International Journal of Pharmaceutics 495 (2015) 710–718

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Glycan-mediated uptake in urothelial primary cells: Perspectives for improved intravesical drug delivery in urinary tract infections

Clara Maria Pichl, Sophie Feilhauer, Rose-Marie Schwaigerlehner, Franz Gabor, Michael Wirth, Lukas Neutsch*

Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstr. 14, 1090 Vienna, Austria

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form 8 September 2015
Accepted 11 September 2015
Available online 14 September 2015

Keywords:
Lectin
UPEC
Urinary tract infection
Instillation
Antibiotic
Internalization

ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections. Despite a wide range of therapeutic options, treatment success is compromised by multiresistance and the efficient mechanism of tissue colonization of uropathogenic *Escherichia coli* (UPEC). In advanced drug delivery systems, a similar, glycan-mediated targeting mechanism may be realized by conjugating the drug to a plant lectin. This may lead to the drug being more efficiently accumulated at the desired site of action, the bacterial reservoirs.

In this study, we aimed at elucidating the potential of this biorecognitive approach. Glycan-triggered interaction cascades and uptake processes of several plant lectins with distinct carbohydrate specificities were characterized using single cells and monolayer culture.

Due to pronounced cytoadhesive and cytoinvasive properties, wheat germ agglutinin (WGA) emerged as a promising targeter in porcine urothelial primary cells. The lectin–cell interaction proved highly stabile in artificial urine, simulating the conditions in actual application. Colocalisation studies with internalized WGA and lens culinaris agglutinin (LCA) revealed that intracellular accumulation sites were largely identical for GlcNAc- and Mannose-specific lectins. This indicates that WGA-mediated delivery may indeed constitute a potent tool to reach bacteria taken up via a FimH-triggered invasion process. Existing pitfalls in intravesical treatment schedules may soon be overcome.

1. Introduction

Urinary tract infections (UTIs) range among the most common bacterial infections and represent a severe burden for the health care system due to high medical and societal costs (Foxman et al., 2000; Litwin et al., 2005; Sivick and Mobley, 2010). Especially in the nosocomial setting, chronic and/or multi-resistant UTIs are a pending problem that is complex to manage. The majority of UTIs (80%) are caused by uropathogenic *Escherichia coli* (UPEC) (Hooton et al., 2004; Kucheria et al., 2005). Though UPEC are often seen as extracellular pathogens, they were observed to invade urothelial host cells via a zipper-like mechanism (Martinez et al., 2000; Mulvey et al., 2000). After this internalization, UPEC can either enter a quiescent state and durably persist within the urinary tract (Mulvey et al., 2000) or, alternatively, can be multiplied in the host cytosol within superficial facet cells of the urothelium, forming large biofilm-like inclusions, so-called intracellular bacterial communities (IBCs) (Anderson et al., 2003; Justice et al., 2004; Mulvey et al., 2001). In a mouse model, UPEC reservoirs could not be effectively eradicated by antibiotics, though the drug concentrations detected in urine exceed the minimal inhibitory doses by far. This was attributed to the poor penetration of antibiotics being impeded by the rigid urothelial barrier (Blango and Mulvey, 2010; Hvidberg et al., 2000; Kerrn et al., 2005). Moreover, the release of large numbers of UPEC and the following infection of surrounding cells are assumed to trigger recurrent UTIs (Schilling et al., 2001).

Numerous antibiotics have shown to be effective at ameliorating the clinical symptoms, but recurrent and chronic infections continue to afflict many individuals (Nicolle, 2003). In catheterized patients, the intravesical instillation of antibiotic agents can present an effective salvage therapy (Arap and Petrou, 2003), but treatment impact is limited by constant washout processes and the dilution with freshly secreted urine. However, the management of UTIs might become complicated since frequent retreatment or

* Corresponding author.
E-mail addresses: clara.maria.pichl@univie.ac.at (C.M. Pichl), franz.gabor@univie.ac.at (F. Gabor), michael.wirth@univie.ac.at (M. Wirth), neuc@zhaw.ch (L. Neutsch).

http://dx.doi.org/10.1016/j.ijpharm.2015.09.017
0378-5173/

long-term prophylaxis can lead to the problem of antibiotic resistance (Hooton et al., 2004; Mehnert-Kay, 2005; Schilling et al., 2002).

The limitations of currently established therapy schedules and the difficulties in eradicating the causative pathogen require the development of innovative strategies for an improved delivery of antibiotics. To date, methods that aim to optimize drug uptake by exploiting the interaction between a targeting ligand and complementary epitopes at the urothelial cell surface are scarce. The potential of such strategies is impressively demonstrated by the invasion mechanism of UPEC: by aid of adhesive filaments called type 1 pili that are produced by more than 80% of all UPEC (Langermann et al., 1997), the gram-negative bacterium is able to ascend the urinary tract and colonize the bladder. The subunit FimH, located at the pilus tip binds to mannose-containing glycoprotein host receptors (Eto et al., 2007; Hung et al., 2002) and thereby facilitates attachment to and invasion of uroepithelial cells (Hung et al., 2002; Thankavel et al., 1997). A drug's ability to follow the bacteria via the same uptake route could greatly improve treatment outcome. The key function of FimH, which is necessary for this, may be imitable by using carbohydrate-binding proteins, so called lectins.

The adhesion to mannose or other sugar residues of the cellular glycocalyx might thus be a promising biorecognitive delivery approach. The resultant increase in drug–cell interaction could lead to prolonged residence times and enhanced accumulation at the urothelial target. This might improve the efficacy of drugs and overcome natural limitations in the urinary bladder such as dilution processes and loss of drug by urinary excretion (Gabor et al., 2004; Neutsch et al., 2012).

For the development of such advanced delivery strategies, *in vitro* models are a very useful tool to gain first biological insights, since they are easy to maintain, allow for high-throughput screening, and offer straightforward comparability to animal experiments (Hartung and Daston, 2009).

In this study, we aimed to elucidate the potential of lectins as delivery vectors in the context of UTI treatment. A thorough characterization of the involved mechanistic determinants was performed using primary porcine urothelial cells as a model system, due to the marked anatomical and physiological similarities to the human bladder (Desgrandchamps et al., 1997; Ludwikowski et al., 1999). In total, six plant lectins with distinct carbohydrate specificity were tested for their binding behavior and selectivity for certain sugar moieties on both, single cells and monolayer samples. Moreover, the mechanisms determining uptake and intracellular fate of selected lectins were assessed. By showing that biorecognitive interaction may indeed prove utilizable to follow bacteria into their protected reservoirs, new therapeutic approaches for more efficient eradication of UPEC can be envisioned on basis of this approach.

2. Materials and methods

2.1. Chemicals

Fluorescein-labelled lectins from *Triticum vulgare* (WGA, molar ratio fluorescein/protein (F/P) = 4.5), *Arachis hypogaea* (PNA, F/P = 10.1), *Lens culinaris* (LCA, F/P = 2.7), *Galanthus nivalis* (GNL, F/P = 1.9), *Dolichus biflorus* (DBA, F/P = 2.3) and *Ulex europaeus* (UEA, F/P = 3.2) were purchased from Vector Laboratories (Burlingame, USA). N,N′,N″-triacetylchitotriose, *N*-acetyl-D-galactosamine, D-galactosamine, L-fucose, D-mannose and monensin were from Sigma (St. Louis, USA). K-SFM (keratinocyte serum-free medium), BPE (bovine pituitary extract), EGF (epidermal growth factor), Alexa Fluor® 594/647 conjugate of WGA (aWGA) and Alexa Fluor® 594/647 conjugate of albumin from bovine serum (aBSA) were bought from Life Technologies (Carlsbad, California). Trypsin inhibitor was purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany).

Artificial urine was prepared according to the protocol of Chutipongtanate and Thongboonkerd, by dissolving 2.427 g of urea, 0.034 g of uric acid, 0.090 g of creatinine, 0.297 g of $Na_3C_6H_5O_7{\cdot}2H_2O$, 0.634 g of NaCl, 0.450 g of KCl, 0.161 g of NH_4Cl, 0.089 g of $CaCl_2{\cdot}2H_2O$, 0.100 g of $MgSO_4{\cdot}7H_2O$, 0.034 g of $NaHCO_3$, 0.003 g of $Na_2C_2O_4$, 0.258 g of Na_2SO_4, 0.100 g of $NaH_2PO_4{\cdot}H_2O$ and 0.011 g of Na_2HPO_4 in 200 ml of distilled water (Chutipongtanate and Thongboonkerd, 2010).

2.2. Culture of porcine urothelial cells

Porcine bladders obtained from a local abattoir were processed according to an optimized protocol to isolate urothelial cells (Southgate et al., 2002).

Isolated primary cells were further maintained in K-SFM medium containing EGF (5 ng/ml), BPE (50 μg/ml), cholera toxin (30 ng/ml) and gentamycine (60 μg/ml) and subcultured by trypsinization. For passaging, cells were incubated with 5 ml of PBS containing 0.1% EDTA (w/v) for 5 min at 37 °C, resulting in cell rounding. After aspiration of the PBS, the cells were incubated with 1 ml Trypsin/EDTA 0.25% for 5 min at 37 °C in order to detach the cells from the surface. Trypsinization was terminated by addition of 5 ml K-SFM containing 2.5 mg trypsin inhibitor. After centrifugation (1000 rpm, 7 min, RT) and aspiration of the supernatant, the cell pellet was resuspended in 2 ml of K-SFM. For further cultivation, cells were seeded on gelatin-coated 75 cm^2 tissue flasks at a concentration of 2×10^6 cells.

2.3. Flow cytometry and fluorimetry

Flow cytometric measurements were carried out on a Gallios flow cytometer (485/525 nm; Beckman Coulter, Brea, California). Cell-bound fluorescence intensities were determined using a forward versus side scatter gate to include the single cell population and exclude cell aggregates and debris. The mean channel number of the logarithmic fluorescence intensities of individual peaks was used for further calculations. At least 3000 cells were accumulated per analysis run and all experiments were carried out in triplicate.

For monolayer studies, cells were seeded on tissue culture-treated 96-well microplates at a density of 5×10^4 cells/well and grown to confluence. Cell-associated fluorescence intensity was detected at 485/525 nm (ex/em) using a fluorescence microplate reader (Infinite M200, Tecan, Grödig, Austria). All assays were carried out in quadruplicate.

2.4. Determination of the lectin binding capacity of porcine urothelial cells

Interaction between the cell surface and the targeting ligand was assessed for all lectins shown in Table 1 using both single cells and monolayer culture.

Single cells were processed immediately after harvesting by trypsinization. 50 μl of cell suspension (2.5×10^5 cells) were incubated with 50 μl of lectin solution (16–4000 pmol/ml, serial dilution) for 30 min at 4 °C. The cell suspension was resuspended in 1 ml of particle-free PBS plus Ca^{2+}/Mg^{2+} (PBS) and the RFI was determined via flow cytometry. Unlabelled cells were included for estimation of the autofluorescence of the cells and the acquired values were subtracted from all data.

Confluent monolayers were washed twice with 100 μl of PBS prior to incubation with 50 μl of lectin solution (3–3200 pmol/ml, serial dilution) for 30 min at 4 °C. Unbound lectin was removed in