

MORPHOLOGICAL STUDIES OF *ESCHERICHIA COLI* IN THE URINE OF PATIENTS WITH ACUTE SIMPLE CYSTITIS TREATED WITH AZTOREONAM

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The effects of aztreonam (AZT) on the morphology of *Escherichia coli* in the urine of 5 patients with acute simple cystitis were studied by differential interference contrast microscopy. The urine specimens were collected via catheter before, 5, 15 and 30 minutes after intravenous administration of 1.0gm AZT. The minimum inhibitory concentration of *E. coli* against AZT was 0.05 $\mu\text{g/ml}$. The mean urinary concentrations at 5, 15 and 30 minutes after administration of AZT were 481.6 $\mu\text{g/ml}$, 1168.2 $\mu\text{g/ml}$ and 993.4 $\mu\text{g/ml}$, respectively. In 4 patients, the urinalysis became normal within 30 minutes after the administration. Filamentous cells were observed at low and high urinary concentrations of AZT. They had vacuole-like formations. These findings were characteristic morphological changes caused by AZT. In the other patient, spheroplasts and ovoid cells were observed. AZT in the urine of patients with acute simple cystitis is suggested to have a high affinity for penicillin-binding protein (PBP) 3 and moderate affinity for PBP 1a.

Key words: Morphological study, Acute simple cystitis, Aztreonam

A novel monobactam, Aztreonam (AZT), is specifically active against gram-negative aerobic bacteria and stable to most beta-lactamases^{1,2}.

The morphological effects of the antibiotic on the organisms must be examined to understand the mode of actions of antibiotics, especially monocyclic beta-lactam drugs in the aspects of binding affinity of penicillin-binding protein (PBP). We studied the morphological response of *Escherichia coli* (*E. coli*) in urine treated with AZT to investigate the antibacterial mechanism of AZT in practice.

MATERIALS AND METHODS

E. coli was isolated from the urine of 5 female patients with acute simple cystitis

who were administered AZT for treatment at our clinic. AZT of 1.0gm. was intravenously injected and urine specimens were collected with the catheter before, 5, 15 and 30 minutes after the injection.

The urinary concentrations were measured by bioassay method with *E. coli* ATCC27166 as a test organism. The urine was centrifuged by 1,000 rpm/min for 10 minutes.

A drop of the urinary sediments was spread over a thin film of Trypticase soy agar medium (BBL) on a slide glass and covered with a coverglass which was then fixed with liquid paraffin. The morphological effects were observed and recorded photographically by means of a differential interference contrast micro-

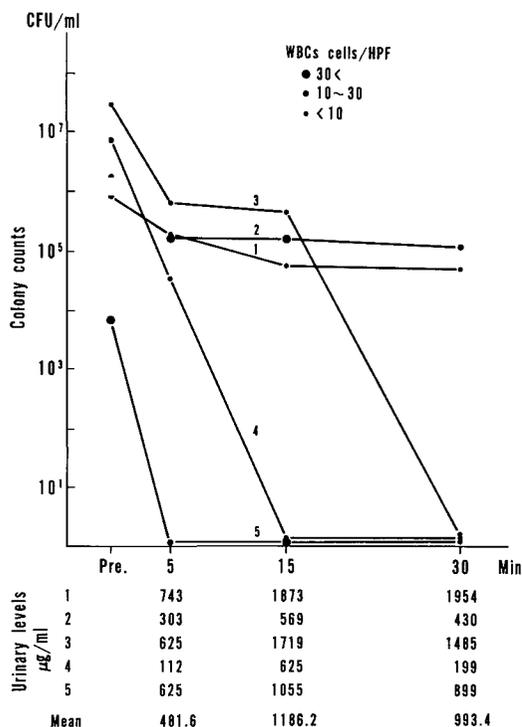


Fig. 1. The urinary levels of AZT, white blood cells and colony forming units of *E. coli* in urine after intravenous administration of AZT.

scope (Nikon).

RESULTS

The minimum inhibitory concentration (MIC) of *E. coli* against AZT was 0.05 µg/ml. The urinary excretion was good and the mean urinary concentration at 5, 15 and 30 minutes after administration of 1.0g AZT was 481.6 µg/ml, 1,168.2 µg/ml and 993.4 µg/ml, respectively (Fig. 1).

The white blood cells in the urine tended to decrease within 30 minutes. In 4 patients, the urinalysis became normal within 30 minutes after the administration (Fig. 1).

Filamentous cells were observed at low and high urinary concentrations of AZT. These findings were characteristic morphological changes caused by AZT (Fig. 2. A~D). In the other patient, spheroplasts and ovoid cells were observed and filamentous changes with vacuole-like formations were noticed (Fig. 3. E~H).

DISCUSSION

Monobactams are monocyclic beta-lactam antibiotics characterized by the 2-oxyoazetidine-1-sulfonic acid moiety which have been recently isolated from gram-negative bacteria³⁾. AZT and related monobactams bound specifically to PBP 3, a peptidoglycan transpeptidase involved in septation¹⁾, in *E. coli* and other susceptible organisms. The PBP profile of AZT indicated a very high affinity for PBP 3, and moderate affinity for PBP 1a, and poor affinity for PBP 1b, PBP 2, PBP 4 and PBP 5/6⁵⁾.

In morphological studies, AZT induced filament formation at a low and high concentration. It was a characteristically morphological change attributable to the high affinity of AZT for PBP 3. Spheroplast formation and cell lysis at a high concentration of AZT were observed in 1 of the 5 specimens. The vacuole-like formations in filamentous cells were found simultaneously. These changes were possibly attributable to the affinity of AZT for PBP 1a.

In this series, *E. coli* disappeared in 3 of the 5 specimens and the urinalysis became normal in 4 of the 5 specimens within 30 minutes after the administration. The white blood cells in urine tended to decrease as the organisms decreased.

In *E. coli*, AZT caused filamentation characteristically. It is suggested that AZT in the urine of patients with acute simple cystitis has the same high affinity for penicillin-binding proteins 3 as *in vitro*.

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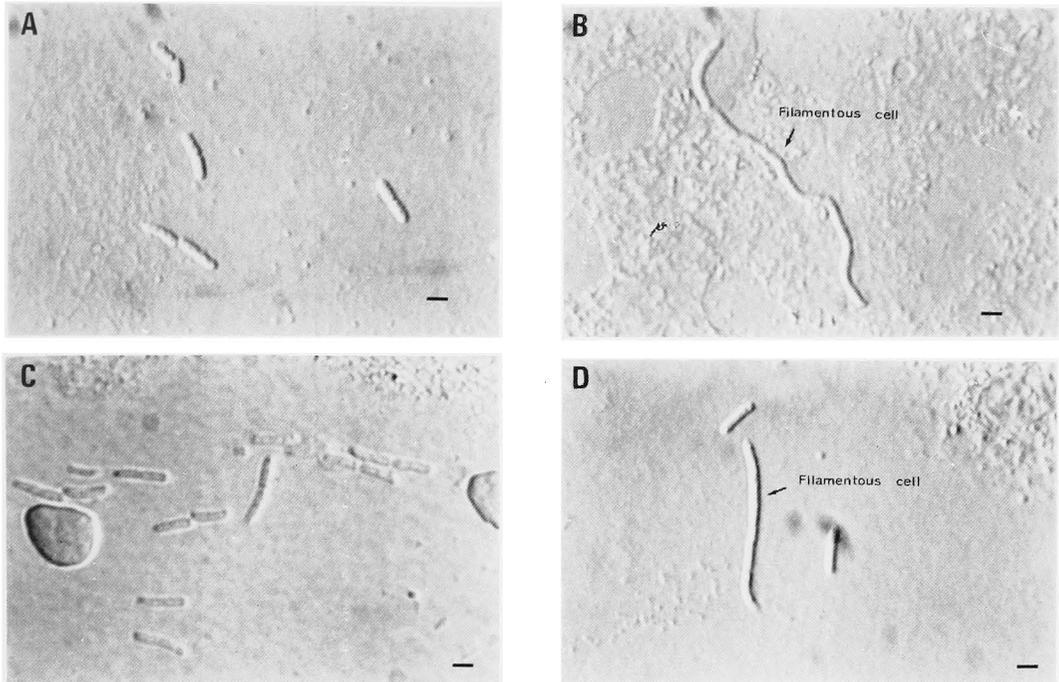


Fig. 2. Morphological changes of *E. coli* in urine after intravenous administration of AZT (Case No. 1 and 2.)
 Case No. 1 A: before B: 5 minutes Case No. 2 C: before D: 5 minutes
 Forming filamentous cells were observed.

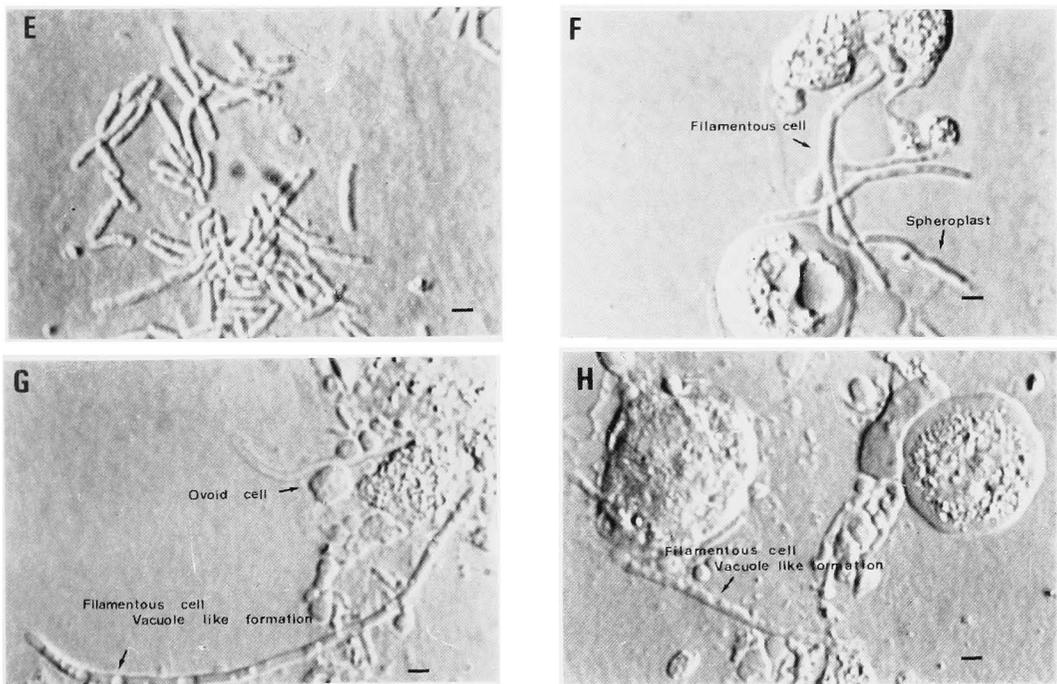


Fig. 3. Morphological changes of *E. coli* in urine after intravenous administration
 Case No. 3. E: before F: 5 minutes G: 15 minutes H: 30 minutes
 Filamentous changes with vacuole-like formations, spheroplasts and ovoid cells were observed.

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和文抄録

Aztreonam による急性単純性膀胱炎患者の *Escherichia coli* の形態学的変化について

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急性単純性膀胱炎の5症例に Aztreonam (AZT) を投与し、透過型微分干涉顕微鏡を用いて大腸菌の形態学的変化について検討した。検体の尿は AZT 1.0 g の静注前、後 5, 15, 30分にカテーテルを通して採取した。AZT に対する大腸菌の最小発育阻止濃度は 0.05 $\mu\text{g/ml}$ であった。AZT 投与後 5, 15, 30分の尿中 AZT 濃度の平均は、それぞれ 481.6 $\mu\text{g/ml}$, 1168.2 $\mu\text{g/ml}$, 993.4 $\mu\text{g/ml}$ であった。本剤投与後30分以内に5例中4例の尿所見は正常化していた。大腸

菌の形態学的変化としては、フィラメント像が AZT の低濃度、高濃度のいずれの場合にも観察された。またフィラメント化した大腸菌の中には空胞様の変化が認められた。このような所見は AZT による特徴的な形態変化であった。5例中1例の尿中にスフェロプラストや卵形細胞が観察された。これらの形態変化から急性単純性膀胱炎患者の尿において AZT はペニシリン結合性蛋白質 (PBP) 3 に強い新和性を有し、PBP 1a に対しては中等度であると考えられた。