PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF BETA-LACTAM ANTIBIOTICS IN VOLUNTEERS AND PATIENTS WITH CYSTIC FIBROSIS

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PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF BETA-LACTAM ANTIBIOTICS IN VOLUNTEERS AND PATIENTS WITH CYSTIC FIBROSIS

PHARMACOKINETISCHE EN PHARMACODYNAMISCHE STUDIES VAN BETA-LACTAM ANTIBIOTICA IN VRIJWILLIGERS EN PATIENTEN MET CYSTIC FIBROSIS

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ACHILLES: I know the rest of you won't believe this, but the answer to the question is staring us all in the face, hidden in the picture. It is simply one word- but what an important one: "MU"!

CCRAB: I know the rest of you won't believe this, but the answer to the question is staring us all in the face, hidden in the picture. It is simply one word- but what an important one: "HOLISM"!

[.....]

ANTEATER: I know the rest of you won't believe this, but the answer to the question is staring us all in the face, hidden in the picture. It is simply one word- but what an important one: "REDUCTIONISM"!

(DR Hofstadter,
Gödel, Escher, Bach)

Aan mijn ouders
Voor Carla

CONTENTS

LIST OF ABBREVIATIONS

AUC	Area Under the time-concentration Curve
ß	Elimination rate constant
BMI	Body Mass Index
CFU	Colony Forming Units
CI	Continuous Infusion
Cl_u	Urinary clearance
DHP-1	Dehydro-Peptidase I
FEV1	Forced Expiratory Volume during the first second
II	Intermittent Infusion
LBM	Lean Body Mass
MIC	Minimal Inhibitory Concentration
MRT	Mean Residence Time
PAE	Post Antibiotic Effect
$T_{1/2\alpha}$	Half-life during distribution phase
$T_{1/2\beta}$	Half-life during elimination-phase
T_{MIC}	Time above the MIC
TBC	Total Body Clearance
Vd_β	Volume of distribution during elimination phase
Vd_{ss}	Volume of distribution during steady state

ONE

INTRODUCTION AND OVERVIEW OF THE STUDIES

CHAPTER 1

INTRODUCTION AND OVERVIEW OF THE STUDIES

Cystic fibrosis (CF) is the most common fatal autosomal recessive disease amongst Caucasians. The heterozygous carrier frequency has been estimated to be approximately 1:25, leading to 1:2500 live births affected with the disease. Children manifesting the disease were described at the beginning of this century, but the first publication of the ailment as a separate entity was in 1938 by Andersen. The major characteristic of the disease is the formation of abnormal exocrine gland secretions. The pancreas and the lung are the organs mainly affected.

The basic defect of the disease has been elucidated, and involves a mutation in the gene encoding the CF transmembrane conductance regulator (CFTR). More than two hundred mutations have been described in the last few years, and additional ones are still being discovered. Most evidence points to a gene defect effecting a disfunction in the Cl^- channels of the exocrine glands.

The diagnosis of the disease used to be based on the sweat test. A high Cl^- content of the sweat, due to a disfunction of the Cl^- channels, ascertains the diagnosis cystic fibrosis. During recent years, other methods have become available. Both direct measurement of the channel function in vitro and DNA analysis are now used as complementary tests.

Clinically, the disease is manifested by obstruction of the hollow organs by viscous secretions, leading to pancreatic insufficiency and chronic obstructive pulmonary disease. In the lungs, the poor clearance of the viscous mucus promotes colonization and infection by micro-organisms, and bacterial lung infections determine the prog-

nosis for most CF patients. The decrease of lung function, due to destruction of lung tissue by infections, eventually leads to death at a relatively early age.
The antibacterial therapy of these recurrent infections is difficult for several reasons. Increasing resistance to antibiotics of the infectious organisms and host-bacterium interactions are among the major problems confronted by patients and physicians. Furthermore, these patients display altered pharmacokinetics, characterized by an increased clearance for several antibiotics and other drugs. Higher doses and more frequent dosing compared to non-CF patients are therefore often necessary. These problems are discussed extensively in *Chapter 2*.
It has become clear from several studies, that the CF population is heterogeneous with respect to their pharmacokinetic parameters. Not only is there a large inter-individual variation, also the intraindividual variation is considerable. For optimal treatment, for instance with drugs with a narrow therapeutical index, individual drug monitoring is therefore necessary. In *Chapter 3*, individual drug monitoring is discussed with special emphasis on CF patients.
Thus, the Chapters 2 and 3 outline the major problems in the antibacterial treatment of CF patients.

In the studies presented in this thesis, the problems encountered in antibacterial therapy were approached from different angles; the objectives of the studies were threefold.

The *first objective* concerns the emergence and prevalence of resistance to antibiotics of bacteria isolated from CF patients. Since CF patients are repeatedly treated with antibiotics, emerging resistance is becoming, or is already, one of the major difficulties in treating these patients. In *Chapter 4*, the focus is on *Staphylococcus aureus*. The resistance of *S. aureus* to several antibiotics was surveyed in strains isolated from CF patients. Furthermore, the frequency of tolerant strains was evaluated. Tolerance is a type of resistance in which beta-lactam antibiotics and in some cases vancomycin, inhibit growth of the micro-organisms without killing them. It could be speculated that, due to repeated antimicrobial treatment of CF patients, tolerance of *S. aureus* is

induced, or tolerant strains are selected. In *Chapter 5*, the emergence of resistance in *Pseudomonas aeruginosa* is studied over a period of several years. Although an association between the use of antibiotics and increase of resistance has been repeatedly observed in populations as a whole, few, if any data exist on individual patients. Since CF patients harbour the same strain for several years, it is possible to study emergence of resistance in the individual patient, and correlate this with antimicrobial treatment. Furthermore, to study the linkage between the emergence of resistance to different antibiotics, pattern analysis could be used.

The *second objective* was to study the pharmacokinetics of beta-lactams during continuous and intermittent infusion. As outlined above, an increase of resistance and altered pharmacokinetics both lead to treatment problems. Apart from the use of new antibiotics, improvement of existing therapy is necessary, for instance by optimization of dosing schedules. Studies in vitro and in animals suggest that, for beta-lactams, continuous infusion might be more efficacious than the usual regimen of three times daily. To study the pharmacokinetics of continuous versus intermittent infusion, these regimens were studied in volunteers and CF patients, and the results compared with each other.

In *Chapter 6* the pharmacokinetics of ceftazidime is described in human volunteers during intermittent and continuous infusion. To study the extent of tissue penetration of ceftazidime, suction blister fluid concentrations were also analyzed during these dosing regimens. Especially during intermittent infusion, the timing of the drawing of the blisters might be important for the concentration of ceftazidime in the blister fluid. This was examined by taking samples at different time-points. In *Chapter 7*, the pharmacokinetics and blister fluid penetration of meropenem was studied in human volunteers during intermittent and continuous infusion. Meropenem is a new carbapenem, with a potential use to treat pulmonary exacerbations due to *P. aeruginosa* in CF patients.

As was discussed in Chapters 2 and 3, CF patients display altered pharmacokinetics to several drugs. For ceftazidime there are conflicting results, and studying pharmacokinetics during continuous infusion may help to elucidate the origin of these

discrepancies. Also, if continuous infusion is to be used routinely, it is important to know the pharmacokinetic behaviour of this drug under these circumstances.
In *Chapter 8*, the pharmacokinetics of ceftazidime during continuous and intermittent infusion was studied in CF patients in a cross-over study, and the results are compared with those of healthy volunteers.
During the studies described in the Chapters 6, 7 and 8, there were indications that there may be a circadian rhythm in serum concentrations of ceftazidime and also of meropenem. An evaluation of these results is presented in *Chapter 9*.

The *third part* of this thesis focuses on the interaction between *P. aeruginosa* and ceftazidime in vitro during continuous and intermittent infusion. As indicated above, continuous infusion may be more efficacious than intermittent infusion. However, few in vitro data exist where continuous infusion is compared with intermittent administration. Determinations of the susceptibility of a micro-organism, usually as a minimal inhibitory concentration (MIC) or a killing curve, do not take the fluctuation of antibiotic concentrations due to intermittent dosing into account, as usually occurs when patients are treated.
The first part of *Chapter 10* discusses this problem, and a brief review of the literature describing in vitro pharmacokinetic models is given. In the second part of this Chapter, an in vitro pharmacokinetic model is described, and the results of intermittent and continuous infusion of ceftazidime on the killing of *P. aeruginosa* are presented.

TWO

PRINCIPLES OF ANTIBACTERIAL THERAPY IN CYSTIC FIBROSIS

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CHAPTER 2

PRINCIPLES OF ANTIBACTERIAL THERAPY IN CYSTIC FIBROSIS

INTRODUCTION

Bacterial lung infections determine the prognosis for most cystic fibrosis (CF) patients. The antibacterial therapy is difficult, not only because of the special relationship which exists between host and bacterium, but also because the pharmacokinetics of antibiotics is abnormal in these patients. In this chapter these two features will be discussed in relation to antimicrobial therapy. First, pulmonary infection and its causative agents will be considered. In the second part new insights in the working mechanisms of antibiotics will be examined, which may lead to better treatment results.

PULMONARY INFECTION

The major cause of morbidity and mortality in patients with CF is pulmonary infection (Wood, Boat and Doershuk, 1976; Hoiby, 1982). When the disease was first described by Andersen (1938) most patients died at an early age. Since the introduction of antibiotics, the prognosis has improved dramatically and now many patients live into adulthood. Although this is not solely due to increasing use and efficacy of antibiotic treatment, most clinicians think that antibiotics provide an important contribution to the treatment of CF patients when suffering from an infectious exacerbation.

At birth, the lungs of CF patients are histologically normal or show only minor changes (Bedrossian *et al.*, 1976). Within the first year of life pathological changes

may develop, initially in the bronchioles. Infection and hypersecretion of viscous mucus lead to peribronchiolar inflammation and small airway obstruction. This process may be accelerated by early viral infections (Abman *et al.*, 1988). As the disease progresses, the larger airways become involved and the various complications of bronchopneumonia, micro-abscess formation and bronchiectases develop. Chronic airway obstruction results in a ventilation perfusion mismatch and hypoxemia, which may ultimately lead to pulmonary hypertension and cor pulmonale (Ryland and Reid, 1975).

In CF four bacterial species play an important role as infectious agents: *Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa*, and, on the American continent, *Pseudomonas cepacia*. Other organisms may also be present, but are relatively infrequent (Hoiby, 1977; Petersen *et al.*, 1981; Thomassen, Demko and Doershuk, 1987). The pattern of isolation changes with the age of the patient. In the young, *H. influenzae* and *S. aureus* are isolated most frequently. With increasing age, *P. aeruginosa* is the most common organism cultured. Although there are several theories trying to explain this shift, none of these are satisfactory (Smith *et al.*, 1989).

H. Influenzae

H. influenzae is probably the least harmful of these organisms. It is most often found in relatively young patients. If present, it is seldom cultured from sputum for more than a few months. In the majority of patients the sputum is free of *H. influenzae* after treatment. In a Danish study (Petersen *et al.*, 1981), 14% of acute exacerbations of respiratory symptoms were associated with *H. influenzae*. Most strains are biotype I and nonencapsulated (Hoiby and Kilian, 1976; Watson, Kerr and Hinks, 1985), but the reasons for this are not known. Recently, it has been demonstrated that *H. influenzae* is more often present in sputum then hitherto thought, especially when *P. aeruginosa* is present in large quantities. *H. influenzae* may then be difficult to detect due to overgrowth of *P. aeruginosa* (Terpstra *et al.*, 1988). Using monoclonal antibodies directed against an outer membrane protein and staining with in situ immuno-peroxidase in sputum smears, Moller *et al.* (1992) showed that *H. influenzae*

was present in 54% of CF sputum samples, compared to 23% positivity with standard culture. However, the clinical significance of these findings still has to be resolved.

S. aureus

S. aureus was responsible for most of the lung morbidity and mortality when the disease was first described (Andersen, 1938; Huang *et al.*, 1961; Iacocca, Van Loon and Banbere, 1961). Patients usually died at an early age. When penicillin was introduced the prognosis of CF patients improved remarkably (Andersen, 1949). *S. aureus* is often held responsible for most of the early permanent lung damage. In a Danish study, 9% of the patients were colonized for more than 6 months (Szaff and Hoiby, 1982), and persistent colonization and infection with identical types of *S. aureus* has been described (Goering *et al.*, 1990). Since frequent and prolonged treatment with antimicrobial agents may lead to development of resistance and tolerance, we tested the activity of various antimicrobial agents against 52 *S. aureus* isolates from 52 patients with CF, and investigated whether tolerance to methicillin was present. The results showed that, in general, the MIC's of the drugs tested were within the therapeutic range, and that the proportion of tolerant strains was similar to that found for non-CF populations (Chapter 4).

Although *S. aureus* is one of the predominant organisms isolated in CF patients during childhood, infections can usually be effectively treated with antibiotics. It has been speculated, however, that the lung damage caused by this micro-organism, or *H. influenzae*, predisposes to colonization with *P. aeruginosa*.

P. aeruginosa

Since the 1960's, a significant decrease in the frequency of isolation of *S. aureus* has been noted with an increase in *P. aeruginosa* (May and Hernick, 1972; Mearns, Hunt and Rushworth, 1972). This is probably due to relatively more patients reaching an older age, who later in life become colonized with *P. aeruginosa*. In the Erasmus University Hospital, the prevalence in 1988 was below 40% in children under 5 years of age, but amounted to 90% in the oldest age group (Figure 1).

There seems to be a relationship between the size of the treatment centre and percentage of patients colonized with *P. aeruginosa*. In larger centres, relatively more patients are colonized with this organism (Hoiby and Pedersen, 1989). The prevalence of patients with a chronic *P. aeruginosa* infection may be as high as 70-80% (Isles *et al.*, 1984). The role of treatment centres in acquiring *P. aeruginosa*, however, is not clear. If cross-infection occurs frequently, one would expect that often identical strains are isolated. Epidemiological typing showed that clusters of distinct strains could be found, indicating that cross-infection could have happened (Hoiby and Rosendal, 1980; Speert and Campbell, 1987). In the Danish CF centre, the incidence of acquisition of *P. aeruginosa* decreased after the introduction of hygienic measures (Pedersen *et al.*, 1987). Studies from holiday camps for patients with CF show that the risk of cross-infection is low (Speert, Lawton and Damm, 1982). Siblings with CF sometimes carry the same strain, but this may be due to the same environmental source (Kelly *et al.*, 1982). In the Erasmus University Hospital, the diversity of strains

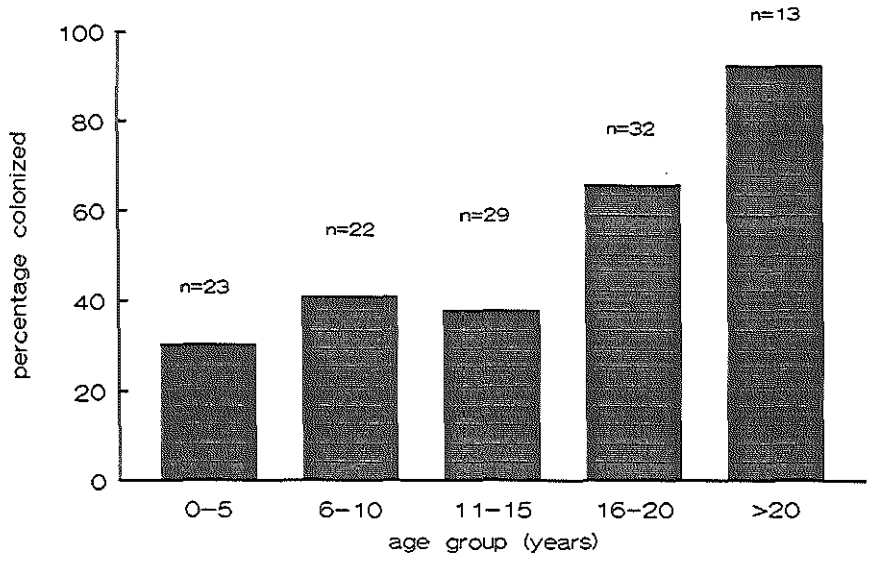

Figure 1. Prevalence of P. aeruginosa in different age-groups of CF patients treated at the Erasmus University Hospital in 1988.

found, as determined by various methods of typing (Horrevorts *et al.*, 1990; Chapter 5), show no evidence of cross-infection being a widespread phenomenon. In conclusion, cross-infection probably does occur, but the frequency is very low (Hoiby and Pedersen, 1989). Once infection has occurred, it is almost impossible to eradicate the micro-organism and most patients harbour it for years. Whether repeated isolation is due to persistent colonization or reinfection with different strains was resolved by using different typing methods. Using a combination of conventional techniques, Horrevorts *et al.* (1990) showed that colonizing strains do as a rule persist for many years. The results of serotyping, pyocin typing and phage typing were consistent with each other. These results were later confirmed (Chapter 5), and new techniques such as restriction enzyme analysis and the use of probes showed similar results (Ogle *et al.*, 1987; Grothues *et al.*, 1988).

It has been observed that *P. aeruginosa* bacteria isolated from sputum in CF patients are often of the mucoid variety (Doggett, 1969). In many CF patients the initial strain is non-mucoid. When a transition to mucoid strains occurs, non-mucoid and mucoid strains may be isolated simultaneously, and gradually mucoid, alginate producing forms predominate (Doggett *et al.*, 1964, Hoiby, 1974). The reason for and function of this are not exactly known. Recently, it was found that two potential conditions in the CF lung, high NaCl and dehydration, may have a profound influence initiating the production of alginate. The underlying molecular mechanism of the transition is beginning to be resolved (May *et al.*, 1991; Russell *et al.*, 1992).

There is still no explanation for the chronicity of the Pseudomonas infection. The basic defect of cystic fibrosis itself, for instance an altered epithelial cell surface favouring adherence of *P. aeruginosa* and other micro-organisms, could play a role (Boat and Cheng, 1983). The mucoid character and the production of alginate of *P. aeruginosa*, however, are often taken as a convincing explanation. The formation of microcolonies (Lam *et al.*, 1980) surrounded by alginate constitute an impregnable barrier for the phagocytes (Doring, Albus and Hoiby, 1988). The alginate layer may thus impair the ability of the immune system to combat *P. aeruginosa* in the CF lung. There is some evidence that the immune system is somehow involved in the impaired bacterial clearance of *P. aeruginosa*, although there is no evidence of a defect in host

immunity (Hoiby *et al.*, 1975; Doring *et al.*, 1988). Pier *et al.* (1987), however, showed a specific defect of opsonophagocytic antibodies to the mucoid exopolysaccharide in a group of CF patients colonized with *P. aeruginosa* when compared to noncolonized CF patients or healthy adults. It was suggested (Pedersen *et al.*, 1989) that high titers of non-opsonic IgG subclasses might interfere with pulmonary macrophage phagocytosis or a change from opsonic to non-opsonic antibodies. The production of proteases which may cleave opsonizing antibodies, complement components and cell receptors on phagocytes (Suter, 1989; Doring, 1989), are probably also important.

P. cepacia

Since the early 1980's, *P. cepacia* has been recognized as a major pathogen in CF patients (Isles *et al.*, 1984; Thomassen *et al.*, 1985), especially in adults. Initially isolated on the American continent only, the organism is now common in Europe as well (Simmonds *et al.*, 1990). Three distinct clinical patterns can be observed: chronic aymptomatic carriage; progressive deterioration over many months with recurrent fever, progressive weight loss and repeated hospital admissions; and rapid, usual fatal deterioration in previously mildly affected patients (Isles *et al.*, 1984). The virulence of the different strains seems to vary considerably, but little is known about the host immune response and pathophysiology of *P. cepacia* infection (Anonymous, 1992). Treatment of *P. cepacia* is often difficult, since the organism is intrinsically resistant to most antibiotics (Nelson *et al.*, 1991).

A cause of concern is cross-infection with *P. cepacia*. Although the transmission route remains a controversial issue (Nelson *et al.*, 1991), cross-infection seems to be important (Lipuma *et al.*, 1990). Since some strains may be very virulent, recommendations with regard to prevention of cross-infection have been published, but the social impact of these guidelines will and can be hard to accept (Anonymus, 1992).

Deterioration of lung function

There is growing evidence that the formation of immune complexes, due to a vigorous response of the host to the chronic infection, and ineffective phagocytosis, possibly due to the formation of microcolonies, lead to an imbalance between polymorphic

mononuclear neutrophil proteinases and their inhibitors (Suter, 1989). This results in proteolytic and oxidative tissue damage (Elborn and Shale, 1990). The production of exoenzymes, such as elastase, by pseudomonas may also contribute to the pathogenesis (Woods and Schol, 1983).

ANTIBIOTIC TREATMENT OF PULMONARY INFECTIONS IN CF

Dosage

The dosage of antibiotics in CF-patients is a controversial subject; few clinicians use the same amount of a drug for treatment (Michel, 1988). Often, dosages are based on in vitro Minimum Inhibitory Concentrations (MIC) and calculated as to obtain serum levels above MIC for most of the dosing interval, rather than based on the specific bactericidal effect of an antibiotic, or on the evaluation of clinical efficacy. Failure of therapy using conventional dosage regimens have lead to an increase in both dose and frequency of antimicrobial agents. There are several reasons why the usual dosage regimens may not be adequate:

1. *Different pharmacokinetics*. The resorption and elimination of drugs in CF patients differ from those in non-CF patients. In general, CF patients have a larger volume of distribution and an increased total body clearance of beta-lactams and aminoglycosides (for reviews, see de Groot and Smith, 1987; Prandota, 1988; Spino, 1991) with, as a result, a smaller area under the curve (AUC), a shorter elimination half-time and lower peak plasma antibiotic concentrations. To obtain the same plasma concentrations as in non-CF patients, dosages have therefore to be increased. Since infections in CF are difficult to treat, drug monitoring in CF may be of particular importance to ensure an optimal dosing regimen (Chapter 3).

2. *Low sputum concentrations*. Although the serum concentrations may be adequate, the site concentrations of the antibiotic may be too low. There are several factors influencing the penetration of the antibiotic in the bronchial secretions, which are mainly dependent on the barriers which the antibiotic has to cross within the lung (Bergogne-Berezin, 1981; Mendelman *et al.*, 1985; Honeybourne and Baldwin, 1992). Physico-chemical properties, degree of bronchial inflammation and route of admini-

stration are all important. Inflammation has been shown to increase the permeability of the bronchial tissue to antibiotics. Because the inflammation in CF is mainly on the luminal side of the bronchi (Jeffery and Brain, 1988), penetration through the airway barrier may be hampered.

In many antibiotics, the sputum concentration in relation to the plasma concentration is not known. Studies are complicated because of the large differences in the amount of sputum produced between and within patients, the variation in the rate of sputum expectoration, salivary contamination and medication (Honeybourne and Baldwin, 1992). For aminoglycosides, sputum concentrations are substantially lower than plasma concentrations (Mendelman *et al.*, 1985). Even when sputum concentrations are determined, it is not certain what this means for the concentration of antibiotic at the site of infection.

3. *Diminished activity of antibiotic in sputum.* Since sputum or mucus contains mucins, proteins, DNA, lipid and cellular debris, these substances could interfere with the activity of the antibiotic in the sputum, for instance by binding. Ramphal *et al.* (1988) did a binding study with standardized concentrations of macromolecules obtained from sputum of CF patients. Their results showed a decreased activity of tobramycin, but not of ceftazidime, which was probably due to increased binding of tobramycin. Differences in ion concentration and pH may also influence activity (Mendelman *et al.*, 1985; Levy, 1986).

4. *Behaviour of micro-organism. P. aeruginosa* in the CF lung form microcolonies (Lam *et al.*, 1983; Govan and Harris, 1986) surrounded by a mucoid anion matrix. In vitro studies have shown that the penetration of antibiotics through this layer is hampered (Slack and Nicols, 1981; Nichols *et al.*, 1988; Bolister *et al.*, 1991). The alginate may thus function as a barrier to antibiotics, thereby rendering the micro-organism less susceptible (Govan and Fyfe, 1978; Slack and Nichols, 1981). However, the clinical significance of these findings is not exactly known (Bollister *et al*, 1991).

5. *Mixed bacterial populations.* The *P. aeruginosa* bacterial population residing in the CF lung is not homogeneous, although belonging to the same strain. When a sputum sample is cultured, it is usually possible to isolate bacteria with different sensitivity patterns (Thomassen *et al.*, 1979; Seale *et al.*, 1979).

6. *Emergence of resistance.* As CF patients are often treated with antibiotics, emergence of resistance during long-term use of antobiotics may occur. We studied the emergence of resistance of *P. aeruginosa* isolated from CF patients by comparing the Minimum Inhibitory Concentration for 15 antibiotics during two time-periods (Chapter 5). From 34 chronically colonized patients, strains isolated before 1987 and in 1990 or later were compared with each other. The pairs of strains showed similar typing patterns for each pair, indicating prolonged colonization with the same strain. Nineteen patients newly colonized in 1990 or later served as a control group. There was a marked increase in resistance between the 34 pairs of strains during the study period. The susceptibilities of the 34 strains isolated before 1987 were comparable to the strains of the control group. There was a significant correlation between the increase in resistance and the number of days spent in hospital and number of admissions to the hospital. Pattern analysis of the emergence of resistance showed linkage within groups of antibiotics. It was concluded that long-term antimicrobial therapy is associated with development of resistance within the individual patient.

Dosing schedules

Discussion concerning the dosing regimens of antibiotic drugs in general, especially the aminoglycosides and the beta-lactams, has been reopened during recent years (e.g. Cars and Craig, 1990). One of the reasons was the demonstration of the post-antibiotic effect (PAE), or lack thereof, of antibiotics on the killing and growth of bacteria (e.g. Vogelman and Craig, 1986). The PAE is the period of time which elapses before micro-organisms start to regrow when no longer exposed to an antibiotic, and differs for each class of antibiotics. Aminoglycosides have been shown to have a PAE on aerobic Gram-negative rods and Gram-positive cocci. Beta-lactams (excluding imipenem), on the other hand, show a PAE on Gram-positive cocci, but not on Gram-negative rods, such as *P. aeruginosa.*

Another important reason to reconsider dosing regimens is the presence or absence of a dose-effect relationship of an antibiotic. Since dosing regimens may directly influence the results of treatment, especially of the difficult to treat pseudomonas

infections in CF patients, these issues will be discussed more extensively for the aminoglycosides and beta-lactams. The quinolones are also considered.

Aminoglycosides. The bactericidal effect and the PAE of aminoglycosides are concentration dependent (Vogelman and Craig, 1986). Several studies have shown a correlation between the peak plasma concentration of aminoglycosides one hour after administration and clinical and bacteriological response (Laskin *et al.*, 1983; Rabin *et al.*, 1980). However, the therapeutic range of aminoglycosides is narrow due to oto- and nefrotoxicity (McRorie *et al.*, 1989). The risk of side effects has been reported to increase if trough concentrations exceed a certain threshold (Jackson, 1983). These characteristics have lately resulted in less frequent dosing with higher dosages, and clinical trials are now in progress to demonstrate the efficacy and safety of these regimens. In CF patients, however, the situation may be different. After a conventional dose, lower plasma concentrations and a shorter half-life are found than in the normal population, which could be explained by an increased total body clearance and larger volume of distribution or both (review: Spino, 1991). This may cause the dose interval to become too long. Horrevorts *et al.* (1987, 1988) therefore adjusted the dosing frequency in individual CF patients and obtained a significantly better clinical outcome than with a standard regimen when treating *P. aeruginosa* infections with tobramycin. Winnie *et al.* (1991) also found a 6-hour dosing interval more efficacious than an 8-hour interval in CF patients. The aim is to keep top levels between 6 and 12 mg/l, while trough levels should fall between 1 and 2 mg/l (Horrevorts *et al.*, 1987; Moore *et al.*, 1982).

Beta-lactams. In contrast to aminoglycosides, a PAE of beta-lactams is absent when treating Gram-negative rod infections (excluding imipenem). Killing of bacteria is time-dependent, but relatively independent of the concentration. Concentrations much higher than the MIC contribute no extra effect. The conventional treatment of three doses daily leads to plasma concentrations below MIC for part of the dosing interval and may then not be effective, while unnecessary high peak concentrations are reached after each dose. A continuous infusion overcomes this problem (Figure 2) and may lead to better clinical results, as has been shown in some reports (Daemen and de Vries-Hospers, 1988; David and Devlin, 1989). This is in agreement with stu-

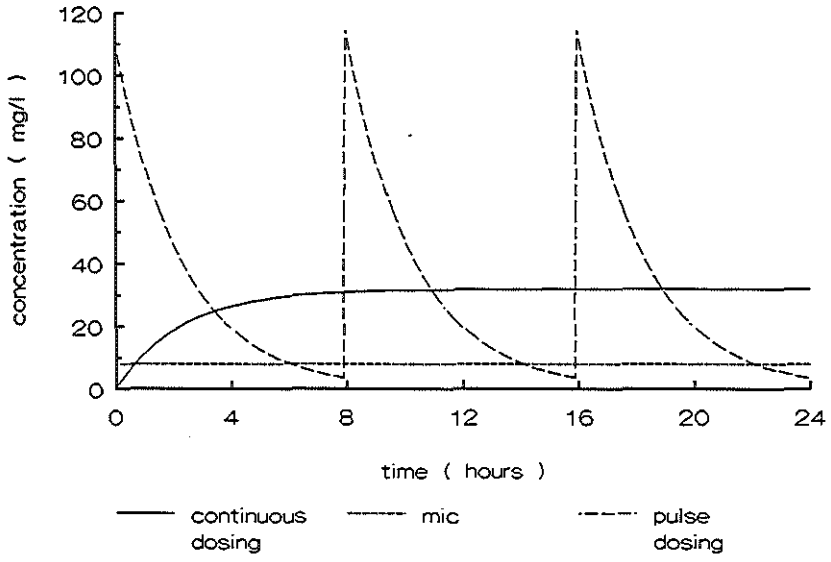

Figure 2. Simulated concentration-time curves of ceftazidime during continuous and intermittent infusion. With continuous infusion of 100 mg/kg/24 h, and a MIC of 8 mg/l, a plasma concentration well above MIC is reached continuously. Using intermittent dosing, the plasma concentration falls below MIC for about one third of the time (Based on data from De Groot and Smith, 1987).

dies in laboratory animals (Roosendaal *et al.*, 1989). In CF patients, there is another argument in favour of a continuous mode of administration when treating *P. aeruginosa* infections. In these patients, the elimination half-time is sometimes shorter than in non-CF subjects (de Groot and Smith, 1987; Spino, 1991), leading to a longer period of time below MIC when dosing intermittently; this subject is more extensively discussed in Chapter 8. Since beta-lactams do show a PAE on Gram-positive cocci, and these generally have a lower MIC, pulmonary exacerbations due to *S. aureus* may be treated using an intermittent dosing schedule.

Quinolones. Ciprofloxacin is one of the newer fluoroquinolones which is effective against *P. aeruginosa.* It has the advantage that it can be given orally, making home therapy feasible. Its bioavailability in CF patient was found to be 70% (Davis *et al.*, 1987) and 80% (Christensson *et al.*, 1992), respectively. In the latter study, it was found that oral availability in CF patients was increased compared to healthy volunteers. Ciprofloxacin can be used as monotherapy, although this may be limited by the development of resistance (Goldfarb *et al.*, 1987). It is also effective against *S. aureus* and *H. influenzae.*

Pharmacokinetic studies of ciprofloxacin in CF patients show conflicting results. LeBel *et al.* (1986) found a shorter half-life in CF patients compared to healthy controls, and advised a dosage given four times daily. Other studies (Davis *et al.*, 1987; Stutman *et al.*, 1987) showed no such differences. Both the tissue penetration and the sputum concentration were reported to be adequate, and with an MIC90 of 1 mg/l for *P. aeruginosa*, appropiate levels may be reached.

The MIC of ciprofloxacin of *P. aeruginosa* often increases with a factor 4 to 16 during therapy (Bosso *et al.*, 1989; Goldfarb *et al.*, 1987; Rubio and Shapiro, 1986). Although usually reversible, resistance to this agent may become a problem in the future. Several trials have shown no difference in treatment results obtained with ciprofloxacin compared to standard regimens (Bosso *et al.*, 1987; Rubio and Shapiro, 1986). In one study the efficacy was slightly better (Hodson *et al.*, 1987), although this may have been caused by the design of the study. In contrast, Jensen *et al.* (1987) showed that the long-term clinical effect of repeated courses of ciprofloxacin was inferior to that of conventional intravenous treatment.

Of the newer quinolones, ofloxacin is increasingly being used. Resorption is close to 100%, while for ciprofloxacin resorption is 70%-80%. However, the MIC's of ofloxacin for *P. aeruginosa* are at least a factor two higher (e.g. chapter 5; Gonzalez and Henwood, 1990). The somewhat higher resorption does not compensate for differences in MIC. Ciprofloxacin still appears to be the first choice among the fluoroquinolones, although clinically good results have been obtained with ofloxacin (Jensen *et al.*, 1987).

Aerosols

Aerosolized antibiotics have been used since the 1940's. The aim is to deliver the drug within the intrapulmonary airways, so that effective local concentrations are obtained with low doses, and systemic side effects are avoided. Several trials have shown a beneficial effect (Hodson *et al.*, 1981; Steinkamp *et al.*, 1989). The number of acute hospital admissions may be reduced, which is advantageous socially as well as in terms of costs, although the aerosols are expensive. The administration, however, can be time-consuming, and most antibiotics have an unpleasant taste. It is unknown what quantity of the dose given is deposited in the lungs, and whether it reaches the infectious foci: in CF patients, the deposition of aerosol is non-uniform and most of the drug reaches the best ventilated areas (Laube *et al.*, 1989).

Three points need further attention: firstly, inhaled antibiotics might lead to an increase in resistant organisms, but this has not been shown to be injurious (Hodson, 1988; Steinkamp *et al.*, 1989). Secondly, hypersensitivity might be induced, but has seldom been found. Bystanders, however, may suffer from hypersensitivity reactions when beta-lactam aerosols are used. Lastly, hypertonic nebulized antibiotics may cause bronchoconstriction (Chua *et al.*, 1990). In the Danish CF Centre, a combination of colistin inhalation and ciprofloxacin orally resulted in a delay of onset of chronic *P. aeruginosa* infection (Valerius *et al.*, 1991). However, colistin inhalation therapy has been suggested to be a risk-factor in the acquisition of *P. cepacia* (Anonymous, 1992).

Indications for the use of aerosols are mild exacerbations which can be treated at home. Aerosols can also be used as maintenance treatment, preferably in combination, or alternating, with another antibiotic such as ciprofloxacin. Maintenance treatment is sometimes necessary in patients with pseudomonas infections who need continuous suppression of their flora.

An important issue when administering aerosols, is the use of the proper equipment and technique. A jet-nebulizer should be used to produce particles of 2-4 mum (Sterk *et al.*, 1984), preferably with a mouthpiece. The patient should be well instructed. In normal lungs slow deep breathing results in a uniform deposition throughout the lung with limited aerosol depositon in the larger airways.

ANTIBIOTIC TREATMENT OF PULMONARY INFECTIONS IN CF: INDICATIONS AND CONSIDERATIONS

Since CF patients are chronically colonized with micro-organisms, and bacteria more often cultured than not, it is not always easy to determine whether an infectious exacerbation is present. Many indices of an infectious exacerbation are used (Table I) but no consensus has been reached on which parameters should be employed to define an exacerbation. Therefore, it is difficult to compare the results of clinical trials especially with regard to anti-pseudomonas therapy (Michel, 1988).

Table I. Indices of infectious exacerbations

Subjective parameters	Objective parameters
increase in cough	lung function: decrease in FEV1
increase in sputum production	weight loss
dyspnea	pulmonary X-ray
malaise	oxygen saturation
decrease in appetite	sputum production
	bacterial counts in sputum
	infection markers in blood
	serology: number of precipitins
	(fever)

Some variables, however, should be measured at regular intervals in order to follow the progression of the disease and could be used to decide whether antibacterial therapy is warranted, namely: lung function, in particular FEV1; weight; pulmonary X-ray and, in more advanced disease, oxygen tension and/or saturation and carbon-dioxide tension. The number of precipitins against *P. aeruginosa* may be of value (Hoiby, 1977).

Several markers in blood have been sought as indicators for an exacerbation, such as CRP. Generally, these markers can be important to describe the course of an exacerbation in the individual patient, but have limited value as a diagnostic tool. The

FEV1 is the lung function parameter most often used when evaluating clinical trials and treatment, and was also used in the study described in Chapter 8. However, at the end stage of the disease lung damage may be of such severity, that further deterioration is hardly possible; other indicators are then necessary.

A decade ago, out-of-hospital treatment of exacerbations due to *H. influenzae* and *S.aureus* was sometimes possible with oral medication, but antipseudomonal therapy was provided almost exclusively in the hospital. Since then, quinolones have become available, aerosol therapy at home has increased and, more recently home intravenous therapy has been introduced in several countries (Winter *et al.*, 1984; Kane *et al.*, 1988; Bakker *et al.*, 1992). These developments have diminished the sharp distinction that used to be made between an exacerbation due to *P. aeruginosa* to be treated in hospital and no exacerbation; however, the same principles for treatment should be used. After isolation and identification of the causing organism from sputum, its susceptibility for antibiotics should be tested.

Table II. Antibiotics commonly used to treat pulmonary exacerbations due to *P. aeruginosa*

Drug	Dose			
Tobramycin	10 mg/kg/day in three dosages, adjust after three days: peak 8-12 mg/l; trough 1-2mg/l			
in combination with				
	intermittent administration		continuous administration	maximum daily dose
	mg/kg	doses/day	mg/kg/24h	(g)
Ticarcillin	750	4	500	30
Ceftazidime	150-200	3-4	100	12
Imipenem/cilastatin	50-100	4	-	4
Aztreonam	150	4	100	8
and/or				
ciprofloxacin: oral:	1500-2250 mg/day	in 3 doses		
intravenous:	400-800 mg/day	in 2 doses		

Treatment should be started with high dosages of appropriate antibiotics (Table II) and last for at least 14 days and until the lung function has returned to pre-exacerbation level or has reached a plateau. The specific side effects of antibiotics used should be monitored. It is important that, when an exacerbation is treated with antibiotics, guidelines or a certain strategy be followed.

This overview has served to show that many dilemmas still exist concerning anti-microbial therapy. Whereas the choice of therapy is becoming more and more limited (Geddes, 1988) during the course of the disease, the agents still available should be handled in a rational manner and with great care.

REFERENCES

Abman SH, Ogle JW, Butler-Simon N. *et al.*: Role of respiratory syncytial virus in early hospitalizations for respiratory distress of young infants with cystic fibrosis. J Pediatr 113:826-830, 1988

Andersen DH: Cystic fibrosis of the pancreas and its relation to celiac disease. Am J Dis Child 56:344-399, 1938

Andersen DH: Therapy and prognosis of fibrocystic disease of the pancreas. J Pediatr 3:406-413, 1949

Anonymous: *Pseudomonas cepacia*- more than a harmless commensal? Lancet i:1385-1386, 1992

Bakker W, Heyerman HGM, de Graaff CS and de Jonge P.: Home intravenous therapy (HIVT) in cystic fibrosis with ceftazidime continuous infusion using an ambulatory pump. XIth International cystic fibrosis congress, Ireland. Abstract TP24, 1992

Beaudry PH, Marks MI, McDougall D. *et al.*: Is anti-pseudomonas therapy warranted in acute respiratory exacerbations in children with cystic fibrosis? J Pediatr 97:144-147, 1980

Bedrossian CM, Greenberg SD, Singer DB *et al.*: The lung in cystic fibrosis: A quantitative study including prevalence of pathologic findings among different age groups. Human Pathol 7:195-204, 1976

Bergogne-Berezin, E.: Penetration of antibiotics in the respiratory tree. J Antimicrob Chemother 8:171-174, 1981

Boat TF and Cheng PW.: Glycoproteins. In: Textbook of cystic fibrosis (Lloyd-Still JD, ed.). Boston, John Wright, 53-70, 1983

Bollister N, Masker M, Hodges NA and Marriott C.: The diffusion of beta-lactam antibiotics through mixed gels of cystic fibrosis-derived mucin and *Pseudomonas aeruginosa* alginate. J Antimicrob Chemother 27:285-293, 1991

Bosso JA, Black PG and Matsen JM.: Ciprofloxacin versus tobramycin plus azlocillin in pulmonary exacerbations in adult patients with cystic fibrosis. Am J Med 82:180S-183S, 1987

Bosso JA, Allen JE and Matsen JM.: Changing susceptibility of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients with the clinical use of newer antibiotics. Antimicrob Agents Chemother 33:526-528, 1989

Cars O and Craig WA.: General discussion and antibiotic dosing. Scand J Infect Dis (Suppl) 74:282-283, 1990

Chua CHL, Collis GG and le Soeuf PN.: Bronchial response to nebulized antibiotics in children with cystic fibrosis. Eur Respir J 3:1114-1116, 1990

Christensson BA, Nilsson-Ehle I, Ljungberg B, *et al.*: Increased oral availability of ciprofloxacin in cystic fibrosis patients. Antimicrob Agents Chemother 36:2512-2517, 1992

Daemen S and de Vries-Hospers H.: Cure of *Pseudomonas aeruginosa* infection in neutropenic patients by continuous infusion of ceftazidim. Lanceti:937, 1988

David TJ and Devlin J.: Continuous infusion of ceftazidim in cystic fibrosis. Lanceti:1454-1455, 1989

Davis RL, Koup JR, Williams-Warren J, *et al.*: Pharmacokinetics of ciprofloxacin in cystic fibrosis. Antimicrob Agents Chemother 31:915-919, 1987

Doggett RG.: Incidence of mucoid *Pseudomonas aeruginosa* from clinical sources. Applied Microbiol 18:936-937, 1969

Doggett RG, Harrison GM and Wallis ES.: Comparison of some properties of *Pseudomonas aeruginosa* isolated from infection in persons with and without cystic fibrosis. J Bacteriol 87:427-431, 1964

Doring G, Albus A and Hoiby N.: Immunological aspects of cystic fibrosis. Chest 94:109S-114S, 1988

Doring G.: Polymorphonuclear leukocyte elastase: its effect on the pathogenesis of *Pseudomonas aeruginosa* infection in cystic fibrosis. In: *Pseudomonas aeruginosa* infection. (Hoiby N, *et al*; eds.) Basel, Karger, 169-176, 1989

Elborn JS and Shale DJ.: Lung injury in cystic fibrosis. Thorax 45:970-973, 1990

Geddes DM.: Antimicrobial therapy against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Pseudomonas cepacia*. Chest 94:140S-144S, 1988

Goering RV, Bauernfeind A, Lenz W and Przyklenk B.: *Staphylococcus aureus* in patients with cystic fibrosis: an epidemiological analysis using a combination of traditional and molecular methods. Infection 18:57-60, 1989

Goldfarb J, Stern RC, Reed D, *et al.*: Ciprofloxacin monotherapy for acute pulmonary exacerbations of cystic fibrosis. Am J Med 82:174-179, 1987

Gonzalez JP and Henwood JM.: Pefloxacin, a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 37:628-668, 1989

Govan WJRW and Fyfe JAM.: Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants in vitro. J Antimicrobial Chemother 4:233-240, 1978

Govan WJRW and Harris GS.: *Pseudomonas aeruginosa* and cystic fibrosis: Unusual bacterial adaptation and pathogenesis. Microbiol Sci 3:302-308, 1986

Groot de R and Smith AL.: Antibiotic pharmacokinetics in cystic fibrosis: Differences and clinical significance. Clin Pharmacokinet 13:228-253, 1987

Grothues D, Koopman U, van der Hardt K and Tummler B.: Genome finger printing of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. J Clin Microbiol 26:1973-1977, 1988

Hodson ME.: Antibiotic treatment: Aerosol therapy. Chest 94:162S-165S, 1988

Hodson ME, Penketh ARL and Batten JC.: Aerosol carbenicillin and gentamicin treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. Lancet ii:1137-1139, 1981

Hodson ME, Roberts CM, Bulland RJA, *et al.*: Oral ciprofloxacin compared with conventional intravenous treatment for *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. Lancet i:235-237, 1987

Hoiby N.: *Pseudomonas aeruginosa* infection in cystic fibrosis, relationship between mucoid strains of *Pseudomonas aeruginosa* and the human immune response. Acta Pathol Microbiol Scand [B] 82:551-558, 1974

Hoiby N.: *Pseudomonas aeruginosa* infection in cystic fibrosis. Acta Pathol Microbiol Immunol Scand (Suppl):262C, 1977

Hoiby N.: Microbiology of lung infection in cystic fibrosis patients. Acta Paediatr Scand 301(Suppl):33-54, 1982

Hoiby N, Andersen V and Bendixen G.: *Pseudomonas aeruginosa* infection in cystic fibrosis in bacteriologic specimens from patients with cystic fibrosis and patients with other diseases. Acta Pathol Microbiol Scand [B] 83:549-552, 1975

Hoiby N and Kilian M.: Haemophilus from the lower respiratory tract of patients with cystic fibrosis. Scand J Respir Dis 57:103-107, 1976

Hoiby N and Koch C.: *Pseudomonas aeruginosa* infection in cystic fibrosis and its management. Thorax 45:881-884, 1990

Hoiby N and Pedersen SS.: Estimated risk of cross-infection with *P. aeruginosa* in Danish cystic fibrosis patients. Acta Paediatr Scand 78:395-404, 1989

Hoiby N and Rosendal K.: Epidemiology of *Pseudomonas aeruginosa* infection in patients treated at a cystic fibrosis centre. Acta Pathol Microbiol Scand [B] 88:125-131, 1980

Honeybourne D, Baldwin DR.: The site concentrations of antimicrobial agents in the lung. J Antimicrob Chemother 30:249-260, 1992

Horrevorts AM, Borst J, Puyk RJT, *et al.*: Ecology of *Pseudomonas aeruginosa* in patients with cystic fibrosis. J Med Microbiol 31:119-124, 1990

Horrevorts AM, Driessen OMJ, Michel MF, *et al.*: Pharmacokinetics of antimicrobial drugs in cystic fibrosis. Chest 94:102S-103S, 1988

Horrevorts AM, Witte de J, Degener JE, *et al.*: Tobramycin in patients with cystic fibrosis: Adjustment in dosing interval for effective treatment. Chest 92:843-848, 1987

Huang NN, Van Loon EL, Kung T, *et al.*: The flora of the respiratory tract of patients with cystic fibrosis of the pancreas. J Pediatr 59:512-552, 1961

Iacocca VF, Sibinga MS and Banbere GJ.: Respiratory tract bacteriology in cystic fibrosis. Am J Dis Child 106:315-324, 1961

Isles A, Maclusky I, Corey M, *et al.*: *Pseudomonas cepacia* infection in cystic fibrosis: An emerging problem. J Pediatr 104:206-210, 1984

Jackson GG.: Aminoglycoside antibiotics: resistance and toxicity - a summary. Rev Infect Dis 5:S314-S316, 1983

Jeffery PK and Brain APR.: Surface morphology of human airway mucosa: normal, carcinoma or cystic fibrosis. Scanning Microscopy 2:553-560, 1988

Jensen T, Pedersen SS, Nielsen CH, *et al.*: The efficacy and safety of ciprofloxacin and ofloxacin in chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. J Antimicrob Chemother 20:585-594, 1987

Kane RE, Jennison K, Wood C, *et al.*: Cost savings and economic considerations using home intravenous therapy for cystic fibrosis patients. Pediatr Pulmonol 4:84-89, 1988

Kelly NM, Fitzgerald MX, Tempany E, *et al.*: Does Pseudomonas cross-infection occur between cystic fibrosis patients? Lancet ii:688-690, 1982

Lam J, Chan R, Lam K and Costerton JW.: Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. Infect Immun 28:546-556, 1980

Laskin OL, Longstreth JA, Smith CR, *et al.*: Netilmycin and gentamicin multidose kinetics in normal subjects. Clin Pharmacol Ther 34:644-650, 1983

Laube BL, Links JM, LaFrance ND, *et al.*: Homogeneity of bronchopulmonary distribution of 99mTc Aerosol in normal subjects and in cystic fibrosis patients. Chest 95:822-830, 1989

LeBel M, Bergeron MG, Vallee F, *et al.*: Pharmacokinetics and pharmacodynamics of ciprofloxacin in cystic fibrosis patients. Antimicrob Agents Chemother 30:260-266, 1986

Levy J.: Antibiotic activity in sputum. J Pediatrics 108:841-846, 1986

Lipuma JJ, Dasen SE, Nielson DW, *et al.*: Person to person transmission of *P. cepacia* between patients with cystic fibrosis. Lancet ii, 1094-1096, 1990

May JR, Hernick NC and Thompson D.: Bacterial infection in cystic fibrosis. Arch Dis Childh 47:908-913, 1972

May TB, Shinabargen D, Maharai R, *et al.*: Alginate synthesis by *Pseudomonas aeruginosa*: a key pathogenic factor in chronic pulmonary infections of cystic fibrosis patients. Clin Microbiol Rev 4:191-206, 1991

Mearns MB, Hunt GH and Rushworth R.: Bacterial flora of the respiratory tract in patients with cystic fibrosis. Arch Dis Childh 47:902-907, 1972

McRorie TI, Bosso J, and Randolph L.: Aminoglycoside ototoxicity in cystic fibrosis. Am J Dis Chest 143:1328-1332, 1989

Mendelman PM, Smith AL, Levy J, *et al.*: Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis 132:761-765, 1985

Michel BC.: Antibacterial therapy in cystic fibrosis: A review of the literature published between 1980 and February 1987. Chest 94:129S-139S, 1988

Moller AVM, Grasselier H, Ruijs GJ, *et al.*: *Haemophilus influenzae* is a common pathogen in patients with cystic fibrosis. XIthInternational cystic fibrosis congress, Ireland. Abstract WS29, 1992

Moore RD, Smith CR and Lietman PS.: Association of aminoglycoside plasma levels with therapeutic outcome in Gram-negative pneumonia. Am J Med 77:657-662, 1982

Mouton JW and Kerrebijn KF.: Antibacterial therapy in cystic fibrosis. Med Clin North Am 74:837-850, 1990

Nelson JW, Doherty CJ, Brown PH, *et al.*: *P. cepacia* in in-patients with cystic fibrosis. Lancet ii:1525, 1991

Nichols WW, Dorington SM, Slack MPE and Walmsley HL.: Inhibition of tobramycin diffusion by binding to alginate. Antimicrob Agents Chemother 32:518-523, 1988

Ogle JW, Janda JM, Woods DE and Vasil ML.: Characterization and use of a DNA probe as an epidemiological marker for *Pseudomonas aeruginosa*. J Inf Dis 155:119-126, 1987

Pedersen SS, Hoiby N, Shand GH and Pressler I.: Antibody response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. In: *Pseudomonos aeruginosa* infection (Hoiby N, *et al*; eds.) Basel, Karger, 130-153, 1989

Pedersen SS, Jensen T, Hoiby N, *et al.*: Management of *Pseudomonas aeruginosa* lung infection in Danish cystic fibrosis patients. Acta Paediatr Scand 76:955-961, 1987

Petersen NT, Hoiby N, Mordhorst CH, *et al.*: Respiratory infections in cystic fibrosis patients caused by virus, chlamydia and mycoplasma-possible synergism with *Pseudomonas aeruginosa*. Acta Paediatr Scand 70:628-638, 1981

Pier GB, Saunders JM, Ames P, *et al.*: Opsonophagocytic killing antibody to *Pseudomonas aeruginosa* mucoid exopolysaccharide in older noncolonized patients with cystic fibrosis. New Engl J Med 317:793-798, 1987

Prandota J.: Clinical pharmacology of antibiotics and other drugs in cystic fibrosis. Drugs 35:542-578, 1988

Rabin HR, Harley FL, Bryan LE, *et al.*: Evaluation of a high dose tobramycin and ticarcillin protocols in cystic fibrosis based on improved susceptibility criteria and antibiotic pharmacokinetics. In Sturgess JM. Perspectives in Cystic Fibrosis. Proc. 8th International CF Congress, Toronto, 1980, p 370-375

Ramphal R, Lhermitte M, Filliat M and Roussel P.: The binding of anti-pseudomonal antibiotics to macromolecules from cystic fibrosis sputum. J Antimicrob Chemother 22:483-490, 1988

Ryland D and Reid L.: The pulmonary circulation in cystic fibrosis. Thorax 30:285-292, 1975.

Roosendaal R, Bakker-Woudenberg AJM, van den Berghe-Raffe MA, *et al.*: Impact of the dosis schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in *Klebsiella Pneumoniae* pneumonia and septicaemia in leukopenic rats. Eur J Clin Microbiol Infect Dis 8:878-887, 1989

Rubio TT and Shapiro C.: Ciprofloxacin in the treatment of pseudomonas infections in cystic fibrosis patients. J Antimicrob Chemother 18:147S-152S, 1986

Russell JR, Tatnell PJ, and Gacesa P.: The regulation of alginate biosynthesis by mucoid *P. aeruginosa*. In: Cystic fibrosis, basic and clinical research (Hoiby, N and Petersen SS, eds.). Excerpta Medica, Amsterdam, 81-92, 1992

Seale TW, Thirkill H, Tarpay M, *et al.*: Serotypes and antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates from single sputa of cystic fibrosis patients. J Clin Microbiol 9:72-78, 1979

Simmonds EJ, Conway SP, Ghoneim ATM, *et al.*: *Pseudomonas cepacia*: a new pathogen in patients with cystic fibrosis referred to a large centre in the United Kingdom. Arch Dis Child 65:874-877, 1990

Slack MPE and Nicols WW.: The penetration of antibiotics through sodium alginate and through the exopolysaccharide of a mucoid strain of *Pseudomonas aeruginosa*. Lancet ii: 502-503, 1981

Smith AL, Ramsey B, Redding G and Haas J.: Endobrochial infection in cystic fibrosis. Acta Paediatr Scand Suppl 363:31-36, 1989

Speert DP and Campbell ME.: Hospital epidemiology of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J Hosp Infect 9:11-21, 1987

Speert DP, Lawton D, Damm S.: Communicability of *Pseudomonas aeruginosa* in a cystic fibrosis summercamp. J Pediatr 101:227-229, 1982

Spino M.: Pharmacokinetics of drugs in cystic fibrosis. In: Clinical reviews in allergy, Vol. 9 (Gershwin E, ed.) Humana Press Inc.,169-210, 1991

Steinkamp G, Tummler B, Gappa M, *et al.*: Long-term tobramycin aerosol therapy in cystic fibrosis. Pediatr Pulmonol 6:91-98, 1989

Sterk PJ, Plomp A, Vate JF vd and Quanjer PH.: Physical properties of aerosols produced by several jet- and ultrasonic nebulizers. Bull Eur Physiopathol Respir 20:65-72, 1984

Stutman HR, Shalit I, Marks MI, *et al.*: Pharmacokinetics of two dosage regimens of ciprofloxacin during a two-week therapeutical trial in patients with cystic fibrosis. Am J Med 82:142S-145S, 1987

Suter S.: The imbalance between granulocyte neutral proteases and antiproteases in bronchial secretions from patients with cystic fibrosis infection. In: *Pseudomonas aeruginosa* (Hoiby N, *et al.* eds.) Basel, Karger, 158-168, 1989

Szaff M, Hoiby N.: Antibiotic treatment of *Staphylococcus aureus* infection in cystic fibrosis. Acta Pediatr Scand 71:821-826, 1982

Terpstra WJ, Groeneveld K, Eijk PP, *et al.*: Comparison of two nonculture techniques for detection of *Haemophilus influenzae* in sputum. Chest 94:126S-128S, 1988

Thomassen MJ, Demko CA, Boxerbaum B, *et al.*: Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. J Inf Dis 140:873-880, 1979

Thomassen MJ, Demko CA and Doershuk CF.: Cystic Fibrosis: A review of pulmonary infections and interventions. Pediatr Pulmonol 3:334-351, 1987

Thomassen MJ, Demko CA, Klinger JD and Stern RC.: *Pseudomonas cepacia* colonization among patients with cystic fibrosis: a new opportunist. Am Rev Respir Dis 131:791-796, 1985

Valerius NH, Koch C and Hoiby N.: Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. Lancet ii:725-726, 1991

Vogelman B and Craig WA.: Kinetics of antimicrobial activity. J Pediatr 108:835-840, 1986

Watson KC, Kerr EJC and Hinks CA.: Distribution of biotypes of *Haemophilus influenzae* and *H parainfluenzae* in patients with cystic fibrosis. J Clin Pathol 38:750-753, 1985

Winnie GB, Cooper JA, Witson J, *et al.*: Comparison of 6 and 8 hourly tobramycin dosing intervals in treatment of pulmonary exacerbations in cystic fibrosis patients. Pediatr Infect Dis J 10:381-386, 1991

Winter RJD, George RJD, Deacock SJ, *et al.*: Self administered home intravenous antibiotic therapy in bronchiectasis and adult cystic fibrosis. Lancet i:1338-1339, 1984

Wood RE, Boat TF and Doershuk CF.: Cystic Fibrosis. Amer Rev Respir Dis 113:833-878, 1976

Woods DE and Schol PA.: Role of *Pseudomonas aeruginosa* extracellular in lung disease. Clin Invest Med 9:283S-287S, 1983

THREE

ABNORMAL PHARMACOKINETICS: THE NEED FOR MONITORING

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CHAPTER 3

ABNORMAL PHARMACOKINETICS: THE NEED FOR MONITORING

SUMMARY

Optimal use of a drug depends on rational dosing and subsequent therapeutic drug monitoring for effectiveness and toxicity. Drug monitoring is not relevant for all drugs, but is indicated for drugs which have a narrow therapeutic range or show a large inter-individual variation. If the response is not satisfactory or toxic side effects are observed, the regimen has to be adjusted or another drug used. Methods have been developed to establish rational dosing schedules for the individual patient. In previous methods, patient's specific data such as age, length, weight and serum creatinine are integrated with population pharmacokinetic parameters for a drug. This approach is subject to an appreciable margin of error, particularly in patients whose physiology is far from normal. Therapeutic drug monitoring via blood level determination makes it possible to evaluate the patient's individual pharmacokinetic parameters on which a rational dosage regimen can be based.

INTRODUCTION

The list of available antimicrobial drugs is long and making the choice of an appropriate drug for therapy is far from simple. The establishment of a scientific basis for antimicrobial therapy is one of the most important developments in modern medicine, although substances with anti-infective potential have been used empirically for thousands of years (Majno, 1975). During the last forty years, better understanding of pharmacokinetics has made precise administration of drugs possible. This knowledge

is not always easy to apply to routine patient care. However, one aspect of clinical pharmacokinetics has become firmly established, namely therapeutic drug monitoring. This has been based on one, two or multiple compartment models, but model independent pharmacokinetics are becoming increasingly popular for that purpose.

In choosing the appropriate drug, host as well as infecting organism factors must be considered. Optimal use of the chosen agent depends on rational dosing and subsequent therapeutic monitoring for both effectiveness and toxicity. In the last decade it has been shown how important a dosage schedule can be as a determinant of efficacy and toxicity (Bakker-Woudenberg and Roosendaal, 1988; Mattie *et al.*, 1988). For instance, the therapeutic activity of beta-lactam agents against gram-negative organisms depends upon sustained serum concentrations that are in excess of the MIC rather than transient high peaks at intervals. On the other hand, aminoglycosides appear to be more toxic when given by continuous infusion, while their efficacy depends upon peak serum levels and the area under the curve, irrespective the method of administration, i.e. continuous or intermittent.

Different methods have been developed to establish rational dosing schedules for the individual patient. These comprise a) nomograms and b) the use of pharmacokinetic (mainly one- or two-compartment) computer models. However, these methods are limited in their application. Both are based on population kinetics and usually assume the volume of distribution to be a fixed fraction of the bodyweight, whereas between patients volume of distribution as fraction of the body weight can vary widely for a given drug (Zaske *et al.*, 1982). In general, data for an individual patient such as age, sex, length, weight and serum creatinine are integrated with the population pharmacokinetic parameters for a drug. These are used to calculate the dose or dosing interval necessary to reach desired concentrations. Sheiner and Beal (1982) have proposed an alternative system which is based on Bayesian statistics. Briefly, prior data on the population pharmacokinetic parameters is combined with that of the individual patient, including actual drug concentrations. Integration of these data can be used to predict volume of distribution and total body clearance for a particular patient. A rational dosage regimen can be based on these data.

ABNORMAL PHARMACOKINETICS: THE NEED FOR MONITORING

General pharmacokinetic parameters of drugs are usually obtained from studies in healthy volunteers. However, in clinical practice drugs are often used in patients who are in poor health, for instance those with impaired absorption (whereby less of the administered drug is available), abnormal renal or liver function (whereby more or less of the drug will be excreted or metabolized), an altered serum protein content (whereby the fraction of free drug is decreased or increased) or an abnormal relative extracellular volume (as in ascites, oedema or dehydration and pregnancy). These could all influence the pharmacokinetics of the drug. Since pharmacokinetics deals with drug distribution and changes of drug concentration in the human body, any alteration in absorption, distribution, metabolism and elimination will result in abnormal pharmacokinetics.

Drug monitoring, i.e. determination of the actual serum concentrations, is only of value when a meaningful relationship has been established between plasma levels and efficacy and/or toxicity. In particular, drugs with a low therapeutic index are ineffective if concentrations are too low, but toxic when levels are too high. Most clinicians are familiar with decreased drug elimination due to impaired renal function. We would like to focus attention on the individual patient with a larger volume of distribution and/or total body clearance and how these effect the pharmacokinetic profile of a particular drug.

INCREASED TOTAL BODY CLEARANCE AND/OR VOLUME OF DISTRIBUTION

The process of creating an appropriate dosage regimen should involve the administration of a loading dose followed by maintenance dosing. A loading dose is given in order to reach a desired level immediately and should be considered whenever the half-life of the chosen drug is relatively long. The volume of distribution is important when calculating the loading dose. Since volume of distribution and total body clearance determine the half-life, both are important for establishing the maintenance

dose. The volume of distribution is often expressed as a fixed percentage of the body weight and the total body clearance is derived from a putative relation with serum creatinine. These simplifications do not always reflect the real situation.
When blood levels are plotted against time, a curve can be constructed from which the area under the curve (AUC) and the terminal elimination rate constant (ß) are derived. These are then used for calculating total body clearance (TBC) and volume of distribution (Vd) by the following equations.

$$TBC = Dose / AUC \quad (1)$$

$$Vd_{ß} = TBC / ß \quad (2)$$

An increased total body clearance leads to a smaller area under the curve which implies lower plasma concentrations. The elimination rate constant depends on total body clearance and volume of distribution according to equation 2. Since volume of distribution and total body clearance are independent variables, an increase in volume of distribution (TBC unchanged) results in a lowering of the elimination rate constant and therefore in an increase in half-life because this parameter is inversely related to the elimination rate constant. An increase in the volume of distribution could be present in patients with an increased extracellular volume, for instance patients with ascites. Patients with cystic fibrosis (CF) provide another example. The pharmacokinetics of some antimicrobial agents appear to be different in these patients than in normal individuals (de Groot and Smith, 1987; Prandota, 1987; Prandota, 1988). Most studies report an increase in total body clearance and/or volume of distribution. The total body clearance (Figure 1) and volume of distribution (Figure 2) of ceftazidime were shown to be increased in our cystic fibrosis patients as compared to healthy volunteers when these parameters were adjusted for body weight (Mouton *et al.*, 1990). The absolute values did not differ between the two groups, as did the elimination rate. Thus, patients with CF have a higher total body clearance and a larger volume of distribution per kg of body weight. The larger volume of distribution is probably due to an altered body mass composition. Moreover, there are wide variations in pharmacokinetics between patients with CF, and even in the same patient

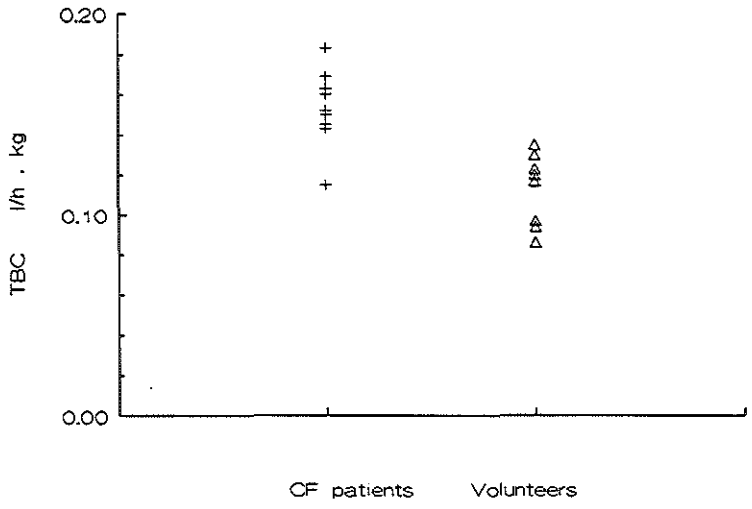

Figure 1. Total body clearance of ceftazidime standardized to kg of body weight during continuous infusion in healthy volunteers and patients with cystic fibrosis ($p<0.05$)

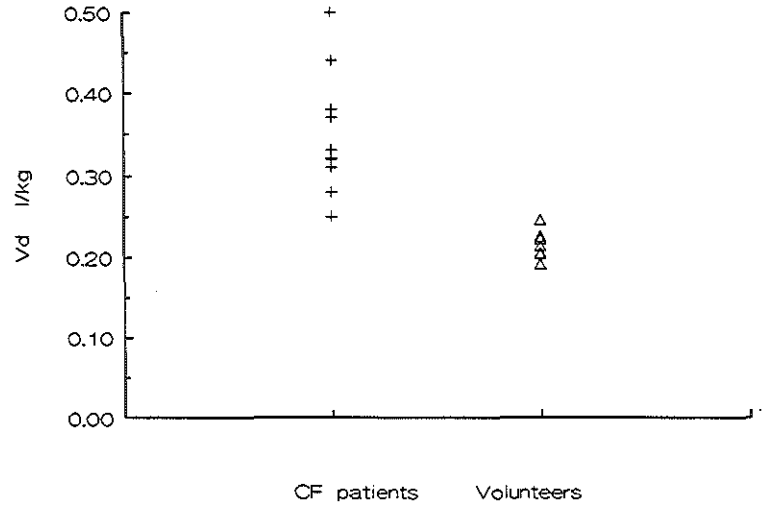

Figure 2. Volume of distribution of ceftazidime standardized to kg of body weight during intermittent infusion in healthy volunteers and patients with cystic fibrosis ($p<0.05$).

at different stages of the disease for some but not all drugs. Consequently, the amount of drug required to successfully control an exacerbation varies from patient to patient and also in the individual patient during the course of the disease (Mann *et al.*, 1985; Horrevorts *et al.*, 1985). Therefore, most centres use serum concentrations as a basis for calculating a dosing regimen in order to achieve a better clinical outcome than is observed with conventional dosing schedules (Horrevorts *et al.*, 1987; Winnie *et al.*, 1991).

When designing a rational dosing regimen for the individual patient, the pharmacokinetic profile is of importance. Conventional nomograms or computer programmes, which are based on population pharmacokinetics, are therefore not always applicable to patients with an increased body clearance or volume of distribution. Individual pharmacokinetic parameters can be obtained by monitoring serum levels.

REFERENCES

Bakker-Woudenberg IAJM and Roosendaal R.: Impact of dosage regimens on the efficacy of antibiotics in the compromised host. J Antimicrob Chemother 21:145-147, 1988

Groot R de and Smith AL.: Antibiotic pharmacokinetics in cystic fibrosis; differences and clinical significance. Clin Pharmacokinet 13:228-253, 1987

Hallynck T, Soep HH and Dettli L.: Influence of age and renal disease on aminoglycoside dosage. J Antimicrob Chemother 8(SupplA):1-9, 1981

Horrevorts AM, de Witte J, Degener JE, Dzoljic-Danilovic G, Hop WCJ, Driessen O, Michel MF and Kerrebijn KF.: Tobramycin in patients with cystic fibrosis: Adjustment in dosing interval for effective treatment. Chest 92:844-848, 1987

Horrevorts AM, Degener JE, Dzoljic-Danilovic G, Michel MF, Kerrebijn KF, Driessen O and Hermans J.: Pharmacokinetics of tobramycin in patients with cystic fibrosis: Implications for the dosing interval. Chest 88:260-264, 1985

Hull JH and Sarubbi FA.: Gentamicin serum concentrations; pharmacokinetic predictions. Ann Int Med 85:183-189, 1976

Majno G. The healing hand: Man and wound in the ancient world. Cambridge, Mass, Harvard Unversity Press, 1975

Mann HJ, Canafax DM, Cipolle RJ, Daniels CE, Zaske DE and Warwick WJ.: Increased dosage requirements of tobramycin and gentamicin for treating Pseudomonas pneumonia in patients with cystic fibrosis. Pediatr Pulmonol 1:238-243, 1985

Mattie H, Craig WA and Pechere JC.: Determinants of efficacy and toxicity of aminoglycosides. J Antimicrob Chemother 24:281-293, 1989

Mouton JW, Horrevorts AM, Mulder PGH, Prens EP and Michel MF.: Pharmacokinetics of ceftazidime in serum and suction blisters fluid during continuous and intermittent infusion in healthy volunteers. Antimicob Agents Chemother 34:2307-2311, 1990

Prandota J.: Drug disposition in cystic fibrosis. Progress in understanding pathophysiology and pharmacokinetics. Pediatr Infect Dis J 6:1111-1126, 1987

Prandota J.: Clinical pharmacology of antibiotics and other drugs in cystic fibrosis. Drugs 35:542- 578, 1988

Ritschel WA.: Handbook of basic pharmacokinetics including clinical applications. Drug Intelligence Publications, Hamilton, IL (ISBN 0-914768-43-3), 1986

Sheiner LB and Beal SL.: Bayesian individualization of pharmacokinetics: Simple implementation and comparison with non-Bayesian methods. J Pharm Sci 71:1344-1348, 1982

Winnie GB, Cooper JA, Witson J, Cowan RG, Mayer M and Lepow M.: Comparison of 6 and 8 hourly tobramycin dosing intervals in treatment of pulmonary exacerbations in cystic fibrosis patients. Pediatr Infect Dis J 10:381-386, 1991

Zaske DE, Cipolle RJ, Rotschafer JC, Solem LD, Mosier NR and Strate RG.: Gentamicin pharmacokinetics in 1640 patients: Methods for control of serum concentrations. Antimicrob Agents Chemother 21:407-411, 1982

FOUR

SUSCEPTIBILITY TO VARIOUS ANTIMICROBIAL AGENTS AND TOLERANCE TO METHICILLIN OF STAPHYLOCOCCUS AUREUS ISOLATES FROM CYSTIC FIBROSIS PATIENTS

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CHAPTER 4

SUSCEPTIBILITY TO VARIOUS ANTIMICROBIAL AGENTS AND TOLERANCE TO METHICILLIN OF STAPHYLOCOCCUS AUREUS ISOLATES FROM CYSTIC FIBROSIS PATIENTS

Pseudomonas aeruginosa, Staphylococcus aureus and *Haemophilus influenzae* are the pathogenic bacterial species most frequently detected in the respiratory tract of patients with cystic fibrosis (CF). To minimize progression of pulmonary destruction, acute exacerbations in CF demand intensive antimicrobial chemotherapy (Mouton and Kerrebijn, 1990). Since persistent colonization and infection with identical types of *S. aureus* have been described (Goering *et al.*, 1990), frequent and prolonged treatment with various antimicrobial agents may influence resistance and tolerance in strains. Tolerance to penicillin has been proposed as one possible explanation for the poor response of some streptococcal infections to penicillin therapy (Goering *et al.*, 1990). In this study we tested the activity of various antimicrobial agents against *S. aureus* isolates from patients with CF. In addition tolerance to methicillin in the isolates was investigated.

Fifty-two isolates of *S. aureus* were recovered from sputum of 52 patients with CF. The strains were identified biochemically by means of the API-STAPH identification system anad stored at -70 °C until tested. Additional tests included a slide coagulase test, a catalase test and mannitol fermentation. Detection of beta-lactamases was performed as follows. Nitrocefin (0.1 ml) was placed in microdilution well and a bacterial suspension yielding 10^7 cfu/ml was added. Production of beta-lactamases was established if the solution turned a deep red within ten minutes.

Antimicrobial agents were obtained as a standard laboratory powder from the manufacturers and stored and handeld as recommended. MICs were determined by an agar dilution technique. Freshly prepared serial twofold dilutions of antibiotic were

incorporated in Diagnostic Sensitivity Test agar (Oxoid, UK). Agar supplemented with 4% NaCI was used to define methicillin susceptibility (Jorgens, 1986). Final concentrations ranged from 0.006 to 25.6 mg/l (for penicillin up to 410 mg/l) Plates were inoculated with a multipoint inoculator, which delivered a final inoculum of 10^4 cfu per spot (1 μl of liquid) from overnight Mueller-Hinton broth (Oxoid) cultures adjusted to 10^8 cfu/ml using a barium silphate standard and diluted 10^{-1}. Inoculated plates were incubated at 35 °C for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent which inhibited visible growth. *S. aureus* strains ATCC 25923 and ATCC 29213 were included as control organisms in all sets of inoculations, and the MICs were consistent with established values within one twofold dilution.

Tolerance to methicillin was determined using the gradient technique of Kim and Anthony (Kim and Anthony, 1983). Briefly, 40 ml of Mueller-Hinton agar (Difco) suplemented with 20 mg/l methicillin was allowed to gel in a 15x15 1.5 cm square petri dish which was placed at an angle to form a wedge. Once cooled, 60 ml of the same agar, but without antibiotics, was overlaid, and allowed to set on a level surface resulting in graduated concentrations nominally ranging from 0 to 20 mg/l. Late-logarithmic cultures were prepared and applied undiluted using cottom wool swabs. Up to six strains together with a non-tolerant and a tolerant control were inoculated as parallel streaks beginning at the lowest concentration along the axis of the gradient. As expected, there was no apparent growth after 24 hours and an impression of the surface was made using a sterile velvet pad which was applied to a methicillin - free plate. This was incubated for 24 hours and the result growth was compared with that of the controls. Tolerance was defined as any growth which occurred beyond that of the non-tolerant control along the line of inoculation. The results are summarized in Table I and Table II. Fourty-nine isolates produced beta-lactamases. The penicillin and methicillin MIC modes for these strains were 12.8 and 1.6 mg/l respectively. Methicillin resistant strains of *S. aureus* were not found. On a weight basis imipenem and rifampicin were the most active of the agents tested, inhibiting 100% of the isolates at 0.025 mg/l and 0.05 mg/l respectively. At clinically attainable serum concentrations flucloxacillin, cefamandole and cefuroxime had similar activity.

Table I. In vitro activity of penicillin against 52 isolates of *Staphylococcus aureus* obtained from 52 patients with cystic fibrosis.

Production of beta-lactamases	Penicillin MIC (mg/l)		Methicillin MIC (mg/l)	
	Range	Mode	Range	Mode
Negative (n=3)	0.05	0.05	1.6-3.2	1.6
Positive (n=49)	0.8-102.4	12.8	0.8-12.8	1.6

Table II. In vitro activity of various antimicrobial agents against 52 isolates of *Staphylococcus aureus* obtained from 52 patients with cystic fibrosis.

Antimicrobial agent	Cumulative percentage of isolates inhibited at given concentration (mg/l)										
	≤0.006	0.012	0.025	0.05	0.1	0.2	0.4	0.8	1.6	3.2	≥6.4
Flucloxacilin					13	73	100				
Imipenem		60	100								
Cefamandole						23	94	100			
Cefuroxime					6	27	100				
Gentamicin			2	2	10	96	100				
Erythromycin				2	10	96	96	96	98	98	100
Clindamycin			2	12	96	98	98	98	98	98	100
Rifampicin		8	87	100							
Fusidic acid				46	98	98	98	98	98	98	100
Vancomycin							10	100			
Teicoplanin						4	13	100			
Ciprofloxacin					2	4	87	100			
Fleroxacin						6	98	100			

With the exception of erythromycin, clindamycin and fusidic acid, MIC ranges were within one (imipenem, vancomycin) to four (ciprofloxacin, gentamicin) twofold dilutions. However, it should be noted that the wider MIC ranges observed for erythromycin, clindamycin and fusidic acid were due to a single isolate. The gentamicin MIC for the 52 isolates was 0.4 mg/l or less. In general, MICs of the drugs tested were within the therapeutic range. Six strains (11.2%) appeared to be tolerant to methicillin. The proportion of tolerant strains identified in this study, i.e. 11.2%, is similar to that of 12.5% reported by Goessens *et al.* (1984) in non-CF populations of *S. aureus*. We therefore conclude that frequent therapy in CF does not result in higher tolerance. Whether tolerance plays a role or not in the clinical outcome in CF has never been determined. Using the simple and reliable method described here it should be possible to determined this.

REFERENCES

Goering RV, Bauernfeind A, Lenz W and Przyklenk B.: *Staphylococcus aureus* in patients with cystic fibrosis: an epidemiological analysis using a combination of traditional and molecular methods. Infection 18:57-60, 1990

Goessens WHF, Fontijne P, Raffe M van and Michel MF.: Tolerance percentage as a criterion for the detection of tolerant *Staphylococcus aureus* strains. Antimicrob Agents Chemother 25:575-578, 1984

Jorgens, JH.: Laboratory and epidemiologic experience with methicillin resistant *Staphylococcus aureus* in the USA. Eur J Clin Microbiol 5:693-696, 1986

Kim KS and Anthony BF.: Use of penicillin-gradient and replicate plates for the demonstration of tolerance to penicillin in streptococci. J Infect Dis 148:488-491, 1983

Mouton JW and Kerrebijn KF.: Antibacterial therapy in cystic fibrosis. Med Clin North Am 74:837-850, 1990

FIVE

EMERGENCE OF ANTIBIOTIC RESISTANCE AMONGST PSEUDOMONAS AERUGINOSA ISOLATES FROM PATIENTS WITH CYSTIC FIBROSIS

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CHAPTER 5

EMERGENCE OF ANTIBIOTIC RESISTANCE AMONGST PSEUDOMONAS AERUGINOSA ISOLATES FROM PATIENTS WITH CYSTIC FIBROSIS

SUMMARY

We investigated the emergence of resistance to 15 anti-pseudomonal antibiotics amongst *Pseudomonas aeruginosa* isolates from 34 chronically colonized patients with cystic fibrosis by comparing the susceptibilities of strains isolated before 1987 and after 1989 from the same patients. Strains obtained after 1989 from a further 19 patients who were newly colonized served as controls.

The 34 pairs of isolates demonstrated a marked increase in resistance which could not be accounted for by a general increase during the intervening years since the susceptibility patterns of strains isolated before 1987 were similar to those of strains isolated from patients in the control group. There was a strong correlation between this increase in resistance and both the frequency of admissions to and the number of days spent in hospital. Cluster analysis of the changes in susceptibility for individual antibiotics revealed four distinct patterns of resistance: the fluoroquinolones with the exception of ofloxacin; the aminoglycosides; the ureidopenicillins and aztreonam; and the carbapenems, cephalosporins and carboxypenicillins as well as ofloxacin.

We conclude that longterm administration of anti-pseudomonal antibiotics to patients who are chronically colonized with *P. aeruginosa* is associated with the development of resistance.

INTRODUCTION

Pulmonary infection caused by *Pseudomonas aeruginosa* is one of the major causes of morbidity and mortality in cystic fibrosis (CF) patients, most of whom will receive antibiotics at least once a year (Wood, Boat and Doershuk, 1976; Hoiby, 1982). An association between the introduction and use of an antimicrobial agent and the subsequent emergence of resistant isolates has been demonstrated in several studies (Mouton, Glerum and Van Loenen, 1976; Cross, Opal and Kopecko, 1983; Levine *et al.*, 1985; Courcot, Pinkas and Martin, 1989), including one of CF patients (Bosso, Allen and Matsen, 1989). However, since these data were based on populations of patients only a few, if any, of whom will have suffered relapses caused by the same organisms, such data cannot be extrapolated to the individual. As CF patients may be colonized with the same strain(s) for several years (Grothues *et al.*, 1988; Horrevorts *et al.*, 1990), they provide a unique opportunity to assess the impact of antimicrobial therapy both on the individual patient and on the population as a whole. We have therefore adopted an alternative approach for monitoring the emergence of resistance by comparing the MIC's of anti-pseudomonal antibiotics for *P. aeruginosa* strains isolated from 34 chronically-colonized patients during two time periods separated by at least three years.

MATERIALS AND METHODS

Patients. All CF patients were treated at the Erasmus University Hospital Rotterdam Dijkzigt. The 34 patients who made up the study population (group 34) had a mean age of 19.6 years (range: 6-28) and had been admitted to hospital up to 28 occasions (median six) for a median duration of 114 days (range 0-590).

During the study period patients were treated with a combination of ticarcillin and tobramycin, both administered intravenously, as first-line therapy (Mouton and Kerrebijn, 1990). Ceftazidime and ciprofloxacin were introduced in 1984 and 1986 respectively, and were used as second-line agents. Aztreonam, piperacillin, imipenem and amikacin, all of which were prescribed occasionally, were the only other anti-

pseudomonal antiobiotics administered.

Bacterial isolates. Since 1981, *P. aeruginosa* strains isolated from sputum of CF patients have been routinely stored at -70°C. Pairs of strains, one strain isolated from a specimen of sputum collected from each of the 34 patients in the study group before the end of 1986 (Group 34A) and the second from a specimen obtained from the same patients after 1 January 1990 (Group 34B) were recovered. The specimens had been collected either a the time of an out-patient visit or during an admission to hospital for an acute exacerbation, in both cases before intravenous therapy had been initiated. Strains of *P. aeruginosa* were also isolated after 1 january 1990 from the sputa of 19 newly colonized patients (group 19NC). If two or more morphologically distinct strains were identified in a specimen, only one, which was selected arbitrarily, was used for further study.

Typing. The 34 pairs of strains of group 34 were characterized by serotyping and active pyocin typing as described by Horrevorts *et al.*, (1990). O-antisera were obtained from Diagnostics Pasteur (Marne-la-Coquette, France).

Antibiotics. The following antibiotics were supplied as standard reference powders by their respective manufacturers: azlocillin and ciprofloxacin (Bayer); aztreonam (Squibb); amikacin and cefepim (Bristol-Meyers); cefpirome (Roussel); ceftazidime (Glaxo); imipenem and norfloxacin (Merck, Sharp and Dome); meropenem (ICI); pefloxacin (Rhône Poulenc); piperacillin (Lederle); ofloxacin (Hoechst); tobramycin (Eli Lilly); ticarcillin (Smith-Kline-Beecham). Stock solutions were prepared according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1990).

MIC Determination. MIC's were determined by a standard agar dilution method (Sahm and Washington, 1991) as described previously (Horrevorts, 1989). Freshly-prepared, serial two-fold dilutions of each antibiotic in concentrations ranging from 0.03 to 2048 mg/l were incorporated into DST agar (Oxoid, Hampshire, England). Overnight Mueller-Hinton broth cultures of the isolates were adjusted to a turbidity equivalent to a 0.5 McFarland standard and an inoculum of 10^4 CFU was delivered to the surface of the agar plates with a multipoint inoculator. The MIC was defined as the lowest concentration of each antibiotic which inhibited visible growth after

incubation for 18 h at 37°C. *P. aeruginosa* ATCC 27853 was included as a control with each batch; the MICs for this organism were required to be within one two-fold dilution of the reported values (NCCLS, 1990).

Data analysis. The antibiotic concentrations inhibiting 25%, 50%, 75% and 90% of isolates in each of the three groups (34A, 34B and 19NC) were calculated from the MICs. The SAS CLUSTER procedure (SAS, 1990) was used to perform cluster analysis which made it possible to determine if a change in the susceptibilities of the Group 34 isolates to each of the 15 antibiotics was associated with changes in the susceptibilities to any of the other antibiotics over the same period of time. The MICs were therefore converted into logarithms and the resulting values for the strains belonging to each pair were substracted one from the other to yield the difference in susceptibility for each of the 15 antibiotics. Cluster analysis of these differences was performed according to Ward 's method with standardized values.

To quantify the increase in resistance for each pair of isolates, the 15 differences in susceptibility were averaged to yield a resistance increase score. Spearman's rank correlation coeffficient was then used to determine whether there was a relationship between this score and the number of admissions to and duration of stay in hospital.

RESULTS

Typing. Both of the strains which comprised 17 of the 34 pairs of isolates (1-17) belonged to the same serotype and pyocin type (Table I). For nine additional pairs (18-26), the serotypes of both strains were the same but one or both were non-typable by pyocin typing; minor differences in the serotypes of the strains which made up two of these pairs (25 and 26) were attributed to polyagglutinability. Conversely, the strains comprising five further pairs (27-31) shared the same pyocin type but one or both of the isolates which formed four of these pairs were non-typable by serotyping; the serotypes of the two strains which made up the fifth pair (31) were different. The strains comprising pair 32 were non-typable according to both typing methods and for pairs 33 and 34, the constituent strains belonged to different serotypes while one of the strains making up pair 33 and both strains making up pair 34 were non-typable by

pyocin typing.

Susceptibility. The MIC_{25}s, MIC_{50}s, MIC_{75}s and MIC_{90}s for the three groups of isolates are shown in Table II. During the years which separated the two collection periods there was an increase in MICs of all 15 antibiotics for the Group 34 strains; the increase in the MIC_{90}s varied from two- to 32-fold depending on the antibiotic. The decline in susceptibility was uniform, as demonstrated by the concordance of the MIC_{25}s, MIC_{50}s, MIC_{75}s and the MIC_{90}s. The MIC_{25-90}s for the 34 strains isolated before 1987 (34A) were broadly similar to those for the control group (19NC), although the MIC_{25-90}s of the aminoglycosides for the control strains more closely resembled those of the post-1989 isolates (34B). The high MIC_{90}s of the quinolones for the control group resulted from the inclusion of two exceptonally resistant strains.

Pattern analysis. Four distinct patterns of resistance were identified at the 0.1 level (Figure). Of these, the first two comprised the quinolones, with the exception of ofloxacin, and the aminoglycosides respectively. Aztreonam was grouped together with the ureidopenicillins, piperacillin and azlocillin, and the fourth cluster included the remaining antibiotics (the cephalosporins, the carboxypenicillin, ticarcillin, the carbapenems and ofloxacin).

As results may vary according to the technique employed for performing cluster analysis, other procedures, including maximum likelihood analysis, were carried out for comparison. However, because the results obtained by the various methods were so similar, they have not been shown.

Resistance increase score. The resistance increase scores were used to demonstrate that increase in antibiotic resistance of the 34 strains isolated from the study patients correlated significantly with both the number of hospital admissions ($r=0.498$, $p<0.0005$) and the number of days spent in hospital ($r=0.573$, $p<0.0005$).

Table I. Typing results of 34 pairs of *P. aeruginosa* strains isolated from 34 chronically colonized CF patients.

Number	isolate 1 (34A group) before 1987		isolate 2 (34B group) after 1989	
	serotype	pyocintype	serotype	pyocintype
1	3/13	45c	3/13	45c
2	13	45c	13	45c
3	3	74c	3	74c
4	3	46c	3	46c
5	16	45c	16	45c
6	15	74c	15	74c
7	13	35b	13	35b
8	6	45c	6	45c
9	14	84b	14	84b
10	1/3	78d	1/3	78d
11	1/3/10	35b	1/3/10	35b
12	6	84c	6	84c
13	6	54c	6	54c
14	8	74b	8	74b
15	6	85c	6	85c
16	3	85c	3	85c
17	13	82d	13	82d
18	11	11d	11	NT
19	6	41d	6	NT
20	13	NT	13	NT
21	3	NT	3	NT
22	13	NT	13	NT
23	1/3	NT	1/3	NT
24	1	NT	1	NT
25	1/3	NT	1/10	NT
26	1	NT	1/6	NT
27	NT	34c	NT	34c
28	3	65c	NT	65c
29	NT	85c	NT	85c
30	3	74c	NT	74c
31	6	85c	5	85c
32	NT	NT	NT	NT
33	6	66c	3	NT
34	13	NT	16	NT

Table II. MICs of three sets of *P. aeruginosa* isolates.

antibiotic	MIC_{25}			MIC_{50}			MIC_{75}			MIC_{90}		
	34A	34B	19NC	34A	34B	19NC	34A	34B	19NC	34A	34B	19NC
azlocillin	4	8	2	8	16	8	8	64	8	32	512	16
piperacillin	2	8	1	4	8	4	8	32	4	8	64	16
ticarcillin	8	16	4	16	64	16	32	256	32	64	512	64
aztreonam	2	4	1	4	8	4	8	32	8	16	32	32
cefipime	1	2	1	1	4	2	4	32	4	8	32	8
cefpirome	2	4	2	2	8	2	4	32	8	16	256	16
ceftazidime	2	2	0.5	2	2	2	4	8	2	4	32	4
imipenem	1	1	0.5	2	2	2	2	8	4	4	16	32
meropenem	0.25	0.5	0.03	0.5	1	0.25	1	4	0.5	2	16	1
ciprofloxacin	0.13	1	0.25	0.25	2	0.5	0.5	16	2	0.5	16	64
norfloxacin	0.5	4	0.5	1	4	1	2	16	4	4	16	32
ofloxacin	2	8	2	2	8	2	4	16	8	8	32	32
pefloxacin	1	8	1	2	8	2	4	16	4	8	64	16
amikacin	2	8	8	4	16	16	8	64	64	16	64	128
tobramycin	0.5	1	1	0.5	2	2	1	8	4	2	16	64

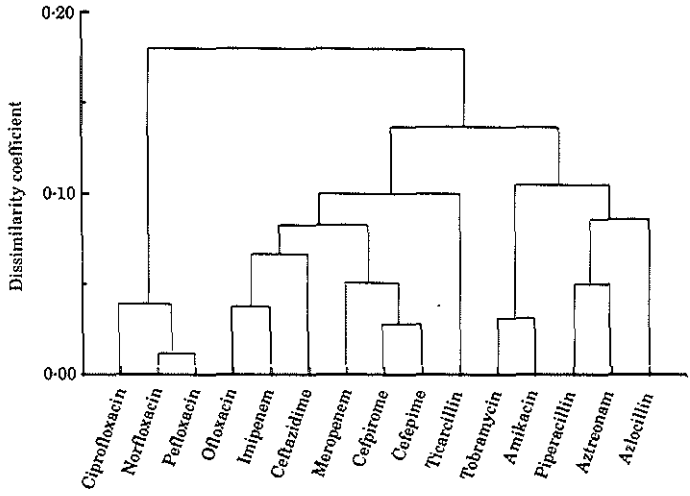

Figure 1. Patterns of resistance to the 15 antibiotics for 34 pairs of P. aeruginosa isolates determined by cluster analysis. The more closely the antibiotics are grouped together (e.g. norfloxacin and pefloxacin), the more closely they are related with respect to the emergence of resistance.

DISCUSSION

Exacerbations of pulmonary disease caused by *P. aeruginosa* occur frequently in CF patients. Treatment is made difficult for several reasons, not the least of which is the lack of consensus concerning optimal antimicrobial therapy (Geddes, 1988; Michel, 1988). Our practice is to administer a combination of antibiotics (Mouton and Kerrebijn, 1990) but since most patients are treated at least once a year (on up to 28 occasions in the present study) resistance is an important consideration.

The results of this study confirm that, with time the development of resistance to anti-pseudomonal agents is inevitable in patients with CF. The data also suggest that this

resistance is directly related to the administration of antibiotics to individual patients, as opposed to a general increase in resistance in this patient population. The reduction in susceptibility of *P. aeruginosa* isolates applied to all of the antibiotics tested and resistance correlated significantly with both the number of admissions to and the duration of stay in hospital. However, the coefficients of correlation approximated 0.5, indicating that exposure to antibiotics in hospital only partially accounted for the increase in resistance; other factors, for instance the domiciliary use of antimicrobials may also play a role.

The aminoglycosides did not follow the general trend observed for other antibiotics in that the MIC's of these agents for the strains in the control group resembled those for the organisms isolated from Group 34B patients in the latter phase of the study more closely than those for strains isolated in the earlier phase. Although we are unable to offer an explanation for this paradox, it cannot be accounted for by a general increase in the level of antibiotic resistance in our hospital. Nor can it be attributed to cross-infection since CF patients tend to retain their own strains and a concurrent reduction in the susceptibility of the control isolates to other groups of antibiotics would have been expected if this had been the explanation.

Virtually all CF patients eventually become colonized with strains of *P. aeruginosa*, which usually persist for long periods of time (Ogle *et al.*, 1987; Grothues *et al.*, 1988; Horrevorts *et al.*, 1990). Although morphologically distinct colonial types can be isolated from a specimen of sputum sample, various typing methods have been used to demonstrate that they are often variants of a single strain. The use of sertyping and pyocin typing techniques in the present study have confirmed that the same strain may continue to colonize a patient for several years.

In the course of examining specimens which yield multiple isolates, the selection of strains for further study may be arbitrary. In CF patients, this is further complicated by the fact that strains which are indistinguishable may subsequently produce morphologically variants with different susceptibility patterns (Seale *et al.*, 1979; Thomassen *et al.*, 1979). By selecting representative isolates on an arbitrarily basis there is the potential to underestimate the extent to which resistance has developed, particularly in patients who have received multiple courses of antibiotics. However,

this should not influence the outcome of cluster/pattern analysis.

The emergence of resistance to antibiotics belonging to one or more unrelated groups has been described as a general phenomenon in CF patients (Bosso *et al.*, 1989). We have made a similar observation, but the isolation of *P. aeruginosa* strains from the same patient allowed us to use pattern analysis on the changes in susceptibility of the 15 antibiotics. It was anticipated that an increase in MICs of agents related by chemical structure and modes of action and degradation would have been clustered together and indeed, this was the case for the aminoglycosides and three of the four quinolones tested. However, aztreonam was found to be more closely related to the ureidopenicillins than to the cephalosporins, which themselves shared a common cluster with the carboxypenicillin ticarcillin and the carbapenems. Finding ofloxacin in the latter cluster, rather than with the other quinolones, was also unexpected. Ofloxacin was not available in our hospital during the study. The association between ofloxacin and imipenem could indicate cross-resistance between these drugs. A similar relationship between imipenem and ciprofloxacin (quinolone-imipenem cross-resistance) has been described previously (Aubert, Pozzetto and Dorche, 1992). In that report, in-vitro exposure of a strain of *P. aeruginosa* to ciprofloxacin and other fluoroquinolones selected quinolone- and imipenem-resistant variants but the converse, quinolone-imipenem cross-resistance after exposure to imipenem, was not detected. Resistance to several of the other antibiotics included amongst the 15 agents tested was also noted although some of them had not been prescribed during the study period; this tends to support the theory that cross-resistance is the most likely explanation for this phenomenon. With the exception of ofloxacin, quinolone resistance occurred relatively independent of other groups of antibiotics.

The results of the present study confirm that the efficacy of antibiotics in individual CF patients will be diminished with time. This limitation wil depend in part on the frequency with which these agents are administered. We conclude that *P. aeruginosa* strains in chronically-colonized CF patients remain fairly constant over long periods, and that repeated exposure to antibiotics during treatment for exacerbation of pulmonary disease and other unindentified factors are associated with the emergence of resistance in individual patients. Moreover, the development of resistance to

antibiotics belonging to groups which are unrelated to those which the patient has received and to which the patient's own strain has not previously been exposed is a common occurrence.

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REFERENCES

Aubert G, Pozzetto B and Dorche G.: Emergence of quinolone-imipenem cross-resistance in *Pseudomonas aeruginosa* after fluoroquinolone therapy. J Chemother 29:307-312, 1992

Bosso JA, Allen JE and Matsen JM.: Changing susceptibility of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients with the clinical use of newer antibiotics. Antimicrob Agents Chemother 33: 526-528, 1989

Courcol RJ, Pinkas M and Martin GR.: A seven year survey of antibiotic susceptibility and its relation ship with usage. J Antimicrob Chemother 23:441-451, 1989

Cross AS, Opal S and Kopecko DJ.: Progressive increase in antibiotic resistance of Gram-negative bacterial isolates. Walter Reed Hospital: Specific analysis of gentamicin, tobramycin and amikacin resistance. Arch Internal Med 143:2075-2080, 1983

Geddes DM.: Antimicrobial therapy against *S. aureus, P. aeruginosa* and *P. cepacia.* Chest 94(Suppl):141-144, 1988

Grothues D, Koopmann U, von der Hardt H and Tummler B.: Genome fingerprinting of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. J Clin Microbiol 26 1973-1977, 1988

Hoiby N.: Microbiology of lunginfections in cystic fibrosis patients. Acta Paediatr Scand 301(Suppl):33-54, 1982

Horrevorts AM.: In vitro activity of new antimicrobial agents against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 6:323-324, 1987

Horrevorts AM, Borst J, Puyk RTJ, de Ridder CM, Degener J, Djolic-Danilovic G et al.: Ecology of *Pseudomonas aeruginosa* in patients with cystic fibrosis. J Clin Microbiol 31:119-124, 1990

Levine JF, Maslow MJ, Leibowitz RE, Pollock AA, Schaefler BA et al.: Amikacin resistant Gram-negative bacilli: correlation of occurrence with amikacin use. J Infect Dis 151:295-300, 1985

Michel BC.: Antibacterial therapy in cystic fibrosis. Chest 94(Suppl):129-140, 1988

Mouton JW and Kerrebijn KF.: Antibacterial therapy in cystic fibrosis. Med Clin North Am 74:837-850, 1990

Mouton RP, Glerum JH and van Loenen AC.: Relationship between antibiotic consumption and frequen cy of antibiotic resistance of four pathogens-a seven year survey. J Antimicrob Chemother 2:9-19, 1976

National Committee for Clinical Laboratory Standards.: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2 National Committee for Clinical Laboratory Standards Villanova Pa, 1990

Ogle JW, Jada JM, Woods DE and Vasil ML.: Characterization and use of a DNA probe as an epidemiological marker for *P. aeruginosa*. J Infect Dis 155 119-26, 1987

Sahm DF and Washington JA.: Antibacterial susceptibility tests: dilution methods In Manual of Clinical Microbiology. (Balows A Hausler WJ Hermann KL Isenberg HD and Shadomy HJ Eds) pp 1105-1116 American Society for Microbiology Washington DC, 1991

SAS Institute.: SAS User's guide. SAS Institute Inc, Cary NC, 1990

Seale TW, Thirkill H, Tarpay M, Flux M and Rennert OM.: Serotypes and antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates from single sputa of cystic fibrosis patients J Clin Microbiol 9 72-78, 1979

Thomassen MJ, Demko CA, Boxerbaum B, Stern RC and Kuchenbrod PJ.: Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. J Infect Dis 140:873-880, 1979

Wood RE, Boat TF and Doershuk CF.: Cystic fibrosis. Am Rev Respir Dis 113:833-78, 1976

SIX

PHARMACOKINETICS OF CEFTAZIDIME IN SERUM AND SUCTION BLISTER FLUID DURING CONTINUOUS AND INTERMITTENT INFUSION IN HEALTHY VOLUNTEERS

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CHAPTER 6

PHARMACOKINETICS OF CEFTAZIDIME IN SERUM AND SUCTION BLISTER FLUID DURING CONTINUOUS AND INTERMITTENT INFUSION IN HEALTHY VOLUNTEERS

SUMMARY

The pharmacokinetics of ceftazidime were investigated during intermittent (II) and continuous (CI) infusion in 8 healthy male volunteers in a crossover fashion. The total daily dose was 75 mg/kg of body weight per 24 h in both regimens, given in three doses of 25 mg/kg/8 h (II) or 60 mg/kg/24 h with 15 mg/kg as a loading dose (CI). After the third dose (II) and during continuous infusion serum and blister fluid samples were taken. Seven new blisters were raised for each timed sample by a suction blister technique. Blisters took 90 min to form. Samples were then taken from four blisters (A samples) and one hour later were taken from the remaining three (B samples). The concentration of ceftazidime was determined using a HPLC method. After intermittent infusion the serum concentrations immediately after infusion (t=30 min) and 8 h after the start of the infusion were 137.9 (standard deviation [sd] 27.5) and 4.0 (sd 0.7) mg/l, respectively. The half-life at α phase ($T_{1/2\alpha}$) was 9.6 min (sd 4.6), $T_{1/2\beta}$ was 94.8 min (sd 5.4), area under the concentration-time curve (AUC) was 285.4 mg.h/l (sd, 22.7), total body clearance (TBC) was 0.115 liter/h.kg (sd, 0.022), and volume of distribution at steady state was 0.178 l/kg (sd, 0.023). The blister fluid (A) samples showed a decline in concentration parallel to the serum concentrations during the elimination phase with a ratio of 1:1. The $T_{1/2}$ of the A samples was 96.4 min (sd, 3.2). The concentration of ceftazidime in the B blister fluid samples was significantly higher (27%) than in the A samples over time. This shows that blisters may behave as a separate compartment, and establishes the need to raise new blisters for each timed sample. The mean AUC/h during continuous infusion was 21.3 mg.h/l

(sd 3.0). The TBC was 0.113 l/h.kg (sd 0.018), the urinary clearance 0.105 l/h · kg (sd 0.012) and the ceftazidime/creatinine clearance ratio was 0.885. The mean AUC of blister fluid per hour was 84.5% (18.0 mg.h/l; sd, 3.6) compared with that of serum. The A samples did not differ significantly from the B samples. The implications of continuous infusion of beta-lactams for treatment of serious infections are discussed.

INTRODUCTION

Time-kill studies of beta-lactam antibiotics on aerobic Gram-negative rods show a bactericidal activity which is slow, proceeds with time and is maximal at relatively low concentrations (Vogelman and Craig, 1986). The killing is exclusively related to the time that serum and tissue levels exceed a certain threshold, whereas higher concentrations contribute no extra effect. When levels fall below this threshold, bacterial growth is immediately resumed. This interaction of beta-lactams with Gram-negative rods has consequences for the way in which these drugs should be administered. It has been shown, in experimental infections, that a continuous infusion of ceftazidime leads to better results than intermittent administration (Roosendaal *et al.*, 1989). This effect was most pronounced in severe infections. There are a few clinical reports that ceftazidime given as a continuous infusion may lead to a better outcome (Daemen and de Vries-Hospers, 1988). Since beta-lactam antibiotics are usually given intermittently, most pharmacokinetic data are based on this form of administration (De Klerk *et al.*, 1983; Lebel *et al.*, 1985; Lebel and Spino, 1988; Naber *et al.*, 1983; Tjandramaga *et al.*, 1982; Walstad *et al.*, 1983; Warns *et al.*, 1983; Wise *et al.*, 1981). To study the pharmacokinetics of ceftazidime in serum and tissue fluid during continuous infusion, the pharmacokinetics of ceftazidime in eight volunteers were investigated in a crossover fashion during intermittent and continuous administration. Suction blister fluid samples were drawn as a measure of ceftazidime concentrations in tissue fluid. In addition, we investigated whether the concentration of ceftazidime in suction blister fluid is affected by the lifetime of the blister.

MATERIALS AND METHODS

Volunteers. Eight healthy male volunteers, aged 20-29 years, participated in the study. Body weight ranged from 63-103 kg. Before entering the study, each volunteer underwent a complete physical examination and was screened biochemically (Na, K, urea, creatinin, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, total protein, albumin, total bilirubin, and glucose) and haematologically (haemoglobin, haematocrit, packed cell volume, erythrocyte indices, leukocyte count, and platelet counts). A standard urine screening test was performed. The study was approved by the Medical Ethical Committee of the Erasmus University of Rotterdam and informed consent was obtained from each volunteer.

Design. The pharmacokinetics of ceftazidime in each volunteer were studied during and after intermittent and continuous administration in a crossover fashion. For each volunteer, a minimum of four weeks was allowed to elapse between the two treatment regimens.

Drug. Ceftazidime was kindly provided by Glaxo (The Netherlands). The drug was reconstituted according to the manufacturer's instructions and further diluted to obtain the necessary concentration for each volunteer. The total volume used amounted to 50 ml per dose for intermittent administration. For continuous administration, the flow of the infusor was adjusted per individual to obtain the necessary dose per time-unit.

Dosing. The total daily dose in both regimens amounted to 75 mg/kg of body weight. For intermittent administration volunteers received 25 mg/kg every 8 h over 24 h. The infusion time was 25 min. For continuous administration, 15 mg/kg was given as a loading dose (infusion time 25 min) and 60 mg/kg was given over 24 h with an infusor.

Blood samples. Venous blood samples for ceftazidime assay were obtained from an indwelling needle from the opposite arm to that used for the infusion of the antibiotic. From subjects receiving intermittent infusion, samples were taken prior to the third dose (t=0) and at: 10, 20, 30, 40, 50 and 60 min and 1.5, 2, 4, 6 and 8 h

after the start of the third infusion. From subjects receiving continuous infusion, samples were taken prior to the start of the loading dose and continuous infusion (t=0) and also at 10, 20, 30, 40, 50, 60 min and 1.5, 2, 4, 8, 16, 20 and 24 h after the start of the infusions.

After sampling, blood was allowed to clot on ice for 20 min, centrifuged and the serum was stored at -70° C until assayed.

Blister fluid samples. Blisters were raised from the abdominal skin by the suction blister technique described by Kiistala and Mustakallio (1967). In brief: perspex cups containing 7 perforations with a diameter of 6 mm each, connected with a vacuum source, were used. The cups were placed on the skin and kept in place by applying a negative pressure of 300 mm Hg ($400x10^2$ Pa). Fifteen minutes after positioning the cups, the suction pressure was lowered to -225 mm Hg ($-300x10^2$ Pa). This pressure was maintained until 1.5 h after placing the cups; 7 full blisters, each containing 20-60 μl of fluid, were formed by then. The flow of fluid into the newly formed blister occurred during the last 10-15 min of this 1.5 h period. Blister samples were drawn from four of the seven blisters, yielding a total sample volume of 50-150 μl. These samples were called the A samples. Two and a half hours after placement of the cup, samples from the remaining three blisters, the B sample, were drawn. Samples were stored at -70°C until assayed.

The cups were placed at the following times: 1.5, 2.5, 4.5 and 6.5 h after the third infusion when dosing intermittently and 17.5, 18.5, 20.5 and 22.5 h after the start of the infusion continuous doses.

Urine samples. Volunteers were encouraged to drink and urine samples were collected whenever possible, the volume of each void was measured and an aliquot of 1 ml stored at -70° C until assayed to determine the urinary clearance. The urinary clearance during continuous administration was determined during the last 8 h of infusion.

Analysis. Samples were assayed by a HPLC method, using an ODS column (Chrompack, Middelburg, The Netherlands) with a 0.05 M ammonium diphosphate solution (ph=2.0) containing 11.5% v/v acetonitrile as mobile phase. A perchloric acid solution, containing 50 mg/l cefaclor as an internal standard, was added to an

equal volume of sample, centrifuged, and the supernatant was filtered through a membrane filter (Millipore Corp., type HV, 0-45 μm). The lower limit of assay sensitivity was 0.5 mg/l. The between sample between day variation was less then 7%. All samples were assayed in duplicate. Control runs were performed regularly.

Protein binding. Blister fluid samples of the 8 volunteers, obtained after intermittent administration, were pooled for each time point, yielding to four pooled "A" and four pooled "B" blister fluid samples. The serum samples were also pooled for the last four timed samples. In one part of each pooled sample the total ceftazidime concentration was determined as described above. In the remnant the non-protein bound ceftazidime concentration was determined using the Amicon micropartition system (MPS-1, 4010, Amicon, Mass. USA). Protein binding is expressed as 1 minus the ratio free ceftazidime/total ceftazidime * 100%.

Other assays. A 24 h creatinin urinary clearance was performed on each study day to compare with the ceftazidime urinary clearance.

Pharmacokinetic and statistical analysis. Serum concentrations were plotted versus time in a semilogarithmic plot. Pharmacokinetic parameters were estimated using the SAS NLIN computer program package using a two-compartment open model with a weighted least squares adjustment (SAS Institute, Inc., 1982). The equations used were according to Allen *et al.* (1982). The Wilcoxon matched pairs test was used to compare results between treatments (Snedecor and Cochran, 1980). Repeated measurements analysis of variance from the BMDP 5V program package (University of California Press, 1988) was used to obtain the half-lives and to test the difference between A and B blister fluid samples. The concentration in blister fluid/concentration in serum ratio of the A blister fluid samples during continuous infusion was determined from the area under the concentration-time curve (AUC) ratio during the time period at which the blister fluid concentrations were known, i.e. the last 5 h of infusion.

The results are expressed as arithmetic means $\pm$ standard deviations (sd), except for the half-lives which are expressed as harmonic means $\pm$ sd.

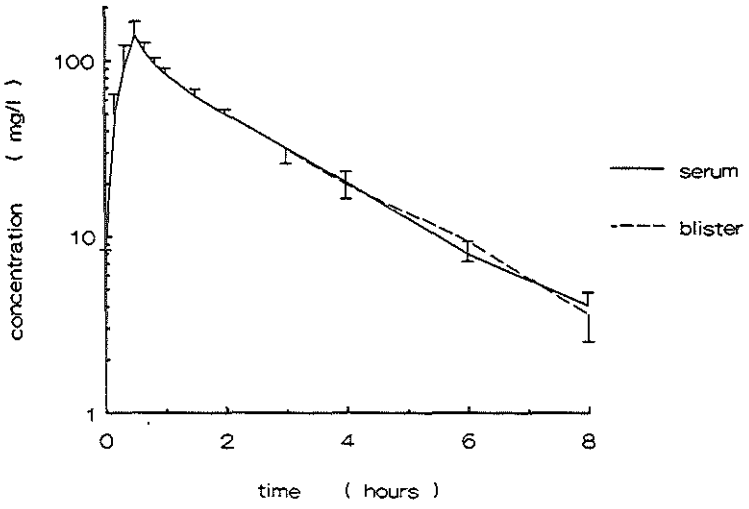

Figure 1. Mean (± sd) ceftazidime concentrations in serum and blister fluid plotted versus time after intermittent dosing (25 mg/kg per dose)

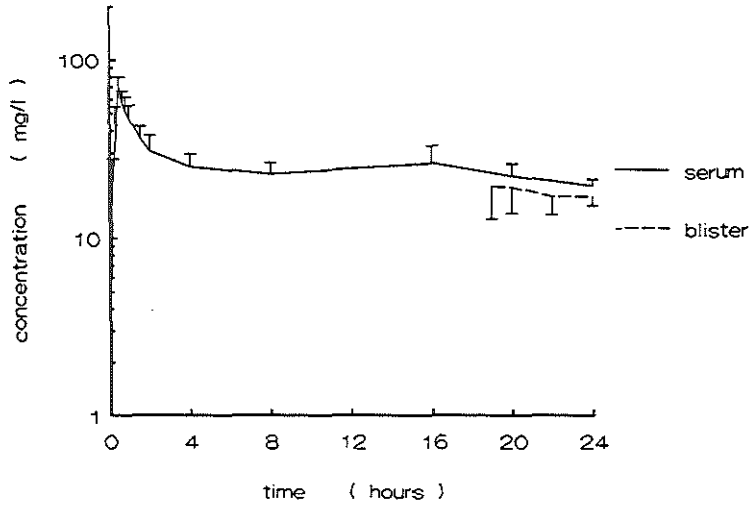

Figure 2. Mean (± sd) ceftazidime concentrations in serum and blister fluid plotted versus time during continuous administration (60 mg/kg/24 h and 15 mg/kg as a loading dose)

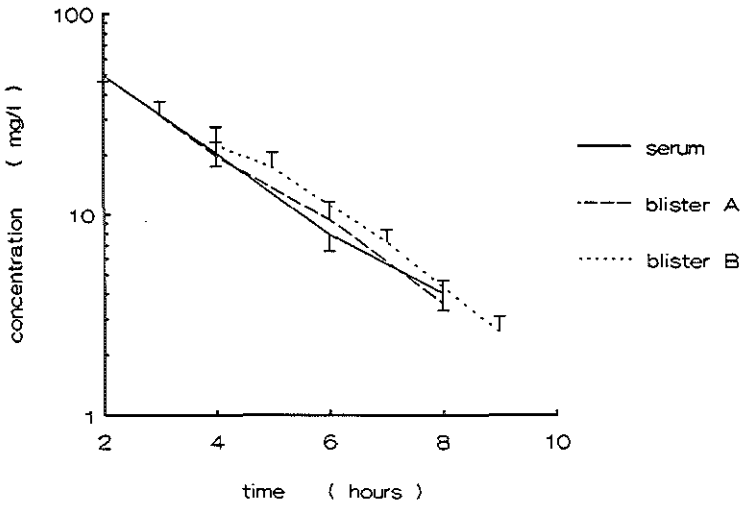

Figure 3. Mean (± sd) ceftazidime concentrations (plotted versus time) in serum and blisterfluid A and B samples during the elimination phase.

RESULTS

Concentrations in serum and blister fluid are plotted versus time in Figure 1 (intermittent administration) and Figure 2 (continuous administration). Concentrations in blister fluid declined parallel to the concentrations in serum (Figure 1). The decline in concentration in the blisters is usually lagging slightly in time and the concentration itself was thus relatively higher at the same time point when compared with concentrations in serum. The penetration in tissue of less than 100% seems to compensate the time lag almost exactly. There might also be a very short time lag due to the mere drawing of the blisters.

To determine whether ceftazidime diffuses freely between blisters and tissue, B blister samples were drawn 1 h after the original (A) samples from as-yet-intact blisters. After we tested that the time trend does not differ significantly between A and B blis-

Table I. Pharmacokinetic parameters of ceftazidime after intermittent administration.

Volunteer data				Intermittent administration					
No	Age	Weight	Length	$T_{1/2\alpha}$	$T_{1/2\beta}$	AUC	TBC	Vdss	Blister/serum concentration
	(years)	(kg)	(cm)	(min)	(min)	(mg.h/l)	(l/h·kg)	(l/kg)	(%)
1	25	76.5	175	7.0	94.9	257.8	0.124	0.193	96.0
2	24	103.5	190	12.3	92.8	273.4	0.158	0.178	139.8
3	22	61.0	176	9.7	100.6	313.9	0.081	0.180	80.8
4	28	81.5	182	13.7	103.4	306.9	0.111	0.169	96.2
5	23	81.0	189	17.7	94.8	261.5	0.129	0.215	115.2
6	21	73.0	183	4.0	87.2	283.4	0.107	0.160	93.3
7	21	76.0	181	6.2	88.7	312.3	0.101	0.139	89.7
8	25	74.0	182	6.1	95.8	274.0	0.113	0.193	101.3
mean	23.6	78.3	182.3	9.6	94.8	285.4	0.115	0.178	101.5
sd	2.4	12.0	5.0	4.6	5.4	22.7	0.022	0.023	18.3

see text for abbreviations

ter samples, the concentration of ceftazidime in the B blister samples was established to be 27% higher (95% confidence interval, 24% to 31%, repeated measurements analysis of variance) than in the A samples Figure 3. This may indicate that blisters behave as a separate compartment.

Since protein binding might be responsible for the differences found between serum and blister fluid ceftazidime concentrations, this binding was measured in serum and blister fluid. Protein binding in serum was found to be 18.7% $\pm$ 2.9%, and 14.9% $\pm$ 4.9% and 21.0% $\pm$ 6.4% in the A and B blister fluid samples respectively.

The concentration-time curve for serum after intermittent infusion showed a biphasic decline, representing a distribution phase and an elimination phase with mean half-lives of 9.6 $\pm$ 4.6 min at α phase and 94.8 $\pm$ 5.4 min at ß phase, respectively. The mean concentration in serum obtained immediately after infusion was 137.9 $\pm$ 27.5 mg/l, and the mean concentration at the end of the dosing interval was 4.0 $\pm$ 0.7

Table II. Pharmacokinetic parameters of ceftazidime during continuous administration.

volunteer no	TBC (l/h.kg)	Cl_u (l/h.kg)	$Cl_{ceftazidime}/Cl_{creatinin}$ (%)	mean serum AUC (mg.h/l)	Blister AUC/ serum AUC (%)
1	0.123	0.118	89.9	19.8	75.4
2	0.094	0.086	92.9	24.0	85.0
3	0.086	0.104	81.4	27.1	78.6
4	0.135	0.121	89.0	18.4	76.4
5	0.097	0.094	87.8	22.0	99.1
6	0.120	0.098	84.4	19.8	86.0
7	0.117	0.108	95.1	20.9	102.3
8	0.130	0.112	87.6	18.2	72.8
mean	0.113	0.105	88.5	21.3	84.5
sd	0.018	0.012	4.4	3.0	11.0

see text for abbreviations

mg/l. The mean AUC was 285.4 ± 22.7 mg.h/l, the total body clearance (TBC) was 0.115 ± 0.022 l/h.kg and the volume of distribution at steady state was 0.178 ± 0.023 l/kg (Table I).

The concentrations in blister fluid (A samples) showed a decline similar to that in serum with a mean half-life of 96.4 ± 3.2 min (repeated measurements analysis of variance), and a blister fluid/serum concentration ratio of 101.5% ± 18.3% during the elimination phase.

Pharmacokinetic parameters obtained after continuous infusion are summarized in Table II. The TBC was 0.113 ± 0.018 l/h.kg, the urinary clearance was 0.105 ± 0.012 l/h.kg during the last 8 h of infusion and the ratio ceftazidime/creatinin clearance 88.5% ± 4.4%. The urinary clearance of the free fraction was 108.9% ± 5.4, which is not significantly different from 100%, indicating that there is probably no net tubular handling of the drug.

The mean AUC/h during continuous infusion was 21.3 mg.h/l and the mean blister fluid AUC was 18.0 mg.h/l, corresponding to 84.5% of the serum (blister/serum).

There was no sigificant difference between A and B blister fluid concentrations during continuous infusion.
The TBC during and after intermittent administration was not significantly different from the TBC during continuous administration.

DISCUSSION

The aim of this study was to determine pharmacokinetic parameters of ceftazidime during continuous infusion compared to intermittent administration in serum and in tissue. As a representative of the concentration of ceftazidime in tissue, suction blister fluid concentrations were measured. Suction blisters were drawn as originally described by Kiistala and Mustakallio (1967). Schreiner *et al.* (1978) demonstrated its use for studies on extravascular antimicrobial activity. We used a modification of this technique by forming new blisters before every sample to eliminate, as far as possible, a possible diffusion barrier between blister and tissue, and thus reducing the time-lag of the concentrations in blisters versus tissue to almost zero during intermittent administration.
The penetration of ceftazidime in the blisters during intermittent infusion was 101% during the elimination phase. This is in agreement with results of Wise *et al.*. (1981), who found a penetration of nearly 100% using cantharide blisters. Walstad *et al.*. (1983), who also used suction blisters, found a penetration of 80%. Investigators who used subcutaneous threads found lower values, i.e. 50% (Hoffstedt and Walder, 1981; Ryan *et al.*, 1981). The difference between these values is rather high, and probably depends on the method used (Ryan *et al.*, 1982; Wise, 1986). The determination of the penetration in blisters in steady state should be independent of the method used. We found a penetration of 84.5%. This can not be explained by the protein binding, as the difference between the protein binding in serum and blister fluid was found to be rather small.
To study the effect of a possible diffusion barrier on the concentration of ceftazidime in the blisters, a second sample (B samples) was drawn 1 h after the first one (A samples) from the same set of, but other, blisters. If no diffusion barrier existed, the

concentrations in the second sample would equal those in serum as they did in the first drawings. In fact, they were 27% higher. This demonstrates that the blisters may behave as a separate compartment and establishes the need to raise new suction blisters for each timed sample. This view is supported by a persistently high concentration of ceftazidime in blister fluid from one volunteer, drawn 2 and 3 h after the collection of the A blister samples (results not shown). The half-lives of ceftazidime are not significantly different in serum and the two blister samples, and are log-linear. In earlier studies, the time concentration curves in blisters seemed to be bi-exponential (Walstad *et al.*, 1983). In these studies, the blisters were drawn at the onset of the study and the blisters from which the later samples were taken could have undergone histological changes and thus formed a diffusion barrier, explaining the relatively high concentrations found at the later time points. Also, the elimination of ceftazidime from the blisters is dependent on the volume of the blister with respect to the surface area.

One possible explanation of the discrepancy between serum and blister fluid concentrations might be lower protein binding in the blister fluid samples due to a lower protein content of blister fluid (Vermeer *et al.*, 1979). Although the protein binding in the A samples was slightly lower, this can not fully explain the differences found. Also, the higher values found in the B samples can not explain the relatively higher concentrations in the B samples.

The pharmacokinetic data in serum obtained during intermittent administration are in agreement with earlier studies (De Klerk *et al.*, 1983; Lebel *et al.*, 1985; Naber *et al.*, 1983; Tjandramaga, 1982; Walstad *et al.*, 1983; Warns *et al.*, 1983; Wise *et al.*, 1981) The data best fit a two-compartment open model.

The bactericidal action of beta-lactam antibiotics on Gram-negative rods is related to the duration of exposure above a certain threshold (Vogelman and Craig, 1986). In conventional treatment regimens, beta-lactams are as a rule given intermittently. This may lead to plasma concentrations below this threshold during part of the dosing interval, and thus impair efficacy. In tissue, this effect may be even more marked, as antibiotic concentrations in tissue are generally lower. During continuous infusion, a

level above this threshold may be easily maintained. A continuous infusion of ceftazidime has been shown to be more efficacious than intermittent administration in leucopenic animal models when treating Gram-negative rod infections (Roosendaal *et al.*, 1989). A decrease in the dosing interval was shown to be more efficacious for ticarcillin (Gerber, 1983; Mordenti *et al.*, 1985). In humans, there are a few reports that continuous infusion may lead to a better outcome. Daemen and de Vries-Hospers (1988) were able to cure serious infections by *P. aeruginosa* with a continuous infusion of ceftazidime whereas the same drug had failed to do so when given intermittently. Bodey *et al.*. (1979) found that continuous infusion of cefamandole, administered together with carbenicillin, was more efficacious than intermittent infusion when treating febrile episodes in cancer patients. Since the antibiotic activity of ceftazidime on Gram-negative rods is related to the duration of active drug levels present at the site of infection, this should have consequences for the way in which ceftazidime, or any beta-lactam, should be administered. The present paper provides pharmacokinetic data which may serve as a model as to which doses should be applied. More clinical trials are needed to determine the efficacy of continuous dosing versus intermittent dosing in humans.

REFERENCES

Allen L, Kimura K, MacKichan J and Rittschel WA.: Manual of symbols, equations and definitions in pharmacokinetics. J Clin Pharmacol 22(Suppl):1S-23S, 1982

Bodey GP, Ketchel SJ and Rodriguez V.: A randomized study of carbenicillin plus cefamandole or tobramycin in the treatment of febrile episodes in cancer patients Am J Med 67:608-616, 1982

Daemen S and de Vries-Hospers U.: Cure of *Pseudomonas aeruginosa* infection in neutropenic patients by continuous infusion of ceftazidime. Lancet i:937, 1988

De Klerk S, Walters L, van Wyck M, Harding SM, Paton AM and Ayrton J.: Pharmacokinetics of ceftazidime in male and female volunteers. Antimicrob Agents Chemother 23:892-896, 1983

Gerber AU, Craig WA, Brugger HP, Feller C, Vastola AP and Brandel J.: Impact of dosing intervals on activity of gentamicin and ticarcillin against *Pseudomonas aeruginosa* in granulocytopenic mice. J Infect Dis 147(Suppl):910-917, 1983

Hoffstedt B and Walder M.: Penetration of ceftazidime into extracellular fluids in patients. J Antimicrob Chemother 8(Suppl B):289-293, 1981

Horrevorts AM.: In vitro activity of new antimicrobial agents against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 6:323-324, 1987

Kiistala U and Mustakallio KK.: Dermo-epidermal separation with suction. J Investigative Dermatol 48:466-477, 1967

Klingeren B van.: Antibiotic resistance in *Pseudomonas aeruginosa, Haemophilus influenzae and Staphylococcus aureus.* Chest 94(Suppl):103-108, 1988

Lebel M and Spino M.: Pulse dosing versus continuous infusion of antibiotics. Clin Pharmacokinet 14:71-95, 1988

Lebel M, Barbeau G, Vallee F and Bergeron MG.: Pharmacokinetics of ceftazidime in elderly volunteers. Antimicrob Agents Chemother 28:713-715, 1985

Mordenti JJ, Quintiliani R and Nightingale CH.: Combination antibiotic therapy: comparison of constant infusion and intermittent bolus dosing in an experimental animal model. J Antimicrob Chemother 15(Suppl):313-321, 1985

Naber KG, Kees F and Grobecker H.: Ceftazidime: pharmacokinetics in young volunteers versus elderly patients and therapeutic efficacy with complicated urinary tract infections. J Antimicrob Chemother 12(Suppl A):41-45, 1983

Roosendaal R, Bakker-Woudenberg IAJM, van den Berghe-van Raffe M, Vink-van den Berg JC and Michel MF.: Impact of the dosage schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in *Klebsiella pneumoniae* pneumonia and septicemia in leukopenic rats. Eur J Clin Microbiol Infect Dis 8:878-887, 1989

Ryan DM, Mason U and Harding SM.: The penetration of ceftazidime into extravascular fluid. J Antimicrob Chemother 8(SupplB):283-288, 1981

Ryan DM, Hodges B, Spencer GR and Harding SM.: Simultaneous comparison of three methods for assessing ceftazidime penetration into extravascular fluid. Antimicrob Agent Chemother 22:995-998, 1982

SAS user's guide. Caig, USA; SAS Inst Inc, ISBN 0-917382-32-3, 1982

Schreiner A, Hellum KB, Digranes A and Bergman I.: Transfer of penicillin G and ampicillin into human skinblisters induced by suction. Scan J Infect Dis 14(Suppl):223-237, 1978

Snedecor GW and Cochran WG.: Statistical Methods, 7th ed Ames, IOWA: The Iowa State University Press, 1980

Tjandramaga TB, van Hecken A, Mullie A, Verbesselt R, de Schepper PJ and Verbist L.: Comparative pharmacokinetics of ceftazidime and moxalactam. Antimicrob Agents Chemother 22:237-241, 1982

University of California Press.: BMDP statistical software manual University of California Press, Berkeley, 1988

Vermeer JV, Reman FC, and van Gent CM.: The determination of lipids and proteins in suction blister fluid. J Invest Dermatol 73:303-305, 1979

Vogelman B and Craig WA.: Kinetics of antimicrobial activity. J Pediatr 108:835-840, 1986

Walstad RA, Hellum KB, Blika S and Dale LG *et al.*: Pharmacokinetics and tissue penetration of ceftazidime: studies on lymph, aqueous humour, skinblister, cerebrospinal and pleurol fluid J Antimicrob Chemother 12(Suppl A):275-282, 1983

Warns H, Lode H, Harnoss CM, Kemmerich B, Koeppe P and Wagner J.: Multiple dose pharmacokinetics and therapeutic results with ceftazidime. J Antimicrob Chemother 12(Suppl A):235-240, 1983

Wise R, Armstrong GC, Brown RM and Andrews JM.: The pharmacokinetics and tissue penetration of ceftazidime and cefamandole in healthy volunteers. J Antimicrob Chemother 8(Suppl B):277-282, 1981

Wise R.: Methods for evaluating the penetration of beta-lactam antibiotics into tissues. Rev Infect Dis 8(Suppl 3):325-332, 1986

SEVEN

MEROPENEM PHARMACOKINETICS IN SERUM AND SUCTION BLISTER FLUID DURING CONTINUOUS AND INTERMITTENT INFUSION

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CHAPTER 7

MEROPENEM PHARMACOKINETICS IN SERUM AND SUCTION BLISTER FLUID DURING CONTINUOUS AND INTERMITTENT INFUSION

SUMMARY

The pharmacokinetics and penetration into suction blister fluid of meropenem was investigated after intermittent (10 mg/kg q6h) and during continuous infusion (10mg/kg/6h) in 8 healthy male volunteers in a crossover fashion. Concentrations in serum, urine and blister fluid were determined by a HPLC method. The pharmacokinetic parameters in serum (±sd) $T_{1/2\beta}$, AUC and clearance after the third dose of intermittent infusion were 62.7 (11.4) min, 43.5 (7.1) mg.h/l and 292.1 (36.8) ml/min respectively. The penetration in blister fluid was rapid and good, with a AUC ratio blister fluid/serum of 84.7 (11.4) %. During continuous infusion, from 12h-18h after the start of the infusion, the mean concentration in serum was 6.3 (0.7) mg.h/l and in blister fluid 87.1 (8.5) % thereof. The clearance was 338.3 (45.6) ml/min for serum. Urinary recovery was 61.6 (8.7) % after intermittent and 61.9 (6.9) % after continuous infusion.

INTRODUCTION

Meropenem is a new carbapenem with a good in vitro activity against a wide range of Gram-positive and Gram-negative bacteria, including *P. aeruginosa* (Jones, Barry & Phillips, 1989; King, Boothman & Phillips, 1989; Moellering, Eliopoulos & Sentochnik, 1989). One of the advantages of meropenem in comparison with imipenem, is its relative stability to hydrolysis by the proximal tubular brush border enzyme dehydro-peptidase I (DHP-I). Thus it can be administered without an inhibitor of DHP-I, such as cilastatin.

In earlier studies, the pharmacokinetics of meropenem have been determined after a 30 minute infusion (Bax *et al.*, 1989; Burman *et al.*, 1990). Meropenem penetration in to inflammatory exudate has also been examined (Wise *et al.*, 1990). In this study, we have investigated the pharmacokinetics of meropenem at steady state, i.e. after three 25 minute infusion doses, and during continuous infusion, in a crossover fashion. The penetration of meropenem into suction blister fluid (Mouton *et al.*, 1990, Walstad *et al.*, 1983) was also determined.

METHODS

Volunteers. Eight healthy male volunteers, aged 20-29 years, participated in the study. Their bodyweight ranged from 65-90 kg. Before entering the study, each volunteer underwent a complete physical examination and was screened biochemically (Na, K, urea, creatinine, AST, alkaline phosphatase, total protein, albumin, total bilirubin, glucose) and haematologically (haemoglobin, haematocrit, packed cell volume, red cell indices, leukocyte count, platelet count). A standard urine screening test was also performed. No drugs were used in the two weeks preceding the study. The study was approved by the Medical Ethical Committee of the Erasmus University of Rotterdam and written informed consent was obtained from each volunteer.

Design. The pharmacokinetics of meropenem were studied in each volunteer during and after intermittent and continuous administration in a randomized crossover fashion. In each volunteer a minimum of four weeks was allowed to elapse between

the two treatment regimens.

Drug. Meropenem was provided by ICI Pharmaceuticals. The drug was reconstituted according to the manufacturer's instructions. The flow of the infusor was adjusted per individual to obtain the necessary dose per time-unit. Infusion bags were changed every 3 hours during the continuous infusion.

The dose solution concentrations were 20 mg/ml (30 mg/ml for one subject) for intermittent and 10 mg/ml for continuous infusion.

Dosing. The total daily dose during both regimens amounted to 30 mg/kg. For the intermittent regimen volunteers received three doses of meropenem of 10 mg/kg per dose. Doses were administered at 0, 6 and 12 h. The infusion time of each dose was 25 min. For the continuous infusion regimen, 0.2 mg/kg was given as a loading dose and 30 mg/kg was given over 18 h using an infusor.

Dose concentration controls. Samples of the dose solutions were taken from each infusion bag of the third intermittent dose, and at the start and end of infusion of the fifth and the sixth infusion bag of the continuous infusion, i.e. spanning the period 12-18 h.

Blood samples. Venous blood samples for meropenem assay were obtained from an indwelling cannula from the arm opposite to that used for the infusion of the antibiotic.

Intermittent: samples were taken: prior to the third dose (t=0) and at: 10, 20, 30, 40, 50, 60 min and 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after the start of the third infusion.

Continuous: samples were taken: prior to the start of the loading dose and continuous infusion (t=0) and further at: 10, 20, 30, 40, 50, 60 min and 1.5, 2, 4, 8, 12, 14, 16, 18, 19, 20, 22, 24 and 26 h after the start of the infusion.

After sampling, blood was left on ice for 10 min, centrifuged at +1 °C and the serum was rapidly frozen using an ethanol/dry ice mixture. Samples were stored at -70° C until assayed.

Blister fluid samples. Blisters were raised from the abdominal skin by the suction blister technique as described earlier (Mouton *et al.*, 1990). In brief: perspex cups containing 3 perforations with a diameter of 6 mm each, connected with a vacuum source, were used. The cups were placed on the skin and kept in place by applying a

negative pressure of 300 mm Hg. Fifteen minutes after positioning the cups, the suction pressure was lowered to -225 mm Hg. This pressure was maintained until 1.5 h after placing the cups. A total 60-200 μl of fluid could be then drawn using a fine needle. Samples were rapidly frozen and stored at -70°C until assayed.

The cups were placed at the following times: -1, -0.5, 0, 0.5, 1, 1.5, 2.5, 4.5 and 6.5 h after the start of third infusion when dosing intermittently and 2.5, 10.5, 12.5, 14.5, 16.5, 18.5, 20.5, 22.5 and 24.5 h after the start of the infusion when dosing continuously.

Urine samples. Urine samples were collected at the following times: 4, 8, 12, 14, 16, 18, 20, 22, 24 and 26 h after the start of the infusion. The volume of each void was measured, an aliquot of 2 ml rapidly frozen and stored at -70° C until assayed to determine the urinary clearance and recovery. The urinary clearance during continuous administration was determined during the last 6 h of infusion.

Analysis. Samples were assayed by ICI Pharmaceuticals using an HPLC method (Bax *et al.*, 1989). The lower limit of assay sensitivity was 0.18-0.45 mg/l for plasma, 0.15 mg/l for urine and for blister fluid 0.17 mg/l. The coefficient of variation for plasma was $\leq$ 6% from 0.5-20 mg/l and for urine $\leq$ 5%. Blister fluid yielded the same linear response as plasma or urine.

Other assays. A 24 h creatinine urinary clearance was performed on each study day to compare with the meropenem urinary clearance.

Pharmacokinetic and statistical analysis. Serum concentrations were plotted versus time in a semilogarithmic plot. Pharmacokinetic parameters were estimated using the SAS NLIN computer program package (SAS Institute, Inc., 1989) using a two-compartment open model. The equations used were according to Allen *et al.* (1982). The Area Under the time-concentration Curve (AUC) after the third dose of intermittent infusion was determined using the log-linear trapezoideal rule with extrapolation to infinity. Blister fluid concentration/serum concentration ratio was determined from the AUC ratio. AUC, total body clearance (TBC) and urinary clearance during continuous infusion were determined during the time period 12-18 h. The Wilcoxon matched pairs test was used to test differences between treatments and between concentrations in the infusion bags.

Table I. Pharmacokinetic parameters of meropenem after intermittent administration.

Volunteer data				Intermittent administration					
No	Age (years)	Weight (kg)	Length (cm)	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	AUC (mg.h/l)	TBC (ml/min)	Vdss (l/kg)	Urinary recovery (%)
1	24	80	194	11.7	74.4	42.7	312.0	0.325	73.2
2	23	90	192	13.7	66.6	52.8	284.3	0.261	73.6
3	21	71	184	17.0	65.3	41.9	282.3	0.250	64.0
4	29	80	184	6.3	51.0	53.6	248.6	0.235	51.3
5	26	72	175	17.0	63.2	45.7	262.7	0.290	56.3
6	25	76	182	6.6	44.6	34.8	363.6	0.236	53.9
7	23	68	188	23.7	78.5	41.7	272.0	0.302	61.1
8	20	65	187	20.4	57.5	34.8	311.5	0.313	59.9
mean	23.9	75.3	185.8	14.6	62.7	43.5	292.1	0.276	61.6
sd	2.9	8.0	6.0	6.2	11.4	7.1	36.8	0.036	8.7

see text for abbreviations

The results are expressed as arithmetic means $\pm$ standard deviations (sd), except for the half-lives which are expressed as harmonic means $\pm$ sd.

RESULTS

Concentrations in serum and blister fluid are plotted versus time in Figure 1 (intermittent administration) and Figure 2 (continuous administration). As can be seen from the 0.5 h time point in Figure 1, the penetration of meropenem in blister fluid is rapid. Blister concentrations decline parallel to the serum concentrations during the first 4 hours. There seems to be a difference in concentrations at the 6 h sampling point. The mean serum concentration obtained immediately after intermittent infusion was 32.1 $\pm$ 2.9 mg/l and the mean concentration at the end of the dosing interval (i.e. 18h) was 0.61 $\pm$ 0.15 mg/l. The mean AUC was 43.5 $\pm$ 7.1 mg.h/l, the

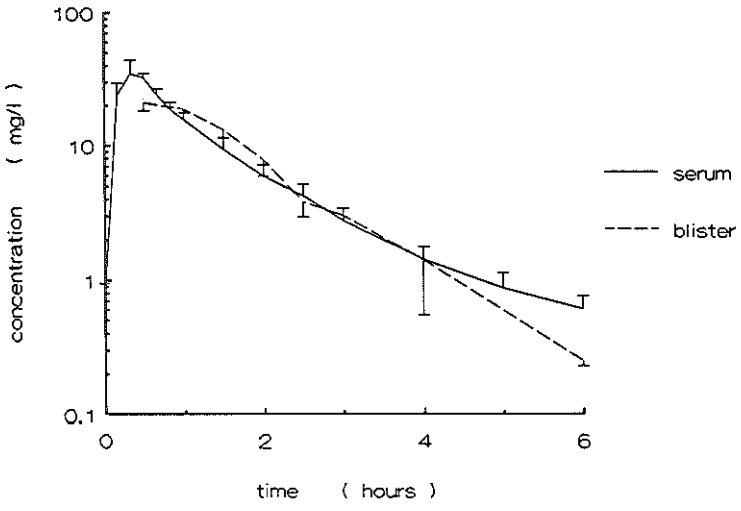

Figure 1. Time-concentration plot of meropenem after intermittent infusion.

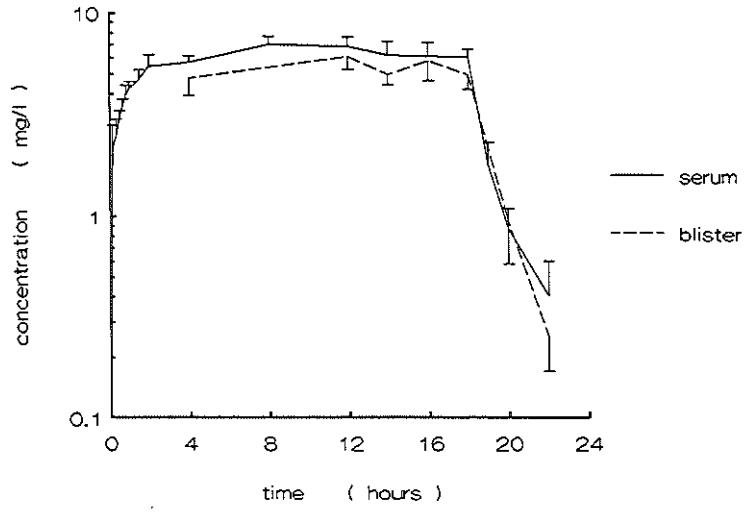

Figure 2. Time-concentration plot of meropenem during continuous infusion.

Table II. Pharmacokinetic parameters of meropenem in blister fluid.

Volunteer	Intermittent administration		Continuous infusion	
No	AUC (mg.h/l)	Blister AUC/ Serum AUC (%)	Mean blister fluid concentration (mg/l)	Blister AUC/ Serum AUC (%)
1	34.8	81.3	4.6	77.0
2	38.2	72.4	5.6	82.8
3	33.0	78.7	4.8	88.8
4	38.3	71.4	5.8	102.2
5	41.4	90.7	5.8	80.4
6	36.8	105.5	5.0	80.9
7	35.3	84.7	6.7	95.5
8	32.4	93.1	5.0	89.0
mean	36.3	84.7	5.4	87.1
sd	3.0	11.4	0.7	8.5

see text for abbreviations

TBC 292.1 ± 36.8 ml/min and the volume of distribution in steady state (Vdss) 0.276 ± 0.036 l/kg (Table I). The urinary recovery was 61.6 ± 8.7 %.

The blister fluid concentrations show a decline parallel to serum and the mean blister fluid/serum concentration (AUC) ratio was of 84.7 ± 11.4% (Table II).

Pharmacokinetic parameters obtained after continuous infusion are summarized in Table III. Continuous infusion at 30 mg/kg over 18 hours produced a steady state meropenem concentration of 6.3 ± 0.7 mg/l. The TBC was 338.3 ± 45.6 ml/min, the urinary clearance was 209.0 ± 35.4 ml/min during the last 6 h of infusion. The urinary recovery was 61.9 ± 6.9 %.

The mean blister fluid concentration during the last 6 h of continuous infusion was 5.4 ± 0.7 mg/l, corresponding to 87.1% of the serum (blister/serum, Table II).

As also observed after the 25 minute infusion dose at the 6 h sampling point, there is a discrepancy between serum and blister fluid levels at the 22 h sampling point. The TBC and recovery during and after intermittent administration were not significantly

Table III. Pharmacokinetic parameters of meropenem during continuous infusion.

Volunteer No	TBC (ml/min)	Cl_u (ml/min)	$Cl_{meropenem}/Cl_{creatinine}$ (%)	Mean serum concentration (mg/l)	Urinary recovery (%)
1	369.7	261.7	148.2	6.0	70.5
2	368.7	213.8	142.9	6.8	57.4
3	366.5	216.9	131.3	5.4	59.1
4	393.5	251.3	135.5	5.7	63.9
5	279.8	191.3	135.0	7.2	68.3
6	341.3	203.1	122.7	6.2	60.9
7	267.7	177.3	134.8	7.1	66.3
8	319.2	156.4	108.4	5.7	49.1
mean	338.3	209.0	132.4	6.3	61.9
sd	45.6	35.4	112.3	0.7	6.9

see text for abbreviations

Table IV. Concentration control samples of meropenem

Infusion bag	Mean concentration (mg/ml)	sd
3rd intermittent infusion	19.4*	0.7
before 5th infusion	9.9	0.4
after 5th infusion	9.7	0.8
before 6th infusion	10.2	0.3
after 6th infusion	9.4	1.1

* normalized from 30 mg/ml to 20 mg/ml for one volunteer

different from the TBC and recovery during continuous administration.

Control samples were taken of the third infusion dose of the intermittent administration and at the start and the end of the infusions from the fifth and the sixth infusion bags for the continuous infusion in order to determine stability of

meropenem under hospital conditions (Table IV). The deviation from the calculated 20 mg/ml in the intermittent infusion bag was 3.2%, which is not statistically significant. For continuous infusion, the concentrations after the infusion were 4.8% lower than the before infusion values, but this is also not significant. During both treatment regimens, no adverse effects were experienced by the volunteers, and no significant changes in biochemical or haematological parameters were found.

DISCUSSION

The pharmacokinetics of meropenem after intermittent dosing are generally in agreement with earlier studies (Bax *et al.*, 1989, Wise *et al.*, 1990), and confirmed that meropenem may be administered without the need for an inhibitor of DHP-1. The recovery in urine was more comparable with the 65.4% found by Wise *et al.* (1990), than the 79% reported by Bax *et al.* (1989). The figure reported by Burman *et al.* (1990) amounted to 72%. The recovery after continuous infusion was also in the same range, 61.9%. There appears to be a large variation between subjects with regard to the meropenem recovery. This variation could be due to the extent of meropenem renal metabolism, as is suggested by the data of Burman *et al.* (1990). They found a strong negative correlation between recovery of the main metabolite of meropenem, ICI 213,689, and meropenem in urine. This phenomenon could not be explained by plasma pharmacokinetics. The meropenem urinary clearance was significantly larger than the clearance of creatinine, indicating tubular secretion. This has been confirmed by a probenicid interaction study in which renal clearance of meropenem was reduced by approximately one-third, to values compatible with glomerular filtration rates in normal healthy males. Co-administration of drugs competing for tubular excretion mechanisms, will probably have little value (Bax *et al.*, 1989).

The penetration of meropenem into suction blister fluid is rapid. The values found are comparable with those found for ceftazidime (Mouton *et al.*, 1990, Walstad *et al.*, 1983) and slightly higher than for cefepime (Nye *et al.*, 1989), but the penetration was slightly lower than found by Wise *et al.* (1990) for meropenem into inflammation blister fluid. However, the chemically elicited inflammatory reaction caused by

cantharidin results in blister fluid with a relatively high protein content, and higher antibiotic penetration ratios are generally found in these blisters compared to suction blisters (Wise, 1986). Also, as indicated in their discussion, their value of 110% may be influenced by a high value for one volunteer. The values for blister fluid penetration found in our study during intermittent and continuous infusion are comparable, 84.7% vs 87.1%. After both intermittent and continuous administration of meropenem, the serum concentration of the later time points appeared higher than would be expected for the log-linear decline seen at previous times. This may represent a positive bias of the assay in serum at low concentrations, although a thorough review did not identify such a bias. Interestingly, the decline of the concentration in serum as depicted in the time-concentration plots in the reports of Wise *et al.* (1990) and Bax *et al.* (1989) seems to indicate a similar trend below 1 mg/l. This discrepancy at low concentrations may also be the cause of the relatively large errors found in the regression analysis when using a weighted procedure (results not shown).

Meropenem exhibits good stability during typical hospital conditions. Even after three hours the decline in concentration was minimal, and not statistically significant. If meropenem is given as a continuous infusion clinically, dose solutions in infusion bags could probably be used for periods greater than the 3 hours used in this study.

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REFERENCES

Allen L, Kimura K, Macmihan J and Rittschel WA.: Manual of symbols, equations and definitions in pharmacokinetics. J Clin Pharmacol 22(Suppl):1-23, 1982

Bax RP, Bastain W, Featherstone A, Wilkinson DM, Hutchinson M and Haworth SJ.: The pharmacokinetics of meropenem in volunteers. J Antimicrob Chemother 24(Suppl A):311-20, 1989

Burman LA, Nilsson-Ehle I, Hutchinson M, Haworth SJ and Norrby SR.: Pharmacokinetics of meropenem and its metabolite ICI213,689 in healthy subjects with known renal metabolism of imipenem. J Antimicrob Chemother 27:219-24, 1990

Jones RN, Barry AL and Thornsberry C.: In-vitro studies of meropenem. J Antimicrob Chemother 24(Suppl A):9-30, 1989

King A, Boothman C and Phillips I.: Comparative in-vitro activity of meropenem on clinical isolates from the United Kingdom. J Antimicrob Chemother 24(Suppl A):31-45, 1989

Moellering RC, Eliopoulos GM and Sentochnik DE.: The carbapenems: new broad spectrum beta-lactam antibiotics. J Antimicrob Chemother 24(Suppl A):1-7, 1989

Mouton JW, Horrevorts AM, Mulder PGH, Prens EP and Michel MF.: Pharmacokinetics of ceftazidime in serum and suction blister fluid during continuous and intermittent infusions in healthy volunteers. Antimicrob Agents Chemother 34:2307-11, 1990

Nye KJ, Shi YG, Andrews JM and Wise R.: Pharmacokinetics and tissue penetration of cefepime. J Antimicrob Chemother 24:24-8, 1989

SAS Institute, Inc. SAS Users guide SAS institute inc, Cary, NC, 1989

Walstad RA, Hellum KB, Blika S, Dale LG, Frederiksen T, Myre KI and Spencer GR.: Pharmacokinetics and tissue penetration of ceftazidime: studies on lymph, aqueous humour, skinblister, cerebrospinal and pleural fluid. J Antimicrob Chemother 12 (Suppl A):235-40, 1983

Wise R.: Methods for evaluating the penetration of beta-lactam antibiotics into tissues. Rev Inf Dis 8(Suppl 3):325-32, 1986

Wise R, Logan M, Cooper M, Ashby JP and Andrews JM.: Meropenem pharmacokinetics and penetration into an inflammatory exudate. Antimicrob Agents Chemother 34:1515-17, 1990

EIGHT

PHARMACOKINETICS OF CEFTAZIDIME DURING CONTINUOUS AND INTERMITTENT INFUSION IN ADULT CYSTIC FIBROSIS PATIENTS

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CHAPTER 8

PHARMACOKINETICS OF CEFTAZIDIME DURING CONTINUOUS AND INTERMITTENT INFUSION IN ADULT CYSTIC FIBROSIS PATIENTS

SUMMARY

The pharmacokinetics of ceftazidime were investigated during intermittent (II) and continuous infusion (CI) in 9 Cystic Fibrosis (CF) patients (4 males and 5 females) during a pulmonary exacerbation due to *P. aeruginosa* in a crossover fashion. The total daily dose was 100 mg/kg/24 h in both regimens, given in three doses of 33.3 mg/kg/8 h or 100 mg/kg/24 h. After at least two days of therapy serum samples were taken. The concentration of ceftazidime was determined using a HPLC method. The $T_{1/2\beta}$ was 1.53 h (sd 0.21), AUC 214.3 mg.h/l (sd 45.3), Total Body Clearance (TBC) 149.2 ml/min (sd 37.2), the Vd_{β} 19.2 l (sd 4.2), the Mean Residence Time 1.98 h (sd 0.19) and the urinary recovery 78.7 % (sd 11.1). The AUC during continuous infusion was 220.7 mg.h/l (sd 30.3), the TBC was 140.0 ml/min (sd 35.9) and the urinary recovery 84.0 % (sd 6.1). The TBC (II) was negatively correlated with the urinary recovery (II), and significantly lower than in healthy volunteers, as was the urinary recovery (CI). In contrast, the TBC during continuous infusion was similar to that found in the volunteers, but significantly different when expressed per kg bodyweight. The serumconcentrations were relatively lower in the CF patients.

INTRODUCTION

The major cause of morbidity and mortality in patients with cystic fibrosis (CF) is pulmonary infection (Wood, Boat and Doershuk, 1976). As patients grow older, most become chronically colonized with *Pseudomonas aeruginosa*, (Marks, 1981; Hoiby, 1982). Pulmonary exacerbations due to this organism are difficult to treat, since the strains are often less susceptible or resistant to common antibiotics. Apart from the use of new effective antibiotics such as the quinolones, the dosage regimens of existing antimicrobial agents may be optimized. During the last decade, it has been shown how important a dosage schedule can be as a determinant of efficacy and toxicity (Mattie, Craig and Pechere, 1989; Cars and Craig, 1991). Time-kill studies of beta-lactam antibiotics on *P. aeruginosa* show a bactericidal activity which is slow, and proceeds with time. There is no concentration dependent killing above the MIC (Vogelman and Craig, 1986). For beta-lactams, the time above MIC may therefore be a determinant in clinical outcome (Schentag, 1991). Animal studies have shown that a continuous infusion of ceftazidime leads to a better efficacy as compared to ceftazidime given four times daily (Roosendaal *et al.*, 1989). A similar observation was made for ticarcillin (Mordenti *et al.*, 1985). It has been suggested that continuous infusion of ceftazidime may lead to a better clinical outcome as compared to the usual regime of 3-4 times daily administration when treating pulmonary exacerbations in CF patients caused by *P. aeruginosa* (Mouton and Kerrebijn, 1990), especially so since it has been suggested by several authors that the elimination of antibiotics in CF is enhanced as compared to non-CF individuals (Spino, 1991). As part of a larger clinical study, the pharmacokinetics of ceftazidime were determined during continuous and intermittent infusion in a randomized cross-over fashion, and the results are compared with healthy volunteers (Mouton *et al.*, 1990).

MATERIALS AND METHODS

Patients. CF patients treated at the Erasmus University Hospital Dijkzigt Rotterdam were eligible for the study. Inclusion criteria were pulmonary exacerbation due to *P.*

aeruginosa, sensitivity of *P. aeruginosa* to ceftazidime and decline of FEV1 of more than 10% as compared to base-line values for each individual patient as measured between exacerbations. The study was approved by the Medical Ethical Committee of the University Hospital Dijkzigt, Erasmus University of Rotterdam and informed consent was obtained from each patient.

Design. The pharmacokinetics of ceftazidime were studied in each patient during intermittent and continuous administration in a randomized cross-over fashion during two pulmonary exacerbations due to *P. aeruginosa*. The mean duration between exacerbations was 259 days (range: 74-515 days).

Drug. Ceftazidime was used as its commercial preparation. The drug was reconstituted according to manufacturer's instructions.

Dosing. The total daily dose amounted to 100 mg/kg. For intermittent administration, patients received 33.3 mg/kg per dose every 8h. The infusion time was 30 min. For continuous administration, 100 mg/kg was given over 24 h with an infusor.

Blood samples. Venous blood samples for ceftazidime assay were taken after at least 2 days therapy and obtained from an indwelling cannula from the arm opposite to that used for the infusion of the antibiotic, or from capillary puncture. Samples were drawn at the following time points: For intermittent administration prior to the dose (t=0, 8.00 am) and at: 30, 40, 50, 60 min and 1.5, 2.5, 4, 6 and 8 h after the start of the infusion; for continuous infusion at t=0 (8.00 am) and at 2, 4, 6, 8 h. A control sample was taken the previous day at 12.00 am. After sampling, blood was left on ice for 20 min, centrifuged and the serum was stored at -70° C until assayed.

Urine samples. Urine samples were collected between 8.00 am and 4.00 pm. Before dosing, at 8.00 am aswell as at 4.00 pm, the patients were asked to void.

Analysis. Samples were assayed by an HPLC method as described before (Mouton *et al.*, 1990).

Pharmacokinetic and statistical analysis. The SAS program (SAS, 1990) was used to analyze the results. Pharmacokinetic parameters were estimated using the SAS NLIN procedure with a two-compartment open model with a weighted least squares adjustment, with the exception of subjects 1, 2 and 6 for which a one-compartment model was used (Yamaoka, Nakagawa and Uno, 1978). Equations used were those as

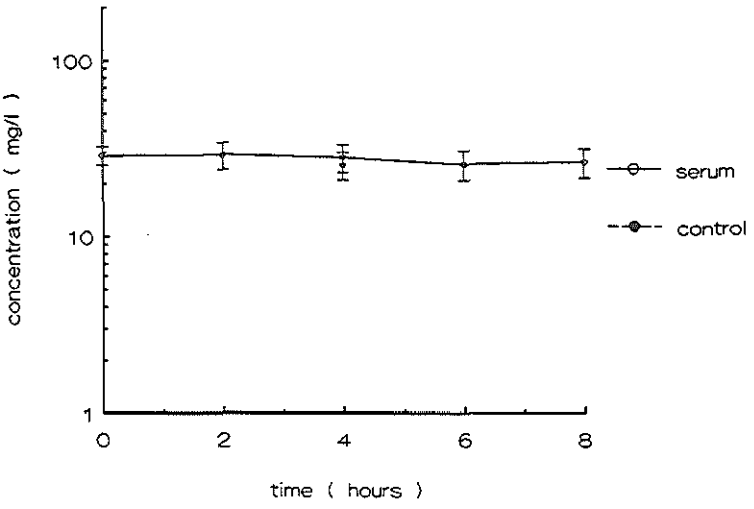

Figure 1. Mean (± sd) serum concentrations of ceftazidime plotted against time during continuous administration (100 mg/kg/24h). A control sample was taken the day before at 12.00 a.m.

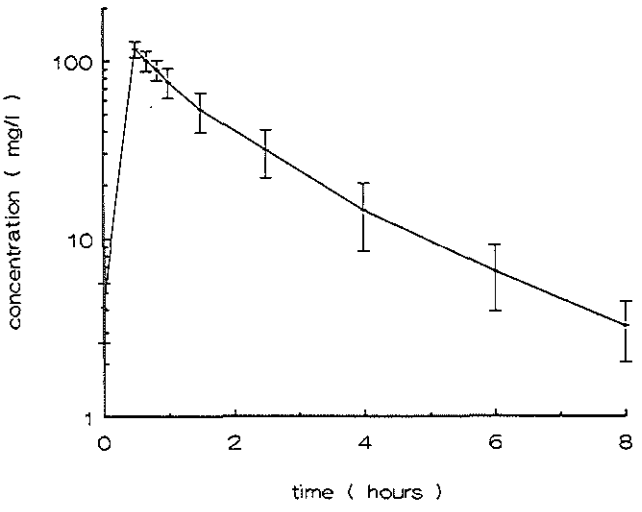

Figure 2. Mean (± sd) serum concentrations of ceftazidime plotted against time during intermittent administration (33.3 mg/kg/dose).

described by Allen *et al.* (1982). The Area Under the time-concentration Curve (AUC) was determined using the trapezoideal rule. AUC, Total Body Clearance (TBC), Mean Residence Time (MRT), Volume of distribution (Vd_β and urinary recovery were determined during the time-period 8.00 am to 4.00 pm. The Body Mass Index (BMI) was calculated as weight/length^2. The Lean Body Mass was calculated as described by Hallynck *et al.* (1981). The Wilcoxon matched pairs test was used to test differences between treatments, and the Mann-Whitney test was used to test differences between groups. For correlation analysis, the Spearman correlation coefficient was used.

Volunteers. To compare data with those of healthy volunteers (mean age 23.6 $\pm$ 2.4 years, mean weight 78.3 $\pm$ 12 kg, Mouton *et al.*, 1990), the AUC and TBC after intermittent infusion of the latter group was recalculated over the same time-period (i.e. 0-8 h) as the CF group using corresponding time-points.

Since the CF group consisted of both males and females, we analyzed the data separately for the males aswell. For the non-standardized results, only the males were used to compare with the volunteers.

RESULTS

From nine CF patients pharmacokinetic data were obtained during intermittent (II) and continuous infusion (CI). Patient characteristics are summarized in Table I. Five patients received intermittent infusion and four continuous infusion during the first episode. The FEV1 and bodyweight at admission were not significantly different between the two treatment modes. Concentrations in serum are plotted versus time in Figure 1 during continuous and Figure 2 during intermittent infusion. The mean concentration during continuous infusion at 12.00 h the day before sampling was 25.4 mg/l (sd 4.5) and not significantly different from the values of the day of sampling. Pharmacokinetic parameters during intermittent treatment are summarized in Table II, and during continuous administration in Table III. The AUC, TBC and urinary recovery were not significantly different between the two treatments, but there appears to be large variation not only between patients, but also within the same

Table I. Patient Characteristics

No.	Sex	Wt (kg)	Length (m)	Age (y)	LBM (kg)	randomization	FEV1 (CI) (l/sec)	FEV1 (II) (l/sec)
1	m	70.0	1.86	38	58.9	c/i	0.98	0.86
2	m	64.1	1.78	19	53.9	c/i	3.00	2.73
3	m	66.2	1.76	37	54.7	i/c	1.95	1.94
4	m	50.0	1.76	24	44.7	c/i	1.44	1.36
5	f	40.0	1.66	21	34.2	c/i	0.54	0.63
6	f	52.2	1.62	19	40.5	i/c	1.41	1.21
7	f	49.0	1.63	20	39.1	i/c	1.54	1.59
8	f	39.4	1.64	22	33.6	i/c	2.24	1.51
9	f	63.0	1.75	23	48.2	i/c	1.08	1.37
Mean		54.9	1.72	24.8	45.3		1.58	1.47
Sd		11.4	0.08	7.4	9.2		0.74	0.61

abbreviations see text

Table II. Pharmacokinetic parameters of ceftazidime after intermittent administration

No.	$T_{1/2\beta}$ (h)	AUC (mg.h/l)	TBC (ml/min)	Vd_β (l)	MRT (h)	urinary recovery (%)
1	1.56	231.0	169.6	22.7	2.07	75.9
2	1.29	169.3	215.7	23.5	1.75	63.9
3	1.68	261.2	140.4	20.5	2.23	64.2
4	1.64	178.8	158.4	22.0	1.89	89.9
5	1.37	231.0	115.5	11.4	1.87	86.8
6	1.53	292.2	97.0	13.1	2.16	94.2
7	1.82	228.5	120.4	18.8	2.12	86.0
8	1.71	163.3	137.8	19.8	2.00	71.2
9	1.19	173.1	192.6	20.8	1.69	75.9
Mean	1.53	214.3	149.2	19.2	1.98	78.7
Sd	0.21	45.3	37.2	4.2	0.19	11.1

abbreviations see text

Table III. Pharmacokinetic parameters during continuous administration

No.	AUC_{ss} (mg.h/l)	TBC (ml/min)	urinary recovery (%)
1	204.6	190.1	83.7
2	218.8	159.6	90.1
3	182.3	159.0	77.8
4	222.8	96.2	87.6
5	230.7	97.5	89.2
6	288.8	158.5	91.6
7	196.9	138.2	73.7
8	233.2	92.9	80.5
9	208.3	168.0	81.5
Mean	220.7	140.0	84.0
Sd	30.3	35.9	6.1

abbreviations see text

patient.

The correlation between the TBC and the LBM was positive ($r=0.83$) and significant ($p=0.005$) during continuous infusion, but failed to reach significance during intermittent infusion ($r=0.65$, $p=0.06$). The TBC (II) was positively correlated with the Vd_β (II) ($r=0.83$, $p<0.01$). The LBM was significantly correlated with the Vd_β (II) ($r=0.70$, $p=0.04$). The TBC and urinary recovery during intermittent infusion are negatively correlated ($r=-0.53$, $p=0.046$).

The correlation between the TBC and the BMI was positive ($r=0.65$) and significant ($p=0.02$) during continuous infusion, but not during intermittent infusion ($r=0.28$, $p=0.30$). The BMI was not significantly correlated with the Vd_β (II) ($r=0.33$, $p=0.22$)

In table IV, the pharmacokinetic data of the CF patients are compared with those of 8 healthy volunteers (Mouton *et al.*, 1990). The TBC/kg of CF patients is significantly higher during both continuous infusion and intermittent infusion, but the TBC of the males is significantly higher during intermittent infusion only. The mean serum concentration during continuous infusion was significantly lower in the CF patients

Table IV. Pharmacokinetic parameters of CF patients compared to volunteers

		Volunteers (N=8)		CF patients (N=9)		CF males (N=4)	
	unit	mean	Sd	mean	Sd	mean	sd
TBC/kg, continuous	ml/min.kg	1.88	0.30	2.56*	0.32	2.39*	0.33
TBC/kg, intermittent	ml/min.kg	1.58	0.10	2.68*	0.55	2.70*	0.55
TBC/kgLBM, continuous	ml/min.kg	2.37	0.39	3.09*	0.52	2.81	0.46
TBC/kgLBM, intermittent	ml/min.kg	1.99	0.220	3.26*	0.62	3.19*	0.61
TBC, continuous	ml/min	147.6	28.45	140.0	35.9	151.2	39.5
TBC, intermittent	ml/min	124.8	25.07	149.2	37.2	170.0*	30.3
Concentration/mg dose/kg	mg/l	9.09	1.55	6.63*	0.88	7.16*	0.98
Weight#	kg	78.3	12.0	54.9*	11.4	62.2*	8.7
LBM#	kg	62.3	6.8	45.3*	9.2	53.0*	6.0
Body Mass Index#	kg/l^2	23.5	2.7	18.5*	2.7	19.8*	2.2
Recovery, continuous	%	94.6	12.0	84.0*	6.1	84.8	5.4
MRT, intermittent	h	2.03	0.12	1.98	0.19	1.98	0.21
$T_{1/2\beta}$, intermittent	min	94.8	5.4	91.8	12.6	92.4	10.8
Vd_β/kg, intermittent	l/kg	0.22	0.02	0.35*	0.08	0.36*	0.06
Vd_β, intermittent	l	17.04	3.29	19.18	4.20	22.2*	1.28

during continuous infusion
* $p<0.05$, CF vv Volunteers

when expressed as concentration reached per mg dose/kg given.

The urinary recovery during continuous infusion, the LBM, the BMI and weight were significantly higher in the volunteers ($p<.05$), and the Vd_β/kg (II) was significantly lower.

DISCUSSION

In this study, we compare the pharmacokinetics of ceftazidime during continuous and intermittent infusion in CF patients during a pulmonary exacerbation due to *P. aeruginosa*, and also compare these results with those obtained from healthy

volunteers. Generally, the pharmacokinetic parameters during continuous infusion and intermittent infusion are similar, and not significantly different from each other, but there appears to be a large variation between patients aswell as within the same patient. The urinary recovery during intermittent infusion was somewhat lower, but this is mainly due to the low values of patients 2 and 3. The $T_{1/2\beta}$ found after intermittent infusion is comparable to results of earlier studies (Leeder *et al.*, 1984; Blumer *et al.*, 1985; Permin *et al.*, 1983; Turner *et al.*, 1984). We found no difference in half-life or MRT between the CF patients and the volunteers, in contrast to Leeder *et al.* (1984).

Low serum-concentrations have been reported for a number of drugs in CF patients (de Groot and Smith, 1987; Prandota, 1988; Spino, 1991). The low serum concentrations of ceftazidime have been explained by a larger volume of distribution and an increased clearance (de Groot and Smith, 1987), due to increased renal clearance. During continuous infusion, in contrast to intermittent infusion, lower serum concentrations can not be explained by a larger volume of distribution. However, we found the serum concentrations of ceftazidime as accomplished during continuous infusion relatively lower in the CF patients as compared to the volunteers. The lower values could be explained by the relatively larger LBM as compared to the total body weight in the CF patients and thus, since we dosed per kg, resulting in a lower dose per kg LBM. However, when the data are recalculated taking the LBM into account, the concentration reached per mg/kg LBM is still significantly lower in the CF group. Since the TBC is determined from the dose divided by the AUC, the approximately identical values of the TBC during continuous infusion in the volunteers and the CF patients can thus be explained by a relatively lower dose in the CF patients due to their lower weight, and a relatively lower AUC (expressed here as the concentration reached in serum per mg dose/kg given), balancing out for the differences. This is supported by the significant difference in TBC when expressed per kg bodyweight or kg LBM. We found no evidence of increased renal clearance during continuous infusion; in contrast, the recovery in the CF patients was significantly lower than in the volunteers, indicating increased non-urinary clearance in these patients.

The TBC during intermittent infusion is negatively correlated with the recovery, also

indicating increased non-urinary elimination in some patients. In contrast to the results found during continuous infusion, was the significantly higher TBC during intermittent infusion in the CF male patients. The volume of distribution is also increased and thus offsets the increased clearance, resulting in minimal changes in $T_{1/2\beta}$. The difference in TBC values found between continuous and intermittent infusion suggest that, for ceftazidime, altered volume of distribution may account at least partly for the discrepancy found with the volunteers during intermittent infusion. The higher TBC/kg, or relatively lower AUC, during continuous infusion may have consequences for dosing: if a dose would be determined from the plasma levels of volunteers, it would be too low if the dose were given per kg and should be adjusted.

REFERENCES

Allen L, Kimura K, MacKichan J and Ritschel W.: Manual of symbols equations and definitions in pharmacokinetics J Clin Pharmacol 22(Suppl):1-22, 1982

Blumer JL, Stern RC, Klinger JD, *et al.*: Ceftazidime therapy in patients with cystic fibrosis and multiple drug-resistant Pseudomonas. Am J Med 79(Suppl 2A):37-46, 1985

Cars O and Craig WA.: General discussion on antibiotic dosing. Scand J Infect Dis 74(Suppl):282-282, 1991

Groot de R and Smith AL.: Antibiotic pharmacokinetics in cystic fibrosis: differences and clinical significance. Clin Pharmacokin 13:228-253, 1987

Hallynck TH, Soep HH, Thomis JA, *et al.*: Should clearance be normalized to body surface or to lean bodymass? Brit J Clin Pharmacol 11:523-525, 1981

Hoiby N.: Microbiology of lunginfections in cystic fibrosis patients. Acta Paediatr Scand 301(Suppl):33-54, 1982

Leeder JS, Spino M, Isles AF, *et al.*: Ceftazidime disposition in acute and stable cystic fibrosis. Clin Pharmacol Ther 36:355-62, 1984

Marks MI.: The pathogenesis and treatment of pulmonary infections in patients with cystic fibrosis. J Pediatr 98:173-79, 1981

Mattie H, Craig WA and Pechere JC.: Determinants of efficacy and toxicity of aminoglycosides. J Antimicrob Chemother 24:281-293, 1989

Mordenti JJ, Quintiliani R and Nightingale CH.: Combination antibiotic therapy: comparison of constant infusion and intermittent bolus dosing in an experimental animal model. J Antimicrob Chemother 15(Suppl A):313-321, 1985

Mouton JW, Horrevorts AM, Mulder PHG, Prens EP and Michel MF.: Pharmacokinetics of ceftazidime in serum and suction blister fluid during continuous and intermittent infusion in healthy volunteers Antimicrob Agents Chemother 34:2307-2311, 1990

Mouton JW and Kerrebijn KF.: Antibacterial therapy in cystic fibrosis. Med Clin North Am 74:837-850, 1990

Permin H, Koch C, Hoiby N, *et al.*:Ceftazidime treatment of chronic *Pseudomonas aeruginosa* respiratory tract infection in cystic fibrosis. J Antimicrob Chemother 12(Suppl A):313-323, 1983

Prandota J.: Clinical pharmacology of antibiotics and other drugs in cystic fibrosis. Drugs 35:542-578, 1988

Roosendaal R, Bakker-Woudenberg IAJM, van den Berghe-Raffe M, Vink-van den Berg JC and Michel MF.: Impact of the dosage schedule on the efficacy of ceftazidime gentamicin and ciprofloxacin in *Klebsiella pneumoniae* pneumonia and septicaemia in leukopenic rats. Eur J Clin Microbiol Infect Dis 8:878-887, 1989

SAS Institute.: SAS User's guide. SAS Institute Inc Cary NC, 1990

Schentag JJ.: Correlation of pharmacokinetic parameters to efficacy of antibiotics: relationship between serum concentrations MIC values and bacterial eradication in patients with Gram-negative pneumonia. Scand J Infect Dis 74(Suppl):218-234, 1991

Spino M.: Pharmacokinetics of drugs in cystic fibrosis. In: Clin Rev Allergy vol 9 (E Gershwin ed) 169-210, 1991

Turner A, Pedler SJ, Carswell F, Spencer GR and Speller DCE.: Serum and sputum concentrations of ceftazidime in patients with cystic fibrosis. J Antimicrob Chemother 14:521-527, 1984

Vogelman BS and Craig WA.: Kinetics of antimicrobial activity. J Pediatr 108:835-840, 1986

Wood RE, Boat TF and Doershuk CF.: Cystic fibrosis. Am Rev Respir Dis 113:833-78, 1976

Yamaoka K, Nakagawa T and Uno T.: Application of Akaike's in-formation criterion (AIC) in the evaluation of linearpharmacokinetic equations. J Pharmacokin Biopharmacol 6:165-175, 1978

NINE

CIRCADIAN VARIATION IN SERUM CONCENTRATIONS OF CEFTAZIDIME AND MEROPENEM

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Submitted for publication

CHAPTER 9

CIRCADIAN VARIATION IN SERUM CONCENTRATIONS OF CEFTAZIDIME AND MEROPENEM

SUMMARY

Serum concentrations were determined during continuous and intermittent infusion over 24 hours for meropenem in healthy volunteers and for ceftazidime in healthy volunteers and cystic fibrosis patients. Serum concentrations during continuous infusion of meropenem and ceftazidime were significantly higher in the early morning as compared to concentrations in the evening and the late afternoon. During intermittent infusion, trough levels of ceftazidime in the healthy volunteers in the early morning were higher than those in the late afternoon. These results indicate a circadian variation of ceftazidime and meropenem serum concentrations, which may be due to variation of kidney function.

INTRODUCTION

Time-kill studies of beta-lactam antibiotics on gram-negative rods show a bactericidal activity which is slow, proceeds with time and is maximal at relatively low concentrations (Vogelman & Craig, 1986). Administration by continuous infusion, to accomplish maintained serum concentrations, may therefore be more efficaceous than conventional intermittent treatment, as has indeed been shown in animal studies (Roosendaal *et al.*, 1989). During three studies, we investigated the pharmacokinetics of ceftazidime and the new carbapenem meropenem during continuous infusion as compared to intermittent infusion in healthy volunteers and cystic fibrosis patients. Although infusion pumps deliver a constant dose of antibiotic, serum levels may vary considerably during the day (e.g. Sharma *et al.*, 1979; Jonkman *et al.*, 1988; Elting *et al.*, 1990). During the three studies, we had the opportunity to observe time-related differences of serum concentrations.

MATERIALS AND METHODS

In two studies, the pharmacokinetics of ceftazidime and meropenem were studied during continuous and intermittent infusion in eight healthy volunteers in a cross-over fashion. Details have been published elsewhere (Mouton *et al.*, 1990; Mouton & Michel, 1991). Briefly, the start of the ceftazidime infusions was at 4.30 p.m. (75 mg/kg total dose, given as 25 mg/kg q8h or 60 mg over 24 hours with 15 mg/kg as a loading dose), and the start of the meropenem infusions at 7.30 p.m. (30 mg/kg total dose, given as 10 mg/kg q6h or 30 mg/kg over 18 hours). During a third study, the pharmacokinetics of ceftazidime was studied in Cystic Fibrosis patients during a pulmonary exacerbation due to *P. aeruginosa*. After at least two days of therapy (100 mg/kg total daily dose, given as 33.3 mg/kg q8h or 100 mg/kg over 24 h), serum samples were taken. Intermittent infusions were given at 8 a.m., 4 p.m. and 12 p.m. Serum samples were taken at appropiate time-points, and analyzed by HPLC. The Wilcoxon matched pairs test was used to determine differences between serum levels (Blalock, 1981).

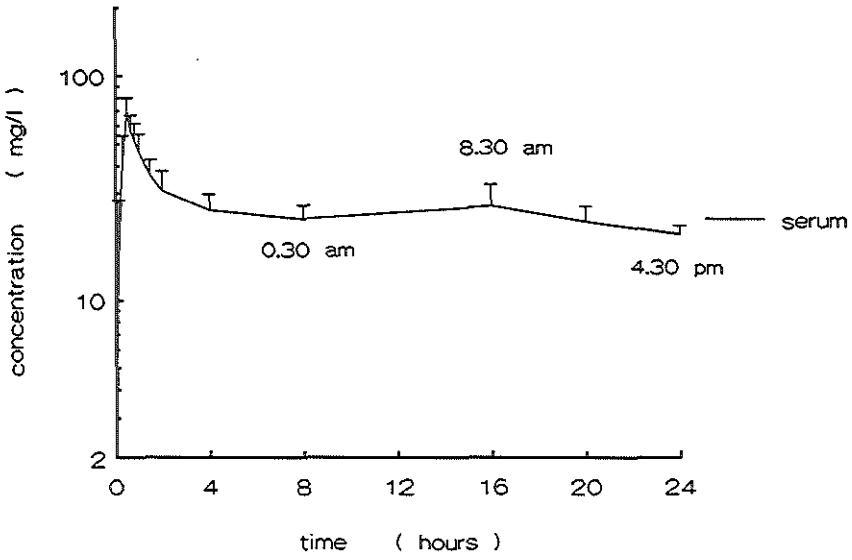

Figure 1. Mean serum concentrations of ceftazidime over 24 hours (60 mg/kg/24 h and 15 mg/kg as a loading dose) in 8 volunteers. The 8.30 a.m. concentrations are significantly higher than those at 0.30 a.m. and 4.30 p.m..

RESULTS

In Table I, the mean values of ceftazidime and meropenem serum levels are shown during continuous and intermittent administration at night, in the early morning and in the late afternoon. For ceftazidime given to healthy volunteers, the concentrations during continuous infusion were significantly higher in the early morning compared to the late afternoon and at night. Figure 1 shows the rise and fall of the ceftazidime serum levels over 24 hours, with the highest concentration at 8.30 a.m.. During intermittent infusion, the trough levels in the early morning were significantly higher than those at 4.30 p.m.. The serum concentrations of meropenem during continuous infusion were significantly higher in the early morning as compared to the late afternoon and at night. During intermittent infusion, there was no significant difference. In

Table I. Influence of the time of the day on serum concentrations (sd) of ceftazidime and meropenem

Healthy volunteers, ceftazidime (n=8)	mean ceftazidime level (sd) (mg/l)		
	0.30 am	8.30 am	4.30 pm
Continuous, 2.5 mg/kg/h	22.8 (3.6)[@]	26.3 (6.6)	19.6 (1.8)[@]
Intermittent, 25 mg/kg q8h	nd	7.1 (1.2)	4.0 (0.7)[@]

Healthy volunteers, meropenem (n=8)	mean meropenem level (sd) (mg/l)		
	11.30 pm	7.30 am	1.30 pm
Continuous, 1.66 mg/kg/h	5.7 (0.4)[@]	6.8 (0.8)	6.0 (0.6)[@]
Intermittent, 10 mg q6h	nd	0.7 (0.2)	0.6 (0.2)

Cystic Fibrosis patients	mean ceftazidime level (sd) (mg/l)	
	8.00 am	4.00 pm
Continuous, 4.16 mg/kg/h (n=13)	29.4 (3.8)	26.3 (4.9)[@]
Intermittent, 33.3 mg/kg q8h (n=9)	4.1 (1.5)	3.3 (1.2)[*]

[@] $P < 0.05$ and [*] $P < 0.1$ as compared to early morning sample (Wilcoxon matched pairs test)

the cystic fibrosis patients, ceftazidime concentrations in serum in the early morning were significantly higher as compared to the afternoon during continuous infusion, during intermittent infusion the difference did not reach significance.

DISCUSSION

Rhythmic changes in pharmacokinetic parameters in men dependent upon dosing time have been reported for many drugs (Reinberg, Levi & Smolenski, 1984; Brugueroll, 1987), but only for a few antibiotics (Dettle & Spring, 1966; Kabasakalian *et al.*, 1970; Di Santo, Chodos & Halberg, 1975; Sharma *et al.*, 1979; Jonkman *et al.*, 1988; Elting *et al.*, 1990). Differences in serum levels may be related to variation in absorption, metabolism or excretion (Reinberg *et al.*, 1984; Reinberg, Smolenski & Labrecque, 1985). Ceftazidime and meropenem were both given intravenously, so differences in absorption do not have to be considered. Ceftazidime is almost completely excreted by the kidneys (Mouton *et al.*, 1990; Welage, Schultz & Schentag, 1984). Kidney function, as measured by urine volume and glomerular filtration rate, is known to increase during the day and decrease during the night, and be independent of fluid intake (Wesson & Lauler, 1961), and thus offers the most likely explanation for the differences of ceftazidime serum levels. A similar explanation was given by Elting *et al.* (1990), who found circadian variations in serum amikacin levels. However, the authors discuss two possible confounding factors in their study. The first was differences in specimen handling, which in our studies was similar for all samples. Their second confounding factor was possible interference with other antibiotics, since the concentrations were measured during combination therapy, while during our studies monotherapy was given. Meropenem is excreted via the kidneys for 60-70% as the unchanged compound (Harrison *et al.*, 1989; Mouton & Michel, 1991), and although kidney function probably may explain the results, it can not be excluded that metabolization also varies during the day (Reinberg *et al.*, 1984).

Our findings suggest that there are variations in serum levels of ceftazidime and meropenem, and that these are higher in the early morning as compared to late evening and late afternoon concentrations. Although these differences are probably not clinically relevant for these compounds, the time of the day should be considered when interpreting serum concentrations.

REFERENCES

Blalock HB.: Social Statistics. McGraw-Hill, 1981.

Bruguerolle B.: Donnees recentes en chronopharmacokinetique. Pathol Biol 35:925-934, 1987

Dettle L and Spring P.: Diurnal variations in the elimination rate of a sulfonamide in man. Helvetica Medica Acta 33:291-306, 1966

Di Santo A, Chodos D and Halberg F.: Chronobioavailability of three erythromycin test preparations assessed by each of four indices: Time to peak, peak, nadir and area. Chronobiologia 2(Suppl 1):17, 1975

Elting L, Bodey GP, Rosenbaum B and Fainstein V.: Circadian variation in serum amikacin levels. J Clin Pharmacol 30:798-801, 1990

Harrison MP, Moss SR, Featherstone A, Fowkes AG, Sanders AM and Case DE.: Disposition and metabolism of meropenem in laboratory animals and man. J Antimicrob Chemother 24:265-277, 1989

Jonkman JHG, Reinberg A, Oosterhuis B, de Noord OE, Kerkhof FA, Motohashi Y, Levi F, Dammacco F and Carandente F.: Dosing time and sex-related differences in the pharmacokinetics of cefodizime and in the circadian cortisol rhythm. Chronobiologia 15:89-102, 1988

Kabasakalian P, Katz P, Rosenkrants B and Townley E.: Parameters affecting absorption of griseofulvin in human subjects using urinary metabolic excretion data. J Pharmacol Sci 59:596-600, 1970

Mouton JW and Michel MF.: Pharmacokinetics of meropenem in serum and suction blister fluid during continuous and intermittent infusion. J Antimicrob Chemother 28:911-918, 1991

Mouton JW, Horrevorts AM, Mulder PHG, Prens EP and Michel MF.: Pharmacokinetics of ceftazidime in serum and suction blister fluid during continuous and intermittent infusions in healthy volunteers. Antimicrob Agents Chemother 34:2307-2311, 1990

Reinberg A, Levi F and Smolenski M.: Chronopharmacokinetique clinique. J Pharmacol 15(Suppl 1):95-124, 1984

Reinberg A, Smolenski HS and Labrecque G.: New aspects in chronopharmacology. Ann Rev Chronopharmacol 2:3-26, 1985

Roosendaal R, Bakker-Woudenberg IAJM, Berghe-van Raffe M, Vink-van den Berg JC and Michel MF.: Impact of dosage schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in *Klebsiella pneumoniae* pneumonia and septicemia in leukopenic rats. Eur J Clin Microbiol Infect Dis 8:878-887, 1989

Sharma SD, Deshpande VA, Samuel MR and Vakil BJ.: Chronobioavailability of ampicillin. Chronobiologia 6:156, 1979

Vogelman B and Craig WA.: Kinetics of antimicrobial activity. J Pediatr 108:835-840, 1986

Welage LS, Schultz RW and Schentag JJ.: Pharmacokinetics of ceftazidime in patients with renal insufficiency. Antimicrob Agents Chemother 25:201-204, 1984

Wesson LG and Lauler DP.: Diurnal cycle of glomerular filtration rate and sodium and chloride excretion during responses to altered salt and water balance in man. J Clin Invest 40:1967-1977, 1961

TEN

PHARMACOKINETICS AND KILLING OF BACTERIA IN VITRO

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CHAPTER 10

PHARMACOKINETICS AND KILLING OF BACTERIA IN VITRO

10.1

GENERAL INTRODUCTION AND REVIEW

To determine the susceptibility of a micro-organism, usually a Minimal Inhibitory Concentration (MIC) is determined. This method has two distinct disadvantages: (1) the endpoint is growth or no-growth after a fixed point of time without taking the growth or killing kinetics into account and (2) the medium in which the micro-organism grows contains a steady state concentration of antibiotic, while in the body there is a fluctuation of antibiotic concentrations as a result of intermittent dosing and clearance of the antibiotic.

To overcome the first problem, killing-curves are sometimes used, and it has been shown in several studies that the killing of the bacteria by various classes of antibiotics does indeed differ. The classical paper of Vogelman and Craig (1986) describes the effect of ticarcillin and tobramycin on *Pseudomonas aeruginosa*. Killing of bacteria by ticarcillin was slow, continued in time, and high concentrations contributed no extra effect. In contrast, tobramycin exerted a fast killing, which was concentration dependent. The results of a similar study on ceftazidime and tobramycin are shown in Figure 1a and 1b respectively. For ceftazidime, the killing curves at concentrations much higher than the MIC do not essentially differ from each other. Killing starts almost immediately and continues in time, as can been seen from the steady decrease in surviving bacteria. For tobramycin concentration dependent killing is evident, and at the highest concentration used killing is almost immediate. Killing curves thus provide information on the pharmacodynamics of an antibiotic, which an MIC does not.

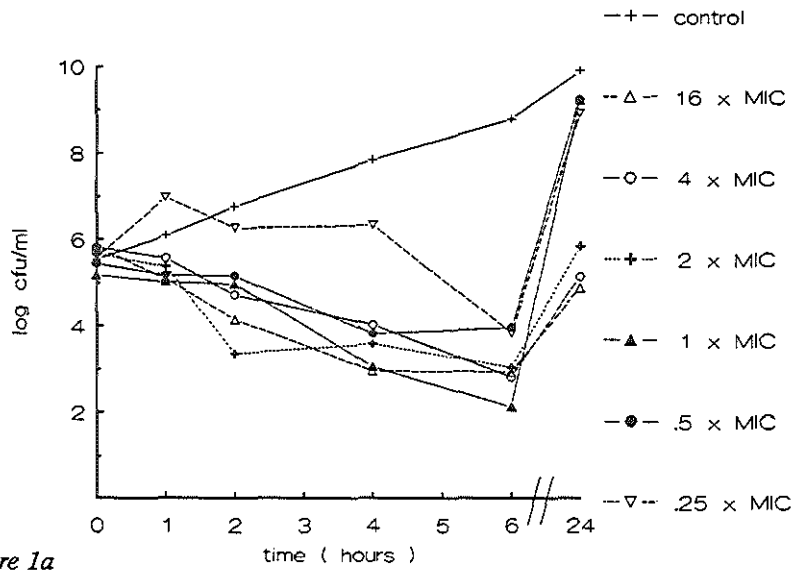

Figure 1a

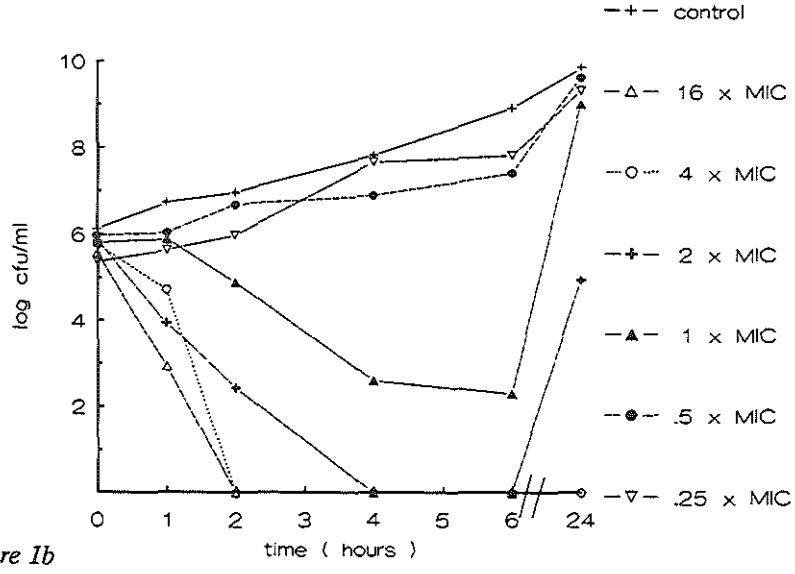

Figure 1b

Figure 1a and 1b. Killing curves of ceftazidime (above, Figure 1a) and tobramycin (below, Figure 1b). Inocula of 5 x 10^5 cfu/ml P. aeruginosa were incubated with ceftazidime or tobramycin for 24 hours in a range from .25 x MIC to 16 x MIC.

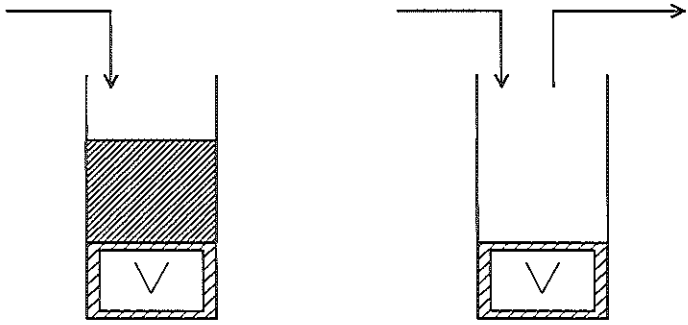

Figure 2. Principle of models based on dilution. The culture is diluted with fresh broth to obtain a concentration gradient of the antibiotic. Left: simple dilution. Right: diluted culture is eliminated to retain an equal volume (V).

The second problem, concerning fluctuation of antimcrobial concentrations, was recognized many years ago, and several models have been evolved to simulate plasma concentrations in humans. In 1968, Sanfilippo and Morvillo developed an in vitro kinetic model, that mimicked plasma concentrations as observed in vivo. The apparatus consisted of a series of flasks containing a bacterial culture to which an antibiotic was added. Using a peristaltic pump to control the flowrate, sterile diluent broth was added to simulate decreasing concentrations of antibiotic. In this way, it was possible to study the effect of decreasing concentrations on the killing of bacteria (Figure 2). Two disadvantages were recognized in this system. One was an increase in culture broth during dilution and the amount of broth needed to dilute the culture, which increased exponentially in time. The second drawback was the pump flow rate which, with increase in time, yielded concentrations which did not decrease exactly exponentially. This was solved in subsequent studies by keeping the culture volume constant, for instance by keeping the flow out of the culture flask the same as the diluent flow (Otaya, Ozawa and Goto, 1976; Bergan *et al.*, 1980). Although most of the problems encountered with the diluent method were resolved technically by

Table I. In vitro pharmacokinetic models based on dilution.

authors	*model description*	*regimens used*
O'Grady & Pennington, 1966	dilution, model to study cystitis	dilution effect on growth of bacteria
Sanfilippo & Morvillo, 1968	reproduction plasma levels, series of flasks, flow control not very reliable, much diluent needed	sulfonamides on S. aureus
Otoya et al., 1976	pressure pump to keep central compartment at the same volume	cefalothin, erythromycin and kanamycin div. spp.
Grasso et al., 1978	pressure pump, mono and biexponential concentration-time curves, continuous monitoring	cefazolin against E. coli and Klebsiella sp. one dose.
Nishida, 1978	stepwise dilution	cefazolin, cefaloridine and cefalothin against E. coli
Greenwood & Grady, 1978	full automated system of a simulation of the bladder	
Leitner et al., 1979	stepwise dilution	cephalosporins against div spp.
Bergan et al., 1980	two pressure pumps, continuous monitoring	
Murakawa et al., 1980	two-compartment open model, continuous decline concentration	cefazolin, cefamandole, cefuroxime, cefoxitin, one dose. PAE?
Shah, 1980	filter to prevent removal of bacteria	
Klaus et al., 1981	model of Grasso et al., 1978	amoxicillin on E. coli, beta-lactamase production
Greenwood & Tupper, 1982	two chambers, one with bacteria, one with a changing antibiotic concentration, diffusion through membrane	
Gerber et al., 1982	model of Grasso et al., 1978	gentamicin, 2 doses vv continuous infusion. no difference. PAE.

Table I, continued

authors	*model description*	*regimens used*
Schneider et al., 1982	filter	cefroxadine, cefalexine, cefradine on Gram-negatives
Bauernfeind, 1982	stepwise dilution	ceftriaxone and netilmicin on P. aeruginosa. synergism.
Haller, 1982	stepwise dilution	azlocillin and sisomycin on P. aeruginosa
Van Etta et al., 1982	model of Murakawa et al., 1980	cefapirin, one dose. effect of ratio of surface area to volume on the penetration of antibiotics in extravascular spaces.
Shah, 1983	model of Shah, 1980	cephalosporins and netilmicin on Gram-negatives
Tosch and Schnell, 1984	dilution and filter	comparison of dilution models and models with filter. results were overlapping
Wiedemann & Seeberg, 1984	model of Grasso et al., 1978	cefotiam, one dose. influence of beta-lactamases on killing kinetics. correlation between killing ability and MIC only for MIC > 16 mg/l.
Seeberg & Wiedemann, 1985	model of Grasso, et al., 1978	ampicillin, amoxycillin, cephalosporins. 3 doses. development of resistance.
White et al., 1985	model of Grasso et al., 1978 and Murakawa et al., 1980	amoxicillin, ticarcillin and clavulanic acid against P. aeruginosa. gentamicin enhances effect
Sous & Hirsch, 1985	model of Grasso et al., 1978	phenoxymethylpenicillin against S. aureus. one dose.
Haag et al., 1986	model of Grasso et al., 1978	artifacts in dilution pharmacokinetic models
White et al., 1989	model of Grasso et al., 1978	ampicillin against E. coli
Bauernfeind, 1992	stepwise dilution	teicoplanin, mezlocillin, netilmicin and ciprofloxacin alone and in dual combinations against E. faecalis

Table II. In vitro pharmacokinetic models based on diffusion or dialysis.

authors	*model description*	*regimens used*
Al-Asadi et al., 1979	two tubes separated by membrane, one of the chambers with declining antibiotic concentration. blocking of pores by bacteria.	gentamicin against E. coli
Drugeon et al., 1979	dialysis unit	aminoglycosides against div. spp.
Zinner et al., 1981	artificial capillary unit with polysulphone fibers	azlocillin against P. aeruginosa
Toothaker et al., 1982	hemodialysis unit	ampicillin against E. coli
Blaser et al., 1985	two compartment model, multiple artificial capillary units	-
Blaser et al., 1985	two compartment model, multiple artificial capillary units	-
Zinner et al., 1985	two compartment model, multiple artificial capillary units, antibiotic combinations	piperacillin with thienamycin or amikacin, azlocillin with netilmicin against P. aeruginosa
Reeves, 1985	hollow fiber dialyser	ciprofloxacin against S. marcescens. four doses.
Guggenbichler et al., 1985	dialyser	various antimicrobials against div. spp.
Blaser et al., 1985	dialysis	netilmicin intermittent and continuous on P. aeruginosa. intermittent favorable
Blaser et al., 1985	dialysis	ceftazidime-netilmicin combinations on P. aeruginosa. synergism.
Shah, 1985	membrane filter	imipenem against Gram-negative rods in blood
Bergan and Carlsen, 1985	membrane filter	various antimicrobials against div. spp.
Zinner & Blaser, 1986	dialysis	discrepancy synergism in vitro pharmacokinetics and other methods

Table II, continued

authors	*model description*	*regimens used*
Navashin et al., 1989	membrane filter	sisomycin on E. coli
Firsov et al., 1990	membrane filter	sisomycin on P. aeruginosa, E.coli and K. pneumoniae
Silley et al., 1990	membrane filter	cefuroxime axetil kill kinetics
Vergeres and Blaser, 1991	membrane filter	various antimicrobials against P. aeruginosa and S. epidermidis in biofilm

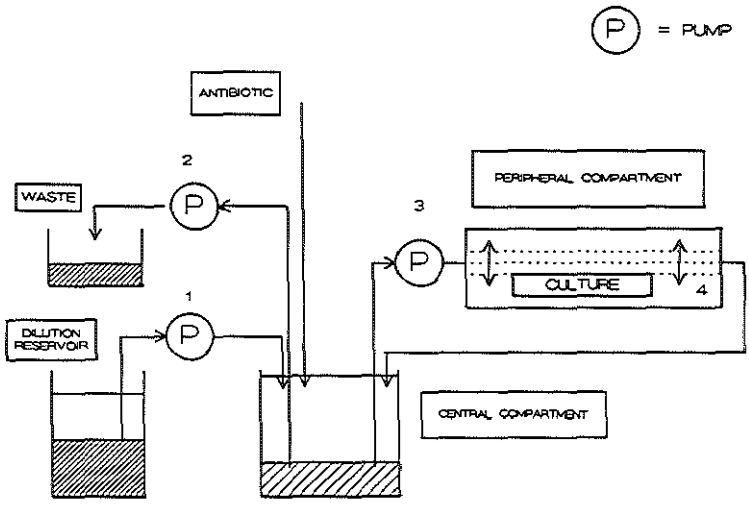

Figure 3. Principle of models based on diffusion. The broth with antibiotic in the central compartment is diluted with fresh broth from the dilution reservoir (1), while at the same time the same amount is eliminated (2). The volume thus remains constant and a log-linear concentration gradient is obtained. The contents in the central compartment are pumped through diffusive capillaries or along a membrane (3), communicating freely with the bacterial culture through the membrane (4), thus creating a concentration gradient in this compartment as well. The size of the pores of the capillaries allow only small molecules to pass through. It is possible to place several peripheral compartments in series, and thus to study several cultures simultaneously.

various authors (Table I), the major disavantage still remained dilution of not only the antibiotic, but also of the bacterial culture (Figure 2). To overcome this problem, Shah (1980) inserted a micro-glass filter before the outflow to prevent removal of bacteria.

The models described so far were all based on dilution of the original culture. In 1979 Al-Asadi, Greenwood and O'Grady, as well as later authors, described a model based on diffusion (Table 2). Two tubes were separated by a membrane, one tube contained the antibiotic solution, the other the culture. By diluting the antibiotic solution a gradient was obtained, and as the broth with antibiotic was free to cross

the membrane, a gradient was obtained in the culture as well. One of the drawbacks of the model however, was that the bacteria blocked the membrane pores, thereby impeding diffusion. This problem was solved in several ways. Drugeon, Maurisset and Courtieu (1979), described a model based on a dialysis unit, which appeared to be very efficient. In 1981 Zinner, Husson and Klasterky described a device based on an artificial capillary unit, which was later perfected by Blaser (1985) and Blaser, Stone and Zinner (1985a,b; Figure 3). The model ultimately consisted of serially placed bacterial compartments interfacing with a central compartment through artificial capillaries. The antibiotic gradient obtained in the central compartment through dilution, was reflected in the peripheral compartment. The model also allowed simultaneous elimination kinetics of two drugs with different half-lives, to study antibacterial effects of drug combinations.
The model which was chosen for the studies described below is a modification of the model as described by Blaser (1985).

purpose of the study

Although there are several studies describing the efficacy of beta-lactams during decreasing concentrations in vitro, there are no studies which compare the effect of a continuous versus an intermittent dosing regimen over several dosing intervals. To allow us to study the killing kinetics of ceftazidime on *P. aeruginosa* during continuous and intermittent infusion, we developed an in vitro pharmacokinetic model, and applied these dosing regimens on *P. aeruginosa*. Several strains with different MICs were used to determine the interaction between susceptibility and dosing regimen.

10.2

KILLING OF PSEUDOMONAS AERUGINOSA DURING CONTINUOUS AND INTERMITTENT INFUSION OF CEFTAZIDIME IN AN IN VITRO PHARMACOKINETIC MODEL

SUMMARY

An in vitro pharmacokinetic model mimicking human serum concentrations was used to study the efficacy of continuous infusion and intermittent administration of ceftazidime over a period of 36 hours. The model consisted of a central compartment interfacing with dialyser units containing the bacterial cultures. The daily dose was 300 mg/l/24 hours given either as a continuous infusion or as three bolus doses. The concentrations of ceftazidime were determined by HPLC, and efficacy was determined by bacterial counts. The dosing regimens yielded top and trough concentrations after the fourth dose of 92.3 $\pm$ 8.0 and 1.4 $\pm$ 0.9 mg/l, respectively. Continuous administration yielded concentrations of 20 mg/l. To study efficacy, three *P. aeruginosa* strains, ATCC 27853, CF224 and CF224M were used. The MIC for ceftazidime of these strains was 1, 4 and 16 mg/l, respectively. Strain CF224M was killed initially during both regimens, and then started to regrow. At the end of the fourth dosing interval, i.e. after 32 hours, there was no difference between the regimens. Regrowth was due to the emergence of resistant strains as demonstrated by a higher MIC and the presence of beta-lactamase in the medium. The strains ATCC 27853 and CF224 were killed initially during both dosing schedules, and after the first dosing interval viable counts were similar. However, after the fourth interval, there was a marked difference between bacterial counts during continuous and intermittent infusion, being 2.2 and 3.8 ^{10}log respectively, demonstrating a higher efficacy during continuous infusion.

The results indicate that, in the absence of other factors, a sustained level of cef-

tazidime around or slightly above the MIC is not high enough to maintain efficacy over more than one (8 h) dosing interval, and probably should be around 4 to 5 times the MIC. When sustained concentrations higher than 4 times the MIC are employed, continuous administration in this model is more efficacious than intermittent dosing.

INTRODUCTION

Time-kill curves of beta-lactam antibiotics on *P. aeruginosa* show time dependent killing which is maximal at relatively low concentrations (Vogelman and Craig, 1986). Concentrations much higher than the MIC contribute no extra effect. From these experiments, it can be deduced that continuous serum concentrations above the MIC of the antibiotic used to combat the micro-organism in question, should be more efficacious than declining concentrations as observed after intermittent dosing (Cars and Craig, 1991; Craig and Ebert, 1992). During the latter regimen, concentrations fall below the MIC during part of the dosing interval. It has been shown in several animal models that continuous infusion is indeed more efficacious than intermittent dosing (Gerber *et al.*, 1983; Mordenti *et al.*, 1985; Roosendaal *et al.*, 1989). However, the half-life of most drugs is severely shortened in the animals studied and conclusions regarding the pharmacodynamics in humans are difficult to make (Gerber *et al.*, 1991). For, if the shortened half-life is taken into account, the time below MIC applies for almost the entire dosing interval, which is contrary to the situation as observed in humans. This has, however, partly been overcome by fractional dosing to mimick human pharmacokinetics (Gerber *et al.*, 1986).

In vitro simulation of human pharmacokinetics may thus give additional information with respect to killing kinetics of micro-organisms. Several in vitro pharmacokinetic models have been described to simulate human pharmacokinetics, based on either dilution or diffusion (see 10.1). We developed an in vitro pharmacodynamic model based on a dialyser unit, which is described here. This model was subsequently used to study the efficacy of continuous versus intermittent infusion of ceftazidime against *P. aeruginosa* over a period of 36 hours. Although there are some reports suggesting a better efficacy in vitro when dosing beta-lactam antibiotics continuously (Gerber *et*

al., 1982), in these studies they were given in combination with an aminoglycoside. In other reports the MIC is compared with the results obtained in the pharmacodynamic model (Klaus *et al.*, 1981; Seeberg and Wiedemann, 1985; White *et al.*, 1989), but circumstances during which an MIC is determined differ from those in the model. Furthermore, all these studies were based on dilution models, and although correction for dilution of the bacteria (White *et al.*, 1987) can be made, artifacts due to dilution have to be precluded (Haag *et al.*, 1986).

In the present study, we used a standard dose yielding concentrations comparable to those observed in humans during treatment. Three *P. aeruginosa* strains with different MIC's were examined.

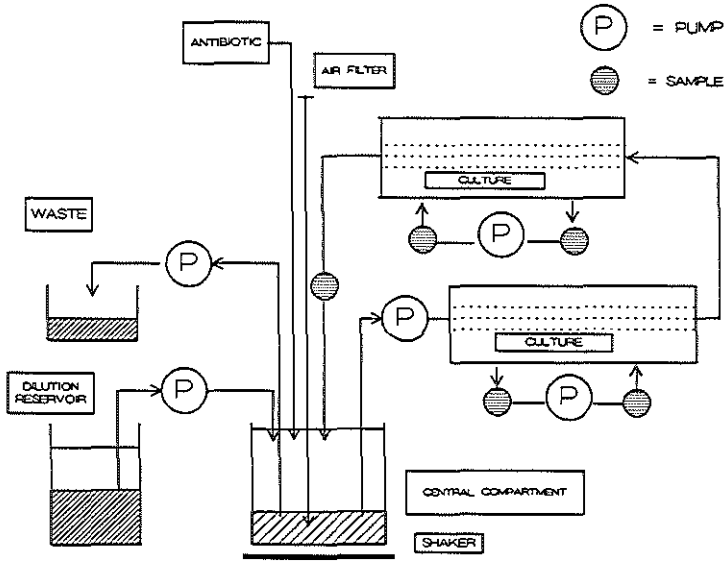

Figure 4. Schematic diagram of the two-compartment model. Antibiotic is added to the central compartment by bolus injection or by use of a pump. The volume of the central compartment is kept constant by setting the pumps from the dilution reservoir and to the waste reservoir at the same rate, yielding an exponential decline of the concentration of the antibiotic. The central and the peripheral compartment communicate through the artificial capillaries. Two peripheral compartments are shown here; in experiments up to four were placed in series.

METHODS

Model. A two-compartment model consisting of one central compartment and several peripheral compartments was designed to expose bacteria to changing antibiotic concentrations mimicking human pharmacokinetics. Figure 4 shows a schematic diagram of the model. Antibiotic was added to a central compartment containing broth. This central compartment was diluted from a diluent reservoir using a peristaltic pump (Biorad econo pump, Veenendaal, NL). Antibiotic containing broth was pumped from the central compartment to the elimination reservoir at the same pump rate, thus keeping the central compartment at a constant volume. The peripheral compartments consisted of disposable dialyser units (ST23, Baxter, Utrecht, NL) with a pore diameter of 2.8 nm. Up to four serially placed dialysers were used to allow simultaneous cultures to be studied. A peristaltic pump (Biorad econo pump, 17.5 ml/min) was used to circulate the culture in each unit. Inoculation and sampling was done through silicone injection points (BSM-V01 S, Hospal, Uden, NL) with sterile needles. Each peripheral unit contained 150 ml bacterial culture. The central compartment was pumped through the artificial capillaries of the dialyser unit by a peristaltic pump (Rhône Poulenc Medical 440, 125 ml/min). The central and peripheral compartments were placed on a shaking apparatus (125 rpm) to provide optimum dilution of the injected antibiotic and to accelerate bacterial growth in the peripheral compartments. Air was blown into the broth of the central compartment through an air filter. The complete system was placed in a room at 37°C ambient temperature.

Media. Mueller-Hinton Broth (Difco, A'dam, NL) supplemented with Ca^{2+} (22 mg/l) and Mg^{2+} (12 mg/l) was used in all experiments (MHB_s).

Strains. *P. aeruginosa* AT1 (ATCC 27853), *P. aeruginosa* CF4 (CF224) and *P. aeruginosa* CF16 (CF224M) were used. Strain CF4 was originally isolated from a cystic fibrosis patient. CF16 is a laboratory mutant of strain CF4. The MIC's for ceftazidime were 1, 4 and 16 mg/l for *P. aeruginosa* AT1, CF4 and CF16 respectively. Both CF strains showed the same growth characteristics as strain AT1 (see below).

Antibiotic. Ceftazidime was obtained from Glaxo (Zeist, NL). Stock solutions were prepared according to the guidelines of the National Committee for Clinical Labora-

tory Standards (NCCLS, 1990).

MIC and growth curves. Minimal Inhibiting Concentrations (MIC) were determined by both a standard agar dilution method and a microdilution method (NCCLS, 1990). Growth curves were made as follows: one colony from a fresh overnight culture on ISO-sensitest agar (Oxoid CM 471) was suspended in MHB_s and incubated overnight on a shaking apparatus (200 rpm) at 37°C. The inoculum was obtained by diluting this culture to approximately $5*10^5$ cfu/ml and incubation at 37°C while shaking. Samples of this log-culture were taken at t = 0, 1, 2, 4, 6 and 24 h and diluted serially tenfold in cold sterile saline on ice. 100 μl of each dilution was plated on ISO-sensitest agar and incubated overnight at 37°C. Growth curves were also determined in the model described above, only without injection of antibiotic. At t=0 each of the the peripheral compartments were filled with a log-culture of approximately $5*10^5$ cfu/ml. Samples were taken at t = 0, 1, 2, 4, 6, 24h for cfu/ml counts as described above.

Pharmacokinetic and killing curves. The in vitro activity of changing ceftazidime concentrations against three strains was tested in the model described above. For the intermittent infusion experiment at t=0 (immediately after the inoculation of the log-cultures into the peripheral compartments) ceftazidime solution (100 mg/l/dose) was injected in the central compartment. This was repeated after each 8th hour, continuing for 36 h. Samples were taken at t = 0, before and 10' and 30' after each dose and further at every hour until t=10 h, then every two hours until t=16 h, and at t=25 h, 28 h, 33 h and 36 h. For the continuous infusion experiment at t=0 an infusor (Braun, NL) was used providing the same daily dose of ceftazidime as during intermittent infusion (300 mg/l/24h). Every 12 h the ceftazidime solution in the infusor was replaced. Samples were taken at t = 0, 10', 30', 1 h every hour until t=10 h and then every 2 h until t=14 h, t=24, 26, 28, 30, 32 and 36 h. The samples used for viable counts were immediately washed (3*) with sterile saline on ice before the tenfold dilutions were made. Samples taken for ceftazidime assay were stored at -70°C. Samples were assayed for ceftazidime by high performance liquid chromatography (HPLC) as described earlier (Mouton *et al.*, 1990).

Other assays. Beta-lactamase production was determined qualitatively in the original samples from t=0, 8, 14, 24, 32 and 36 h with a standard iodometric method (Neu,

1980). Briefly, 100 µl sample was added to 100 µl freshly prepared penicillin G solution (6000 mg/l). Two drops of starch solution (1%) were added and incubated at room temperature for 30-60'. After addition of iodine reagent, disappearance of the then blue colored mix was considered positive.

The MIC's of the isolated strains at these time points and pH of the medium were also determined.

Analysis. The growth rate µ was determined during log-fase (Carlberg, 1986). Pharmacokinetic analysis of the time-concentration curves was done using the equations of Allen *et al.* (1982). Pharmacokinetic parameters were estimated using the SAS NLIN computer program package (SAS, 1990) using a one-compartment open model. The Area Under the time-Concentration Curve (AUC) was determined over the first 32 h using the log-linear trapezoidal rule.

RESULTS

Pharmacokinetic curves. A typical example of the pharmacokinetic curves obtained during intermittent and continuous infusion are shown in Figure 5. The curves were reproducible, and the pharmacokinetic parameters in each of the dialyser units were comparable (Table III). The top and trough concentrations during intermittent infusion as well as the half-lives did not differ significantly over the five doses (Table III). The AUC from 0 to 32 h was slightly higher during intermittent infusion (678.8 ± 117.8 mg.h/l) compared to continuous infusion (609.5 ± 13.4 mg.h/l). The mean concentration of ceftazidime was 19.8 ± 1.6 mg/l at the end of the fourth dosing interval (Table III).

Growth curves. The growth rates µ (± sd, means of four curves) of the strains AT1, CF4 and CF16 were 1.07 ± 0.27, 0.83 ± 0.28 and 1.01 ± 0.22 1/h respectively and not statistically significantly different from each other. The growth rates in the model were 1.18 ± 0.24, 1.03 ± 0.18 and 1.02 ± 0.24 1/h respectively.

Killing curves. Killing curves of the *P. aeruginosa* strains exposed to ceftazidime are shown in Figure 6. During intermittent infusion, bacteria were killed initially but started to regrow after 7h. After each dose, there was some killing or suspension of

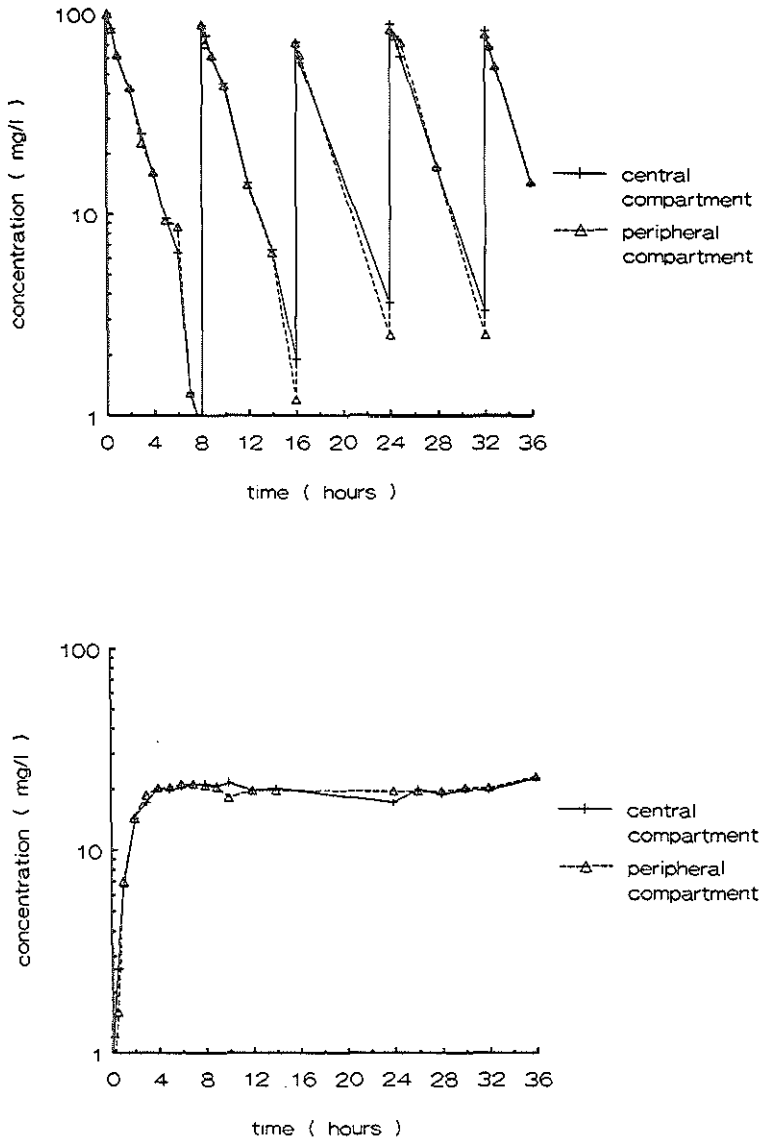

Figure 5. Typical example of time-concentration curves of ceftazidime during intermittent (above) and continuous (below) infusion (300 mg/l/24h).

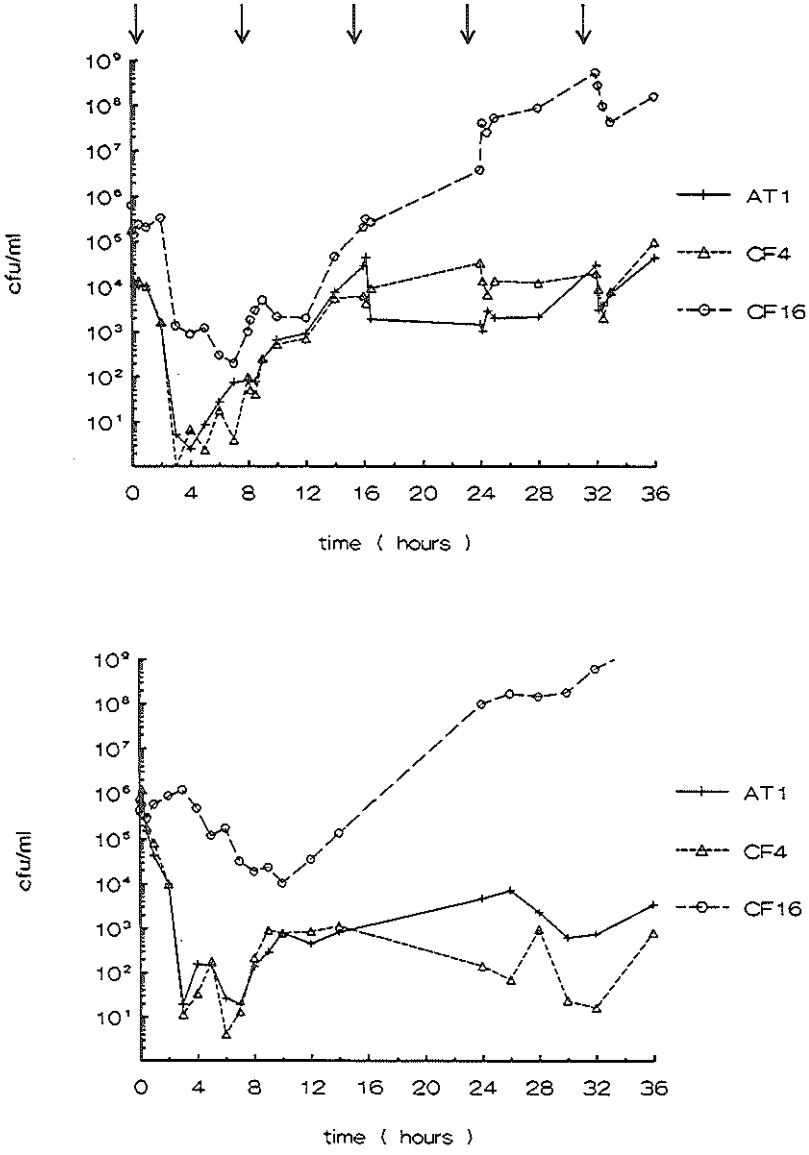

Figure 6. Killing curves of three strains of P. aeruginosa during intermittent (above) and continuous (below) infusion. Data shown are geometric means of at least two experiments.

Table III. Pharmacokinetic parameters during intermittent and continuous infusion for each of the five doses. All values are means ± sd of seven curves.

parameter	dose 1	2	3	4	5
trough (mg/l)	0	0.62±0.16	2.2±1.6	1.4±0.9	2.2±0.8
peak (mg/l)	96.3±7.8	102.3±14.8	84.5±9.8	92.3±8.0	86.9±7.1
$T_{1/2}$ (h)	1.50±0.23	1.49±0.23	-	1.67±0.32	1.56±0.17
mean concentration at end interval (continuous, mg/l)	21.1±2.0	19.8±1.1	19.7±2.3	19.8±1.6	21.2±1.9

growth, but then growth was resumed; this was most apparent for strain CF16. During continuous infusion, strains AT1 and CF4, with an MIC of 1 and 4 mg/l, respectively, were killed initially and then started to regrow for a few of hours, where upon the number of bacteria/ml remained constant during remainder of the experiment. After the first dosing interval of 8 h there was no difference between killing by continuous and intermittent infusion for strains AT1 and CF4. In contrast, after each subsequent dosing interval, there was an increasing difference between efficacy of intermittent and continuous infusion in favour of the latter, which was most marked after the last dosing interval, i.e. after 32h (Table IV). The difference was somewhat lower for strain AT1 than for strain CF4 due to the emergence of a resistant mutant in one experiment whereby the MIC of ceftazidime increased to 64 mg/l (see below). Strain CF16 was killed initially, but during continuous infusion numbers of bacteria were not reduced to the same extent as during intermittent infusion. Regrowth started at approximately the same time during both regimens and after 32 h the numbers of CFU

were equal (Table IV).

Emergence of resistent strains. To determine whether resistent strains emerged in the course of time, the MIC's of strains isolated during the experiments were determined. The MIC of the AT1 strain remained constant, except for one experiment during continuous infusion. The MIC of the strains isolated during the experiments with CF4 and CF16 increased during both continuous and intermittent infusion, and amounted to 128 mg/l after 24 hours. Thereafter no strains with higher MIC's could be isolated. Determination of beta-lactamase in the medium of the CF16 strain revealed that a detectable amount of beta-lactamase was present at the same time as isolation of the resistant strains, but not before. Beta-lactamase could also be detected after the emergence of the resistant AT1 strain during continuous infusion. In contrast, beta-lactamase could not be detected in the samples of the experiments with strain CF4, either during continuous or intermittent infusion.

pH measurements of the samples showed no deviating values.

Table IV. Changes in bacteria concentrations between 0 and 8 hours (after 1 dosing interval) and 0 and 32 hours (after 4 dosing intervals) of treatment with intermittent and continuous dosing regimens of ceftazidime.

Strain (MIC mg/l)	Interval (h)	Change in bacterial concentration*		
		Intermittent	Continuous	Difference
ATCC 27853	8	3.3	3.7	0.4
(1)	32	0.7	2.9	2.2
CF4	8	3.3	3.6	0.3
(4)	32	0.9	4.7	3.8
CF16	8	2.8	1.3	-1.5
(16)	32	-2.9	-3.2	-0.3

* Given as change in ^{10}log CFU/ml between t=0 h and t=32 h (geometric mean of at least 2 experiments)

DISCUSSION

Several models have been used to mimic plasmaconcentrations (see 10.1). The model developed here, based on diffusion, enables to study killing kinetics during continuous and intermittent dosing regimens. The model proved to adequately reproduce pharmacokinetic profiles.

In the present study, the model was used to determine whether continuous administration of ceftazidime is more efficacious than intermittent dosing using dosing regimens yielding concentrations comparable to those observed in vivo. The half-life chosen for these experiments was approximately 1.5 h. This is in accordance with values found in vivo (Tjandramagra *et al.*, 1982; De Klerk *et al.*, 1983; Mouton *et al.*, 1990). The daily dose chosen yielded concentrations comparable to those found in vivo. The killing curves of *P. aeruginosa* show that, during the time-course of the experiment, continuous infusion was more efficacious than intermittent infusion for the strains AT1 and CF4. Although a bolus dose exerted killing initially, the *P. aeruginosa* strains started to regrow after several hours. At each new dose growth was halted for some time, or some killing was achieved, but then the bacteria resumed growing. A similar observation was made for amoxicillin (Klaus *et al.*, 1981; Seeberg and Wiedeman, 1985) and ampicillin (Toothaker *et al.*, 1982; White *et al.*, 1989) on *E. coli*. After the start of continuous infusion bacteria were killed as well, but regrowth was substantially less or hardly present during continuous infusion compared to intermittent infusion. This is in agreement with the hypothesis and some observations that Gram-negatives exposed to beta-lactams resume growth when concentrations fall below a certain level (Craig and Ebert, 1991) and that high concentrations do not exert an additional effect as for instance for the quinolones (Blaser *et al.*, 1987).

The most consistent index of bactericidal activity found by White *et al.* (1989), when studying efficacy of ampicillin on *E. coli*, was the AUC and not the time above the MIC (T_{MIC}). The T_{MIC} did not covary strongly with the MIC, and the authors suggest that the high frequency of emergence of resistant strains may have masked the importance of the T_{MIC}. Since during continuous infusion the T_{MIC} was incessant when related to the strains AT1 and CF4 at the start of the experiments, and continuous

infusion was more efficacious than intermittent infusion, we conclude that the T_{MIC} is an important parameter. The suggestion of White *et al.* (1989) that their failure to find T_{MIC} to be an important parameter may have been caused by the high frequency of emergence of resistant strains may, therefore, be correct. Studying efficacy of beta-lactams in patients with pneumonia, Schentag (1991) also found the T_{MIC} to be important.

It has been argued that the the emergence of resistance, or the continued isolation of strains is possibly an artifact of in vitro systems due to the adherence of bacteria to the walls of the incubation flasks (Haag *et al.*, 1986). This observation was made in dilution models, and control experiments in these type of models can rule this out (Haag *et al.*, 1986; White *et al.*, 1989). In the diffusion model used in the present study, the importance of adherence of bacteria is not easily assessable. However, if adherence were a main determinant of the outcome of the studies, one might expect that there would have been no difference in the outcome of the dosage regimens.

To determine whether the MIC itself may be of influence on the interaction between bacteria and antibiotic, we examined three strains. The *P. aeruginosa* strains CF4 and CF16 chosen were fourfold and sixteenfold less susceptible for ceftazidime, respectively, than strain AT1, and the growth rates were comparable with each other. Although the concentration during continuous infusion was maintained at approximately 20 mg/l ceftazidime, which is higher than the MIC of strain CF16, this particular strain started to regrow after some time. This was due to the appearance of strains with MIC's up to 128 mg/l, a phenomenon that was also observed during intermittent infusion. Regrowth due to resistant subpopulations has also been observed in other studies with a comparable model. However, in these studies the bacteria were exposed to enoxacin and gentamicin, which belong to another group (Blaser *et al.*, 1987; Zinner and Blaser, 1985). Beta-lactamase, not detectable in the earlier samples, was present in the medium from approximately the same time onwards, possibly facilitating regrowth. It also indicates that the apparent resistance was probably due to the production of beta-lactamase by *P. aeruginosa*. The regrowth during continuous administration, despite continuous concentrations above the MIC, imply that these concentrations are not high enough to prevent regrowth.

In contrast to this observation for strain CF16 was the appearance of resistant strains of CF4 with a high MIC during both continuous as well as during intermittent infusion. These strains were highly resistant as determined by their MIC, yet appreciable growth did not occur. The absence of growth of the isolated mutants of CF4 is not easy to explain. It could be that, if the experiment had lasted longer, regrowth would have been observed, although this did not occur during the 12 hours that the strain was present. Presence of beta-lactamase could not be detected, suggesting that resistance was cell wall mediated in these cases.

The results of these experiments suggest that, when the antibiotic is the only agent present to exert efficacy, as is the case in neutropenic patients, a sustained level around or slightly above the MIC is not high enough. The strains exposed to a sustained concentration of 5 times the MIC or more were inhibited in their growth, indicating that a concentration 4 to 5 times the MIC are effective. When sustained concentrations higher than 5 times the MIC are employed, continuous administration in this model is more efficacious than intermittent dosing.

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REFERENCES

Al-Asadi MJS, Greenwood D and O'Grady F.: In vitro model simulating the form of exposure of bacteria to antimicrobial drugs encountered in infection. Antimicrob Agents Chemother 16:77-80, 1979

Allen L Kimura K, Macmihan J and Rittschel WA.: Manual of symbols equations and definitions in pharmacokinetics. J Clin Pharmacol 22(Suppl):1-23, 1982

Bauernfeind A, Jungwirth R and Petermuller C.: Simultaneous simulation of the serum profiles of two antibiotics and analysis of the combined effect against a culture of *Pseudomonas aeruginosa*. Chemotherapy 28:334-340, 1982

Bauernfeind A.: Bactericial activity of antibiotics alone and in combination against *Enterococcus faecalis* in a pharmacodynamic model. Clin Infect Dis 15:481-485, 1992

Bergan T, Carlsen IB and Fuglesang JE.: An in vitro model for monitoring bacterial responses to antibiotic agents under simulated in vivo conditions. Infection 8(Suppl 1):96-102, 1980

Bergan T and Carlsen IB.: Effect of antibiotics eliminated by first order kinetics. J Antimicrob Chemother 15(Suppl A):147-52, 1985

Blaser J.: In vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. J Antimicrob Chemother 15(Suppl A):125-130, 1985

Blaser J, Stone BB and Zinner SH.: Efficacy of intermittent versus continuous administration of netil micin in a two-compartment in vitro model. Antimicrob Agents Chemother 27: 343-49, 1985a

Blaser J, Stone BB and Zinner SH.: Two compartment kinetic model with multiple capillary units. J Antimicrob Chemother 15(Suppl A):131-137, 1985b

Blaser J, Stone BB, Groner MG and Zinner SH.: Impact of netilmicin regimens on the activities of ceftazidime-netilmicin combinations against *Pseudomonas aeruginosa* in an in vitro pharmacokinetic model. Antimicrob Agents Chemother 28:64-68, 1985

Blaser J, Stone BB, Groner MG and Zinner SH.: Comparative study with enoxacin and netilmicin in an in vitro pharmacokinetic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob Agents Chemother 31:1054-1060, 1987

Carlberg DM.: Determining the effects of antibiotics on bacterial growth by optical and electrical methods. In: Antibiotics in Laboratory Medicine (Lorian V ed) Williams and Wilkins London 64-92, 1986

Cars O and Craig WA.: General discussion on antibiotic dosing Scand J Infect Dis 74(Suppl):282-283, 1991

Craig WA and Ebert SC.: Killing and regrowth of bacteria in vitro: a review. Scand J Infect Dis 74(Suppl):63-70, 1991

Craig WA and Ebert SC.: Continuous infusion of beta-lactam antibiotics. Antimicrob Agents Chemother 36:2577-2583, 1992

De Klerk S, Walters, Ivan Wyck M, Harding SM, Paton, AM and Ayrton J.: Pharmacokinetics of ceftazidime in male and female volunteers. Antimicrob Agents Chemother 23:892-896, 1983

Drugeon HB, Maurisset B and Courtieu AL.: Bactéricidie des aminosides dans un système statique et dans un modéle dynamique. La Nouvelle Presse Médicale 8:3403-3407, 1979

Etta LL van, Peterson LR, Fasching CE and Gerding DN.: Effect of the ratio of surface area to volume on the penetration of antibiotics into extravascular spaces in an in vitro model. J Infect Dis 146:423-428, 1982

Firsov AA, Chernykh VM and Navashin SM.: Quantitative analysis of antimicrobial effect kinetics in an in vitro dynamic model. Antimicrob Agents Chemother 34:1312-1317, 1990

Gerber AU, Wiprächtiger P, Stettler-Spichiger U and Lebek G.: Constant infusions vs intermittent doses of gentamicin against *Pseudomonas aeruginosa* in vitro. J Infect Dis 145 554-560, 1982

Gerber AU, Craig WA, Brugger HP, Feller C, Vastola AP and Brandel J.: Impact of dosing intervals on activity of gentamicin and ticarcillin against *P. aeruginosa* in granulocytopenic mice. J Infect Dis 147: 910-917, 1983

Gerber AU, Brugger H, Feller C, Stritzko T and Stalder B.: Antibiotic therapy of infections due to *P. aeruginosa* in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J Infect Dis 153:90-97, 1986

Gerber AU, Stritzko T, Segessenmann C and Stalder B.: Simulation of human pharmacokinetic profiles in mice and impact on antimicrobial efficacy of netilmicin ticarcillin and ceftazidime in the peritonitis-septicaemia model. Scand J Infect Dis 74(Suppl):195-203, 1991

Grasso S, Meinardi G, Carneri I de and Tamassia V.: New in vitro model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. Antimicrob Agents Chemotherapy 13:570-576, 1978

Greenwood D and O'Grady F.: An in vitro model of the urinary bladder J Antimicrob Chemother 4:113-120

Greenwood D and Tupper H.: New in vitro device for examining the response of bacteria to changing drug concentrations In: Current Chemotherapy and Immunotherapy (Periti P and Grassi GG eds), pp 100-102, 1982

Guggenbichler JP, Semenitz E and König P.: Kill kinetics and regrowth pattern of bacteria exposed to antibiotic concentrations simulating those observed in vivo. J Antimicrob Chemother 15(Suppl A):139-146, 1985

Haag R, Lexa P and Werkhauser I.: Artifacts in dilution pharmacokinetic models caused by adherent bacteria. Antimicrob Agents Chemother 29:765-768, 1986

Haller I.: Combined actions of decreasing concentrations of azlocilin and sisomicin on *Pseudomonas aeruginosa* as assessed in a dynamic in vitro model. Infection 10(Suppl 3):229-233, 1982

Klaus U, Henninger W, Jacobi P and Wiedermann B.: Bacterial elimination and therapeutic effectiveness under different schedules of amoxicillin administration. Chemotherapy 27:200-208, 1981

Leitner F, Goodhines RA, Buck RE and Price KE.: Bacterial activity of cefadroxil cephalexin and cephradine in an in vitro pharmacokinetic model. J Antibiotics 17:718-726, 1979

Mordenti JJ, Quintiliani R and Nightingale CH.: Combination antibiotic therapy: comparison of constant infusion and intermittent bolus dosing in an experimental animal model. J Antimicrob Chemother 15(Suppl A):313-321, 1985

Mouton JW, Horrevorts AM, Mulder PHG, Prens EP and Michel MF.: Pharmacokinetics of ceftazidime in serum and suction blister fluid during continuous and intermittent infusion in healthy volunteers. Antimicrob Agents Chemother 34:2307-2311, 1990

Murakawa T, Sakamoto H, Hirose T and Nishida M.: New in vitro kinetic model for evaluating bac tericidal efficacy of antibiotics. Antimicrob Agents Chemother 18:377-381, 1980

National Committee for Clinical Laboratory Standards.: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically Approved Standard M7A2. National Committee for Clinical Laboratory Standards Villanova Pa USA, 1990

Navashin SM, Fomina IP, Firsov AA, Chernykh VM and Kuznetsova SM.: A dynamic model for in vitro evaluation of antimicrobial action by simulation of the pharmacokinetic profiles of antibiotics. J Antimicrob Chemother 23:389-399, 1989

Neu HC.: Antibiotic inactivating enzymes and bacterial resistance. In: Antibiotics in Laboratory Medicine (Lorian V ed) Williams and Wilkins London pp 454-473, 1980

Nishida N, Murakawa T, Kamimura T and Okada N.: Bactericidal activity of cephalosporins in an in vitro model simulating serum levels. Antimicrob Agents Chemother 14:6-12, 1978

O'Grady F and Pennington JH.: Bacterial growth in an in vitro system simulating conditions in the urinary bladder. British J Exp Pathol 47:152-57, 1966

Otoya H, Ozawa A and Goto J.: Mode of action of chemotherapeutic agents against bacteria growing in a continuous flow culture with special reference to the antibacterial effect of cephalothin, erythromycin and kanamycin. In Chemotherapy (Williams JD and Geddes AM eds) pp 205-210 Plenum Press New York, 1976

Reeves DS.: Advantages and disadvantages of an in vitro model with two compartments connected by a dialyser: results of experiments with ciprofloxacin. J Antimicrob Chemother 15(Suppl A):159-67, 1985

Roosendaal R, Bakker-Woudenberg IAJM, van den Berghe-Raffe MA *et al.*: Impact of the dosis schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in *Klebsiella Pneumoniae* pneumonia and septicaemia in leukopenic rats. Eur J Clin Microbiol Infect Dis 8:878-887, 1989

Rowe EL and Morozowich W.: A simple dilution analog computer for simulation of drug distribution processes. J Pharmaceut Sciences 58:1375-8, 1969

Sanfilippo A and Morvillo E.: An experimental model for the study of the antibacterial activity of the sulfonamides. Chemotherapy 13:54-60, 1968

SAS Institute Inc.: SAS Users guide. SAS Institute Inc., Cary NC, 1990

Schentag J.: Correlation between pharmacokinetic parameters to efficacy of antibiotics: relationships between serum concentrations MIC values and bacterial eradication in patients with Gram-negative pneumonia. Scand J Infect Dis 74(Suppl):218-234, 1991

Schneider P, Tosch W, Mauer M and Zak O.: Antibacterial effects of cefroxadine, cephalexin and cephradine in a new in vitro pharmacokinetic model. J Antibiotics 35:843-849, 1982

Seeberg AH and Wiedemann B.: Application of in vitro models: development of resistance. J Antimicrob Chemother 15(Suppl A):241-49, 1985

Shah PM.: An improved method to study antibacterial activity of antibiotics in an in-vitro model simulating serum levels. Exp Clin Pharmacol 4:171-176, 1980

Shah PM.: In vitro activity of cefotetan, other ß-lactams and netilmicin. J Antimicrob Chemother 11(Suppl A):73-78, 1983

Shah PM.: Activity of imipenem in an in vitro model simulating pharmacokinetic parameters in human blood. J Antimicrob Chemother 15(Suppl A):153-157, 1985

Silley P Monsey D and Harris AM.: Model simulation of a single oral dose of cefuroxime axetil and the related in vitro kill kinetics against four bacterial species. J Antimicrob Chemother 25:83-90, 1990

Sous H and Hirsch I.: Bactericidal activity of phenoxymethylpenicillin in an in vitro model simulating tissue kinetics. J Antimicrob Chemother 15(Suppl A):233-239, 1985

Tjandramaga TB, van Hecken A, Mullie R, Verbesselt PJ, de Schepper and Verbist L.: Comparative pharmacokinetics of ceftazidime and moxalactam. Antimicrob Agents Chemother 22:237-241, 1982

Toothaker RD, Welling PG and Craig WC.: An in vitro model for the study of antibacterial dosage regimen design. J Pharmaceut Sciences 71:861-864, 1982

Vergères P and Blaser J.: Amikacin ceftazidime and flucloxacillin against suspended and adherent *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* in an in vitro model of infection. J Infect Dis 165:281-289, 1992

Vogelman B and Craig WA.: Kinetics of antimicrobial activity. J Pediatr 108:835-840, 1986

White AR, Stokes DH, Slocombe B and Sutherland R.: Bactericidal effects of amoxy-cillin/clavulanic acid and ticarcillin/clavulanic acid in in vitro kinetic models. J Antimicrob Chemother 15(Suppl A): 227-232, 1985

White CA, Toothaker RD, Smith AL and Slattery JT.: Correction for bacterial loss in in vitro dilution models.Antimicrob Agents Chemother 31:1859-1860, 1987

White CA, Toothaker RD, Smith AL and Slattery JT.: In vitro evaluation of the determinants of bactericidal activity of ampicillin dosing regimens against *Escherichia coli*. Antimicrob Agents Chemotherapy 33:1046-1051, 1989

Wiedemann B and Seeberg AH.: The activity of cefotiam on ß-lactamase producing bacteria in an in vitro model. J Antimicrob Chemother 13:111-19, 1984

Zinner SH and Blaser J.: Efficacy of DNA-gyrase inhibitors in experimental animal studies and in pharmacokinetic in vitro models. Research Clin Forums 6:45-54, 1985

Zinner SH and Blaser J.: In vitro studies of antibiotic combinations with special emphasis on the evaluation of newly developed methods. J Antimicrob Chemother 17(Suppl A):1-5, 1986

Zinner SH, Blaser J, Stone BB and Groner MC.: Use of an in vitro kinetic model to study antibiotic combinations. J Antimicrob Chemother 15(Suppl A):221-226, 1985

Zinner SH, Dudley MN and Blaser J.: In vitro models in the study of combination antibiotic therapy in the neutropenic patient. Am J Med 80(Suppl 6B):156-160, 1986

Zinner SH Husson M and Klastersky J.: An artificial capillary in vitro kinetic model of antibiotic bactericidal activity. J Infect Dis 144:583-587, 1981

SUMMARY

SUMMARY

Pulmonary infections are still the major cause of morbidity and mortality in patients with cystic fibrosis (CF). Although the possibilities of treatment have greatly increased over the years, the decline in lung function, as a result of destruction of lung tissue by infections, ultimately leads to death at a relatively early age. The purpose of the studies presented in this thesis was threefold. In the first part (Chapters 2-5), the problems which emerge during antibacterial treatment in CF patients are discussed. The second part (Chapters 6-9) presents the results of several studies which were all based on the assumption that continuous infusion of beta-lactam antibiotics may lead to better treatment results compared to the usual intermittent dosing regimens. Studies in vitro and in animals, as well as a few clinical observations indicate that continuous infusion may be more efficacious than intermittent dosing. In the third part (Chapter 10), an in vitro pharmacokinetic model is described in which the pharmacodynamics of ceftazidime against *Pseudomonas aeruginosa* were studied during continuous and intermittent treatment regimens.

Chapter 2 reviews the difficulties which may be encountered in antimicrobial treatment of CF patients. Two problems, abnormal pharmacokinetics and emergence of resistance respectively, are discussed in more detail.

CF patients display abnormal pharmacokinetics as compared to the general population. It has been observed in numerous studies that the elimination of various drugs, including some antibiotics, is enhanced in CF patients. The cause of this phenomenon, however, is not well understood, and may differ for individual drugs. Several mechanisms may be responsible, including increased metabolization and increased renal clearance, either passive or active. In addition, the body composition, the proportion fatty mass/free fatty mass or an anomalous circulating volume (first compartment) in relation to the total extracellular volume can also play a role. So-called 'deep' compartments are either known to exist or are speculated upon for several drugs. Another factor is the large inter- and intra group variation within the CF patient population. This last issue has implications for dosing regimens and is considered in more detail in Chapter 3, where individual

monitoring of antimicrobial agents is discussed with emphasis on CF patients.

The second problem is the emergence of resistance against antimicrobial agents. In Chapter 4 the susceptibility to antibiotics of 52 *Staphylococcus aureus* isolates, isolated from 52 CF patients is described. Forty-nine of the 52 isolates produced beta-lactamases, but methicillin resistant strains of *S. aureus* were not found. In general, MIC's of the drugs tested were within the therapeutic range. Since frequent antimicrobial treatment could lead to the emergence of tolerant strains, this was also studied. The frequency of tolerant strains found (11.2%), however, was not different from the frequency observed for the general population.

In Chapter 5, the emergence of resistance among strains of *P. aeruginosa* isolated from 34 chronically colonized CF patients was investigated by comparing in each patient the susceptibility for antibiotics in pairs of strains isolated before 1987 or after 1990. Strains isolated from a further 19 patients who had become newly colonized served as controls. There was a marked increase in resistance between the 34 pairs of isolates during the study period, which was not explained by a general increase over the 3-year interval, since strains isolated before 1987 exhibited similar susceptibilities as those cultured from the control group. There was a strong correlation between this increase and both the number of days in hospital and the frequency of admissions. Pattern analysis of the emergence of resistance indicated four separate groups comprising the fluoroquinolones except ofloxacin, the aminoglycosides, the ureidopenicillins together with aztreonam, and the carbapenems, cephalosporins and carboxypenicillins as well as ofloxacin. It was concluded that long-term antimicrobial therapy is associated with the development of resistance within the individual patient.

The problems encountered when treating CF patients with antibiotics, i.e. abnormal pharmacokinetics and emergence of resistance, infer that optimization of therapy is necessary. Except for the use of new antimicrobial agents, the employment of existing antibiotics can be improved. It is known, that killing of aerobic Gram-negatives by beta-lactams continues in time and is relatively concentration independent. Continuous infusion of beta-lactams could, therefore, be more efficacious than the usual dosing regimen of three times daily when treating pulmonary exacerbations due to *P. aeruginosa* in CF patients. In the latter regimen, the high concentrations reached immediately after

infusion do not particularly contribute to efficacy, while after several hours concentrations have fallen low enough to permit regrowth of the bacteria. Chapters 6-10 describe the pharmacokinetics of two beta-lactams during continuous and intermittent infusion in a cross-over setting. The studies were performed in volunteers, CF patients and in vitro. The beta-lactams ceftazidime and meropenem, a new carbapenem, were studied in volunteers. Apart from serum concentrations, the penetration in blister fluid was also analyzed. Blisters were obtained by applying negative pressure. It appeared that the concentration profile in the blisters was concurrent with that in serum, and that penetration was fast, although the concentrations in blister fluid were somewhat lower for both antibiotics. In the ceftazidime study, the effect of the age of the blister on the concentration profile was determined. This was done by taking samples from older blisters and comparing these concentrations with those obtained in younger blisters. It appeared that the concentration in suction blister fluid is dependent on the age of the blister, and that this factor should be taken into account when drawing conclusions from data obtained from suction blister fluid samples. During continuous infusion, there was no difference between concentrations found in older and younger blisters.

The pharmacokinetics of ceftazidime during continuous and intermittent infusion was also studied in CF patients. The area under the time-concentration curve, total body clearance (TBC) and urinary recovery were not significantly different between the two treatment regimens, but these parameters showed a large variation not only between patients, but also within the same patient. The results were also compared with those observed in volunteers. During intermittent infusion, the half-lives were not significantly different, but the TBC was significantly higher and the volume of distribution was significantly larger in the CF patients. During continuous administration, the serum concentrations were relatively lower in the CF patients, even if the higher lean body mass per kg bodyweight in the CF patients is taken into account. This could not be explained by a higher urinary clearance, since the urinary recovery was lower in the CF patients than in the volunteers. A comparison of the results of continuous and intermittent infusion showed that differences in the pharmacokinetic parameters between volunteers and CF patients can be explained in part by a difference in volume of distribution.

During the studies described above, it was noted that serum concentrations of both cef-

tazidime and meropenem were significantly higher in the morning than in the evening; this circadian rhythm is described in Chapter 9.

In Chapter 10, a model is described to simulate concentration profiles of antibiotics in vitro. The model was developed to study killing of *P. aeruginosa* during continuous and intermittent dosing in a dynamic situation. The results show that the model is useful to simulate in vivo concentration profiles, and that the model can be used to study bacterial killing in vitro. The efficacy of continuous and intermittent infusion (q8h) of ceftazidime with a total daily dose of 300 mg/l/24h, mimicking human serum concentrations, was studied over a period of 36 hours. Three *P. aeruginosa* strains, ATCC 27853, CF224 and CF224M were studied, with MIC's of 1, 4 and 16 mg/l, respectively. The results showed that the efficacy of continuous infusion was higher for the strains with an MIC of 1 and 4 mg/l. After the fourth dosing interval, i.e. after 32 hours, there was a marked difference between bacterial counts during continuous and intermittent infusion for these strains, 2.2 and 3.8 ^{10}log respectively. During both regimens, the strain with an MIC of 16 mg/l started to regrow after approximately 10 hours. This was due to the emergence of resistant strains with a higher MIC. Measurement of beta-lactamase in the medium revealed that, in most cases, this could be attributed to the emergence of beta-lactamase producing strains. However, beta-lactamase could not be detected during emergence of resistant subpopulations of strain CF224.

The conclusion of the experiments was that, in the absence of other factors, a sustained level of ceftazidime around or slightly above the MIC is not high enough to maintain efficacy over more than one (8h) dosing interval, and should probably be around 4 to 5 times the MIC. When sustained concentrations higher than 4 times the MIC are employed, continuous administration in this model is more efficacious than intermittent dosing.

DIRECTIONS FOR FUTURE RESEARCH.

The main problem when treating CF patients with antimicrobial agents, namely the emergence of resistant strains, is difficult to solve. Such is nature. However, the problem may be avoided, or at least the risk minimized by using antibiotics in a rational manner,

with care and only when required. The question that immediately rises is: when is the use of antimicrobial therapy really necessary? It cannot be denied that a pulmonary exacerbation needs antimicrobial treatment, and there are several parameters indicating when its use is mandatory. However, as noted in Chapter 2, during recent years new approaches to antimicrobial treatment are being used, such as intravenous treatment at home, and spraying with aerosolized antibiotics. In addition, antibiotics effective against *P. aeruginosa* which can be taken orally have become available. Furthermore, new ideas to prevent colonization with *P. aeruginosa* are becoming accepted. Although these approaches are probably effective on the short term few, if any, studies have been performed to determine the long-term effects of these modes of treatment. It is necessary, therefore, to initiate such studies, or at least monitor emergence of resistance in such a way that conclusions regarding the importance of emerging resistance during non-hospitalized antibiotic treatment can be made.

As was indicated in the Introduction, optimization of existing therapy is one way of expanding therapeutic possibilities. In the studies presented in this thesis, several were based on the assumption that continuous infusion of beta-lactams may be more efficacious than intermittent infusion. Although the studies in human volunteers showed that penetration of beta-lactams in blister (interstitial) fluid is fast and concentrations only slightly less than in serum, the concentration of antibiotics at the site of the *P. aeruginosa* infection is not known and might be lower. Also, it is not known what the influence of continuous or intermittent infusion would be at the site of those infections. If concentrations at the site of infection are dependent on factors other than diffusion alone, for instance presence of beta-lactamases, it could be that intermittent infusion leads to higher concentrations near the micro-organism, albeit for only a short period of time. Alternatively, a combination of continuous and intermittent infusion could be beneficial. Methods developed recently to study the site concentrations of antibiotics (Honeybourne and Baldwin, 1992) could help to shed more light on this issue.
The results of the experiments in the in vitro model showed that continuous infusion was more efficacious than intermittent infusion for strains with an MIC 4 to 5 times lower than the concentration maintained during continuous infusion. However, the strain with

an MIC of 16 mg/l started to regrow after 10 hours even though the concentration was maintained above 16 mg/l. This was due to the evolvement of resistant strains. However, the strain with an MIC of 4 mg/l originally also showed increasing resistance, but did not grow. The reasons for this are not yet clear and elucidating this mechanism could help to understand what happens in vivo. For instance, from sputa of CF patients, resistant strains can often be isolated without apparent problems for the patient at the time of isolation. Although the immune system and other factors play a role here as well, comparable mechanisms as just described in vitro may be similarly important. In this context it is interesting that *P. aeruginosa* strains isolated from CF patients often not only grow slower than control strains, but also often do not grow beyond 4 or 5 ^{10}log cfu/ml. It could be that factors in the medium, whether absent or present, inhibit growth. Alternatively, the cost for the bacterium to maintain its resistance could be too high to permit a higher growth rate.

The model which was developed is suitable to study the effect of antibiotic combinations. For instance, the efficacy of antibiotics with different half-lives can be studied. It would also be interesting to study the efficacy of continuous or intermittent infusion with a beta-lactam and an aminoglycoside, or a quinolone. Still another option would be to study the timing of dosing when using two antimicrobial agents. There are some reports suggesting that the efficacy of a beta-lactam in combination with an aminoglycoside can be improved by optimization of the time period between the dosing of the two agents.

Reports on the efficacy of continuous infusion in humans compared to intermittent administration are still scarce. Only two randomized clinical trials have compared the efficacy of beta-lactams during these two dosing regimens , but definite conclusions from these trials could not be made (Bodey *et al.*, 1979; Lagast *et al.*, 1983). In another study performed in humans, it was shown that the time above the MIC of the micro-organism to combat is a major determinant in the outcome of treatment (Schentag, 1991). Further clinical trials are needed to demonstrate the validity of continuous treatment with beta-lactams. It may then appear that equal efficacy of continuous and intermittent infusion may be obtained with relatively lower doses during continuous infusion.

REFERENCES

Bodey GP, Ketchel SJ and Rodriguez V.: A randomized study of carbenicillin plus cefamandole or tobramycin in the treatment of febrile episodes in cancer patients. Am J Med 67:608-616, 1979

Honeybourne D and Baldwin DR.: The site concentrations of antimicrobial agents in the lung. J Antimicrob Chemother 30:249-260, 1992

Lagast H, Meunier-Carpentier F and Klasterski J.: Treatment of Gram-negative bacillary septicaemia with cefoperazone. Eur J Clin Microbiol 2:554-558, 1983

Schentag J.: Correlation between pharmacokinetic parameters to efficacy of antibiotics: relationships between serum concentrations MIC values and bacterial eradication in patients with Gram-negative pneumonia. Scand J Infect Dis 74(Suppl):218-234, 1991

SAMENVATTING

Longinfecties vormen nog steeds de belangrijkste oorzaak van morbiditeit en mortaliteit bij patienten met cystische fibrose (CF). Ondanks het feit dat de behandelingsmogelijkheden in de loop der jaren enorm zijn toegenomen, leidt de achteruitgang van de longfunctie, als gevolg van destructie van het longweefsel door infecties, uiteindelijk tot een vroegtijdige dood. Het doel van de studies zoals die beschreven staan in dit proefschrift, is drieledig. In het eerste deel (hoofdstukken 2-5) wordt een overzicht gegeven van de infectie- en behandelingsproblematiek die een rol spelen bij de behandeling van de longinfecties van CF patienten. In het tweede deel (hoofdstukken 6-9) worden een aantal studies beschreven, die als uitgangspunt hadden dat, gezien de noodzaak tot optimale antimicrobiele therapie bij CF patienten, continue infusie van beta-lactams wellicht een beter effect zouden kunnen hebben dan de tot op heden gebruikelijke drie- of vierdaagse dosering. In vitro en dierexperimentele studies, alsmede enkele anecdotische therapeutische resultaten bij patiënten leken daarop te wijzen. In het laatste deel, hoofdstuk 10, wordt een in vitro farmacokinetisch model beschreven, waarin de farmacodynamiek van ceftazidime ten opzichte van *Pseudomonas aeruginosa* werd bestudeerd gedurende continue en intermitterende infusie.

In hoofdstuk 2 wordt een algemeen overzicht gegeven van de behandeling van longinfecties van CF patienten. Verschillende problemen komen hier aan de orde en worden ter discussie gesteld. Twee vraagstukken, afwijkende farmacokinetiek en ontwikkeling van resistentie, worden nader toegelicht en deze vormen ook de basis van de drie daaropvolgende hoofdstukken.
De farmacokinetiek bij CF patienten is afwijkend ten opzichte van de algemene populatie. Uit vele studies is gebleken dat CF patienten een versnelde eliminatie vertonen van een aantal farmaca, waaronder verschillende antibiotica. Waaraan dit precies moet worden toegeschreven is niet goed bekend. Het zou zowel een verhoogde metabolisatie kunnen betreffen, als een verhoogde renale klaring, zowel passief als actief. Een afwijkende lichaamssamenstelling ten opzichte van niet CF patienten zou eveneens een rol kunnen spelen. Zowel een andere verhouding vet/vet vrije massa, als een andere ver-

houding circulerend volume (eerste compartiment)/resterend volume (tweede compartiment) kunnen hierbij van belang zijn; ook zijn van een aantal middelen zogenaamde diepe compartimenten bekend of er wordt over gespeculeerd. Binnen de groep CF patienten bestaat er wat dat betreft ook een grote variatie. In hoofdstuk 3 wordt dit nog eens nader beschouwd, waarbij tevens wordt ingegaan op de noodzaak tot 'individual monitoring' van de CF patiënt, juist vanwege de grote individuele spreiding.

Het tweede vraagstuk is de ontwikkeling van resistentie tegen antibiotica. In hoofdstuk 4 wordt de gevoeligheid voor een aantal antibiotica van 52 *Staphylococcus aureus* stammen beschreven. Deze werden geisoleerd uit sputa van 52 CF patienten. Negenenveertig vand de 52 isolaten produceerden beta-lactamase, maar methicilline resistente stammen werden niet gevonden. De MRC's van de geteste antibiotica lagen in het algemeen binnen de therapeutische grenzen. Tevens wordt hier bekeken of tolerantie een rol speelt bij CF patienten. Omdat frequente antimicrobiele therapie zou kunnen leiden tot een verhoogde frequentie van voorkomen van tolerante stammen, werd dit ook bestudeerd. De frequentie van tolerante stammen die gevonden werd (11.2%) was echter niet afwijkend ten opzichte van bevindingen bij de algemene populatie.

In het daaropvolgende hoofdstuk 5 worden de resultaten beschreven van een studie waarin het toenemen van isolatie van resistente *P. aeruginosa* stammen bestudeerd werd. Van 34 CF patienten werden van elke patiënt gepaarde *P.aeruginosa* isolaten, geïsoleerd voor 1987 of na 1990, uit het sputum met elkaar vergeleken door ze te typeren en de gevoeligheid voor een aantal antibiotica te testen. De tussenliggende periode tussen de isolaten binnen elk paar was minimaal 3 jaar. Als controle groep fungeerde een groep van 19 patienten die pas recent waren gekoloniseerd met *P. aeruginosa*. Het bleek dat de resistentie van de stammen binnen elk van de 34 paren over de jaren sterk was toegenomen. Dit kon niet verklaard kon worden door een algemene toename in resistentie, omdat de stammen van de controle groep in het algemeen eenzelfde resistentie patroon lieten zien als de 34 stammen geïsoleerd voor 1987. Er was een goede correlatie tussen de toename in resistentie en het aantal opnames in het ziekenhuis. Deze laatste kan als maat worden gezien van het aantal malen dat intraveneuze therapie aan de patiënt gegeven is. Patroon analyse van de toename in resistentie liet vier groepen zien, bestaande uit de fluoroquinolonen behalve ofloxacin, de aminoglycosiden, de ureidopeni-

cillinen samen met aztreonam en tenslotte de carbapenems, cefalosporines, carboxypenicillines en ofloxacin.

Naar aanleiding van bovengeschetste problematiek, i.e. een afwijkende farmacokinetiek bij CF patienten en een toename van resistentie van de bacterien, is het duidelijk dat een optimale antimicrobiele therapie veelal noodzakelijk is. Behalve de toepassing van nieuwe middelen kan tevens gezocht worden naar de meest optimale toedieningsvorm van een antibioticum. Van beta-lactams is bekend dat de doding van Gram-negatieven door deze middelen doorgaat in de tijd en relatief concentratie onafhankelijk is, mits boven een bepaald minimum. Het zou daarom zinvol kunnen zijn beta-lactams in een continu infuus toe te dienen, in tegenstelling tot de gebruikelijke drie daagse dosering, waarbij hoge topspiegels geen extra effect bewerkstelligen, en anderzijds de spiegel na een aantal uren zodanig is gedaald dat geen effect meer optreedt. De studies in de overige hoofdstukken beschrijven de farmacokinetiek van enkele beta-lactams gedurende continue en intermitterende toediening bij de zelfde dagdosis in een cross-over setting. De studies werden uitgevoerd bij vrijwilligers, CF patienten en in vitro.

Bij vrijwilligers werden de beta-lactams ceftazidime en meropenem bestudeerd, waarvan de resultaten beschreven staan in hoofdstuk 6 en 7. Meropenem, een carbapenem, is een nieuw antibioticum met een goede antipseudomonale activiteit. Om tevens een indruk te verkrijgen van bereikte weefselconcentraties van deze middelen, werden blaren opgewekt door middel van negatieve druk, en de concentraties in de blaarvloeistof gemeten. Het bleek dat het concentratie verloop in de blaarvloeistof goed overeenkwam met die in het bloed, en er een snel evenwicht ontstaat. Zowel bij ceftazidime als bij meropenem werden iets lagere spiegels in de blaren aangetroffen. In de ceftazidime studie werd tevens gekeken of de ouderdom van de blaar van invloed was op de gemeten spiegels. Dit werd gedaan door van oudere blaren monsters te trekken en de concentraties daarin te vergelijken met die van jongere blaren. Het bleek dat de ouderdom van de blaar inderdaad van invloed is op de gemeten concentratie bij intermitterende toediening. De conclusie was, dat dit soort blaren, als ze een indruk willen geven van de concentratie in het weefsel, steeds nieuw getrokken moeten worden. Bij continue infusie werd geen verschil in concentratie aangetoond tussen oude en jongere blaren.

De farmacokinetiek van ceftazidime gedurende continue en intermitterende infusie werd

tevens bestudeerd in CF patienten (hoofdstuk 8). De oppervlakte onder de tijd concentratie curve, de totale lichaamsklaring (TBC) en terugvondst in urine waren niet significant verschillend tussen de twee behandelingsschema's, maar er was een grote intra-individuele als ook inter-individuele spreiding. De resultaten werden ook vergeleken met die van de vrijwilligers. Er was geen kortere halfwaardetijd, maar zowel het verdelingsvolume als de klaring waren signifikant hoger bij de CF patienten tijdens intermitterende toediening. Gedurende continue toediening waren de serum concentraties relatief lager in de CF patienten, ook als de relatief hogere verhouding Lean Body Mass/kg lichaamsgewicht daar in betrokken werd. Een vergelijking tussen continue en intermitterende infusie liet zien, dat een deel van gevonden verschillen van farmacokinetische parameters tijdens intermitterende infusie bij vrijwilligers en CF patienten verklaard kan worden door een verschil in verdelingsvolume.

In hoofdstuk 9 worden enkele bevindingen beschreven naar aanleiding van de hiervoor beschreven studies. Er werd gesignaleerd dat zowel de ceftazidime als de meropenem serum concentraties 's ochtends relatief hoger waren dan 's avonds. Hoewel het hier een beschrijving van een fenomeen gaat, kan geen uitspraak worden gedaan over de oorzaak van dit circadiaanse ritme.

Werden in het voorgaande continue en intermitterende infusie in vivo bestudeerd, in hoofdstuk 10 wordt een model beschreven waarmee het mogelijk is om het spiegelverloop van intermitterende en continue infusie in vitro na te bootsen. Het model werd opgezet met de bedoeling om de doding van bacterien te kunnen bestuderen onder een dynamisch verlopende spiegel, in tegenstelling tot de statische spiegel zoals die gehanteerd wordt in MRC bepalingen en killing-curves. Met het uiteindelijke model konden spiegelverlopen goed reproduceerbaar nagebootst worden. Het model werd vervolgens gebruikt om de doding van verschillende *P. aeruginosa* stammen te bestuderen tijdens continue en intermitterende infusie (q8h) van ceftazidime gedurende 36 uur in een dosering van 300 mg/l/24h. Dit resulteerde in een concentratie verloop van ceftazidime dat vergelijkbaar was met dat wat met adequate therapie gemeten wordt. De bestudeerde *P. aeruginosa* stammen ATCC 27853, CF224 and CF224M hadden een MRC voor ceftazidime van respectievelijk 1, 4 en 16 mg/l. Het bleek, dat de doding van *P. aeruginosa* tijdens continue infusie effectiever was dan bij intermitterende infusie voor de stammen

met een MRC van 1 en 4 mg/l bij dezelfde dagdosis. Het verschil na vier doseringen, i.e. na 32 uur, bedroeg uitgedrukt in verschil in concentraties bacterien respectievelijk 2.2 en 3.8 ^{10}log. De stam met een MRC van 16 mg/l hergroeide echter na 10 uur gedurende beide regimes. Dit bleek in beide regimes om een beta-lactamase vormende mutant te gaan. Beta-lactamase kon echter niet aangetoond worden gedurende het optreden van resistente sub-populaties van stam CF224. De uiteindelijke conclusie was, dat een continue spiegel ter hoogte of iets boven de MRC in dit model niet voldoende was, en een effectieve spiegel ongeveer een factor 4 tot 5 daar boven ligt. De resultaten met dit model toonden ook dat een of twee doserings intervallen te kort is om een effect goed te beoordelen.

LIST OF PUBLICATIONS

Schaik van CP, Amerongen van A and Mouton JW. The organisation of range use in Sumatran long-tailed macaques. In Schaik, CP van: The socioecology of Sumatran long-tailed macaques (Macaca fascicularis). 23-65, 1985

Mouton JW and Kerrebijn KF. Antibacterial therapy in cystic fibrosis. Med Clin North Am 74:837-850, 1990

Daal van GJ, Jong de PT, Tenbrinck R, Mouton JW, Petzoldt K, Bergmann KC and Lachmann B. Oral immunization with bacterial lysate against infection with *Streptococcus pneumoniae* in mice. Respiration 57:229-232, 1990.

Mouton JW, Horrevorts AM, Mulder PGH, Prens EP and Michel MF. Pharmacokinetics of ceftazidime in serum and suction blister fluid during continuous and intermittent infusion in healthy volunteers. Antimicrob Agents Chemother 34:2307-2311, 1990

Daal van GJ, So KL, Mouton JW, Veen van 't A, Tenbrinck R, Bergmann KC and Lachmann B. Oral immunization with a polyvalent bacterial lysate can reduce mortality by infection with *S. pneumoniae* or Influenza A in mice. Pneumologie 44:1180-1182, 1990

Kerrebijn KF and Mouton JW. Pulmonary infection and antibiotic treatment in patients with cystic fibrosis. Acta Univ Carol 36:28-33, 1990

Daal van GJ, Beusenberg FD, So KL, Fievez RBAM, Sprenger MJW, Mouton JW, Veen van't A and Lachmann B. Protection against influenza A virus infection in mice by oral immunization with a bacterial lysate. Int J Immunopharmac 13:831-840, 1991

Horrevorts AM and Mouton JW. Susceptibility to various antimicrobial agents and tolerance to methicillin of *Staphylococcus aureus* isolates from cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 10:785-786, 1991

Kluytmans JAJW, Niesters HGM, Mouton JW, Quint WGV, Ijpelaar JAJ, Rijsoort-Vos JH, Habbema L, Stolz E, Michel MF and Wagenvoort JHT. Performance of a non-isotopic DNA-probe for detection of *Chlamydia trachomatis* in urogenital specimens. J Clin Microbiol 29:2685-2689, 1991

Mouton JW and Michel MF. Pharmacokinetics of meropenem in serum and suction blister fluid during continuous and intermittent infusion. J Antimicrob Chemother 28:911-918, 1991

Horrevorts AM, Mouton JW, Driessen OMJ and Kerrebijn KF. Pharmacokinetische en pharmacodynamische studies bij patienten en vrijwilligers. In: Ontwikkelingen in de behandeling van infectieziekten (Bakker-Woudenberg IAJM, Horrevorts AM eds.), 34-51, 1991

Daal van GJ, Veen van 't A, So KL, Mouton JW, Smit F, Hal van PthW, Bergmann KC and Lachmann B. Oral immunization with polyvalentlysate and infection with *S. pneumoniae*: influence on interferon-gamma and PMN-elastase concentrations in murine bronchoalveolar lavage fluid. Int Arch Allergy Immunol 97:173-177, 1992

Mouton JW and Michel MF. Meropenem pharmacokinetics in serum and suction blister fluid during continuous and intermittent infusion. In: Proceedings ICC "Recent advances in chemotherapy". (Lode H, Adam D eds.) Futuramed verlag, Munchen, 1066-1067, 1992

Mouton JW, Horrevorts AM and Michel MF. Circadian rhythm of ceftazidime and meropenem serum levels during continuous and intermittent infusion. In: Proceedings ICC "Recent advances in chemotherapy". (Lode H, Adam D eds.) Futuramed verlag, Munchen, 1016-1017, 1992

Teunenbroek van A, Muinck Keizer-Schrama de SMPF, Stijnen T, Mouton JW, Blum WF, Mercado M, Baumann G, Drop SLS and the Dutch Working Group on Growth Hormone. Effect of growth hormone administration frequency on 24h growth hormone profiles and levels of other growth related parameters in girls with Turner's syndrome. Clin Endocrinol 39:77-84, 1993.

Horrevorts AM and Mouton JW. Abnormal pharmacokinetics: the need for monitoring. Eur J Clin Microbiol Infect Dis 28(Suppl):58-60, 1993

Vos MC, Vincent HH, Yzerman EPF, Stamkott A, Vogel M and Mouton JW. Drug clearance by continuos hemodiafiltration (CAVHD). II. Results with the AN-69 capillary hemofilter and recommended dose adjustments. submitted.

Veeze HJ, Mouton JW, Halley DJJ, Overbeek SE, Jongste de J and Sinaasappel M. Clinical variability in patients with cystic fibrosis who are non-homozygous for the most common CF mutation. submitted.

Mouton JW, den Hollander JG and Horrevorts AM. Emergence of resistance among *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. J Antimicrob Chemother 31:919-926, 1993

Mouton JW, Horrevorts AM, Overbeek SE, Kerrrebijn KF and Michel MF. Pharmacokinetics of ceftazidime during continuous and intermittent infusion in adult cystic fibrosis patients. submitted.

Mouton JW, Horrevorts AM and Michel MF. Circadian variation in serum concentrations of ceftazidime and meropenem. submitted.

Eijking EP, Gommers D, So KL, Maat de MPM, Mouton JW and Lachmann B. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. Anesth Analg 76:472-477, 1993

Bakker-Woudenberg IAJM, Lokerse AF, Kate ten MT, Mouton JW, Woodle MC and Storm G. Liposomes with prolonged blood circulation and selective localization in *Klebsiella pneumoniae* infected lung tissue. J Infect Dis 168:164-171, 1993.

Bakker W, Vinks AATMM, Mouton JW, de Jonge P, Verzijl JG en Heijerman HGM. Intraveneuze thuis-behandeling bij patienten met cystic fibrosis: continue infusie van ceftazidim via een draagbare pomp. geaccepteerd.

Mouton JW en Vinks AATMM. De antimicrobiële behandeling met beta-lactam antibiotica: is continue infusie zinvol? geaccepteerd.

Kluytmans JAJW, Goessens WHF, Mouton JW, Rijsoort-Vos JH, Niesters HGM, Quint WGV, Habbema L, Stolz E and Wagenvoort JHT. Evaluation of the clearview chlamydia test, the magic lite chlamydia test, a polymerase chain reaction and cell culture for detection of *Chlamydia trachomatis* in urogenital specimens. submitted.

Veen van 't A, Ruyter de HA, Mouton JW, Hartleb M and Lachmann B. Pretreatment with spleenextract enhances survival in influenza A infected mice. submitted.

Goessens WHF, Deventer van AJM, Zeijl van JH, Mouton JW and Michel MF. Discriminative antibody and antigen reactions in immunocompromised patients with systemic candidiasis. submitted.

Mouton JW and Hollander den JG. Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. submitted.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 3 november 1956 te Leiden. In 1975 werd het het diploma Atheneum B behaald aan de Thorbecke Scholengemeenschap te Utrecht. In dat jaar begon hij de studie Biologie aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen biologie B5" (medische biologie) werd in 1979 behaald. In datzelfde jaar werd tevens aangevangen met de studie Geneeskunde, eveneens aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen Geneeskunde werd behaald in 1981. In 1983 werd het doctoraalexamen biologie behaald, met als hoofdvakken microbiële genetica (Prof. dr. G.A. van Arkel en dr. C. van den Hondel), ethologie (Prof. dr. J.A.R.A.M. van Hooff en dr. C.P. van Schaik) en bijvak algemene dierkunde (Prof. dr. F.C.G. van de Veerdonk en dr. P.N.E. de Graan). In het kader van de ethologie werd een jaar doorgebracht in het Ketambe Research Station, Gunung Leuser Reservaat in Indonesie.
Het doctoraalexamen Geneeskunde werd behaald in 1986 en het artsexamen in 1988. In april 1988 trad hij als AIO in dienst van de Erasmus Universiteit te Rotterdam, alwaar een aanvang werd gemaakt met de in dit proefschrift beschreven studies onder leiding van Prof. dr. M.F. Michel, Prof. dr. K.F. Kerrebijn en dr. A.M. Horrevorts. In april 1990 werd de opleiding tot Medisch Microbioloog aangevangen aan het Dijkzigt ziekenhuis te Rotterdam.