Concentration-Dependent Effect On Adherence Of Escherichia Coli To Bladder Epithelial Cells Of Cysticlean Capsules (240 Mg/Capsule Of Proanthocyanidins)

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Citation

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Abstract

Background

The proanthocyanidins (PACs) are the main components of cranberry (fruit of Vaccinium macrocarpon Aiton), and their content determines the effectiveness in the urinary tract infections (UTIs). The activity of PACS is related to inhibition of bacterial adherence. Cysticlean® is a cranberry extract product with a high quantity of proanthocyanidins (240 mg/capsule of PACs). Cysticlean® is used in the prevention and treatment of recurrent UTIs. The aim of this study is the comparison of different concentrations of Cysticlean® capsules on adherence of Escherichia coli to bladder epithelial cells.

Methods

In vitro, E. coli pre-incubated in different concentrations of Cysticlean® capsules was incubated with human urinary bladder carcinoma cells (T24 cells) for 1 hour and the number of bacteria adhered to cells was recorded.

Results

Cysticlean® has demonstrated a dose-dependent inhibition of bacterial adherence. At concentrations of 5, 25, 75 and 137 mg PACs/mL, Cysticlean® decreases the number of bacteria adhered to epithelial cells by 17%, 52%, 76 and 89%, respectively.

Conclusions

Cysticlean® capsules (240 mg/capsule of PACs) induces a dose-dependent significant decrease in the number of E. coli adhered. Previous clinical assays showed that Cysticlean® is a product highly recommended in the prophylaxis and treatment of UTIs. The present study confirms that the amount of proanthocyanidins is very important to achieve greatest effectiveness of the cranberry products.

BACKGROUND

UTIs (Urinary tract infections) are a frequent problem in primary care and it refers to microbial colonization of the urine and infection of the structures of the urinary tract. Adhesion of bacteria to the uroepithelial cells is a key point in the development of urinary tract infection (UTI), and it is a major virulence factor in their complex pathogenesis. Therefore one of the most obvious ways to prevent UTIs is to reduce the adhesiveness of bacterial cells that could cause the infection. Moreover recurrent UTIs are caused by biofilm forming bacteria and there are a significant correlation between bacteria motility and biofilm production.

In a urinary tract infection (UTI), the bacterial adhesion is a critical first step prior to invasion. UTIs are mainly attributed to a highly heterogeneous group of Escherichia coli. Urothelial cell invasion and biofilm formation are two involved mechanisms. Bacterial motility is also associated with virulence of bacterial pathogens. The initiation of this process involves the adhesion of bacteria to the epithelium, allowing them to remain at the urinary tract despite the drag

effect of urinary flow. Those responsible for this adhesion are filamentous appendages (fimbriae or pili) on the surface of the bacteria, although there are also adherent structures, or adhesins, not fimbriated. The antiadherence activity is one of the therapeutic mechanisms to address the treatment and prophylaxis of UTIs. It is know that cranberry (fruit of Vaccinium macrocarpon Aiton) reduces bacteria adhesion, motility by change on bacterial cell morphology and the biomass of a biofilm.

Proanthocyanidins (PACs) are identified as the main responsible for the anti-adherence activity of cranberry. Cranberry PACs has the two types of linkages between epicatechin units, the B-type and the less common A-type. A-type linkages are particularly present in cranberry PACs and may be important structural features in the anti-adhesion process. This action could be related to the ability of proanthocyanidins (PACs) to bind to proteins, such as the adhesins present on these E. coli fimbriae [1-4]. A-type PACs from cranberry differs from B-type PACs found in most berry fruits, and are considered the main active ingredient for inhibiting P-fimbriated E-coli adherence to uroepithelial cells. They have demonstrated anti-adhesion effect of these A-type PACs against Escherichia coli, whereas B-type PACs from other fruits were devoid of anti-adherence properties. Moreover, the content of the PACs determines the effectiveness of a cranberry product in a concentration-dependent manner.

Cranberry products show a significant inhibition of E. coli adherence, in vitro and ex vivo, to uroepithelial cells and other different substrates [5-11].

Cranberry products, with the appropriate amount of PACs, have been clinically proven to be highly effective, as a co-adjuvant treatment, in helping to reduce UTIs recurrence. This use is supported by Crochrane revision (2012), ESCOP monograph, meta-analysis and different published reviews. But it is important to note that the level of the PACs determines the effectiveness of a cranberry product. Clinical evidence supports that the consumption of the PACs of the cranberry products prevents the recurrent UTIs [12-16]. This effect has mainly been studied in women, particularly in women with recurrences, but has also shown a significant reduction in the frequency of these infections in men and in children. Cranberry treatment is a safe, well-tolerated supplement that does not have significant drug interactions [17].

The first review of the Cochrane Library (2001) about cranberry for the prevention and treatment of UTIs concluded that there was preliminary evidence supporting its efficacy. A second Cochrane review of 2008 identified 10 studies on the use of cranberries to prevent UTI and highlighted that cranberries are effective for the prevention of recurrent UTI, especially in young sexually active women [18]. The last Cochrane revision highlights the need for quantified preparations using standardized methods to ensure the potency and enough content of PACs, since, probably the cause of the lack of efficacy observed in some preparations of cranberry juice is lower PACs amount administered [13, 19]. Moreover, meta-analysis was performed using the data from four randomized controlled trials [20-22], where women, subjects with spinal cord injury or the elderly people were involved [18, 23]. And the results show that cranberry products significantly reduced the incidence of symptomatic UTIs in 12 months compared with placebo or control, particularly in women with recurrent UTIs. The Society of Urologic Nurses and Associates has concluded that cranberry products have been posited to prevent or treat UTIs [16].

In preclinical studies, human uroepithelial cells and human urinary bladder carcinoma cell line (T24) are often used in experimental in vitro assays. The use of a bladder epithelial cell line increases the reproducibility of the adherence test by comparison with uroepithelial desquamated cells coming from volunteers or patients [24]. Other studies test for the ability to agglutinate red blood cells using a mannose-resistant human red blood cell (HRBC) assay. Martino et al. [24] developed a bioassay to test the adhesion to the T24 bladder epithelial cell line of bacteria grown in urine samples collected after placebo or cranberry preparation drinking in a double-blind procedure.

The cranberry extract product Cysticlean® is used in the prevention and treatment of recurrent UTIs. Cysticlean® tables and sachets showed a significant inhibition of E. coli adherence, in vitro and ex vivo, to uroepithelial cells. Cysticlean® exhibited in vitro a potent inhibition of adherence of E. coli adherence, pre-incubated in different concentrations of product after the incubation with T24 cells for 1 hour. For these in vitro adherence assays has been used an adaptation of the method proposed by Gupta et al. [25], with T24 cells and using the E. coli strain ATCC 10536. Statistically significant differences were observed between the control group and all the treated groups with different

concentrations of Cysticlean® (5, 25 and 75 mg/mL of PACs) [9].

Additionally, Cysticlean® has shown a beneficial effect as a prophylactic treatment of UTIs in women and in children. Cysticlean® products produces a reduction in habitual recidivism, including recurrent post-coital UTIs, and it is also observed a great improvement in symptoms such as urinary frequency, dysuria, burning during urination, haematuria and leukocyturia in sediment. Moreover, the use of Cysticlean® is safe and well-tolerated [26-30].

The new formulation of Cysticlean as capsules provides 240 mg of PACs (vanillin-HCl modified method). The objective of this study is the comparison of the in vitro effect of different concentrations of Cysticlean capsules on adherence of Escherichia coli to bladder epithelial cells.

METHODS

Cysticlean® samples

The batches 140614 of Cysticlean® capsules (240 mg/capsule of PACs) and 20110428 of the previous Cysticlean® capsules (118 mg/capsule of PACs) have been used.

The test concentrations of PACs have been adjusted in McCoy's 5a modified medium, according to the human doses: 137 mg/mL (for Cysticlean 240 mg/capsule of PACs) and 75, 25 and 5 mg/mL (for Cysticlean 118 mg/capsule of PACs).

Preparation of the cell culture

Human urinary bladder carcinoma cells (T24, ATCC HTB-4TM) (1.1 x 106 cells/mL) were grown in McCoy's 5a modified medium, supplemented with fetal calf serum (10%), L-glutamine (10 M), penicillin (100 U/mL) and streptomycin (100 mg/mL). Cells were grown on chambered glass slides (Chamber slide system, Lab Tekâ) to obtain confluent monolayers, and were incubated at 37ºC, for 72 hours in a humidified atmosphere of 5% CO2.

Preparation of bacterial suspensions and pre-incubation period

E. coli strain ATCC 10536 was grown on TSA plates, at 37ºC. Afterwards, it was collected in PBS. After washing, it was collected in McCoy's 5a modified medium and re-suspended at a concentration of approximately 4 x 108 cfu/mL. This bacterial suspension was re-suspended in different concentrations (volume: 1 mL) for each treatment (in triplicate form). Pre-incubation under agitation at 37ºC for 30 minutes was performed.

Test procedure

To perform the adherence assay, monolayers were washed three times with PBS. Afterwards, the pre-incubated bacterial suspension was added and incubated at 37ºC for 1 hour. Cells treated with McCoy's 5a modified medium alone served as control. After incubation, the supernatants were decanted and the monolayers washed five times with PBS. The cells were fixed with methanol and stained with Giemsa 20% (v/v).

The number of bacteria adhered to the cells was recorded for 30 cells (10 cells for each replicate). The average number of bacteria adhered per cell was calculated for each treatment group. The percentage of inhibition with respect to the control group was also calculated for each concentration of the products tested. The results obtained were compared using a one-way analysis of the variance (ANOVA). Were significant differences were found, the Student-Newman-Keuls test was done $p<0.05$.

RESULTS

In vitro inhibition of the E. coli adherence

The Table 1 shows the mean of the number of bacteria adhered to cells for each concentration of Cysticlean® capsules and the inhibition percentage. The highest number of bacteria adhered per cell was observed in the control group.

Table 1

Effect of Cysticlean® on the adherence of Escherichia coli Results of the in vitro assay used for the evaluation of the effect of Cysticlean® capsules on the adherence of E. coli to T24 uroepithelial cells.

Treatment	Dose (µg PACs/mL)	Mean of the number of bacteria adhered per cell (± SD)	Inhibition (%)
Cysticlean® capsules	5	26.8 ± 0.58	17.3
	25	15.5 ± 0.44	52.2
	75	7.8 ± 0.28	75.9
	137	6.4 ± 1.13	88.6

No bacteria were observed in cells only treated with McCoy's 5a Modified Medium.

Statistically significant differences were observed between the Control Group and groups treated with Cysticlean® at 5,

25, 75 and 137 µg PAC/ml (Student-Newman-Keuls test $p<0.01$).

This anti-adherence activity of Cysticlean® is a dose-dependent effect. The highest inhibitory effect was observed at a concentration of 137 mg/mL of PACs (89%). At the lowest concentration (5 mg/mL of PACs) the inhibition was 17%. Moreover, at the concentration of 25 mg/mL and 75 mg/mL of PACs) the inhibition was 52% and 75%, respectively.

DISCUSSION

One important property of E. coli is its adherence to the host tissue. The bacterial adhesion is a critical first step prior to invasion. In fact, if E. coli cannot attach to the inner urinary wall it cannot colonize and grow. Actually, without adhesion, the bacteria cannot infect the mucosal surface.

Therefore, anti-adhesion is a functional concept for prevention of pathogens, and cranberry has demonstrated its ability to inhibit the bacterial adhesion and colonization. For this objective, robust in vitro assays for bacterial adhesion on host cells have long been used for the screening of potential therapeutic agents for the ability to minimize pathogen colonization of human tissues. Human uroepithelial cells and human urinary bladder carcinoma cell line (T24) are often used in these experimental in vitro assays.

Previous in vitro, showed that Cysticlean® tablets and sachets produces a potent dose-dependent inhibition of E. coli adherence, pre-incubated in different concentrations of product after the incubation with T24 cells for 1 hour. For these in vitro adherence assays has been used an adaptation [9] of the method proposed by Gupta et al. [25], with T24 cells and using the E. coli strain ATCC 10536.

In the present study, with the same experimental assay, Cysticlean® capsules, at concentrations of 5, 25, 75 and 137 mg/mL of PACs, decrease the number of bacteria adhered to uroepithelial cells by 17%, 52% 76% and 89% respectively. It can be observed that the highest inhibition of Cysticlean® capsules was obtained at the concentration of 137 mg/mL of PACs.

Moreover, Cysticlean® have also demonstrated, ex vivo, a dose-effect inhibition of bacterial adherence [9].

The dose-dependent effect of PACs has already been described in several studies, although a range of lower concentrations [24, 31]. In addition, although some laboratories proposed dose of 36 mg/day of PACs, it is interesting that subsequent studies have shown that higher doses of PACs provide greater activity, and have confirmed that the consumption of cranberry dosages containing 72 mg of PACs is more effective than 18 or 36 mg of PACs [32]. However, as already discussed and demonstrated, higher doses as provided by Cysticlean® produce a greater benefit in clinical studies and it can be established the dose of 240 mg as adequate daily amount of PACs.

The mechanism of action implicated in the effect of cranberry of E. coli adherence it has been reported by the interactions between cranberry and the surfaces of E. coli. It is probably related with the decrease in the adhesion forces of the fimbriae between the cell surface and the bacteria [6, 33-35].

Additionally, Cysticlean® has shown a beneficial effect as a prophylactic treatment of UTIs in women [26-27, 29] and in children [28]. These results showed the high efficacy and good tolerance of Cysticlean® in children and women with frequent urinary tract infections. And as has been demonstrated for other cranberry products, the use of Cysticlean® could reduce the antibiotic use. It is also important to know that the anti-adherence activity associated with cranberry consumption is not related to antibiotic sensitivity or resistance [36].

CONCLUSIONS

Previous clinical assays showed that Cysticlean® is a product highly recommended in the prophylaxis and treatment of UTIs. For people with recurrent uncomplicated UTIs, PACs of cranberry products as Cysticleanâ may offer an alternative methodology to antibiotic prophylaxis and an improvement of their symptomatology.

Cysticlean® capsules has been shown a dose-dependent effect on adherence of Escherichia coli to uroepithelial cells, and this activity is related to the PACs content in the cranberry extract.

The present study confirms that the amount of PACs is very important to achieve greatest effectiveness of the cranberry products in the management of UTIs.

Competing interests

The authors declare that they have no competing interests.
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