49

Moreira C[1], Sperandio V[1], Bacsu C[1], Glover M[1], Zimmern P[1]
1. *UT Southwestern Medical Center*

BIOFILMS FROM URINARY AND BLADDER WALL E.COLI ISOLATES FROM PERI-MENOPAUSAL WOMEN WITH RECURRENT URINARY TRACT INFECTIONS ARE SENSITIVE TO A QUORUM SENSING (LED 209) INHIBITORY AGENT.

Hypothesis / aims of study

A promising strategy for developing new drugs against uropathogenic *E.Coli* (UPEC) is to identify agents that inhibit their virulence factors without inhibiting their growth. Bacterial pathogens rely on a membrane signaling kinase, QseC, to promote the expression of these virulence factors. LED 209, a small molecule which is not toxic, can inhibit the binding of signals to QseC [(1)]. We studied the effect of LED 209 on human urinary and bladder wall biofilms from women with recurrent urinary tract infections (rUTI) and used 2 well characterized UPEC bacterial strains as controls.

Study design, materials and methods

Following IRB approval, peri-menopausal women with a longstanding history of rUTI diagnosed with extensive trigonitis [as defined by office cystoscopic findings of inflammation of the trigone including pustules, bullous lesions and/or submucosal calcifications] were scheduled for bladder biopsies and fulguration of trigonitis under anesthesia on an outpatient basis. Deep cold-cup trigonitis biopsies as well as control biopsies from non-infected sites, and urine cultures were systematically obtained. Bladder biopsies were immediately placed in LB medium at pH 7.4, and plated on LB and McConkey agar. Bacterial characterization was obtained with the api® 20 E kit (Biomerieux). The ability of these identified bladder wall bacteria to produce biofilm was confirmed by an established biofilm assay using crystal violet and spectrophotometry.
In addition, the urine samples of another set of perimenopausal women with rUTI and documented positive urine cultures were also studied to isolate UPEC and test their ability to form biofilms. Then, biofilms from UPEC obtained from urine samples and bladder wall biopsies, along with established UPEC strains: UTI89 [(2)] and CFT073 [(3)], were tested with the addition of LED 209 (5 nM) to study its ability to reduce biofilm formation. All measurements were done in triplicates.

Results

Over the past year, 8 Caucasian patients (mean age 67 years, range 49-81) met inclusion criteria. Four grew bacteria (including 2 *E.coli* and 1 *Pseudomonas*) from the trigonitis biopsy site but none from the control sites (Table 1). Biofilm formation from these strains was similar for all UPEC, and markedly elevated for the Pseudomonas isolate as expected (Figure 1). Of 25 perimenopausal women with rUTI, 9 grew *E.Coli* and were tested for biofilm formation, along with UTI89 and CFT073 (Figure 2). The effect of LED 209 on UTI89 along with UPEC and urine sample isolates showed a statistically significant reduction in biofilm formation for 3 *E.Coli* and 3 non *E.Coli* bacterial strains compared to no LED exposure (Figure 3).

Interpretation of results

In the murine model, the presence of intrabacterial communities (IBC) causing recurrent UTI (rUTI) has been established [(2)]. In the few trigonitis biopsy isolates and in the positive urine samples from a separate set of rUTI women, we confirmed the effect of LED 209 to reduce biofilm formation in some strains. This discovery invites further research on the role of LED 209 to inhibit QseC-mediated activation of virulence gene expression, and its possible interaction with antibiotic therapy.

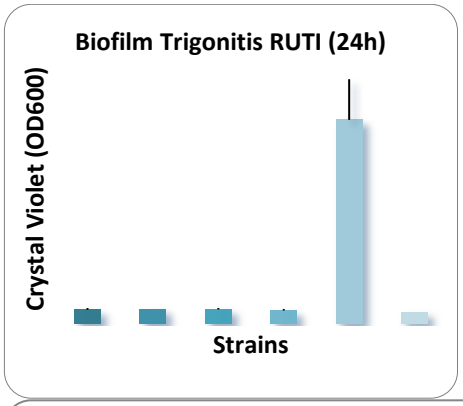
Concluding message

To our knowledge, this study indicates for the first time bacterial growth from the inflamed trigone of women with rUTIs, suggesting a link with the already established mouse model. Some UPEC urinary isolates from perimenopausal women with rUTI responded to LED 209. Reduction in biofilm formation by this anti-virulence factor drug opens new avenues for rUTI therapy.

Table1. Results of trigonitis biopsies.

Patient	Growth on LB Agar	Growth on McConkey agar	Lactose-fermenting	api® 20E Kit	Biofilm Growth
1	**-**	**-**			
2	**-**	**-**			
3	**+**	**+**	**+**	**E. coli**	**Positive**
4	**-**	**-**			
5	**+**	**+**	**-**	**P. aeruginosa**	**Positive**
6	**+ (Gram +)**	**-**			
7	**-**	**-**			
8	**+ (Gram +)**	**-**			

Figure 1: Biofilm Trigonitis RUTI (24h)



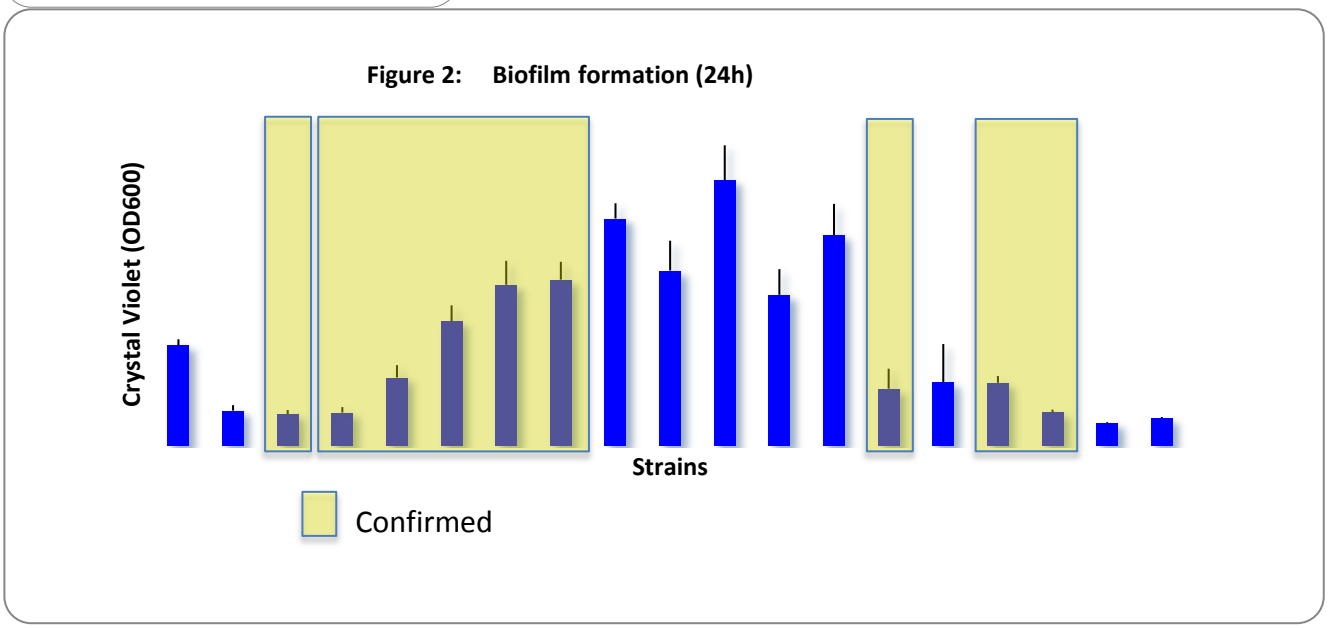
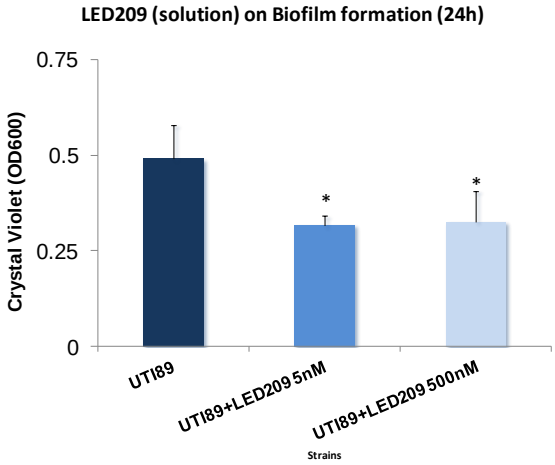
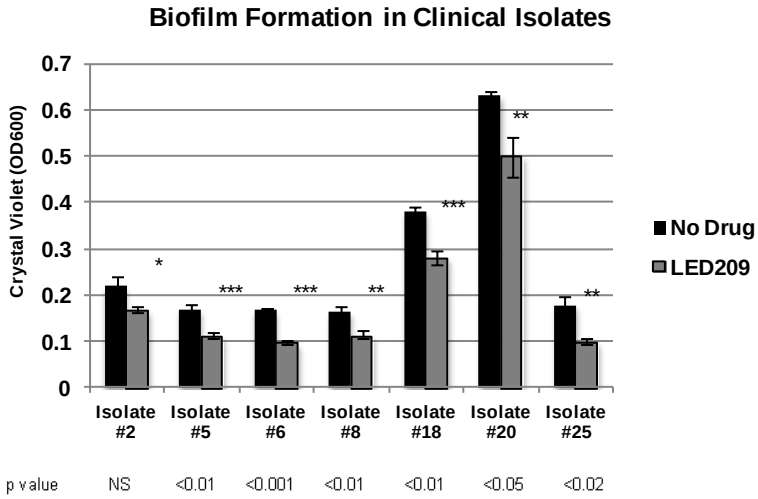


Figure 3 : LED 209 effect on Biofilm formation at 24 hours on UTI89 and selected urinary isolates from rUTI women





References
1. Sperandio, V., et al PNAS, 100:8951, 2003
2. Myrosekar and Hultgren: PNAS, 103:382, 2006
3. Mobley et al. Infect. Immun. 58:1281, 1990

Disclosures
Funding: The Cain Foundation **Clinical Trial:** No **Subjects:** NONE