Intracellular persistence of *Escherichia coli* in urinary bladders from mecillinam-treated mice

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Objectives: It has been suggested recently that intracellular bacteria surviving antibiotic treatment might serve as a reservoir for recurrent infection. The purpose of this study was to directly examine the location of *Escherichia coli* bacteria in the mouse bladder after treatment with mecillinam.

Methods: The bladders were studied by use of colony counts, in situ hybridization and electron microscopy.

Results: The bacterial counts in the bladder remained \( \sim 10^3-4 \) cfu/bladder even after mecillinam treatment had finished, and re-growth in the urine was observed. In the bladder epithelium from treated mice, bacteria cells were occasionally seen, presumably representing intracellularly located bacteria.

Conclusions: This is the first in vivo study indicating that during mecillinam treatment *E. coli* cells can penetrate the mouse bladder epithelium and persist.

Keywords: UTIs, mouse models, antibiotic treatments

Introduction

Acute uncomplicated urinary tract infections (UTIs) are a common problem affecting \( \sim 25\% \) of women, with *Escherichia coli* accounting for \( \sim 80\% \) of the cases.1 Recurrent UTIs occur in about 10%–40% of women.2 Although effective antibiotics are still available, resistance is a problem that has to be taken seriously. Recurrent UTI is correlated with short duration of treatment, with too low doses and with the type of antibiotic.3 Recent studies have shown that *E. coli* can invade the bladder’s epithelial cells, and it has been suggested that intracellularly located bacteria can persist as a reservoir for later re-infection.4

Only a few experiments have been performed in vivo on the persistence of bacteria in the bladder after antibiotic treatment. The purpose of this study was to directly examine the location of *E. coli* bacteria in the mouse bladder after treatment with mecillinam. At the end of treatment, the mice were followed for 2 weeks to find out whether bacteria surviving in the bladder re-established infection.

Materials and methods

Mouse model of urinary tract infection

A type 1 fimbriated *E. coli* (strain 21623884-114), isolated from a patient with UTI susceptible to mecillinam (MIC 0.5 mg/L) was used. The inoculation into the mouse bladder was performed on day 0 leaving the bladder with \( 5 \times 10^7-5 \times 10^8 \) cfu.5 The animal experiments were conducted under the auspices of the Animal Experiments Inspectorate, the Danish Ministry of Justice.

Antibiotic treatment and sampling

Mecillinam solution (Selexid; Leo, Copenhagen, Denmark) was injected subcutaneously, at a dose of 0.5 mg/mouse twice a day, 6 h apart, for 3 days. The first injection was administered the day after inoculation. Further details about treatment and sampling are as previously described.5
doses, respectively. Bacterial count determination was performed on the urine and kidneys. The bladders were fixed and studied by in situ hybridization and electron microscopy. Bacterial count determination was conducted on an additional three mice from each group (on day 4, 12 mice from each group).

**Study B.** For follow-up, six mice per group were sacrificed on days 5, 8, 12 (not control) and 18, and bacterial count determination was performed.

**Bacterial count determination**

The urine and organs were processed immediately after sampling, as described previously.5

**Fixation of bladders**

The bladder (of a killed mouse) was injected with 0.2 mL of Karnovsky fixative (KF) and after 15 min the bladder was isolated and further fixed for 60 min. The bladder was bisected lengthwise and fixed (KF) overnight at 4°C. One half of the bladder was dehydrated and embedded in paraffin for in situ hybridization, whereas the other half was prepared for ultrastructural studies (electron microscopy).

**In situ hybridization**

Sections, 5 μm thick, were mounted on glass slides and treated with xylene three times for 10 min, followed by 10 min of dehydration in 96% ethanol. After drying at room temperature, sections were circumscribed with a hydrophobic pen (Dako S2002; Dako, Glostrup, Denmark). Probe EC1531 (5'-CACCGTAGGTCGTCATCA) specific for *E. coli* 23S rRNA, labelled with CY3, was used. Hybridizations were performed as previously described.6

**Electron microscopy**

The specimens were post-fixed in 1% OsO4 (w/v) in 0.1 M cacodylate buffer, pH 7.2, containing 0.01 M CaCl2 for 90 min, followed by en bloc staining with 2% (w/v) uranyl acetate in barbiturate buffer, pH 7.3, for 60 min. After dehydration and embedding in Epon (glycidyl ether 100; Merck, Darmstadt, Germany), 1 μm sections were stained with Toluidine Blue and areas of interest selected. Thin sections were stained with uranyl acetate and lead citrate. Electron microscopy was carried out using a Philips 201C electron microscope at 60 kV.

**Results**

**Bacterial counts during and after treatment**

The course of the UTI is shown in Figure 1. In urine of the control group, 10^6–7 cfu/mL remained even after 18 days, whereas the treated mice had sterile urine on day 5 followed by re-growth on day 8 up to 6 × 10^4 cfu/mL. The bacteriuria thereafter declined and disappeared after 18 days. The bladders of the control group showed continuous infections, with a median of 10^5 cfu/bladder. In the treated mice, the cfu decreased from 10^5 cfu/bladder after one dose to 10^3 cfu/bladder on day 4 after the sixth dose. At the end of mecillinam treatment, the bacterial counts in the bladder remained with a median of 10^3–4 cfu/bladder even on day 18. In the kidneys of the control group, the amount of bacteria was 10^4 cfu/kidney on day 3 and declined slowly to sterile on day 18.

**Electron microscopy**

The ultrastructure of the bladder epithelium of the untreated mice disclosed a surface layer of cuboidal cells (Figure 2a and b), and a small number of bacteria were found in the lumen of the bladder. The organisms were localized close to the surface of the epithelium, and higher magnification disclosed bacteria with a characteristic Gram-negative cell wall (Figure 2a) lying close to the microvilli of the epithelial cell. In the mecillinam-treated mice, the surface epithelium had well developed microvilli (Figure 2c), and no bacteria were found in the lumen of the bladder. However, (after one mecillinam dose) organisms were found in the cytoplasm of superficial epithelial cells (Figure 2c and d). These cells had a number of vacuoles, thin cisternae and a round nucleus. The internalized bacteria had a Gram-negative
cell wall structure (Figure 2d) and were surrounded by a membrane. No degenerative signs were seen in the organisms.

**Discussion**

Recent in vivo studies have shown that type 1 fimbriated *E. coli* can invade the mouse bladder epithelium, persist within these cells and from here re-emerge into the bladder lumen.\(^4\)\(^7\) Previous studies have shown that bacteria may persist in bladders of antibiotic-treated mice.\(^8\)\(^\text{--}\)\(^\text{10}\) However, direct evidence of bacteria located intracellularly surviving in antibiotic-treated bladders has so far been lacking. In this study, we have shown that in mice with UTI, after 3 days of treatment with mecillinam, the *E. coli* persists in the bladder and causes a re-growth.
of bacteria in the urine. In the bladder epithelial cells of the treated mice, we located intracellular *E. coli* by use of *in situ* hybridization and electron microscopy. This showed that bacteria had been protected from the antibiotic effect of mecillinam.

Rivers & Steck followed mice with *E. coli* UTI over a 2 month period, after 3 days of oral treatment with the combination trimethoprim/sulfamethoxazole (1:5). On day 14, they could still detect culturable bacteria in the bladder and urine, but after the 2 month period they only detected bacteriuria. It may be argued that the lack of bacterial eradication in the bladder is because the antibiotic concentration is too low. The mouse urinary concentration of trimethoprim/sulfamethoxazole is not stated in these reports and can therefore not be evaluated. We have previously measured the urine concentration of mecillinam in humans, and thereafter in treated mice, and adjusted the dose so the \( t > \text{MIC} \) (0.5 mg/L) was \( \sim 6–7 \text{h} \) after one dose of 0.5 mg/mouse.

The phenomenon of persistent bacterial counts in the bladder even after antibiotic treatment has been shown *in vivo* in the experimental mouse model after use of a range of antibiotic classes. As mecillinam and other \( \beta \)-lactam antibiotics are primarily extracellular antibiotics it would be interesting to perform *in vivo* studies using intracellular antibiotics such as fluoroquinolones, tetracycline, trimethoprim or chloramphenicol. Clinical trials evaluating different mecillinam dosing regimens have shown that bacteriological cure rates approach or exceed 90%. The problem with these studies is that the follow-up period is usually 10–14 days after the end of treatment, and as we can see in the mouse model the urinary sample tested at this time point is most often culture-negative. It is not known from the clinical studies whether there were bacteria in the bladder tissue and if these would later establish another UTI. The experimental UTI model in mice or other rodents is perfect for this kind of study. Further studies in the area are needed.

### References


