

Proposed dual antagonist approach for the prevention and treatment of urinary tract infections caused by uropathogenic *Escherichia coli*



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ABSTRACT

Urinary tract infections are among the most common infectious diseases worldwide, primarily caused by uropathogenic *Escherichia coli* (UPEC) strains that harbor type I pili and P pili on the surface. Standard *E. coli* therapy still entails antibiotic consumption, but urinary tract infections tend to recur at a very high rate. Due to the emergence of antibiotic resistant strains of UPEC, as well as high infection recurrence rates, there is a need for new approaches to efficiently treat and prevent urinary tract infections. Since aforementioned adhesive organelles are the principal virulence factors in UPEC, anti-adhesion strategy seems to be the most promising (and hitherto unexplored) treatment option. Here we propose an antiadhesive dual targeting approach towards FimH and PapG adhesive proteins placed on two key virulence factors for UPEC – type I fimbriae and P pili. Such dual antagonists will contain appropriate pharmacophores (mannose and natural cranberry-containing polyphenol) joined together and will more efficiently block the infection and prevent the progression of the disease in comparison to FimH and PapG as isolated targets. More specifically, polyphenol mannosides (due to the structural similarities with the most potent biaryl inhibitors) can act as high-affinity FimH ligands, while cranberry-associated polyphenol moiety can additionally inhibit the PapG-mediated adhesion. Proposed compound may also contribute to the antioxidant capacity of the human organism. In conclusion, this dual-target hypothesis for the prevention and treatment of UPEC infections represents an important foundation for further research on this topic.

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases worldwide and are dominantly caused by uropathogenic *Escherichia coli* (UPEC) [1]. Most of UTIs manifest as a urinary bladder infection, also known as cystitis, which stems from pathogenic bacteria (such as UPEC) colonizing the perineum, subsequently traversing the urethra and infecting the bladder. If left untreated, bacteria may ascend the ureters to the kidneys and establish a secondary infectious process known as acute pyelonephritis, which may give rise to renal scarring and permanent damage of the kidneys. UPEC strains, broadly categorized as extraintestinal pathogenic *E. coli* (ExPEC), have evolved a multitude of virulence factors that enables bacterial growth and persistence within the host urinary tract [2].

Adherence is first step in UTI pathogenesis. During the adherence the specific cell surface-associated proteins, the bacterial adhesins, recognize complementary antigens of the eukaryotic cell and attach to the surface of the urinary epithelium. As already mentioned, UTI typically starts with periurethral contamination by UPEC, followed by colonization of the urethra and migration of the pathogen to the bladder. UPEC-induced exfoliation of the host cells permits UPEC to colonize and persist in the urinary tract. This is one of the main reasons for long-term persistence of UPEC within the urinary tract with a high

recurrence rate. Even though efficient antibiotics (such as beta-lactams and fluoroquinolones) are used as standard treatment for UTI, antibacterial resistance and high recurrence rates emphasize the importance of developing alternative strategies for the prevention and treatment of UTIs. Since adhesive organelles (fimbriae type 1, P, S and F1C pili) are main virulence factors in UPEC, anti-adhesion approach seems to be the most promising alternative therapy option [3]. And indeed, certain anti-adhesive drugs that are promising alternatives to antibiotic treatment of UTIs have already been developed, paving the way for applicable compounds [4].

Hypothesis

Type I fimbriae and P pili are two of the most important adhesive organelles encoded by many UPEC strains and key virulence factors for UPEC. Specific adhesin proteins localized at the distal tip of type I fimbriae and P pilus are FimH and PapG, respectively. Type I fimbriae shows specificity towards mannose and P pili towards phenolic compounds. Our hypothesis is that dual antagonists comprising of mannose and polyphenol subunit would facilitate the suppression of UPEC infection more efficiently by a double-target action.

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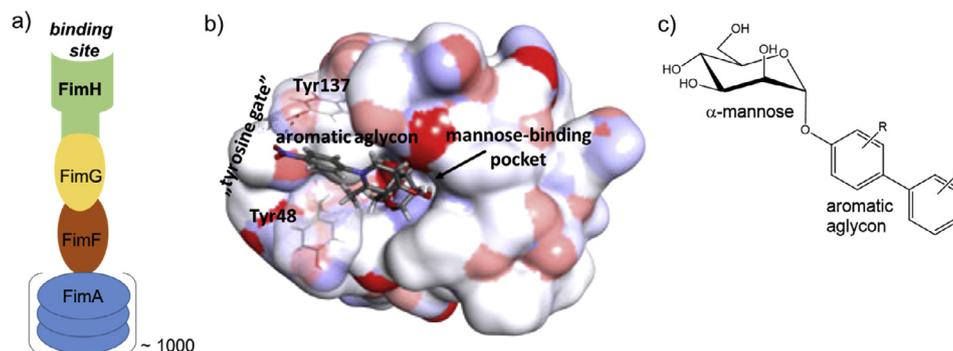


Fig. 1. General structures of: a) type I fimbriae, b) aromatic mannoside in the FimH binding site, c) efficient class of FimH inhibitors.

Evaluation of the hypothesis and the rationale behind it

Type I fimbriae

Type I fimbriae organelles are built of repeating immunoglobulin-like FimA subunits joined to a two adaptor proteins, FimG and FimF, and the adhesin protein FimH located at the top of the pili (Fig. 1a). FimH adhesin specifically recognizes terminal mannose of mannose-ligated receptors on the surface of mammalian bladder epithelial cells including integral membrane glycoprotein called uroplakin 1a (UPa1) present in bladder surface [5–7]. Crystal structure of FimH revealed that binding site of FimH is comprised of a polar mannose-binding pocket and a hydrophobic regions surrounding the polar binding pocket (Fig. 1b). Based on FimH structure, efficient specific ligands have been developed. Key structural characteristics of the most effective anti-adhesive agents are: (i) α -anomeric configuration on mannose and (ii) biaryl aglycon (Fig. 1c). Aromatic aglycon subunit enhance binding affinity to FimH by the additional interactions in the hydrophobic region of FimH called “tyrosine gate” [8–10]. These mannosides are orally bioavailable, effective against multidrug-resistant UPEC (nanomolar affinity range) and known to potentiate antibiotic efficacy [11]. Furthermore, studies in mouse models have confirmed that they significantly reduce bladder colonization and invasion [12].

P pili

P pili are composed of several proteins: PapA, PapK, PapE, PapF and PapG (Fig. 2a). Adhesion protein PapG is placed at the top of the P pili and binds to digalactoside (galabiose) units found on the surface epithelial cells lining urinary tract [13]. Three distinct PapG adhesions have been identified: PapG I, II and III. PapGII is predominantly associated with human pyelonephritis and specifically binds to globotetraosylceramide ligand, globoside (GbO4). GbO4 binds in the binding pocket in V-shape allowing stabilization of receptor-ligand complex by hydrophobic interactions with non-polar part of GbO4 and polar H-bonding with hydroxyl groups of tetra-saccharide GbO4 [14].

Cranberry-associated polyphenols

Previous studies have reported a positive influence of the consumption of cranberries on prevention of UPEC adhesion. Exact mechanism of action is still not known but investigations showed that proanthocyanidins (PAC) of A-type efficiently block the P fimbriae [15–18]. It was indicated also that PAC-metabolites, monomeric cranberry-associated flavonoids, which have higher bioavailability than PACs could be responsible for the antiadhesive effects [19]. General structure of polyphenol backbone present in cranberry bioactives is shown at Fig. 2b.

Additionally, we would like to notice that flavonoids as free radical scavengers could benefit patients taking an UTI therapy due to their

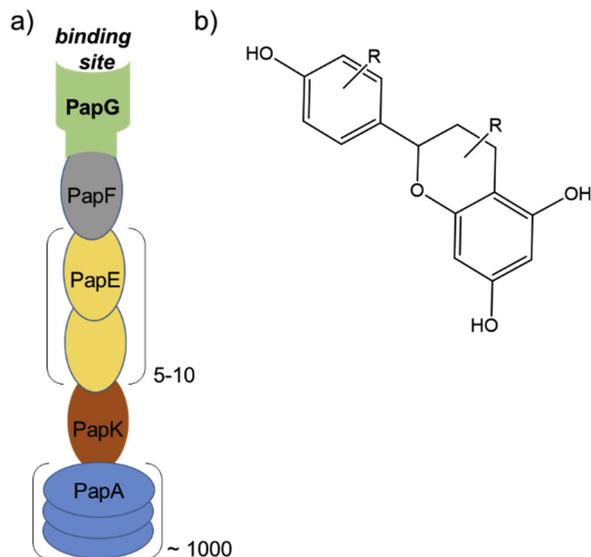


Fig. 2. General structures of: a) P pili, b) polyphenolic backbone of cranberry bioactives acting as inhibitors of PapG adhesion.

anti-oxidant activity which may minimize oxidative stress caused by UTI [20]. There is a reasonable body of evidence showing that the consumption of cranberry juice (or cranberry bioactives for that matter) decreases specific blood markers of oxidative stress, with a plethora of parallel benefits for the healthy functioning of human organ systems [21]. Moreover, cranberry-associated flavonoids have been shown to increase urinary and plasma concentrations of salicylic acid, which in turn affects enzymatic pathways that are activated during inflammatory responses during UTI [21].

Combining pharmacophores for dual-target action

Considering the fact that FimH and PapG proteins are highly conserved targets among *E. coli* and the molecular basis of key interactions for the adhesion process is known, fragment-based drug design seems to be a promising approach for the generation of enhanced antiadhesives with increased affinity for two targets. Therefore, we propose dual-target agents prepared by the methodical combination of pharmacophores from selective single-target ligands as efficient antagonists for FimH and PapG proteins. Dual antagonists will contain appropriate pharmacophores (mannose and natural cranberry-containing polyphenols) joined together. Polyphenol mannosides, due to the structural similarities with the most potent biaryl inhibitors, can act as high-affinity FimH ligands. General structure of proposed dual-target antagonist is presented at Fig. 3. Stabilization of FimH receptor-ligand complex will be enabled by H-bonding and π -stacking between mannoside and residues in FimH binding site. Cranberry-associated

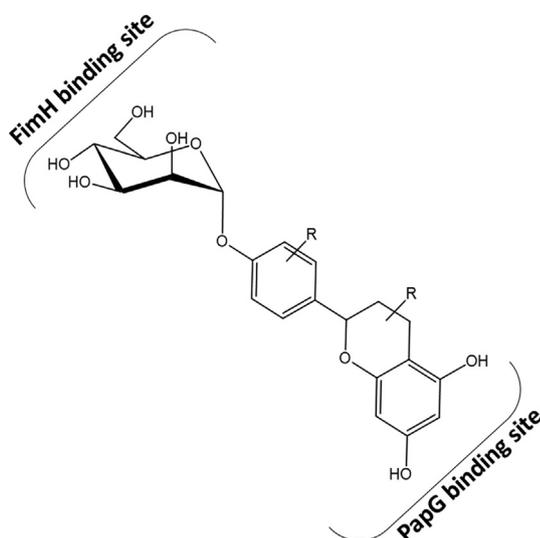


Fig. 3. General structure of proposed antagonists for dual-target action.

polyphenol moiety of dual antagonists can inhibit the PapG-mediated adhesion in addition. We suggest balanced modulation of dual targets which could provide a superior therapeutic effect compared to the action of a selective ligand.

How to test this hypothesis

Novel class of antiadhesive compounds will be synthesized by common methods allowing selective chemical transformations. After detailed structural characterization of prepared compounds the affinity of prepared dual antagonist series toward FimH and PapG adhesins will be screened using functional cell-based assay and adhesion-inhibition assay. The kinetics and thermodynamics of ligand binding will be precisely measured by cell-free assays such as isothermal titration calorimetry (ITC) [22]. A molecular simulation will be applied in order to study binding and conformational changes of inhibitors and adhesins. The results obtained by these assays will be compared with a cocktail of the mannose and corresponding phenolic compounds acting as inhibitors of FimH and PapG adhesins, in order to determine the strength of dual-antagonist effect of conjugates.

Additionally, antioxidant properties of compounds will be explored by antioxidant assay in order to measure the contribution of phenolic subunit toward radical scavenging. In the following phase questions about drug safety should be answered and therefore pharmacokinetic and pharmacodynamics profile of lead compounds will be determined. Study of ADME (absorption, distribution, metabolism, and excretion) and toxicological parameters will be performed using assays such as solubility, rate of dissolution, membrane permeability, active transport, ionisation constant, lipophilicity, chemical stability, metabolic clearance, cytochrome P450 interactions, protein-binding, metabolite identification and metabolic pathways.

Alongside the investigation of antiadhesive properties with aforementioned *in vitro* adhesion assays, *in vivo* studies in BALB/c mice strains will be used as a UPEC infection model (akin to some other studies) [23]. The effect of the dual antagonist on bacterial load in bladder tissue will be monitored in the animals at designated time points, while potential cytotoxic effects and dose dependent anti-adhesive activity will be assessed accordingly. Extensive preclinical toxicity studies will be conducted on animals before entering a phase I clinical trial in humans.

Conclusions

The suboptimal protection against recurring UTIs and the potential

risk of generating resistance among the bacterial pathogens are two pivotal reasons why there is a substantial need for alternative approaches in the management of UTIs. Main virulence factors included in the initiation of UTI are type I pili and P pili located at UPEC surface. Here we propose the development of dual antagonists which will target FimH and PapG adhesins present at type 1 and P pili, respectively. Dual-target binding of mannose and phenolic moieties will block the infection and prevent disease progression more efficiently.

Furthermore, proposed compounds will additionally contribute to the antioxidant capacity of the human organism. Urinary tract infections result in oxidative stress primarily by consuming urinary antioxidant enzymes, and studies have shown that the overproduction of free radicals that are generated during urinary infection lead to low levels of available antioxidant enzymes [24]. Therefore, this dual-target hypothesis for the prevention and treatment of UPEC infections represents an important foundation for further research on this topic.

Conflict of interest statement

All authors declare that there is no conflict of interest in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.01.010>.

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