CHAPTER 2

REVIEW OF LITERATURES

Background

After Florey and his group published their classical paper, "Penicillin as a Chemotherapeutic Agent"(12,13) there was little doubt that penicillin would become an important agent for the treatment of infectious disease. With the passage of time, the beta-lactam antibiotics and their progenitor, penicillin, have exceeded all early expectations. During the last four decades, thousands of semi-synthetic penicillins and cephalosporin have been made. In addition, naturally occurring beta-lactam antibiotics have been derived from bacteria as well as from fungi. From this enormous effort have come many life-saving antibiotics with an array of microbiologic and pharmacologic properties.

Until the late 1960s the developement and introduction of new beta-lactam antibiotics have followed a conservative path. Chemical modification was confined to 6-aminopenicillanic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA), the only two structural variants of the beta-lactam nucleus then known. Spectrum of activity had been expanded to include not only gram positive bacteria but some of the more common gram negative bacteria such as Escherichia coli and Proteus mirabilis. Resistance among staphylococci had been overcome via the cephalosporins and isoxazolyl penicillins but resistance among gram negative bacteria was emerging as a serious problem due to the diversity of beta-lactamase elaborated by this group of antibiotics. What had been gained in this initial period of beta-lactam research, perhaps more than just a number of clinically very useful

antibiotics, was a realization of the tremendous potential of the basic beta-lactam nucleus. This realization efforts led to the explosion of new beta lactams in the 1970S.

The appearance of the cephamycins in 1971 not only expanded the activity spectrum to include anaerobes but also showed that the cephalosporin nucleus could be stabilized to beta-lactamase from gram positive and gram negative bacteria. The second and third generations cephalosporins, also appearing in this decade, reinforced the observation of broad beta-lactamase stability and presented an enhanced activity spectrum to include most of the clinically relevant gram-negative bacteria. The extreme among the broad spectrum agents was found with the carbapenems, as exemplified by compounds like thienamycin. And finally at the end of the 1970S, with the discovery of the monobactams, came the realization that a bicyclic nucleus was no longer an absolute requirement for meaningful antibacterial activity since a suitably activated monocyclic beta-lactam could serve as well.

Aztreonam was derived from the most recently discovered group, the monobactams. Owing to its simplicity of structure, aztreonam is the first beta-lactam antibiotic open to practical chemical synthesis on a large scale. In addition, aztreonam has a directed antimicrobial spectrum, being specifically active against aerobic gram-negative bacteria and showing little or no activity against anaerobic and gram positive bacteria. The entry of aztreonam into the clinic thus provides a demarcation point in the development of beta-lactam antibiotics. Instead of a mere extension on previously discovered penicillins and cephalosporins, aztreonam represents a new therapeutic approach.

Structure-activity relationships

The term monobactam originates from the novel chemical structure and microbiological source of the agent : monocyclic bacterially-produced beta-lactam antibiotics (14). The naturally occurring monobactams, isolated from gram negative soil eubacteria are characterized by an acyl side chain in the $beta_3$-position of the 2-oxoazetidine-1-sulfonic acid moiety and, in the majority of cases, the presence of an $alpha_3$-methoxy group (Figure 1). The simple monocyclic structure of the monobactams is in stark contrast to the bicyclic penicillin and cephalosporin molecules (Figure 2). Unlike their naturally occurring penicillin and cephalosporin counterparts, the natural monobactams exhibit poor antibacterial properties and, as such, cannot be considered as clinically useful antibiotics (15). However, the mechanism of action of the monobactams is closely related to that of other beta-lactam antibiotics. The monobactams interact with certain penicillin-binding proteins (PBP's) of both gram positive and gram negative bacteria (16). The penicillin-binding protein with which the beta-lactam antibiotics interact are almost certainly the enzyme responsible for the biosynthesis of bacterial cell walls.

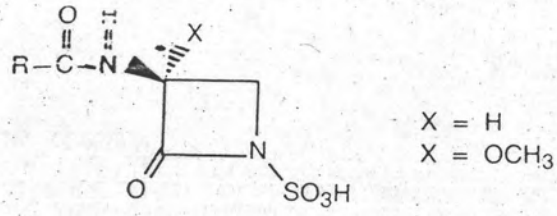

Figure 1 Structure of naturally occuring monobactams.

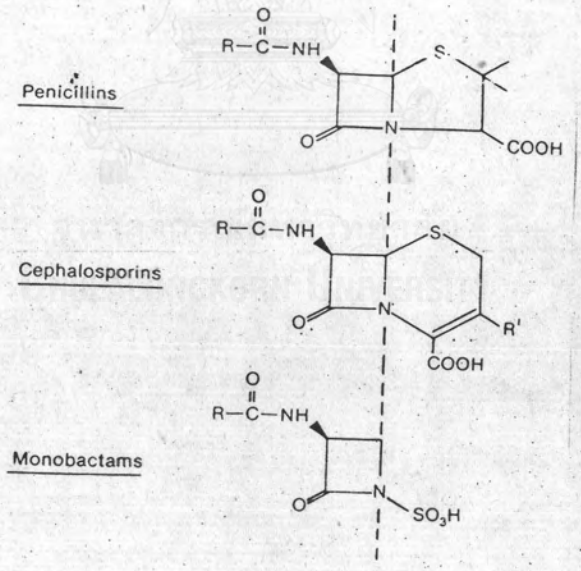

Figure 2 Structures of beta-lactam antibiotics.

Previous experience with side-chain modification in penicillins and cephalosporins has demonstrated that molecular modification around the antibiotic nucleus could have a dramatic impact on the properties of the molecule. The focus of the monobactam development program, therefore, turned toward molecular modifications aimed at increasing the activity of the beta-lactam ring and providing adequate binding of the molecule to the enzyme's active site. Utilizing the synthetic monobactam nucleus, 3-aminomonobactamic acid (Figure 3), aztreonam emerged as the first monobactam for clinical use.

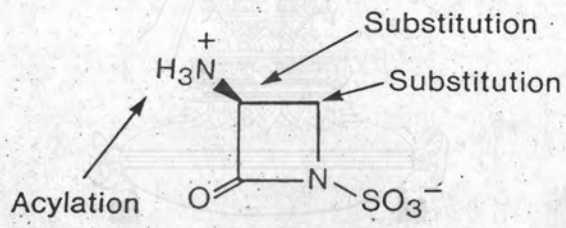

Figure 3 Molecular modification of the monobactam nucleus, 3-aminomonobactamic acid.

The structure-activity relationships in the aztreonam molecule and the influence of the various substituents attached to the monocyclic beta-lactam ring are shown in Figure 4. The 1-sulfonic acid group, attached to the nitrogen of the beta-lactam ring, is responsible for activating the beta-lactam, carbonyl, and the alpha-methyl group at the 4 position enhances the stability of the ring to beta-lactamase attack and provides increased antibacterial activity. The aminothiazole oxime moiety on the acyl side chain is responsible for the potent activity exhibited by aztreonam against aerobic gram negative bacteria. Activity against Pseudomonas spp. is enhanced by the addition of two methyl groups and a carboxylic acid function on the oxime side chain.

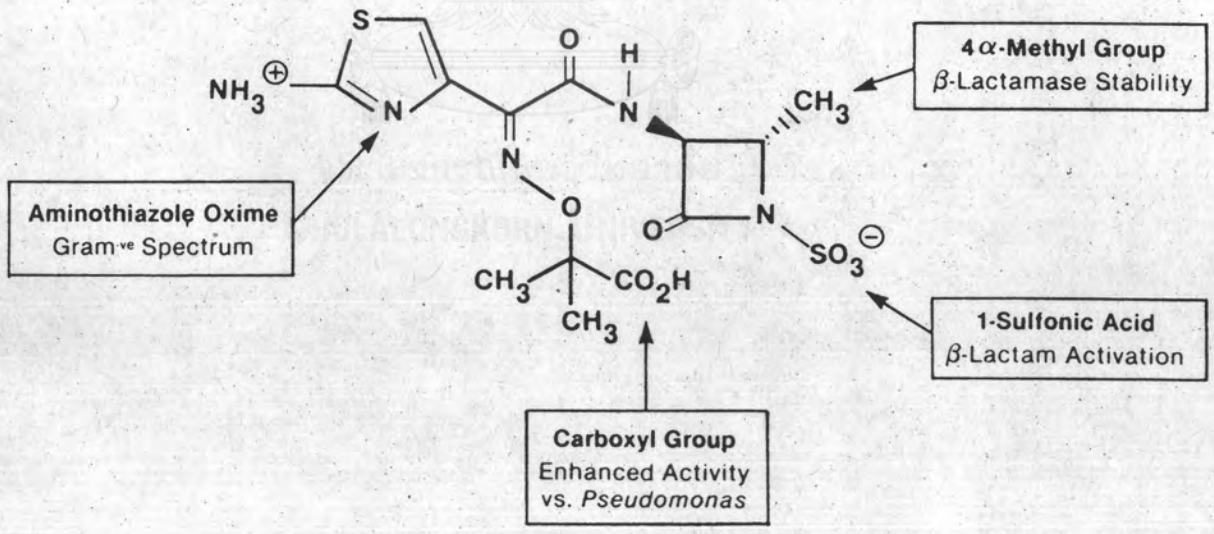

Figure 4 Structure-activity relationships in the aztreonam molecule.

Mode of action

Despite its novel structure, aztreonam acts like other beta-lactam antibiotics and interferes with bacterial cell wall biosynthesis. The PBP selectivity of aztreonam allows for its differential activity spectrum. A relative inactivity against gram positive bacteria and anaerobes is a function of aztreonam interacting poorly with the PBP_5 from these organisms. With gram negative bacteria a very high affinity for PBP_3, the septum peptidoglycan transpeptidase, accounts for the good activity against these organisms. As a consequence of binding to PBP_3 aztreonam cause filamentation, a morphological effect identical to that observed with the cephalosporins (16). After filamentation, breakage of the cell wall occurs, resulting in lysis and death (17). Like most other beta-lactam antibiotics, the bactericidal concentration is closely associated with the inhibitory concentration (18).

Interaction with Beta-Lactamase

Aztreonam is highly resistant to enzymatic hydrolysis by beta-lactamases produced by gram negative and gram positive bacteria. Among the gram negative beta-lactamases, plasmid-mediated enzymes are particularly insidious because the genetic information responsible for TEM-betalactamase production passed freely among the Enterobacteriaceae, Pseudomonas, Neisseria gonorrhoeae and Haemophilus influenza. Beta-lactamases capable of promiscuous behavior are the TEM, OXA, SHV and PSE enzyme (19). Aztreonam demonstrates a high degree of stability to these plasmid-mediated beta-lactamase (20). In contrast to these enzymes, the chromosomally mediated beta-lactamases, which are frequenly produced by species of Enterobacter, Serratia, Proteus and Pseudomonas, are not transferable under normal circumstances. With this diverse group of enzymes, the behavior of aztreonam varies from that of a beta-lactamase inhibitor to that of a poor enzyme substrate (21). The only enzyme of this type that shows any appreciable destruction of aztreonam is the relatively uncommon K_1 beta-lactamase, which is produced by certain strains of Klebsiella oxytoca.

Although poorly active against anaerobes, aztreonam's stability to the Bacteroides enzyme may prove meaningful in the clinical setting of mixed infection. Aztreonam proved inhibitory to E.coli regardless of whether Bacteroides was present in the culture or not (22). In the same experiment, cefotaxime was inhibitory to E.coli in pure culture but in the mixed culture situation, the growth of E.coli was not impeded, reflecting enzymatic breakdown of cefotaxime by the Bacteroides beta-lactamase.

Many of the chromosomally-mediated beta-lactamases are inducible enzymes since their production can be increased in the presence of beta-lactam antibiotics (23). High concentrations of induced enzyme, locate strategically within the periplasmic space, play a major role in resistance to beta-lactam antibiotics. Aztreonam is unique in its inability to induce the production of chromosomally-mediated enzyme (12).

Figure 5 illustrates the induction capacity of several different beta-lactam antibiotics incubated with classl-producing strains of Proteus morganii and Enterobacter cloacae. Cefoxitin is shown to be a potent inducer of both enzyme-producing strains, whereas cefotaxime and ceftazidime induce only the Enterobacter beta-lactamase, and neither cefoperazone nor aztreonam shows much, if any, effect on beta-lactamase production (12).

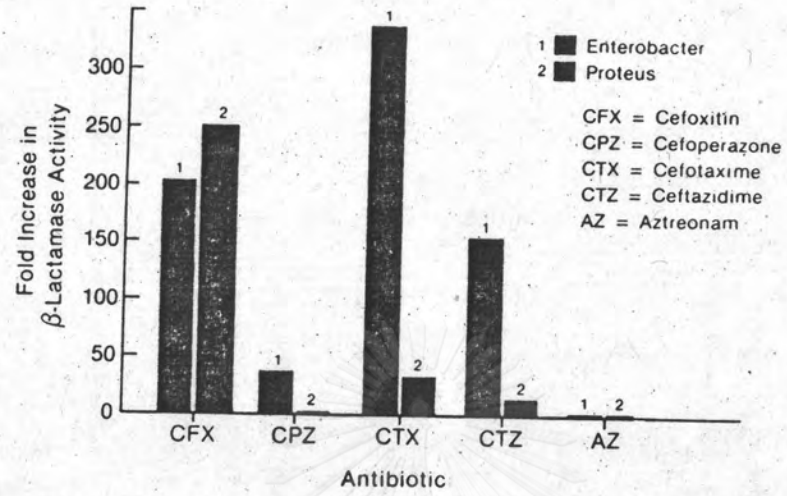

Figure 5 Beta-lactamase induction for which cultures were grown overnight (0.5 percent yeast extract broth, 37°C in shake flasks). A 5 percent transfer was made to fresh broth and inducer was added after two hours' growth to produce inducer levels of 10 μg/ml. After another two hours, cells were harvested, weighed, suspended in 0.1 mol/liter phosphate buffer (pH 7.0), sonicated, and centrifuged. The resulting supernatant was assayed with cephaloridine (spectro-photometrically) to obtain K_m and V_{max} kinetic constants. A Gilford 202 computerized data collection system was used to calculate hydrolysis rates. CFX, cefoxitin; CPZ, cefoperazone: CTX, cefotaxime; CTZ, ceftazidime; AZ, aztreonam.

Antibacterial Activity (18)

The antibacterial spectrum of aztreonam is unique among beta-lactam antibiotics. Unlike the majority of these antibiotics, aztreonam exhibits little or no activity either against gram positive organisms such as staphylococci and streptococci or against anaerobic bacteria in general. The potent antibacterial activity of aztreonam is specifically directed against aerobic gram negative bacteria that are commonly against aerobic gram negative bacteria that are commonly encountered in nosocomial infections (Table 2).

As might have been expected from studies with cell-free beta-lactamase preparations, aztreonam responded only minimally to inoculum changes when tested against beta-lactamase-producing organisms (Table 3). In contrast to results obtained with cefamandole, cefoperazone, and cefotaxime, aztreonam minimum inhibitory concentration values for the beta-lactamase-producing strains tested did not vary significantly with inoculum levels.

Table 2 Antibacterial spectrum of aztreonam (18).

Organism (no. of strains)	MIC_{90} (μg/ml)*			
	Aztreonam	Cefotaxime	Ceftazidime	Cefoperazone
Staphylococcus aureus (12)	>100	3.1	12.5	2.8
Streptococcus pyogenes (11)	12.5	<0.1	0.2	0.1
Streptococcus pneumoniae (11)	>100	<0.1	0.4	<0.1
Streptococcus faecalis (12)	>100	>100	>100	24.4
Bacteroides fragilis (8)	>100	>100	>100	>100
Escherichia coli (79)	0.2	0.2	0.4	>100
Klebsiella pneumoniae (68)	0.3	0.3	1.1	>100
Enterobacter cloacae (29)	12.5	25.0	12.5	100
Enterobacter aerogenes (13)	33.3	42.9	>100	>100
Serratia marcescens (113)	1.6	8.4	1.5	>100
Proteus mirabilis (25)	<0.1	<0.1	0.1	2.4
Proteus vulgaris (11)	<0.1	5.5	<0.1	5.8
Proteus rettgeri (6)	<0.1	0.2	2.3	10.7
Proteus morganii (19)	0.6	1.6	3.1	6.3
Providencia stuartii (15)	<0.1	3.0	4.7	>100
Salmonella species (25)	0.3	0.3	1.4	75.0
Citrobacter freundii (25)	0.7	0.4	2.0	4.7
Acinetobacter calcoaceticus (25)	58.3	24.4	11.4	>100
Pseudomonas aeruginosa (61)	12.0	47.8	3.2	12.0
Haemophilus influenzae Amp^S (18)†	0.2	<0.1	0.2	0.1
H. influenzae Amp^R (18)†	0.2	<0.1	<0.1	>100
Neisseria gonorrhoeae (20)	0.2	<0.1	<0.1	0.2

* MIC_{90} = concentration of antibiotic necessary to inhibit 90% of strains tested at inoculum of 5×10^5 cfu.
† Amp^S = ampicillin sensitive; Amp^R = ampicillin resistant.

Table 3 Antibacterial activity against beta-lactamase producers (18).

Organism		MIC$_{90}$ (μg/ml)									
		Aztreonam		Cefazolin		Cefamandole		Cefoperazone		Cefotaxime	
		10^4*	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6
Escherichia coli TEM+	10,404†	0.1	0.2	6.3	>100	12.5	>100	3.1	>100	0.05	0.1
Citrobacter freundii	10,204	0.1	3.1	>100	>100	1.6	>100	0.2	12.5	6.3	25
Shigella sonnei	10,944	6.3	6.3	25	>100	6.3	50	0.8	50	1.6	>3.1
Enterobacter cloacae P99+	10,435	12.5	25	>100	>100	>100	>100	50	>100	>100	100
Enterobacter cloacae	8,415	0.2	0.2	>100	>100	3.1	12.5	0.2	0.8	1.6	25
Enterobacter cloacae	9,965	0.1	0.2	>100	>100	3.1	50	0.4	0.8	1.6	6.3
Klebsiella pneumonia KI+	10,436	50	>100	>100	>100	>100	>100	>100	>100	6.3	50
Klebsiella pneumonia	11,066	0.8	3.1	12.5	>100	12.5	>100	12.5	>100	0.4	0.8
Proteus rettgeri	8,217	0.05	0.05	>100	>100	6.3	>100	1.6	12.5	0.8	12.5
Proteus rettgeri	11,104	0.1	0.2	>100	>100	25	>100	25	>100	0.8	12.5
Proteus vulgaris	10,950	0.05	0.05	>100	>100	>100	>100	0.4	1.6	0.05	50
Proteus vulgaris	10,951	0.05	0.05	>100	>100	>100	>100	3.1	>100	0.4	50
Pseudomonas aeruginosa	9,545	0.4	0.4	>100	>100	50	>100	0.4	0.8	0.8	1.6
Pseudomonas aeruginosa	8,329	1.6	12.5	>100	>100	>100	>100	3.1	25	25	>100
Pseudomonas aeruginosa	9,546	6.3	12.5	>100	>100	>100	>100	3.1	12.5	6.3	12.5
Serratia marcescens	9,782	0.1	0.4	>100	>100	>100	>100	1.6	12.5	1.6	12.5
Serratia marcescens	8,247	3.1	6.3	>100	>100	>100	>100	25	>100	50	>100
Acinetobacter calcoaceticus	8,333	25	>100	>100	>100	>100	>100	50	>100	50	>100

*Inoculum (colony-forming units).
†Squibb culture collection number.

Antibacterial combination

Positive results have been demonstrated with combination of aztreonam and aminoglycosides (24). For example, a high incidence of synergistic activity against strains of Pseudomonas aeruginosa and to a lesser extent against the Acinetobacter spp. has been shown with aztreonam and gentamicin, tobramycin or amikacin.

However, some penicillin-cephalosporin combinations may be antagonistic against certain pathogens, and it is advisible to determine the nature of any antibiotic interaction by in vitro testing before in a particular clinical situation. The activity of aztreonam is antagonized by cefoxitin or thienamycin against Enterobacter and P.aeruginosa and the clinical use of these combinations should be avoided (25). Similary, chloramphenicol combined with aztreonam or thienamycin can be antagonistic for Klebsiella pneumoniae (26). The in vitro interactions of aztreonam with third-generation cephalosporins (such as moxalactam) or ureidopenicillins (such as piperacillin) against P.aeruginosa and the Enterobacteriaceae is variable and may result in synergy, indifference or antagonism (27). These in vitro interactions of aztreonam are especially significant , since its narrow spectrum of activity will often require that it be combined with other drugs aganist gram positive organisms or anaerobes. Fortunately no adverse in vitro interactions have been reported when aztreonam was combined with nafcillin, vancomycin, ampicllin, clindamycin or metronidazole (12,28).

Disc diffusion studies

Aztreonam was evaluated using agar dilution and disc diffusion test against 255 Gram-negative bacilli including members of the Enterobacteriaceae and strains of P. aeruginosa and Acinetobacter calcoaceticus (12). In the diffusion studies a 30 μg aztreonam disc was employed. Using regression line analysis (Figure 6), Tentative zone standards with MIC breakpoints of $\leq$ 8.0 μg/ml (susceptible) and $\geq$ 32 μg/ml (resistance) were $\geq$ 22 mm and $\leq$ 15 mm , respectively. The intermediate category consisted of organisms having a MIC of 16 μg/ml and yielding zone sizes of 16-21 mm.

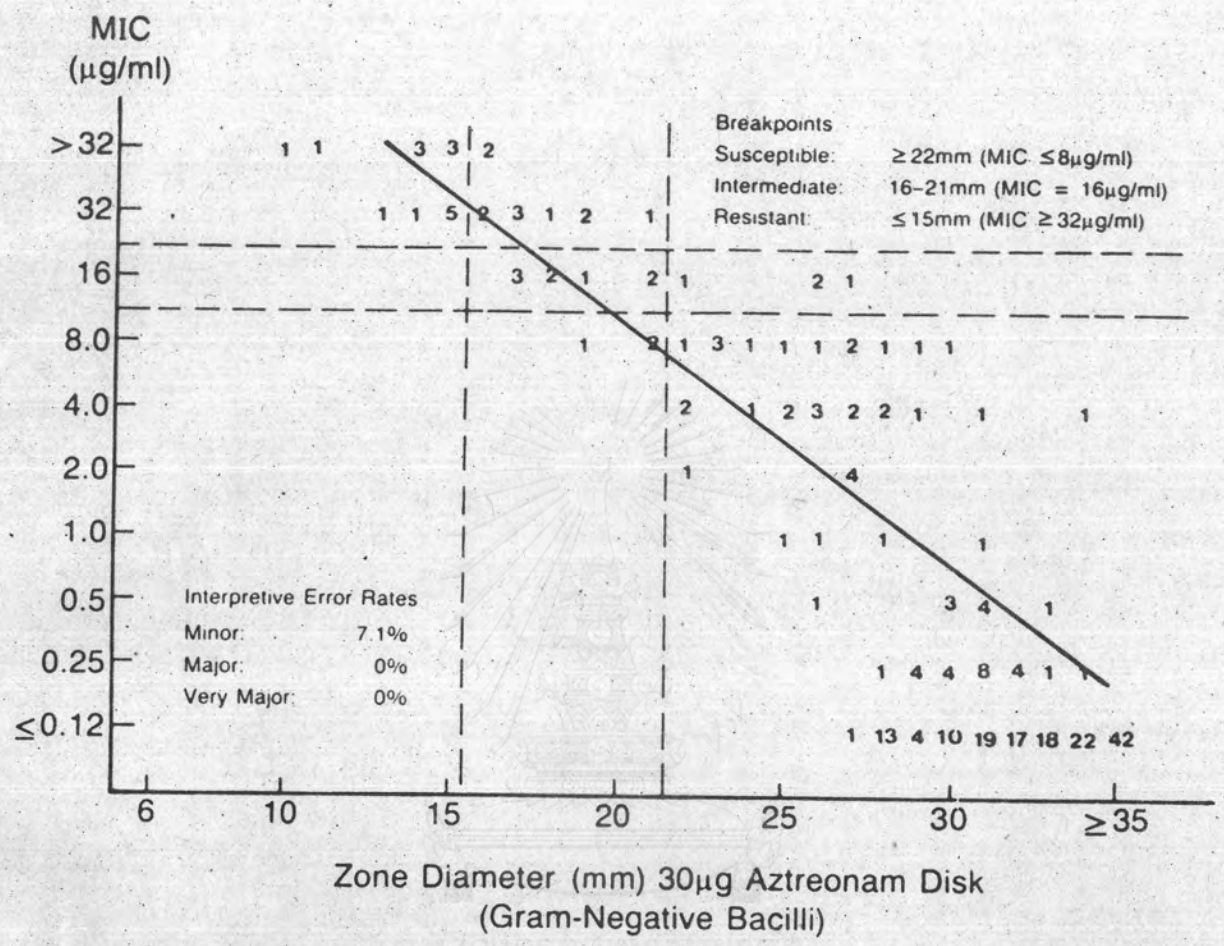

Figure 6 Correlation between inhibitory zone diameters (30 µg aztreonam discs) and minimal inhibitory concentrations with gram negative bacilli. Broken lines represent proposted breakpoints and the solid line represents the regression line (12).

Effects on intestinal microflora

One value of the selective antibacterial spectrum of aztreonam is manifest in its minimal effect on the indigenous host microflora. The use of broad spectrum antibiotics particullarly those demonstrating a high degree of biliary excretion, has resulted in adverse gastrointestinal effects ranging from diarrhea to pseudomembranous colitis (PMC). This latter condition has been associated with the multiplication of Clostridium difficile and other Clostridium spp. in the large intestine depleted of its normal anaerobic bacterial flora.

The lesions of PMC are caused by clostridial cytotoxins. Animal model for antibiotic-associated colitis has been developed(29), in which antibiotics causing PMC in man also cause C. difficile toxin-mediated hemorrhagic caecitis in hamsters (30). When hamsters were given an antibiotic such as cefoperazone, which was both broad spectrum and showed a high level of biliary excretion, the results were dramatic (29). Within 24 hours the intestinal anaerobic flora were substantial decreased (Figure 7). C. difficile invaded and proliferated and within 4 days the hamster died from hemorrhagic caecitis.

In contrast, treatment of the hamster with aztreonam, either orally or parenterally, results in an uneventful course. The animal remain and healthy and caecal examination revealed neither C. difficile nor its toxins. The lack of effect of aztreonam on the intestinal anaerobic microflora (Figure 7) insure the maintenance of colonization resistance.

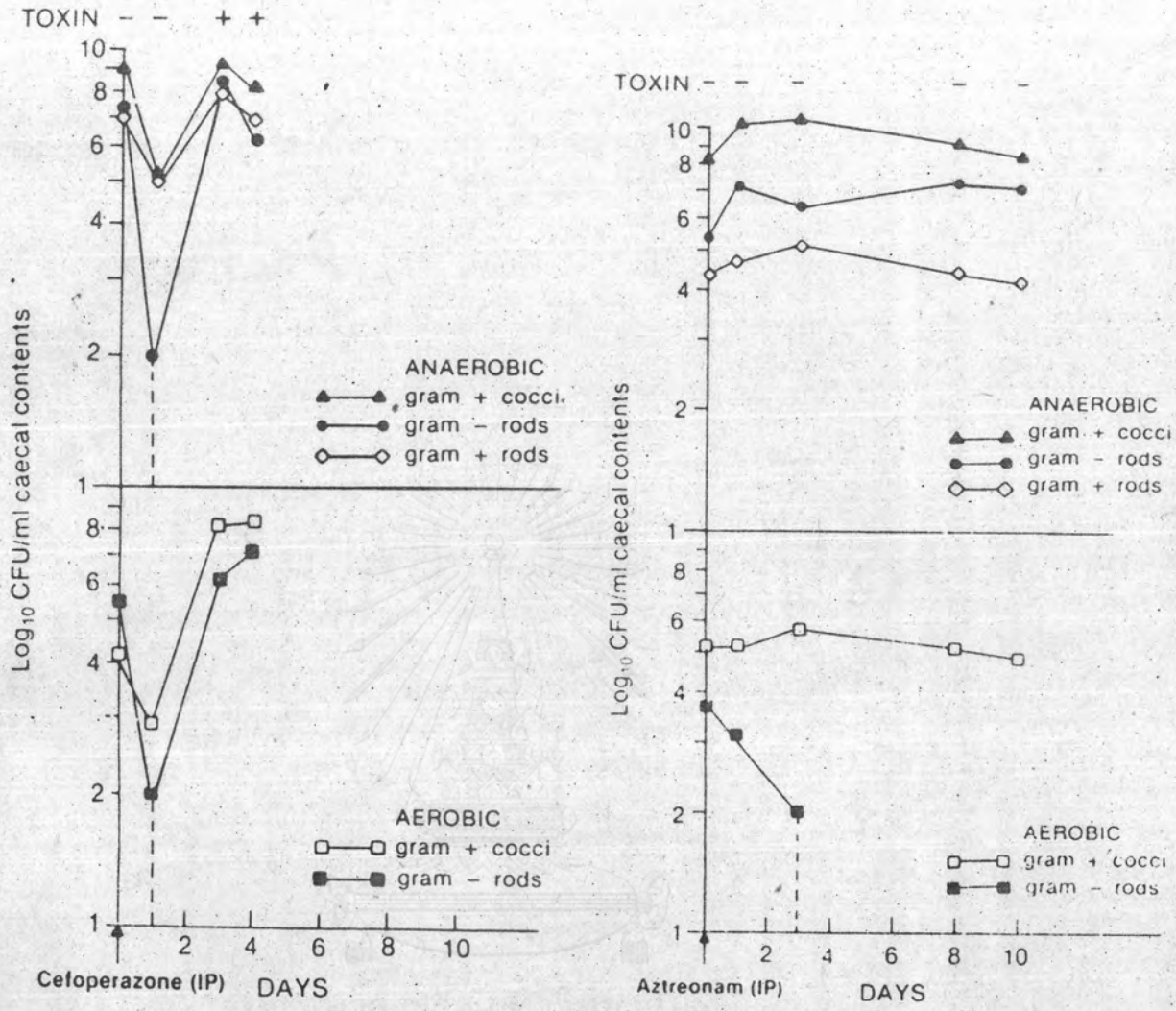

Figure 7 Effect of a 100-mg/kg. ip dose of cefoperazone (left) or aztreonam (right) on hamster caecal flora. Caecal bacteria were quantitated with the use of selective media and microscopy. Specific antiserum - neutralization tests were employed to detect Clostridium difficile toxin (- = Toxin absent, + = Toxin present)(12).

Clinical Pharmacology

Healthy subjects

Microbiological and high-pressure liquid chromatography (HPLC) assay methods give similar values for aztreonam concentration in human serum and urine, indicating the absence of microbiological active metabolite (31). Intravenous dosing achieves the highest serum levels; however, 1 hour after administration of intravenous and intramuscular doses, the serum levels are very similar. All parenteral doses achieve potentially therapeutic levels for Haemophilus, Neisseria and most Enterobacteriaceae for 8 to 12 hours.

A 2-gram intravenous dose gives mean serum concentrations exceeding the MICs 90 of Enterobacter cloacae and Pseudomonas aeruginosa for about 4 to 6 hours.

A 0.5-gram parenteral dose achieves potentially therapeutic urinary levels for 12 to 24 hours.

Aztreonam has been administered as 0.5 or 1 g intravenously or intramusculary every 8 h for 7 days (32). There was no important change in the pharmacokinetic profile of aztreonam, or apparent accumulation of the antibiotic.

Absorption of aztreonam after intramuscular dosing occurs rapidly and completely, in contrast to very poor oral absorption. Aztreonam is rapidly distributed, is 56% bound to serum proteins, and has a steady-state volume of distribution (0.18 L/Kg) comparable to extracellular water space. About two-thirds of a dose of aztreonam is eliminated unchanged in urine, while 6.9 % of a dose is excreted by the kidneys as SQ 26,992, a microbiologically inactive compound resulting from the hydrolytic opening

of the beta-lactam ring, and analogous to the penicilloate metabolite of penicillin (33). Approximately 1 % of a dose of aztreonam is excreted undergoes biliary secretion, the serum half-life for aztreonam(1.7 h.) suggests the feasibility of 6 to 8 h dose intervals.

Under steady state conditions, unbound aztreonam in serum was cleared by

1. renal tubular secretion (two-fifth of serum clearance and partially inhibitable by probenecid)
2. glomerular filtration (two-fifth)
3. non-renal mechanism (one-fifth) (34)

Administration of single 1-gram dose of aztreonam in conjunction with cephradine (1 g), clindamycin (500 mg), gentamicin (80 mg), metronidazole (500 mg) or nafcillin (500 mg) in healthy volunteers did not lead to any major pharmacokinetic interaction between the agents (35).

Patients

Uninfected patients with renal or hepatic insufficiency

The elimination of aztreonam can be significantly impaired in the presence of severe renal insufficiency (36). In anuric patients, the mean serum clearance of aztreonam is about one-fourth normal, and the mean elimination half-life is about three times normal. The mean serum half-life of aztreonam in patients with biliary cirrhosis and alcoholic cirrhosis was 2.2 and 3.2 hours, respectively, compared to 1.9 hours in healthy subject.

Infected patients

Aztreonam pharmacokinetic data in pediatric patients with normal renal function were recently reported (37). In patients 1 month to 12 years old, the serum elimination half-life was similar to that found in adult

patients (1.7 h)(38) but considerably shorter than those observed in low-birth-weight infants less than 7 days old (39,40).

Clinical efficacy and safety

Aztreonam has been evaluated in 3,552 patients with proven or suspected aerobic gram negative infections(41). Clinical and microbiological responses for patients recieving multiple-dose therapy or single dose therapy are shown in table 4. Favorable clinical response ranged from 90 % in patients with intraabdominal infection to 100 % for those with gynecological or bone and joint infections. The microbiologic cure rates ranged from 78 % in patients with lower respiratory tract infection to 100 % for those with gynecological infections. Reinfection ranged from 2 % in lower respiratory tract infection to 7 % in urinary tract infections ; the incidence of microbiologic relapse was approximately 5 %.

Adverse reactions were qualitatively similar to those reported for other beta-lactam antibiotics, i.e., mild gastrointestinal upset, eosinophilia and transient increase in liver enzyme values. The most frequent side effect was phlebitis at the I.V. site, occuring in 2 % of the patients (table 5).

Table 4 Overall clinical and microbiological responses (41).

Diagnosis	Patients (N)	Favorable clinical response (%)	Microbiologic cure (%)
Single dose :			
Acute gonorrhea	209[1]	94	97
Acute cystitis	56	93	84
Multiple dose :			
Urinary tract infection	625[2]	99	85
Skin infection	163[3]	95	87
Lower respiratory tract infection	226[4]	93	78
Gynecologic infection	52	100	100
Intra-abdominal infection	92	90	88
Septicemia	102[3]	94	97
Bone/Joint infection	19	100	89

No clinical response given for : [1] (19 pts.) ; [2](1 pt.) ; [3](2 pts.) ; [4](3 pts.).

Table 5 Clinical adverse reactions multiple-dose aztreonam studies (2,388 patients)(41).

Reaction	Number of patients (% of patients treated)
Dermatologic (2.0 %)	
Rash	31 (1.3%)
Rash with eosinophilia	10 (0.3%)*
Pruritus	4 (0.2%)
Purpura	3 (0.1%)
Gastrointestinal (2.0 %)	
Diarrhea	19 (0.8%)
Nausea/Vomiting	18 (0.8%)
Taste alteration	7 (0.3%)
Jaundice/Hepatitis	4 (0.2%)
Local (2.4 %)	
Thrombophlebitis/Phlebitis	39 (2.0%)**
Discomfort/Swelling at injection site	10 (0.4%)
Miscellaneous (1.1 %)	
Total number of patients experiencing Adverse reactions	163 (6.8%)

* One of these patients, with a history of allergic reactions to other antibiotics, developed urticaria and pharyngeal edema following the tenth dose of aztreonam.

** Based upon the 1,966 patients who received aztreonam intravenously.

The urinary excretion of β_2-microglobulin and renal tubular enzymes, as well as serum creatinine levels and urinary creatinine clearance, show no noteworthy variation during a 7-day multiple-dose study (32). Additional studies indicate that aztreonam may be weakly immunogenic in human, and that there is little cross-reactivity between IgE antibodies to penicillin and aztreonam (42,43,44).

Aztreonam has been evaluated in both open studies and controlled comparative studies in United State, Europe and Japan (45,46,47,48,49). The report from these different parts of the world have shown similar clinical and microbiological responses.