that has no fimbriae, showed the lowest background adhesion force of 0.40 ± 0.26 nN.

When cultured with urine samples collected at different time intervals following CJC consumption, all the clinical *E. coli* strains demonstrated decreasing adhesion forces with time after initial CJC consumption (Fig. 1). The adhesion forces of clinical bacteria cultured with urine collected 0–2 hours after drinking CJC decreased to below 1 nN for all the clinical strains. Adhesion forces continued to decrease for 8 hours, becoming as low as 0.20–0.50 nN. The control strain, HB101, did not show significant changes in the adhesion forces during the 8 hours following CJC consumption.

Water consumption did not affect the adhesion forces of the clinical and laboratory strains, with the exception of B37 (Table 2). Following water consumption, B37 showed an adhesion force of 0.84 ± 0.49 nN at 0–2 hours and 0.75 ± 0.40 nN at 2–4 hours, which were significantly different from the background, but those adhesion forces were still higher than adhesion forces measured after CJC consumption at the same time intervals. For B73, B78, CFT073, BF1023, and J96, the adhesion forces stayed above 1 nN or slightly below 1 nN with no significant differences from the background measurement. The adhesion forces of HB101 remained between 0.30 and 0.50 nN with no significant differences from the background.

Analysis of variance tests showed that for all the clinical strains, adhesion forces after CJC consumption were significantly lower than the background adhesion force (Table 2). The adhesion forces measured after CJC consumption also were lower compared with those measured at the same time intervals after water consumption. B37 showed higher adhesion forces with treatment of urine collected after water consumption, but within 4 hours after consumption, the adhesion forces after water consumption were only slightly

TABLE 2. ADHESION FORCE BETWEEN A SILICON NITRIDE ATOMIC FORCE MICROSCOPY PROBE AND BACTERIAL CELLS AND MEASURED AFTER TREATMENT WITH URINE SAMPLES COLLECTED BEFORE (BACKGROUND) AND AFTER CONSUMPTION OF 16 oz OF WATER OR CRANBERRY JUICE COCKTAIL

Strain	Urine sample treatment	Mean $\pm$ SD adhesion force (nN)				
		Background	0–2 hours	2–4 hours	4–6 hours	6–8 hours
B73	Water	1.30 $\pm$ 0.77	1.42 $\pm$ 0.63	1.15 $\pm$ 0.61	1.13 $\pm$ 0.50	1.26 $\pm$ 0.77
	CJC		0.52 $\pm$ 0.31 ^{*†}	0.42 $\pm$ 0.25 ^{*†}	0.30 $\pm$ 0.17 ^{*†}	0.29 $\pm$ 0.25 ^{*†}
B78	Water	1.30 $\pm$ 0.86	1.37 $\pm$ 0.72	1.33 $\pm$ 0.88	1.44 $\pm$ 0.91	1.33 $\pm$ 0.82
	CJC		0.98 $\pm$ 0.73 ^{*†}	0.73 $\pm$ 0.45 ^{*†}	0.66 $\pm$ 0.35 ^{*†}	0.49 $\pm$ 0.24 ^{*†}
B37	Water	1.07 $\pm$ 0.41	0.84 $\pm$ 0.49 [*]	0.75 $\pm$ 0.40 [*]	1.04 $\pm$ 0.62	0.96 $\pm$ 0.42
	CJC		0.69 $\pm$ 0.30 [*]	0.61 $\pm$ 0.32 [*]	0.47 $\pm$ 0.21 ^{*†}	0.47 $\pm$ 0.22 ^{*†}
CFT073	Water	1.12 $\pm$ 0.75	0.99 $\pm$ 0.70	1.02 $\pm$ 0.60	0.89 $\pm$ 0.50	0.82 $\pm$ 0.47
	CJC		0.77 $\pm$ 0.50 ^{*†}	0.47 $\pm$ 0.36 ^{*†}	0.44 $\pm$ 0.27 ^{*†}	0.38 $\pm$ 0.18 ^{*†}
BF1023	Water	1.68 $\pm$ 1.01	1.61 $\pm$ 1.1	1.82 $\pm$ 1.20	1.75 $\pm$ 1.21	1.64 $\pm$ 0.94
	CJC		0.86 $\pm$ 0.44 ^{*†}	0.72 $\pm$ 0.34 ^{*†}	0.45 $\pm$ 0.25 ^{*†}	0.29 $\pm$ 0.12 ^{*†}
J96	Water	1.25 $\pm$ 0.78	1.29 $\pm$ 0.59	1.29 $\pm$ 0.62	1.35 $\pm$ 0.75	1.30 $\pm$ 0.42
	CJC		0.88 $\pm$ 0.52 ^{*†}	0.48 $\pm$ 0.26 ^{*†}	0.49 $\pm$ 0.19 ^{*†}	0.38 $\pm$ 0.33 ^{*†}
HB101	Water	0.40 $\pm$ 0.26	0.42 $\pm$ 0.31	0.45 $\pm$ 0.32	0.43 $\pm$ 0.36	0.46 $\pm$ 0.37
	CJC		0.41 $\pm$ 0.22	0.35 $\pm$ 0.27	0.43 $\pm$ 0.46	0.41 $\pm$ 0.22

*Statistically significant difference compared with background ($P < .05$).

[†]Statistically significant difference compared with treatment with urine sample collected at the same time point after water consumption ($P < .05$).

CJC, cranberry juice cocktail.

higher than those after CJC consumption, and the difference between the two was not statistically different. After 4 hours the adhesion forces after water consumption increased and became significantly higher than the CJC group. For the majority of clinical strains, namely, B73, B78, CFT073, BF1023, and J96, the difference of adhesion forces between

CJC and water was statistically significant at each time interval, with adhesion forces after water consumption higher than those after CJC consumption.

HRBC agglutination assay

Coculturing clinical strains with urine samples collected 6–8 hours after CJC consumption resulted in a high degree of retention of bacteria on RBCs (Fig. 2). The mean number of attached *E. coli* cells per RBC was >6 , with two strains having especially high retention (11.35 bacteria/RBC for CFT073 and 11.85 bacteria/RBC for BF1023). The retention after culturing with CJC collected at 6–8 hours was significantly lower, being typically below 2 bacteria/RBC. The control strain HB101 demonstrated low retention to RBCs after culturing in urine collected 6–8 hours after consumption of either water (0.15 bacteria/RBC) or CJC (0.35 bacteria/RBC), and there was no statistically significant difference between water and CJC.

DISCUSSION

Adhesion forces of *E. coli*

Antibiotic resistance has been a major problem in the treatment of bacterial infections. CJC is a promising preventive therapy for UTIs because it works as an anti-adhesive instead of a bactericide and thus does not induce the development of antibiotic resistance. In this study, three antibiotic-resistant strains isolated from female patients with cystitis were selected to investigate CJC's effects on these bacteria (Table 1). All of the antibiotic-resistant strains showed little change in adhesion forces after water consumption compared with a significant decrease after CJC

FIG. 1. Adhesion forces of *E. coli* cells measured by atomic force microscopy after incubation with urine samples at 37°C. The urine samples were collected from a volunteer before and at certain time points after CJC consumption. The star symbol indicates a statistically significant difference compared with the mean adhesion forces after treatment of a urine sample collected at the same time after water consumption. All the mean adhesion forces are significantly different from the backgrounds for each strain. Color images available online at www.liebertonline.com/jmf

FIG. 2. Attachment of *E. coli* cells to red blood cells (RBCs). *E. coli* cells and RBCs were incubated separately in urine samples at 37°C for 3 hours. The two were then mixed and incubated at 37°C for 90 minutes to allow bacteria to attach onto RBCs. The number of bacteria retained on RBCs was counted under a light microscope, and the mean number of bacteria attached per RBC was calculated. The difference between CJC consumption (left-hand columns) and water consumption (right-hand columns) is statistically significant for all the clinical strains but not for HB101.

consumption, indicating that cranberry juice is effective in preventing nonspecific adhesion of these antibiotic-resistant *E. coli* strains.

For many years, it has been recognized that uropathogenic *E. coli* strains typically demonstrate mannose-resistant adhesion in MRHA experiments.^{7,8,24} Many phytochemicals in other fruits can inhibit mannose-sensitive adhesion, but cranberry is one of the only fruits found to inhibit mannose-resistant adhesion.^{25,26} Therefore, it is important to investigate the adhesive properties of uropathogenic *E. coli* strains that demonstrate MRHA, especially how oral consumption of cranberry juice affects the adhesion of these strains in a simulated physiological environment (urine collected from a volunteer after CJC consumption). Therefore, strains used in this study are from two MRHA-positive subcategories: B37, CFT073, BF1023, and J96 have P-fimbriae, whereas strains B73 and B78 do not have P-fimbriae but exhibit MRHA.

P-fimbriae are considered the dominant virulence factors in upper UTIs.²⁷ They were named after the P blood group antigens [a family of oligosaccharides with the Gal α (1 $\rightarrow$ 4)Gal β moiety] to which they bind. Although Gal α (1 $\rightarrow$ 4)Gal β -containing receptors are not abundant in the membrane of shed human epithelial cells,²⁸ they are dominant in human renal cell membranes.²⁹ P-fimbriae adhere to epithelial cells in multiple tissues in upper urinary tract,³⁰ explaining the high probability of P-fimbriae-mediated upper UTIs, such as acute pyelonephritis. P-fimbriae also adhere to the epithelial and muscular layers of the bladder,³¹ indicating their involvement in lower UTIs. In addition, human

polymorphonuclear leukocytes in blood only have trace amounts of glycolipids containing Gal α (1 $\rightarrow$ 4)Gal β ,^{32,33} rendering a poor binding of human polymorphonuclear leukocytes with pathogenic P-fimbriated bacteria and thus the killing of these bacteria. We showed a significant decrease in adhesion of P-fimbriated strains (B37, CFT073, BF1023, and J96) after incubation with urine samples that were collected after CJC consumption compared with the non-fimbriated strain HB101, which demonstrated low adhesion forces throughout the 8 hours after CJC consumption. These results indicated that the anti-adhesive components or metabolites of CJC remaining in urine have an inhibitive effect on uropathogenic *E. coli* adhesion.

The adhesins of the other two MRHA-positive strains, B73 and B78, have not been clearly identified yet; they may be Dr adhesins, which belong to the X adhesin family. The X adhesin family consists of Dr adhesins, S fimbriae, F1C fimbriae, M adhesins, G fimbriae, and other unidentified X adhesins.⁵ The Dr adhesins differ from fimbriae because they are not distinct filaments. They can exist as a fine mesh, a coil-like structure, or a filamentous capsular coating.³⁴ The receptors for Dr adhesins are the Dr blood group antigens located on the decay-accelerating factor, a group of cell membrane proteins regulating the complement cascade.³⁵ The binding site of Dr adhesins in the urinary tract include the renal interstitium, Bowman's capsule, tubular basement membrane, ureteral transitional epithelial cells, and exfoliated cells in urine.³⁵

B73 and B78 were isolated from cystitis patients, and the Dr adhesin family constitutes 78% of X adhesin strains among isolates from cystitis patients.³⁴ Therefore, it is likely that B73 and B78 have Dr adhesins. Our results showed that, similar to P-fimbriated strains, B73 and B78 showed decreased adhesion forces after being treated with urine from the volunteer who consumed CJC. Although the molecular binding mechanisms of Dr adhesin and P-fimbriae are different in the urinary tract, CJC can inhibit the adhesion of both, which could be a result of decreased nonspecific adhesion induced by CJC metabolites remaining in urine. Using contact angle measurements, a previous study³⁶ showed that cranberry juice could induce a decrease in nonspecific adhesion between P-fimbriated *E. coli* and uroepithelial cells. Because nonspecific adhesive interactions, including van der Waals forces, electrostatic forces, and hydrophobic interactions, do not involve the specific adhesin-receptor binding, it is likely that CJC metabolites influence the strains without P-fimbriae similar to the way they affect P-fimbriated strains by diminishing nonspecific interactions.^{37,38} It is also possible that CJC metabolites can change the amount and conformation of *E. coli* surface macromolecules and thus the adhesion activity.^{15,39} We showed previously that cranberry juice reduced the equilibrium length of *E. coli* P-fimbriae from ~148 nm to ~48 nm,¹⁵ suggesting the compressing of surface macromolecules on *E. coli* could be a mechanism of decreased adhesion, which could explain the nonspecific adhesion decrease after CJC consumption in both P-fimbriated and non-P-fimbriated *E. coli* strains.

RBC agglutination assay

Although the adhesion forces measured with a silicon nitride AFM probe represent the nonspecific adhesion of *E. coli* cell surfaces, including van der Waals forces and electrostatic and hydrophobic interactions, biological interactions also include specific types of adhesion, such as receptor–ligand bonds. AFM adhesion force measurements represent nonspecific adhesion between the silicon nitride AFM probe and the bacterial surfaces, whereas the RBC agglutination assay represents overall interaction between bacterial cells and human cells, which contains both nonspecific and specific adhesion.

Compared with the control strain HB101, all the clinical strains demonstrated high retention to RBCs after coculturing with the urine sample collected 6–8 hours after water consumption (Fig. 2), which can be explained by the fact that HB101 does not have any adhesins. When cocultured with the urine sample collected 6–8 hours after CJC consumption, the retention of HB101 did not change compared with urine collected 6–8 hours after water consumption. These results suggest that CJC metabolites remaining in urine can inhibit overall adhesion of uropathogenic *E. coli*.

In conclusion, by incubating uropathogenic *E. coli* with urine collected after water or CJC consumption and measuring the resulting change of adhesion force between *E. coli* cell surface and an AFM probe, we were able to demonstrate that the anti-adhesive components in CJC could reach the urinary tract and that these components were active in preventing nonspecific adhesion. We also confirmed that CJC components remaining in urine played a role in inhibiting specific adhesion of *E. coli* by means of the HRBC agglutination assay. In order to further investigate the effects of CJC for longer periods after consumption and the variance between individuals, we are currently working on a study involving 11 volunteers, and the urine samples will be collected over a 48-hour period after CJC or placebo consumption.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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