The morphological response of urinary gram negative bacteria to ceftazidime

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The morphological response of urinary gram-negative bacteria (GNB) to ceftazidime (CAZ) was investigated in five patients with monomicrobial complicated urinary tract infections (UTI). The target bacteria were one strain of *Pseudomonas cepacia* (MIC: 0.39 μg/ml), two strains of *Pseudomonas aeruginosa* (MIC: 1.56 μg/ml for both) and two strains of *Serratia marcescens* (MIC: 50 μg/ml for both). CAZ was efficiently excreted in the urine of four patients. The decrease of urinary CFU were progressive after the first injection, and the urinary viable bacteria were eliminated after the 2nd to 4th injection. Prominent filamentation was characteristically observed, regardless of the MIC, from the morphological responses of the urinary GNB by CAZ. The filamentous cells were markedly uneven in contour under light microscopy. Vacuole-like structures, dissociation of the cell wall from the cell membrane, and debris of bacteriolysis were observed under electron microscopy. We speculate that CAZ binds strongly to penicillin-binding proteins (PBPs), especially PBPs 3, of urinary GNB.

Key words: ceftazidime, Gram negative bacteria, Morphological changes

INTRODUCTION

Ceftazidime (CAZ) is resistant to various of β-lactamases, has a wide anti-bacterial spectrum, and shows potent anti-bacterial effects, especially on *Pseudomonas aeruginosa, Serratia marcescens* and indole-positive *Proteus*. CAZ is not a new type of drug, but has served as a reference drug in the double blind comparison test for the development of new antibiotics because of its high utility and safety[1]. We have used CAZ for the treatment of complicated UTI in our clinical practice and it has shown good results. In the present study, we studied the morphological responses to clarify the anti-bacterial mechanism of CAZ in urine on a clinical setting.

MATERIALS AND METHODS

1. Subjects

Five patients with monomicrobial complicated UTI, who were admitted to our department between April, 1989 and March, 1991 were chosen as the subject of this study; one strain of *Pseudomonas cepacia* (MIC: 0.39 μg/ml), two strains of *P. aeruginosa* (MIC: 1.56 μg/ml for both), and two strains of *S. marcescens* (MIC: 50 μg/ml for both) were isolated from urine samples from the patients. The examination procedure was explained to the patients and their informed consents were obtained. Their drug allergy histories were checked, and a negative skin reaction to CAZ was confirmed prior to CAZ administration.

2. Urinary sampling

A catheter was placed transurethrally and connected to a closed drainage system immediately before the first drug administration. The urine sample obtained at this time was regarded as a control. Sampling was done for 8 hours at 2-hour intervals after the first drug administration, then for 4 hours at 2-hour intervals after the second administration. From the third administration onward, odd numbered administrations were sampled immediately before and 2 hours after. Final sample was obtained 12 hours after the last adminis-

tration. Morphological changes of urinary bacteria were observed up until 2 hours after the third administration. Part of each sample was frozen immediately after collection and stored for determination of drug concentration.

3. Measurement of MIC and urinary drug concentration

The MICs of the GNB were determined according to the standard method proposed by the Japan Society of Chemotherapy[2], and the urinary CAZ concentrations were measured using a bioassay method with *Proteus mirabilis* ATCC 21100 as the test organism.

4. Measurement of urinary CFU

The CFU were quantitatively measured by the agar plate dilution method.

5. Light microscopy

The urine samples were centrifuged at 1,000 rpm/minute for 10 minutes. A drop of urinary sediment was spread over a thin film of Tryptocase soy agar medium (BBN) on a slide glass and covered with a cover glass which was then fixed with liquid paraffin. The morphological changes were observed and recorded photographically by means of a differential interference contrast microscope (Olympus).

6. Electron microscopy

The sediments were further fixed with 3% glutaraldehyde and 1% osmium tetroxide, embedded in Epon 812, cut into ultra-thin sections, double stained with uranyl acetate and lead citrate, and photographed under a transmission electron microscope (Hitachi H-300).

RESULTS

1. Changes of CFU and urinary excretion of CAZ

Changes of the CFU in the patients and the urinary excretion of CAZ are shown in Fig. 1 and in Table 1, respectively. After the first administration, one strain of *P. aeruginosa* (MIC: 1.56 μg/ml) decreased linearly and four strains decreased in step-wise fashion. Urinary excretion was excellent in four patients and poor in one. The reason for poor excretion could not be explained because the patient (a 68-year-old, man) showed a creatinine clearance of 76.5 ml/minute: CAZ is mainly excreted from the kidney. In all five of the patients, two drug administrations were needed to eliminate the urinary GNB.

2. Light microscopy

Prominent filamentation with irregular contours were characteristically noticed in the majority of

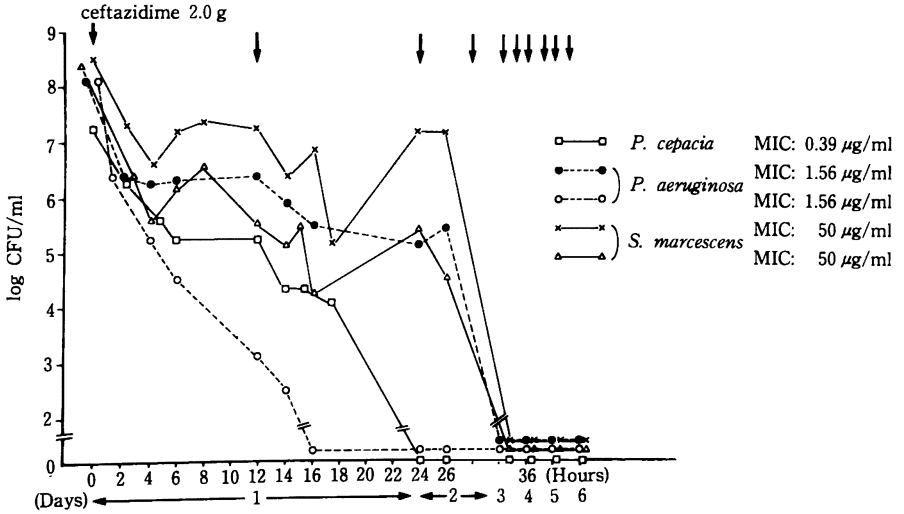

Fig. 1. Bacteriological effects in urine after intravenous administration of ceftazidime (arrows).

Table 1. Urinary levels of ceftazidime (μg/ml)

Time	0 (h)	2	4	6	8	10	12	14	16	18	20	22	24	26	36
Ceftazidime	2.0 g						2.0 g						2.0 g		2.0 g
□—□		1,251	303	228			280	874	881	578					
●--●		1,970	920	780			530	1,790	1,220				750	1,120	
○--○		1,130	350	260			180	1,360	1,350				220	1,800	
×—×			1.2	16.7	61.3	38.8			28.7	43.7					
△—△		724	484	372	186	78		1,456	337		479	586			

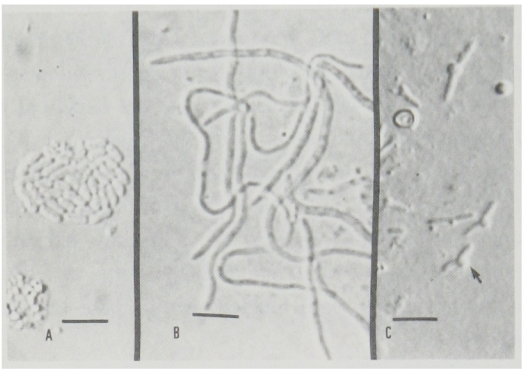

Fig. 2. Light microscopic view of the morphological response of gram negative bacteria in urine before and after administration of ceftazidime (bars represent 10 μm).
A: *Pseudomonas cepacia* before administration of ceftazidime.
B: Prominent filamentous response, with *Pseudomonas cepacia* showing irregular forms 2 hours after.
C: "Rabbit-ear" response (arrow), spheroplast of *Pseudomonas aeruginosa* 2 hours after.

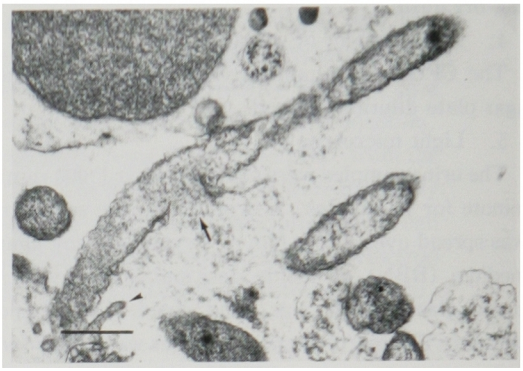

Fig. 3. Transmission electronmicroscopic view of the morphological response of *Pseudomonas aeruginosa* in urine after administration of ceftazidime (bar represents 1 μm).
A gomandized filamentous cell showing bacteriolysis: rupture of cell wall and leakage of cytoplasm (arrow); peusopodium of a phagocyte (arrowhead) 6 hours after.

the strains (Fig. 2-B). Spheroplasts and short filamentous cells were observed in one strain of *P. aeruginosa*, which decreased linearly after CAZ administration (Fig. 2-C).

3. Electron microscopy

The following process of bacteriolysis was observed. The cell surface structure was ruptured and cytoplasmic contents flowed out (Fig. 3). Vacuole-like structures emerged in the cytoplasma as well as the cytoplasma detaching from the surface structure (Fig. 4).

DISCUSSION

Since Spratt's report[3], the interaction between β-lactam antibiotics and PBPs has attracted attention in studies of the antibiotics' action mechanism. As is generally known, PBPs 1, 2 and 3 are related to cell length extension, shape determination, and septum formation, respectively. The morphological changes of the bacterial cells treated with β-lactam antibiotics may be attributed to the differences in their affinities to each PBPs; filamented cells are mainly observed the antibiotics binding to PBPs 3.

The best β-lactam antibiotics are ideally required a potential affinity to PBPs 1[4]. Re-growth of the

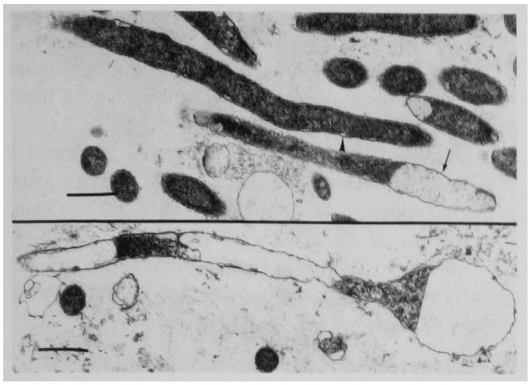

Fig. 4. Transmission electronmicroscopic view of the morphological response of *Serratia marcescens* in urine after administration of ceftazidime (bars represent 1 μm).
A: A spherical pole form on one side of a rod -shaped cell with vacuole-like structure 2 hours after.
B: Filamentous cells with vacuole-like structure (arrow): detachment of the bacterial cell wall and cytoplasmic membrane (arrowhead) 6 hours.

filamentous cells reported by Fujii et al[5] We have carried out investigations similar to this study using other antibiotics[6~9] The antibiotics forming filamentous cells have taken poor clinical effects: they merely elongated bacterial cells and could not eliminated the bacteria from the urine.

In this study, the viable bacterial cells were eliminated from the urine through morphological responses of urinary GNB by CAZ were characteristically filamentous cells with irregular shape. Bacteriolysis of the filamentous cells was confirmed by electron microscopic observation. Similarly, Nakao et al reported the death of the filamentous cells treated with cefmenoxime (CMX)[10] The affinity of CAZ to PBPs was, similarly to CMX, high in the descending order of PBPs 3, 1 and 2, *in vitro*[11]

Changes in the urinary count of viable *P. aeruginosa* were markedly different in the 2 patients examined. This difference could be explained from the morphological changes in urinary bacteria: spheroplasts and filaments were formed in the urine of the patient with a linear decrease and the patient with a step-wise decrease, respectively. The bacteriolysis was more intense in case when spheloplast formed than filaments formed. The outer membrane penetration would be difference between these two strains. However, to make clear the difference of the morphological change was impossible from this study.

In one patient with poor urinary excretion of CAZ, decrease of CFU and filamentous cells were unexpectedly observed at a concentration below the MIC, in this study. Antibacterial agents do not begin to act only when their concentration exceeds the MIC; their actions on bacteria begin at lower concentrations. This is considered that exposure of bacteria to subinhibitory concentration of drugs may cause alterations in bacterial morphology[12]

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Ceftazidimeによる尿中グラム陰性桿菌の形態変化

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複雑性尿路感染症単独感染例5例についてceftazidime (CAZ) 投与後の経時的尿中生菌数の変化と尿中細菌の形態変化を検討した。*Pseudomonas cepacia* 1株 (MIC: 3.13 μg/ml) *Pseudomonas aeruginosa* 2株 (MICはいずれも1.56 μg/ml), *Serratia marcescens* 1株 (MICはいずれも50 μg/ml) であった。CAZの尿中排泄は，4例には良好であった。尿中生菌数は第1回の投与により経時的に減少したが，消失には2～4回の投与を必要とした。細菌の形態は細菌のMICにかかわらずフィラメントを形成する傾向が強かった。多くのフィラメントは光顕では凹凸が著明であり，電顕では空胞形成，細胞壁と細胞質膜の解離，細胞壁と細胞質膜の残骸およびそれらの崩壊像が観察された。CAZは，尿中でもPBP特にPBP3に強く結合することが示唆された。

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