THERAPEUTIC EFFECT OF ANTIBIOTICS IN THE COMPROMISED HOST

AN EXPERIMENTAL STUDY

THERAPEUTISCH EFFECT VAN ANTIBIOTICA IN DE GASTHEER MET VERMINDERDE WEERSTAND

EEN EXPERIMENTELE STUDIE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. A.H.G. RINNOOY KAN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 14 DECEMBER 1988 OM 15.45 UUR

DOOR

ROBERT ROOSENDAAL

GEBOREN TE ROERMOND

Gedrukt bij
Offsetdrukkerij Kanters B.V., Alblasserdam
1988

PROMOTIECOMMISSIE:

Promotor:	Prof. Dr. M.F. Michel
Overige leden:	Prof. Dr. J.W.M. van der Meer
	Prof. Dr. H.J. Neijens
	Prof. Dr. D. van der Waaij
Co-promotor:	Mw. Dr. I.A.J.M. Bakker-Woudenberg

CONTENTS

Other papers related to this thesis

Roosendaal R, Bakker-Woudenberg IAJM, van den Berghe-van Raffe M, Vink-van den Berg JC, Michel MF. Influence of dose frequency on the therapeutic efficacies of ciprofloxacin and ceftazidime in experimental Klebsiella pneumoniae pneumonia and septicemia in relation to their bactericidal activities in vitro.
Pharm Weekbl [Sci] 1987;**9** Suppl:S33-S40

Bakker-Woudenberg IAJM, Roosendaal R. Impact of dosage regimens on the efficacy of antibiotics in the compromised host.
J Antimicrob Chemother 1988;21:145-7

CHAPTER 1

GENERAL INTRODUCTION

Infections are a major cause of morbidity and mortality in patients who are granulocytopenic due to malignant disease or treatment with anticancer chemotherapeutic agents [35, 36, 71, 73, 109]. The course of these infections is fulminant and if not treated adequately early mortality is high [116, 121]. In these cases antibiotics are given empirically and blind at the first signs of fever [121]. At the time therapy is initiated the identity and susceptibility of the causative organism are mostly not known. Therefore antibiotic combinations are used to ensure broad coverage against the possible pathogens [35, 36, 72, 73]. Another consideration for the use of antibiotic combinations forms a possible synergistic interaction of the antimicrobial agents used and the prevention of treatment failures due to monodrug resistance [13, 66].

Antibiotics are generally administered intermittently. Dosage schedules are based on knowledge about the pharmacokinetic properties of the antibiotics used and the in vitro susceptibility of the infecting strain. In addition, dosage has sometimes to be limited to avoid toxicity as is for instance the case with aminoglycosides. However, data about the kinetics of antibacterial activity of antibiotics are less taken into account. The practice of antibiotic dosage mentioned is mostly successful in immunocompetent patients. However, a substantial number of treatment failures are reported in patients with impaired host defenses, especially in case of severe granulocytopenia. Antibiotic treatment is still not always successful in these patients despite the introduction of new and potent antibiotics [37, 73]. So next to the use of highly active antimicrobial agents, more is needed to improve the outcome of antibiotic treatment in these patients. One of the approaches may be intensification of treatment by modification of the dosage schedule. The schedule of drug administration may play an important role in the outcome of antibiotic treatment of serious infections, particularly in the immunocompromised host, as in these patients recovery from infection depends to a high degree on antibiotics. It is not clearly established whether or not repeated high antibiotic serum concentrations of short duration are superior to continuously maintained antibiotic serum concentrations at lower level.

As clinical trials are difficult to perform, experimental infection models

are used, which allow comparison of the efficacy of different antibiotics and antimicrobial treatment schedules under similar conditions of intensity and duration of infection. Already in the early years after the discovery of penicillin infection models were used to study the relevance of the antibiotic dosage schedule for the therapeutic activity. The studies were limited to the effect of penicillins against streptococci in animals with intact host defenses. Since then numerous antibiotics of different classes have been developed for the treatment of various infections.

Various infection models have been used to study the impact of dosage regimens on the therapeutic efficacy of different classes of antibiotics. The experimental studies, especially those in animals with intact host defenses, yielded somewhat contradictory results. The discrepancies observed in individual studies may be explained by differences in the infection model and animal species, in the virulence of the bacterial strains, and in the mode or duration of antibiotic administration. In addition data about the impact of the dosage schedule on the in vivo activity in relation to the class of antibiotic have been mainly derived from studies either performed in infection models with limited clinical relevance or from therapy experiments in which only one class of antibiotic was studied.

The present study was performed in a model of Klebsiella pneumoniae pneumonia and septicemia in rats. This experimental model was used because in leukopenic patients septicemia is a real threat, and K.pneumoniae is one of the pathogens that may be recovered [68, 74, 99, 116, 122, 124]. The experimental design including the infectious disease model is described in chapter 2. The role of the antibiotic dosage schedule as a determinant of therapeutic activity was investigated in relation to various factors such as the severity of infection (chapter 3), the presence of host defense factors (chapter 4), and the kinetics of antibacterial activity in vitro and in vivo for different classes of antibiotics (chapter 5). From the three classes of antibiotics, the ß-lactams, the aminoglycosides and the quinolones, ceftazidime, gentamicin and ciprofloxacin were selected as investigational drug, respectively, because of their antibacterial activity against clinically important pathogens and their broad antibacterial spectrum. In addition, the in vitro susceptibility in terms of MBC values of the K.pneumoniae strain used was similar for the three drugs tested, which facilitated a good evaluation of their therapeutic efficacy. The therapeutic effect of ceftazidime, gentamicin and ciprofloxacin in relation

to the duration of infection and the bacterial growth rate is described in chapter 6. Finally in chapter 7 the experimental results obtained are discussed with reference to the observations of other investigators.
The chapters 3 through 6 represent the appendix papers.

CHAPTER 2

EXPERIMENTAL DESIGN

Bacteria. A Klebsiella pneumoniae strain (ATCC 43816, capsular serotype 2) was used in all experiments. Stationary-phase cultures were prepared by incubation for 16 h at 37°C in Todd-Hewitt broth (Oxoid Ltd., London, England). After proper dilution and reincubation for 2 h at 37°C suspensions of logarithmically growing bacteria were obtained.

Infectious disease model of K.pneumoniae pneumonia and septicemia in rats. Female R strain albino rats (specific pathogen free; 14 to 18 weeks old; weight, 185 to 215 g; bred at REPGO-TNO, Rijswijk, The Netherlands) were used in all experiments.

Experimental pneumonia was produced in the following manner. Rats were anesthetized with fluanisone (Hypnorm^R; Duphar B.V., Amsterdam, The Netherlands) and pentobarbital (Abbott Laboratories, North Chicago, Ill.). The left main stem bronchus was intubated and the left lung was inoculated with 0.02 ml of a saline suspension of K.pneumoniae. The number of CFU (8×10^4 in all therapy studies and most experiments related to the kinetics of bactericidal activity) used to inoculate the left lung in the different experiments was confirmed by plate counts on blood agar. After inoculation the narcotic antagonists Nalorphine bromide and Pentetrazolum (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands) were injected.

Numbers of bacteria in pleural fluid, blood, and left lung were quantitated as follows. Animals were sacrificed, and samples of pleural fluid and blood were taken. Pleural exudate was obtained by washing the chest cavity with physiological saline. Blood clotting was prevented by mixing 0.2 ml of blood with 0.2 ml of 3.8% sodium citrate. Then the left lung was removed after macroscopic examination, weighed, and homogenized in 20 ml of physiological saline for 30 s at 10,000 rpm in a VirTis homogenizer (The Virtis Co., Inc., Gardiner, N.Y.). Volumes of 200 µl of undiluted samples or serial ten-fold dilutions of pleural washing fluid, blood and lung-homogenate in saline were spread on bloodagar plates.

The histological features of the pneumonic lesions were studied. Lungs were fixed by injecting the trachea with 10% formalin under constant pressure to reexpand the lungs. Segments of the left lung were then dehydrated in

ethanol and toluol, embedded in paraffin, sectioned and stained with haematoxylin-eosin or by the Gram stain technique.

The efficacy of inoculation of the left lung with a volume of 0.02 ml after intubation of the left main stem bronchus was verified with Dionosil^R, a contrast medium for bronchography. As shown in figure 2.1 the volume inoculated is rapidly distributed in the left lung exclusively.

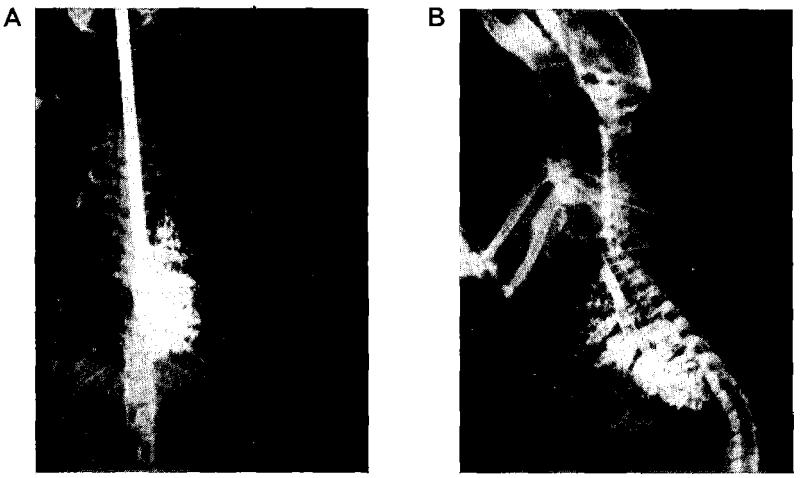


Figure 2.1 Chest roentgenograms of rats after inoculation of the left lung with a volume of 0.02 ml of Dionosil^R a contrast medium for bronchography. The contrast liquid is spread in the intubated left lung exclusively. A. Ventral B. Lateral

After inoculation of the left lung with 0.02 ml of a saline suspension containing 8 x 10^4 viable K.pneumoniae organisms (range 6 x 10^4 - 10 x 10^4), infection developed within 24 h. Macroscopic examination at 72 h after inoculation revealed that large parts of the lung were involved (figure 2.2). In figure 2.3 the course of the untreated infection is shown. Within 5 to 6 days animals died spontaneously from infection. After bacterial inoculation, total body weight decreased constantly, and rectal temperature usually fell below normal values after an initial rise. On days 4 and 5, some rats had increased temperatures, whereas others had subnormal temperatures. Involvement of the lung tissue in the infectious process was reflected by a proportional increase in weight (up to sixfold) of the left lung. On day 5 an average number of 2 x 10^{10} K.pneumoniae organisms were

Figure 2.2 Left lung at 72 h after inoculation with 8 x 10^4 CFU of K.pneumoniae.

cultured from the lung and all rats had bacteria in the blood and pleural fluid. A control experiment revealed that the technique of intubation in itself did not influence total body weights or rectal temperatures. The K.pneumoniae strain used was highly pathogenic for the lung, which is reflected in a 100% lethal dose of 8 x 10^4 CFU after inoculation of lungs as compared to a 50% lethal dose of 7 x 10^6 CFU after intraperitoneal inoculation. Histological examination of the pneumonic lesion in immunocompetent rats revealed that few organisms were present in the alveoli 12 to 14 h after bacterial inoculation. In the hemorrhagic area edema fluid and a light polymorphonuclear infiltrate were present. From day 2 after inoculation, the outer edema zone of the lesion was characterized by a cellular infiltrate composed almost exclusively of polymorphonuclear leukocytes and a few macrophages. More to the center of the lesion, edema-filled alveoli were packed with leukocytes and small lung abscesses demonstrating numerous polymorphonuclear leukocytes and large numbers of gram-negative bacilli were common (figure 2.4).

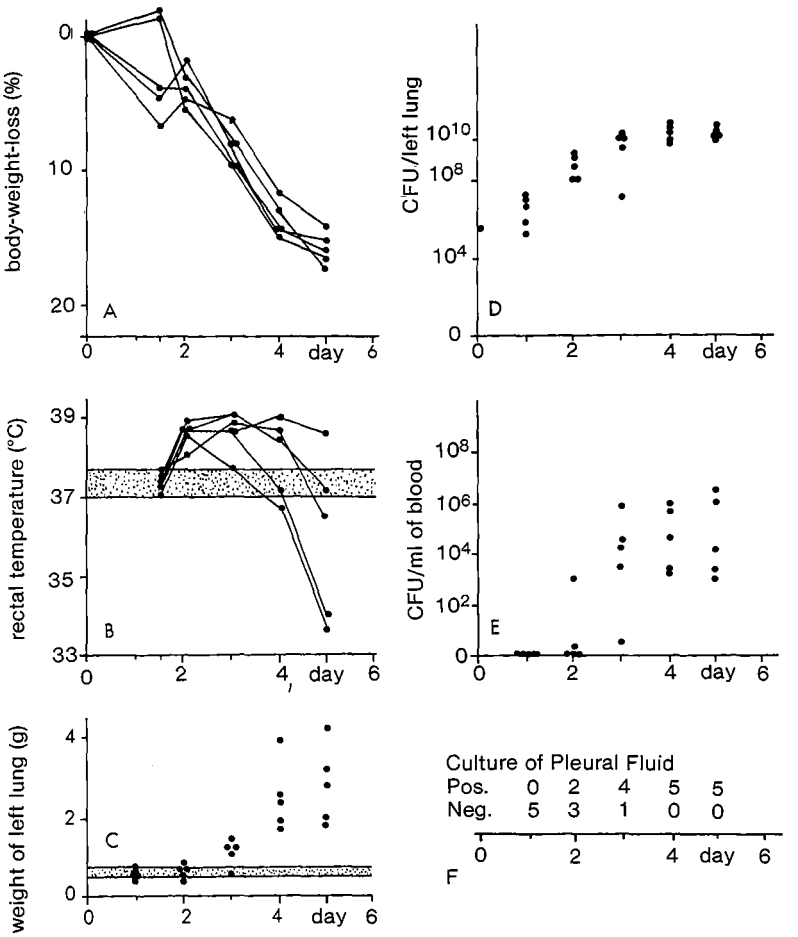
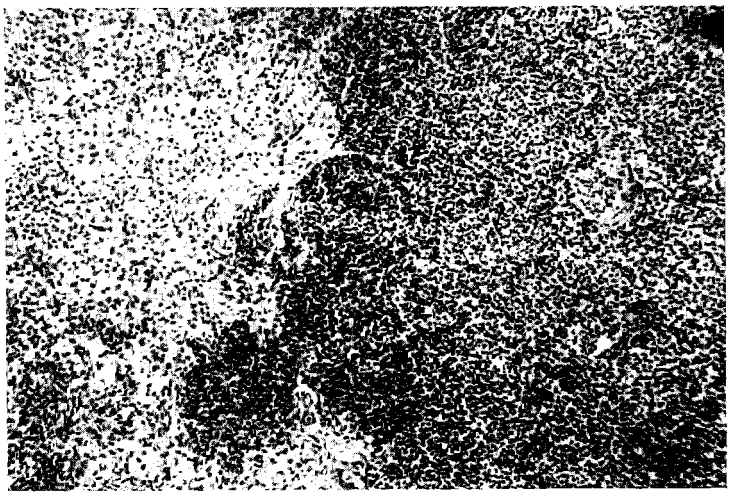
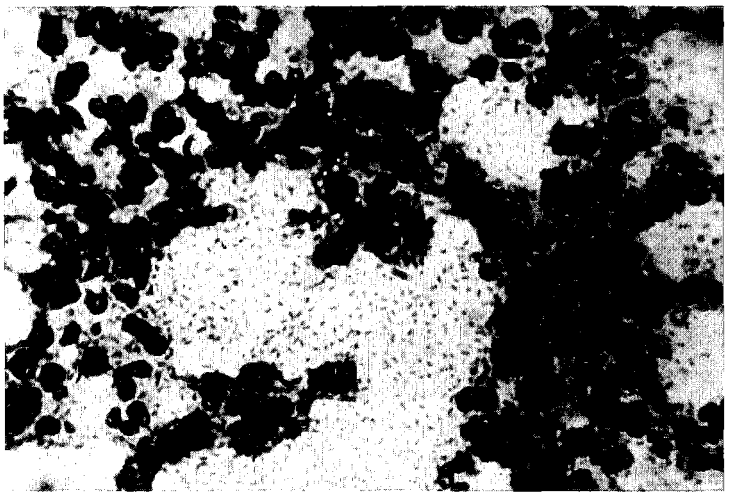


Figure 2.3 Course of K.pneumoniae pneumonia and septicemia in immunocompetent rats after inoculation with 8 x 10^4 CFU of K.pneumoniae at day 0. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of five. (C) Weight of the left lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable K.pneumoniae in the left lung. (E) Number of viable K.pneumoniae per ml of blood. (F) Culture of pleural fluid.



A



B

Figure 2.4 Sections of the left lung from rats 48 h after inoculation with 8 x 10^4 CFU of K.pneumoniae.

A. Section stained with hematoxylin-eosin (x 40).

B. Section stained with gram stain (x 400).

Impairment of host defenses. Leukopenia was induced by intraperitoneal injections of cyclophosphamide (CY) (Koch-Light Limited, Haverhill, Suffolk, England) in two doses of 90 and 60 mg/kg at five days and one day before bacterial inoculation, respectively. Quantitation of blood leukocytes was done in blood samples obtained by orbital puncture under light CO_2 anesthesia from five rats, and collected in polypropylene vials containing 1 mg of disodium EDTA per ml. For total leukocyte counts, blood was diluted 1:10 with Türk solution (0.1% crystal violet in 1% acetic acid), and numbers of leukocytes were determined in duplicate in a Bürker hemocytometer. The total numbers of granulocytes and monocytes were calculated from the total number of leukocytes and differential counts of 400 leukocytes in cytocentrifuge preparations of buffy coats obtained by centrifugation of blood samples for 10 min at 1500 x g in hematocrit tubes.

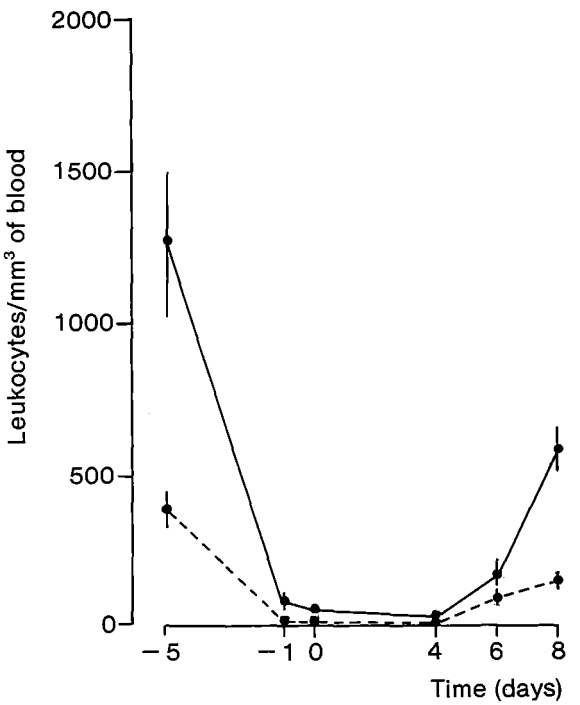


Figure 2.5 Total granulocyte (——) and monocyte (– – –) counts following CY injections given intraperitoneally 5 days (90 mg/kg/day) and 1 day (60 mg/kg/day) before bacterial inoculation at day 0.
Each value represents the mean ± SEM for five rats.

As shown in figure 2.5, CY-treatment resulted in a substantial reduction in the number of blood granulocytes and monocytes to less than 100 and 30/mm^3, respectively, five days after the first CY injection. At that time rats were inoculated with K.pneumoniae. Granulocytopenia and monocytopenia continued for four days, with a return of circulating granulocytes and monocytes after a period of 4 to 6 days. Compared to the course of infection in immunocompetent rats, CY-induced leukopenia resulted in a rapid bacterial multiplication in the lung followed by septicemia at an early stage of the infection (table 2.1). The increase in the numbers of K.pneumoniae in the left lung during the first 5 h after inoculation was similar in both CY-treated and untreated rats. However, from that time the numbers of K.pneumoniae in the left lung increased more rapidly in leukopenic rats than in immunocompetent rats. By 34 h an average number of 5×10^8 was cultured from the left lung of both leukopenic rats and animals with intact host defenses. At 24 h after inoculation K.pneumoniae was cultured from the blood of all leukopenic rats, whereas the blood of immunocompetent rats was sterile. At 29 h and 34 h after bacterial inoculation about half of the leukopenic rats had bacteria in the pleural fluid, whereas the pleural fluid of all normal rats was sterile. The mean time to death ± standard deviation (SD) recorded for CY-treated rats was 2.3 ± 0.3 days, compared with 5.1 ± 1.3 days for untreated rats. CY in itself was not bactericidal as sera obtained from CY-treated rats either 15 min or 1 day after the second CY injection did not affect the growth of K.pneumoniae in broth. Histological studies at 2 days after bacterial inoculation revealed that large numbers of gram-negative bacteria, polymorphonuclear leukocytes, and a few macrophages were present in the left lung of rats with intact host defenses. Lungs of leukopenic rats did not show any substantial cellular infiltrate at the site of infection, whereas large numbers of gram-negative bacteria were present.

Antibiotic susceptibility test. The MICs of the antibiotics were defined as the lowest concentrations that suppressed visible growth after incubation of 5×10^5 CFU/ml for 18 h at 37°C in tubes containing 4 ml of Iso-Sensitest broth (Oxoid Ltd., London, England). The MBCs were defined as the lowest concentrations that killed 99.9% of the original inoculum. MBC was determined by spreading subculture volumes of 200 µl onto Iso-Sensitest agar (Oxoid) plates. The concentrations of the serial dilutions decreased by steps of 0.2 µg/ml.

Table 2.1 Course of K.pneumoniae pneumonia and septicemia in normal and leukopenic rats[a]

h after inoculation	Immunocompetent rats (n = 5)				Leukopenic rats (n = 5)			
	Log CFU in left lung	blood		No. of rats with bacteria in pleural fluid	Log CFU in left lung	blood		No. of rats with bacteria in pleural fluid
		No. of rats positive	Log CFU/ml[b]			No. of rats positive	Log CFU/ml[b]	
5	5.7 (5.3-6.0)	0		0	5.7 (5.7-5.9)	0		0
10	5.8 (4.8-6.3)	0		0	6.5 (6.2-6.7)	0		0
18	5.6 (3.6-5.8)	0		0	7.5 (7.0-7.6)	1	1.5	0
24	6.5 (5.7-7.4)	0		0	8.5 (7.7-8.8)	5	2.3 (1.6-2.2)	0
29	6.3 (5.7-7.3)	1	1.9	0	8.7 (8.2-8.9)	5	3.0 (1.4-3.5)	2
34	8.3 (7.4-9.2)	1	1.9	0	8.9 (8.7-9.4)	5	3.3 (3.1-4.7)	3

[a] Groups of five rats each were studied after inoculation of the left lung with 8×10^4 CFU of K.pneumoniae. The values given are median values with ranges in parentheses. The mean time to death ± SD was 5.1 ± 1.3 and 2.3 ± 0.3 days for normal and leukopenic rats, respectively.

[b] Calculated for positive cultures only.

The effect of the drugs on the short-term growth of K.pneumoniae was studied both in Iso-Sensitest broth and in Hanks balanced salt solution (HBSS) (Oxoid) with 90% normal rat serum at 37°C. A stationary phase culture was diluted in Iso-Sensitest broth to a concentration of 5×10^4 or 2×10^6 CFU/ml, respectively. After reincubation for 2 h at 37°C the number of CFU was 10^6 or 4×10^7 respectively, and antibiotic was added (zero time). Killing experiments were started with an inoculum of 5×10^5 CFU per ml. During incubation, the numbers of viable organisms were then determined at regular intervals by plate counts on Iso-Sensitest agar. Before plating antibiotic concentrations were reduced to an inactive level by centrifugation of 1 ml samples for 2 min at 10,000 g followed by replacement of the drug containing medium by physiological saline.

Antibiotic treatment. Antibiotics were administered in different ways. In experiments related to therapeutic efficacy, antibiotic was administered either intramuscularly into the thigh muscles of the rear legs at regular intervals or by way of continuous infusion. The time of start of treatment is indicated in the individual chapters. Antibiotics were given during four or eight days. Because intravenous infusion techniques did not function properly over a period of four days, an alternative technique developed by Thonus et al. [114] was used. In brief, six weeks prior to its use, a tissue cage constructed from perforated Teflon (length, 40 mm; outer diameter 7 mm; inner diameter, 5 mm; 30% perforation) connected to 50 mm of polyethylene tube was implanted subcutaneously. Rats were anesthetized prior to treatment with antibiotics. The distal portion of the polyethylene tube was recovered via an incision in the neck of the animal and connected to a 12-ml syringe. This syringe was placed on a Varifusor pump (Breda Scientific, Breda, The Netherlands). Drug at the required concentration was infused at a constant rate of 0.113 ml/h. By this technique antibiotic reached the serum from the subcutaneous implanted tissue cage and prolonged steady state levels were reached within 3 h after start of the infusion (figure 2.6).
In experiments related to the kinetics of antibacterial activity antibiotics at various doses were administered intravenously as a single injection into the tail vein, at different times after bacterial inoculation.

Parameters of in vivo activity of antibiotic. In experiments related to therapeutic efficacy response to antimicrobial treatment was evaluated in

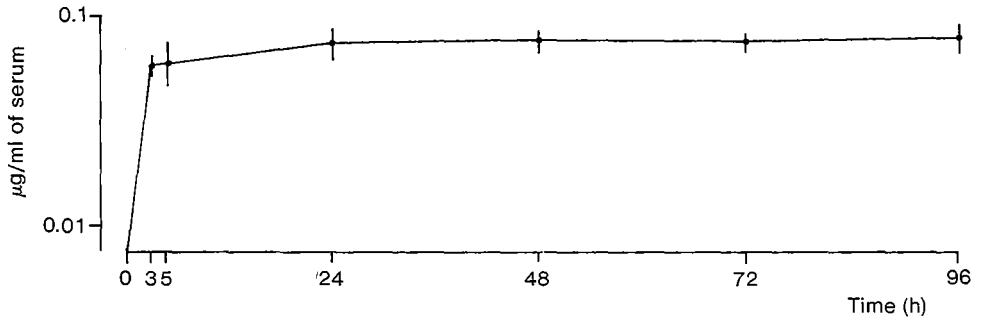


Figure 2.6 Concentration of ceftazidime in serum after administration by way of continuous infusion (infusion rate 0.113 ml/h) at 1.08 mg/kg/day. Each value represents the mean ± SEM for five rats.

different ways. In some experiments the therapeutic efficacy was evaluated with respect to the total daily dose that protected 50% of the animals from death until 16 days after termination of a four-day treatment (PD_{50}). The 50% protective dose was chosen because it is a sensitive parameter which is minimally influenced by incidental survivors or deaths in the individual treatment groups. PD_{50} values were calculated according to the method of Spearman-Kärber as described by Sachs [105]. In short, groups of 10 rats were treated with twofold increasing doses. The lowest dose resulted in 0% survival, whereas the highest dose cured all rats (100% survival). PD_{50} values and standard deviations were calculated by use of the following formulas.

$$m = X_k - d\,(S - 0.5)$$

$$Sm = \frac{d}{100}\sqrt{\frac{\sum p_i\,(100-p_i)}{n_i - 1}}$$

where

m is the estimate of the logarithm of PD_{50}

Sm is the standard deviation

X_k is the logarithm of the smallest dose such that all doses greater than or equal produce 100% reaction

d is the logarithm of the ratio of each consecutive pair of doses

S is the sum of the relative portions of reacting individuals

p_i is the frequency, in percent of reactions with the ith dose (i= 0, 1, 2, ...k)

n_i is the number of test animals tested with the ith dose (i= 0, 1, 2, ...k)

In other experiments therapeutic efficacy was evaluated with respect to the relative number of surviving animals. The Fisher test was then used for statistical analysis.
In experiments related to the kinetics of antibacterial activity response to antimicrobial treatment was evaluated by calculating the number of bacteria in the lung, blood, and pleural fluid at various intervals after administration. For statistical analysis the Mann-Whitney test or two sided analysis of variance was used.

Measurement of antibiotic concentrations in serum and in lungs of infected rats. Blood specimens, obtained by puncture of the retro-orbital plexus under light CO_2 anesthesia were collected from each rat, and serum was separated. For determination of antibiotic concentrations in lung tissue the left lung was removed, exsanguinated, and homogenized in a Potter Elvejhem homogenizer in saline. After centrifugation for 2 min at 10,000 g the supernatant was collected and used for determination of antibiotic concentrations. All tests were done according to a standard large-plate agar diffusion procedure with use of a diagnostic-sensitivity agar (Oxoid) and an Escherichia coli test strain susceptible to 0.025 µg of ciprofloxacin/ml and 0.2 µg of ceftazidime/ml or a Staphylococcus epidermidis strain susceptible to 0.125 µg of gentamicin/ml [5]. Standard samples for determination of antibiotic concentrations in serum were prepared in pooled normal rat serum. Standard samples for determination of drug concentrations in lung tissue were prepared as follows. Antibiotics in standard concentrations were added to homogenates of lungs of untreated rats. After incubation for 1 h at 37°C and centrifugation for 2 min at 10,000 g supernatants were collected. Samples of 100 µl were assayed. For determination of concentrations in serum below 0.2 µg ceftazidime/ml some modifications were introduced in order to increase the sensitivity of the test. The volume of the sample was increased up to 225 µl. This volume consisted of 200 µl of testserum collected from rats to which 25 µl of a ceftazidime solution of 0.8 or 1 µg per ml was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. Determination of samples with known concentrations of ceftazidime in serum with both the original and the modified method yielded similar results. The Mann-Whitney test was used for statistical analysis.

CHAPTER 3

IMPACT OF THE DOSAGE SCHEDULE ON THE THERAPEUTIC EFFECT OF ANTIBIOTIC IN RELATION TO THE SEVERITY OF INFECTION

efficacy of ceftazidime in immunocompetent rats with Klebsiella pneumoniae pneumonia and septicemia

INTRODUCTION

The impact of the dosage schedule of ß-lactam antibiotics on the in vivo efficacy in immunocompetent animals has been studied by several investigators [2, 33, 34, 48, 49, 65, 106, 107, 108, 111, 112, 113, 128]. The experimental studies show somewhat contradictory results. This can be partially explained by the use of different infection models and animal species, and by differences in mode and duration of antibiotic administration. Another important factor may be the variation in severity of infection related to differences in virulence between the individual bacterial strains used and in delay periods before start of treatment.

In clinical practice antibiotic treatment is initiated once infection is suspected or proven. An increase in the time period between the onset of infection and the initiation of treatment is expected to affect the effectiveness of antimicrobial treatment, especially in case of acute infections. In the present study the impact of the dosage schedule of ceftazidime on the therapeutic efficacy in relation to the severity of infection was investigated in an experimental Klebsiella pneumoniae pneumonia and septicemia in immunocompetent rats. Efficacy was evaluated in terms of PD_{50} values obtained after a four-day treatment starting at 5 h or 34 h after bacterial inoculation. Ceftazidime was administered either intermittently at 8-h or 6-h intervals or by continuous infusion.

MATERIALS AND METHODS

The following methods are described in chapter 2: MIC/MBC determination, quantitation of numbers of bacteria in pleural fluid, blood and the left lung, histological examination of the lung, antibiotic administration, evaluation of therapeutic efficacy by calculating PD_{50} values and measure-

ment of antibiotic concentrations in serum.
Antimicrobial treatment with ceftazidime (Glaxo Pharmaceuticals, Ltd., Greenford, England) was started either 5 h or 34 h after bacterial inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae. Antibiotic was administered for a period of 4 days, either intermittently at 8-h or 6-h intervals, or by continuous infusion. Therapeutic response was evaluated with respect to the calculated PD_{50} values.

RESULTS

Course of the untreated infection. The course of the untreated infection, as expressed by the increase in numbers of bacteria in the left lung and the blood is shown in figure 3.1. At 5 h after bacterial inoculation the

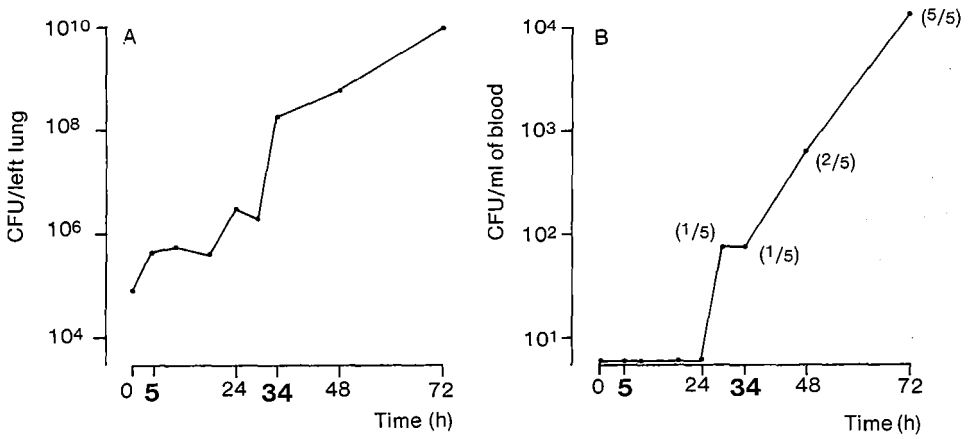


Figure 3.1 Numbers of bacteria in the left lung (A) and blood (B) of immunocompetent rats after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae at 0 h.
Values are median values of five rats.
Within parentheses: numbers of blood cultures positive/numbers tested.

number of K.pneumoniae organisms in the left lung had increased about 7-fold. Blood and pleural fluid were sterile. Histological studies of the pulmonary lesion at that time revealed no signs of tissue necrosis. A moderate number of polymorphonuclear leukocytes and a few macrophages were observed at the site of infection. However, at 34 h after bacterial

inoculation the number of K.pneumoniae organisms in the lung had increased 4,000-fold, up to 2 x 10^8 CFU. One out of five rats had bacteria in the blood. Histological studies of the lung, performed at that time revealed the presence of large numbers of polymorphonuclear leukocytes and gram-negative bacilli and a few macrophages. Tissue necrosis had occurred to some extent.

Therapeutic efficacy of ceftazidime. Ceftazidime was administered in twofold increasing doses to groups of ten rats. A dose range which resulted in a survival rate from 0% up to 100% was given. PD_{50} values were calculated from the survival rates obtained for the individual doses. These are shown in appendix paper I, together with the mean time to death values. Response to the different treatment schedules of ceftazidime is shown in table 3.1. It is demonstrated that when treatment was started at

Table 3.1 Efficacy of ceftazidime treatment schedules[a]

Start of treatment (h)	PD_{50} (mg/kg/day)		
	intermittent administration		continuous administration
	8-h intervals	6-h intervals	
5	1.42	0.35	0.36
34	13.06	3.50	1.08

[a]Ceftazidime was administered over a period of 4 days either as intermittent bolus injections at 8-h or 6-h intervals or by continuous infusion starting at different times after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae at 0 h.

5 h after bacterial inoculation ceftazidime was equally effective when administered at 6-h intervals or by continuous infusion, PD_{50} values being 0.35 and 0.36 mg/kg/day, respectively ($P > 0.05$). Treatment at 8-h intervals was less effective resulting in a PD_{50} value of 1.42 mg/kg/day ($P < 0.001$). Delay of start of treatment until 34 h after bacterial inoculation resulted in a decrease of the therapeutic activity of ceftazidime. However, this decrease was much greater with intermittent than with continuous administration. When ceftazidime was administered at 6-h or 8-h intervals the PD_{50} had

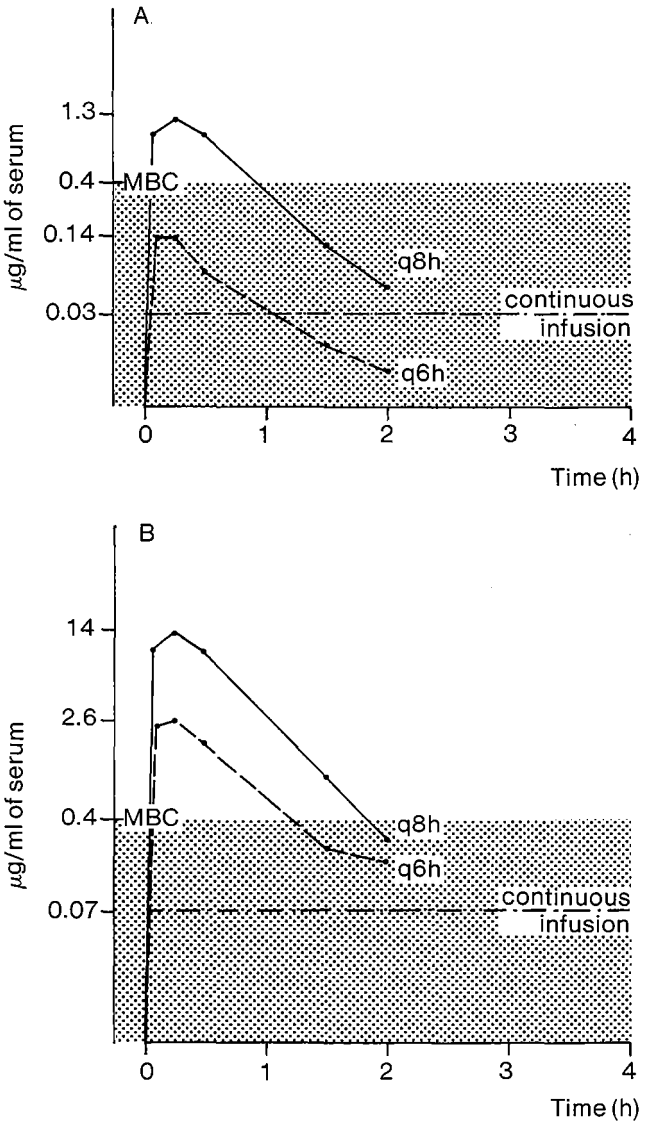


Figure 3.2 Serum concentration-time profiles of ceftazidime in immunocompetent rats after administration of PD_{50} doses obtained when antibiotic treatment was started at 5 h (A) or 34 h (B) after bacterial inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae.

to be increased about a factor 10 up to 3.5 and 13.5 mg/kg/day, respectively. However, continuous infusion of ceftazidime resulted only in a 3-fold increase of the PD_{50} up to 1.08 mg/kg/day ($P < 0.001$).

The ceftazidime serum concentration-time profiles observed after administration of PD_{50} doses are shown in figure 3.2 (mean values ± SD are described in more detail in appendix paper I). Administration of 0.09 and 0.47 mg of ceftazidime/kg (doses corresponding to the PD_{50} values obtained for treatment at 6-h or 8-h intervals, starting at 5 h), resulted in peak concentrations of 0.14 and 1.3 µg/ml of serum, respectively. Time periods of drug concentrations in excess of the MBC of 0.4 µg/ml for the K.pneumoniae strain used were 0 and 60 min, respectively. Injection of 0.88 and 4.35 mg of ceftazidime/kg (doses corresponding to the PD_{50} values obtained for treatment at 6-h or 8-h intervals, starting at 34 h) resulted in peak concentrations in serum of 2.6 and 14 µg/ml, respectively. Time periods of drug concentrations in excess of the MBC were about 65 and 110 min, respectively. Continuous infusion of PD_{50} doses of 0.36 and 1.08 mg of ceftazidime/kg/day (obtained for start of treatment at 5 h or 34 h after inoculation) resulted in steady state levels in serum of 0.03 and 0.07 µg/ml, respectively, both levels being constantly below the MBC of the K.pneumoniae strain.

DISCUSSION

Several investigators have studied the impact of the dosage schedule of ß-lactam antibiotics on the in vivo efficacy in immunocompetent animals [2, 33, 34, 48, 49, 65, 106, 107, 108, 111, 112, 113, 128]. However, the experimental studies show somewhat contradictory results. Due to the use of different infection models and different treatment schedules they are difficult to compare. Therefore in the present study several antibiotic dosage regimens were compared in relation to the severity of infection in one infectious disease model. It was demonstrated that in K.pneumoniae pneumonia and septicemia in immunocompetent rats the efficacy of ceftazidime decreased when start of treatment was delayed. However, this reduction in activity was much greater with intermittent than with continuous treatment, as was expressed by a better efficacy of continuously infused ceftazidime.

An improvement in in-vivo activity obtained by more frequent administra-

tion of ß-lactam antibiotics has also been observed by other investigators for penicillin G in a model of intraperitoneal infection in rats caused by Streptococcus pneumoniae [108] and a Streptococcus pyogenes thigh muscle infection in mice [33], for ampicillin in a Streptococcus faecalis endocarditis in rats [113] as well as for various ß-lactams in a thigh muscle infection in mice caused by Pseudomonas aeruginosa [49]. Our experimental data can not directly be related to those of Jawetz [65], Zubrod [128], and Eagle et al. [34], because in their studies in addition to the variation in dosage frequency also the duration of treatment was varied. In a way our findings correspond to the observations of Eagle et al. in that the in-vivo efficacy of penicillin G in their experiments appeared to be mainly determined by the time period that active drug levels in serum were present during the treatment. Schmidt et al. [107] using penicillin G in a rat model of pneumococcal pneumonia and Gengo et al. [48] using methicillin in a Staphylococcus aureus endocarditis in rats found, applying different treatment schedules, that there was an optimal dosing interval. However, in the study of Schmidt the difference in efficacy between the dosage schedules was small, whereas in the study of Gengo treatment schedules were compared at only one total dose. In contrast to the experimental data discussed, Sande et al. [106] and Taüber et al. [112] treating a meningitis in rabbits caused by S.pneumoniae with penicillin G or ampicillin, respectively, demonstrated that the efficacy of these drugs in sterilizing the CSF did not depend on the frequency of drug administration. However these studies have to be considered with caution as Sande et al. compared different treatment schedules at only one total dose of penicillin G. In addition, experimental data derived from an experimental meningitis in rabbits may be exclusive for this particular model as suggested by Taüber et al. and supported by investigations of this author and others [111]. Finally Bakker-Woudenberg et al. found no improved efficacy of penicillin G when administered by continuous infusion as compared to dosing at 12-h intervals in a pneumococcal pneumonia in immunocompetent rats [2]. The discrepancy between the results obtained by the investigators mentioned are probably due to variations in infection models which differed in respect of bacterial species, experimental animals, route of inoculation, or treatment schedules, in which different antibiotics were given by different schedules and for different periods of time.

From the present study it can also be concluded that ceftazidime cured animals when administered continuously at doses that resulted in steady

state serum levels staying continuously below the MIC of the infecting strain. Even the 100% protective dose obtained for ceftazidime when started at 5 h after inoculation resulted in sub-MIC steady state serum levels (appendix paper II). Several other investigators have also found in experimental infections that substantial survival rates were obtained despite antibiotic concentrations in serum did not reach the MIC [22, 85, 126]. In-vivo efficacy of low concentrations of ß-lactam antibiotics may be explained by effects of antibiotic on the bacterial growth rate or sensitization of bacteria to the phagocytic process as described by several investigators [16, 70, 84, 92] and reviewed by Milatovic [86], Lorian [81], and Atkinson et al. [1].

In conclusion it is demonstrated in an experimental K.pneumoniae pneumonia and septicemia in immunocompetent rats that continuous administration of ceftazidime was more effective than intermittent administration at relatively long intervals, a difference that increased when the start of treatment was delayed. The therapeutic efficacy of ceftazidime decreased with increasing severity of the infection. This decrease was moderate when ceftazidime was administered by continuous infusion but substantial when the drug was given intermittently. It appeared that the activity of ceftazidime was mainly dependent on the maintenance of appropriate antibiotic concentrations during the entire treatment interval.

In order to obtain substantial survival rates antibiotic concentrations in serum needed not to be continuously above the MIC of the infecting strain.

CHAPTER 4

IMPACT OF THE DOSAGE SCHEDULE ON THE THERAPEUTIC EFFECT OF ANTIBIOTIC IN RELATION TO HOST DEFENSE MECHANISMS

efficacy of ceftazidime in immunocompetent versus leukopenic rats with Klebsiella pneumoniae pneumonia and septicemia

INTRODUCTION

It is shown in chapter 3 for immunocompetent rats, that the therapeutic effect of ceftazidime depends mainly on the time an appropriate drug level is maintained. The superiority of continuous administration over intermittent administration at relatively long intervals was more pronounced when start of treatment was delayed. It may be expected that in immunocompromised patients the schedule of antibiotic administration may also be an important determinant for therapeutic efficacy in serious infection. With current antibiotic treatments therapeutic failure still occurs in these patients probably due to the failure of the host defense system to provide adequate support to antibiotic therapy. The question is whether the results of antimicrobial treatment can be improved by intensification of antibiotic treatment as a result of modification of the dosage schedule. It is not clearly established whether or not repeated high antibiotic serum concentrations of short duration are superior to continuously maintained antibiotic serum concentrations at a lower level. Experimental studies in infection models on the impact of the antibiotic dosage schedule on the therapeutic activity of antibiotics in relation to host defense mechanisms are limited [2, 49]. The present study was undertaken first to investigate the impact of the dosage schedule of ceftazidime on its therapeutic efficacy in relation to host defense factors by comparing the activity of different treatment schedules in immunocompetent versus leukopenic rats with Klebsiella pneumoniae pneumonia and septicemia. Efficacy was evaluated in terms of PD_{50} values obtained after a four-day treatment starting at 5 h after bacterial inoculation. Ceftazidime was administered either intermittently at 6-h intervals or by continuous infusion. In addition, the efficacy of treatment with ceftazidime was studied in relation to the duration of leukopenia, as it is not clearly established whether antimicrobial treatment should be continued when leukopenia persists [31, 47, 100, 121].

MATERIALS AND METHODS

The following methods are described in chapter 2: MBC determination, quantitation of numbers of bacteria in the pleural fluid, blood and the left lung, histological examination of the lung, antibiotic administration and evaluation of therapeutic efficacy by calculating PD_{50} values and measurement of ceftazidime concentrations in serum.

Immunocompetent and leukopenic rats were treated with antibiotic as follows. Administration of ceftazidime was started at 5 h after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae. Antibiotic was administered for a period of 4 days, either intermittently at 6-h intervals or by continuous infusion. Therapeutic activity was evaluated with respect to the calculated PD_{50} values.

Experiments on the efficacy of ceftazidime treatments of various duration in relation to the persistence of leukopenia were performed as follows. Leukopenia was induced by intraperitoneal injections of CY in two doses of 90 or 60 mg/kg at five days and one day before bacterial inoculation, respectively. From that time leukopenia persisted for 4 days. Prolonged leukopenia for an additional period of 4 days was obtained by a third intraperitoneal injection of 60 mg CY/kg at 4 days after the second CY injection (figure 4.1; see also figure 2.4). Ceftazidime was administered by continuous infusion for a period of 4 or 8 days, starting 5 h after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae. Therapeutic activity was evaluated with respect to the relative number of surviving animals.

RESULTS

Course of the untreated infection. The course of the untreated infection in immunocompetent and in leukopenic rats is shown in figure 4.2. The number of bacteria in the left lung increased 7-fold within 5 h after inoculation in both leukopenic and immunocompetent rats. However, after that time, due to leukopenia, bacterial numbers in the lung increased more rapidly in CY-treated rats. In addition, in leukopenic rats septicemia occurred at an early stage and was more severe compared to rats with intact host defense. Leukopenic rats died soon as expressed by a mean time to death ± standard deviation of 2.3 ± 0.3 days, compared to 5.1 ± 1.3 days

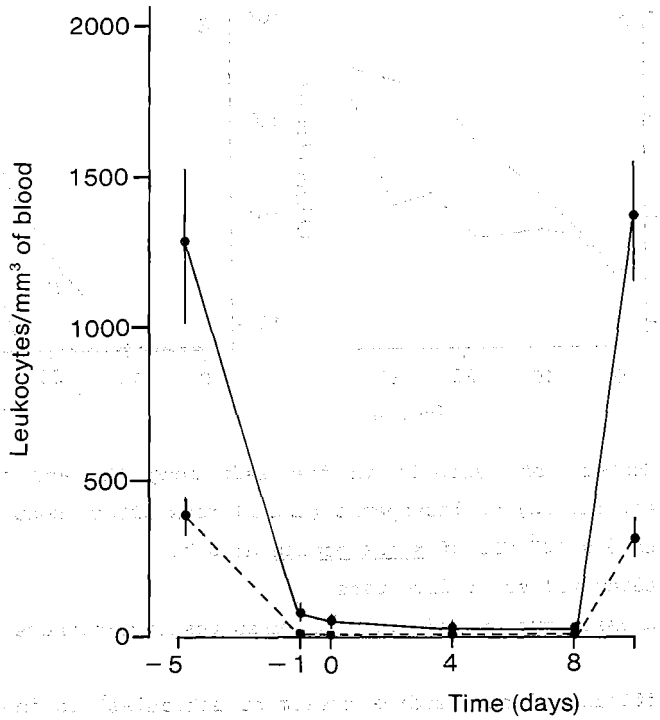


Figure 4.1 Total granulocyte (———) and monocyte (– – –) counts following CY injections given intraperitoneally 5 days before (90 mg/kg), 1 day before (60 mg/kg), and 4 days after (60 mg/kg) bacterial inoculation on day 0. Each value represents the mean ± SEM for five rats.

for immunocompetent rats. Histological studies at 2 days after bacterial inoculation revealed that large numbers of gram-negative bacteria, polymorphonuclear leukocytes, and a few macrophages were present in the left lung of rats with intact host defenses. In contrast large numbers of gram-negative bacteria but no substantial cellular infiltrate was present in lungs of leukopenic rats.

Therapeutic efficacy. Ceftazidime was administered in twofold increasing doses to groups of ten rats. A dose range which resulted in a survival rate from 0% up to 100% was given. PD50 values were calculated from the survival rates obtained for the individual doses. These are shown in appendix paper II together with the mean time to death values. The efficacy of the different ceftazidime treatment schedules is shown in table 4.1. In immuno-

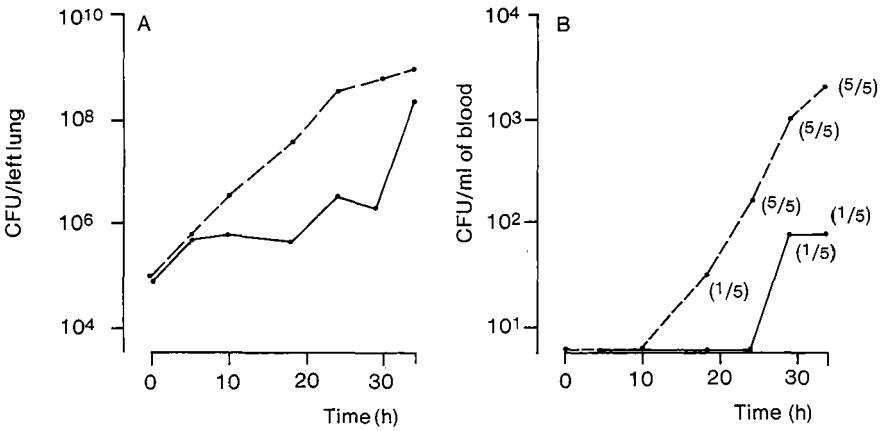


Figure 4.2 Numbers of bacteria in the left lung (A) and blood (B) of immunocompetent (——) or leukopenic (– – –) rats after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae at 0 h.
Values are median values of five rats.
Within parentheses; numbers of blood cultures positive/numbers tested.

Table 4.1 Efficacy of ceftazidime treatment schedules[a] in immunocompetent and leukopenic rats

Rats	PD_{50} (mg/kg/day)	
	intermittent administration	continuous administration
immunocompetent	0.35	0.36
leukopenic	24.37	1.52

[a]Ceftazidime was administered over a period of 4 days, either as intermittent bolus injections at 6-h intervals or by continuous infusion. Treatment was started at 5 h after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae.

competent rats intermittent and continuous administration of ceftazidime were equally effective. PD_{50} values were 0.35 and 0.36 mg/kg per day, respectively (P >0.05). Due to leukopenia the therapeutic efficacy of both treatment schedules decreased. Compared to the PD_{50} values inimmunocompetent rats the PD_{50} in leukopenic rats had to be increased 70-fold when ceftazi-

dime was given intermittently and only 4-fold when administered continuously. In leukopenic rats continuous infusion of ceftazidime appeared to be highly superior to administration at 6-h intervals, PD_{50} values being 1.52 and 24.37 mg/kg per day for the respective treatment schedules ($P < 0.001$).

The ceftazidime serum concentration-time profiles observed after administration of PD_{50} doses are shown in figure 4.3 (mean values ± SD are described in more detail in appendix paper II). Administration of 0.9 and 6.1 mg of ceftazidime/kg (doses corresponding to the PD_{50} values obtained for immunocompetent and leukopenic rats after intermittent dosage) resulted in serum peak levels of 0.14 and 14 µg/ml, respectively. After injection of the respective doses drug concentrations in serum did not reach the MBC of 0.4 µg/ml for the infecting strain or were above the MBC for about 170 min during the treatment interval, respectively. Continuous infusion of PD_{50} doses of 0.36 and 1.52 mg/kg per day (obtained for immunocompetent and leukopenic rats) resulted in steady state levels in serum of 0.03 and 0.19 µg/ml, respectively.

The results of ceftazidime treatments of various duration in relation to the persistence of neutropenia are shown in table 4.2. A total daily dose

Table 4.2 Efficacy of ceftazidime treatments[a] of various duration in relation to the persistence of leukopenia

Persistence of leukopenia (days)	Duration of treatment (days)	No. of survivors	Time to death (days)[b]
4	4	20	
8	4	3	7.3 ± 1.0
8	8	15	8.0 ± 1.0

[a]To groups of 20 rats each 3.75 mg of ceftazidime per kg per day was administered by continuous infusion. Treatment was started 5 h after inoculation of the left lung with 8×10^4 CFU of K.pneumoniae.

[b]Mean ± SD; based on the time of bacterial inoculation (day 0).

of 3.75 mg of ceftazidime per kg administered by continuous infusion over a period of 4 days cured all rats that were leukopenic for only 4 days. This

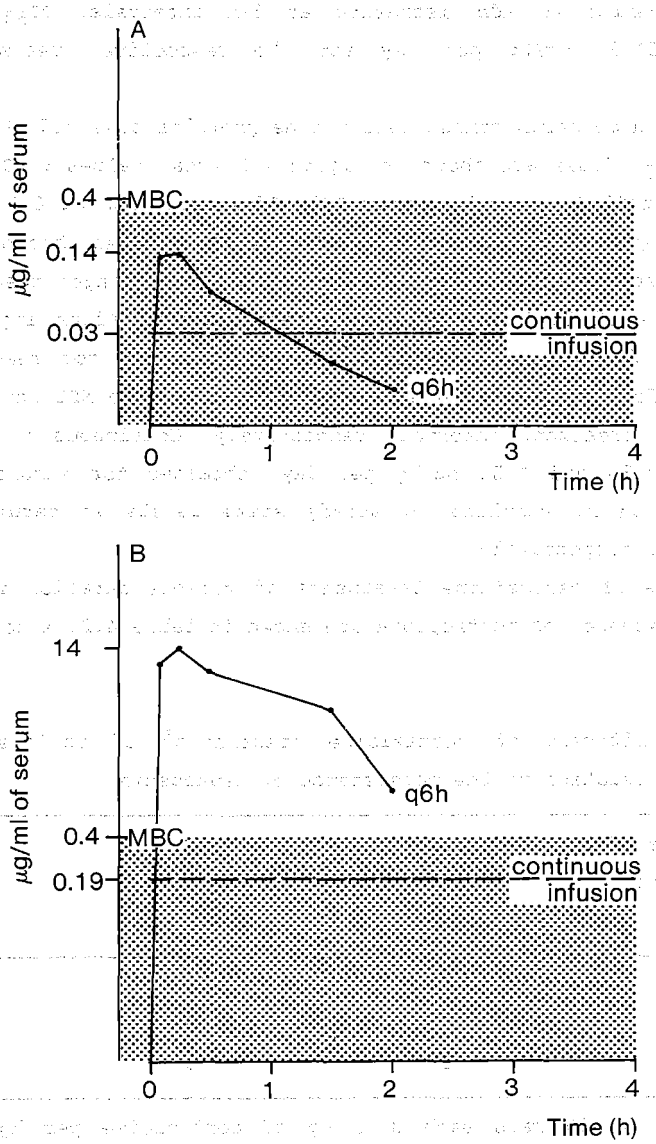


Figure 4.3 Serum concentration-time profiles of ceftazidime after administration of PD_{50} doses to infected immunocompetent (A) or leukopenic (B) rats.

ceftazidime treatment schedule was not successful in rats with leukopenia persisting for 8 days, resulting in significant increase in mortality of rats ($P < 0.05$). On the other hand mortality decreased significantly, when ceftazidime treatment was continued until the return of circulating leukocytes at day 8 ($P < 0.05$). The survival rates of rats treated during the entire leukopenic period of either 4 or 8 days were not significantly different ($P > 0.05$).

DISCUSSION

Studies on the impact of the dosage schedule of β-lactam antibiotics on the therapeutic activity in relation to the host defense factors are limited [2, 49]. The present study demonstrates that ceftazidime was equally effective in treating a K.pneumoniae pneumonia and septicemia in immunocompetent rats when administered either at 6-h intervals or by continuous infusion. However, when host defenses were impaired by means of CY-induced leukopenia treatment by continuous infusion was highly superior as compared to intermittent treatment. As compared to immunocompetent rats the PD_{50} dose in leukopenic rats had to be increased only a factor 4 in case ceftazidime was administered continuously, whereas the PD_{50} had to be increased 70-fold after administration of this drug at 6-h intervals. These results are in agreement with those of Bakker-Woudenberg et al. [2] and Gerber et al. [49]. In immunocompetent rats with pneumococcal pneumonia lungs were sterilized by equal total daily doses of penicillin G administered either at 12-h intervals or by continuous infusion [2]. Rats with impaired phagocytosis were also cured by about the same total daily dose of penicillin provided this drug was administered continuously. On the contrary with intermittent administration the efficacy of penicillin was highly reduced as compared to its activity in rats with intact host defenses. Gerber et al. came to similar conclusions with respect to the treatment of a thigh infection caused by P.aeruginosa in mice with intact host defenses [49]. A regimen in which ticarcillin was injected in small doses at very short intervals was only slightly more effective than the same amount of drug injected as a single bolus injection. On the contrary, in leukopenic mice the fractionated dosage regimen was far more effective in reducing the number of bacteria at the site of infection.

It is not clearly established whether antimicrobial treatment of neutro-

penic patients from whom no pathogen is isolated and who become afebrile after initial therapy, should be continued in case neutropenia persists [31, 47, 100, 121]. Pizzo et al. continued or discontinued antibiotic therapy in cancer patients with prolonged granulocytopenia, who became afebrile during initial antimicrobial treatment [100]. Only in the group in which antimicrobial treatment was discontinued did infectious sequelae develop. They suggested that granulocytopenic cancer patients may profit from the prolongation of antimicrobial treatment when granulocytopenia persists. The data of the present study on the efficacy of ceftazidime treatments of various duration in relation to the persistence of leukopenia in rats also suggest that treatment should be continued until the return of circulating leukocytes. However, prolonged antibiotic treatment may cause an increase in adverse side-effects. This must be outweighed against the risk on development of infections. In addition, the main factor determining the risk on development of serious infection in persistent granulocytopenic patients after stopping antibiotic treatment may be the number of days they are afebrile after initial therapy as recently suggested by DiNubile [31].
In conclusion, it is demonstrated in rats with K.pneumoniae pneumonia and septicemia that the therapeutic efficacy of ceftazidime decreased when host defenses were impaired. This decrease was only moderate when ceftazidime was administered by continuous infusion, but much more pronounced when treatment was intermittent. In other words, in animals with impaired host defenses continuous administration of ceftazidime was far more effective than intermittent administration at relatively long intervals. So it appeared that the activity of ceftazidime was mainly dependent on the maintenance of appropriate antibiotic concentrations during the entire treatment interval. It was also observed that continuation of treatment in granulocytopenic rats with ceftazidime by continuous infusion until the return of circulating leukocytes gave rise to a significant reduction in mortality.

CHAPTER 5

IMPACT OF THE DOSAGE SCHEDULE ON THE THERAPEUTIC EFFECT IN RELATION TO THE KINETICS OF ANTIBACTERIAL ACTIVITY IN VITRO AND IN VIVO FOR DIFFERENT CLASSES OF ANTIBIOTICS

efficacy of ceftazidime, gentamicin, and ciprofloxacin in leukopenic rats with Klebsiella pneumoniae pneumonia and septicemia

INTRODUCTION

From the experimental studies described in chapters 3 and 4 it can be concluded that the antibiotic dosage schedule is an important determinant for the therapeutic activity of the ß-lactam ceftazidime. It appeared that the therapeutic effect of ceftazidime is dependent on the maintenance of antibiotic concentrations in plasma during the entire treatment interval. Data derived from various experimental studies suggest that the role of the dosage schedule in relation to the therapeutic efficacy is dependent on the class of antibiotic [49, 52, 120]. However, these data have been mainly derived from studies performed in infection models with limited clinical relevance or from studies in which only one class of antibiotic was investigated. In the present study the impact of the dosage schedule on the therapeutic efficacy of antibiotics of three different classes was investigated in a Klebsiella pneumoniae pneumonia and septicemia in leukopenic rats. The in vitro susceptibility in terms of MBC values of the K.pneumoniae strain used for the selected drugs ceftazidime, gentamicin and ciprofloxacin was similar. The efficacy of the drugs was evaluated in terms of PD50 doses obtained after a four day treatment, starting 5 h after bacterial inoculation of the lung. Drugs were administered either intermittently at 6-h intervals or by way of continuous infusion. The therapeutic activity observed for the different treatment schedules after a four-day treatment was compared with the kinetics of antibacterial activity of the antibiotics against the K.pneumoniae strain in vitro as well as in lungs of leukopenic rats.

MATERIALS AND METHODS

The following methods are described in chapter 2: MIC/MBC determination, measurement of the effect of antibiotics on short term growth of bacteria, quantitation of numbers of bacteria in the left lung, antibiotic administration, evaluation of in vivo bactericidal activity and therapeutic efficacy by calculating PD_{50} values and measurement of antibiotic concentrations in serum.

The antibiotics used were ceftazidime, gentamicin (Schering Corporation, Kenilsworth N.Y., United States) and ciprofloxacin (Bayer AG, Leverkusen, Federal Republic of Germany).

The postantibiotic effect (PAE) was determined as follows. A stationary phase culture of K.pneumoniae was diluted in Iso-Sensitest broth to a concentration of 5×10^6 CFU/ml. After reincubation for 2 h at 37°C the number of CFU was 10^8 per ml and antibiotic was added. PAE experiments were started at an inoculum of 5×10^7 CFU/ml. After 1 h the cultures containing ceftazidime and gentamicin were diluted 100-fold in fresh prewarmed medium, and the cultures containing ciprofloxacin 250-fold. Bacterial cultures without antibiotic were treated similarly. Control experiments revealed that at the dilutions used the highest residual antibiotic concentrations obtained for all experiments did not affect the growth of logarithmically growing K.pneumoniae. PAE was quantitated as described by Craig and Gudmundsson for PAE measurement by viable counts [25]. They used the following equation to quantify the PAE: PAE = T-C, where T and C represent the times required for the CFU count to increase 1 $\log_{10}$ above the count observed immediately after dilution for the test culture and control culture, respectively.

The antibacterial activity in vivo of the selected drugs ceftazidime, gentamicin, and ciprofloxacin was examined in leukopenic rats in different ways. The kinetics of antibacterial activity was investigated by administration of antibiotic at various doses intravenously at 1 h after inoculation of the left lung with 8×10^4 CFU of logarithmically growing K.pneumoniae. Antibiotic activity was measured with respect to the numbers of CFU in the left lung at various intervals after administration. Statistical analysis was performed by use of two-sided analysis of variance and the Mann-Whitney test. The therapeutic efficacy of the antibiotics was studied by administration of antibiotic for a period of 4 days, starting at 5 h after bacterial inoculation of the left lung with 8×10^4 CFU of K.pneumoniae. Antibiotic

was administered either intermittently at 6-h intervals or by continuous infusion. Therapeutic efficacy was evaluated with respect to the calculated PD_{50} values.

RESULTS

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae **in vitro.** The MICs as well as the MBCs of ceftazidime and gentamicin for the K.pneumoniae strain were both 0.4 µg/ml. The MIC and MBC of ciprofloxacin were both 0.2 µg/ml. Kinetics of bactericidal activity of the three drugs at concentrations of ½, 1, and 2 times the MBC against K.pneumoniae in broth is shown in figure 5.1. Bacterial killing by cefta-

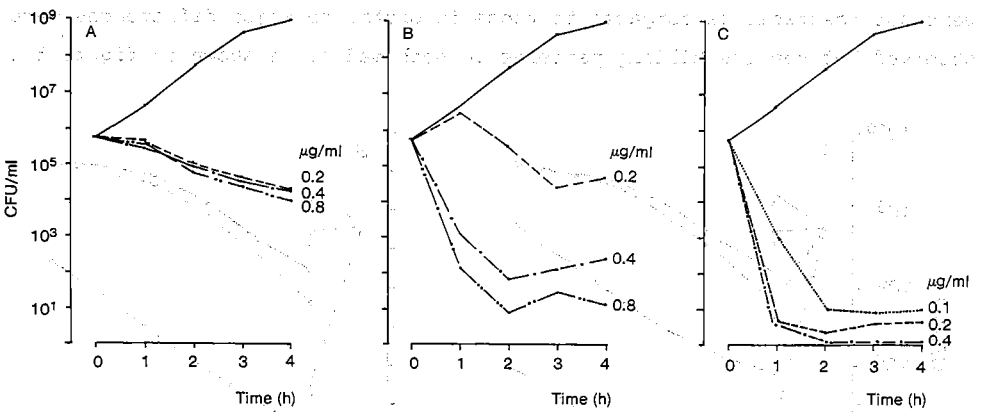


Figure 5.1 Effect of ceftazidime (A), gentamicin (B), or ciprofloxacin (C) at concentrations indicated against K.pneumoniae in broth. Bacterial growth without antibiotic (——).

zidime was slow but continued during the 4 h incubation period, and was relatively independent on the concentration. On the contrary both gentamicin and ciprofloxacin demonstrated a rapid bacterial killing that was related to the concentration of antibiotic. The killing rate by ciprofloxacin was extremely high. Although the bactericidal activities of ceftazidime, gentamicin and ciprofloxacin in HBSS with 90% serum (figure 5.2)

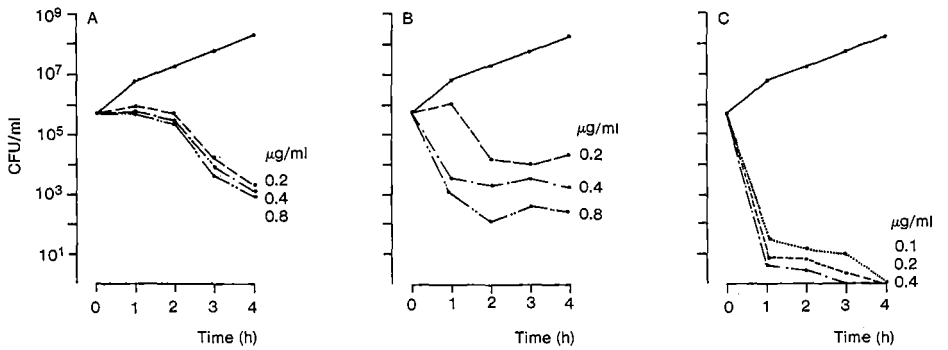


Figure 5.2 Effect of ceftazidime (A), gentamicin (B), or ciprofloxacin (C) at concentrations indicated against K.pneumoniae in HBSS with 90% rat serum. Bacterial growth without antibiotic (——).

were not identical as compared to those in broth, no major differences were observed between the killing patterns in both media. As shown in figure 5.3

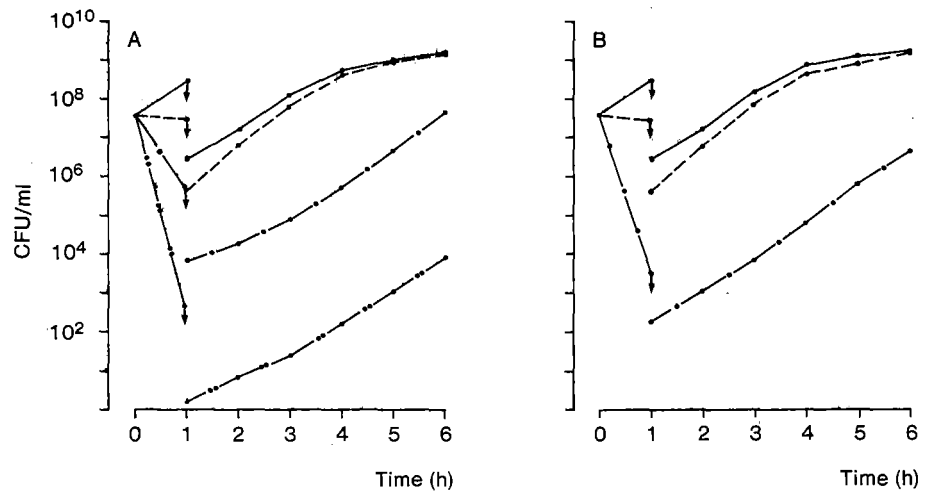


Figure 5.3 PAE determinations of ceftazidime (— — —), gentamicin (—·—), or ciprofloxacin (—··—) after 1 h exposure of K.pneumoniae to drug concentrations of 2 times the MBC (A) or 5 times the MBC (B). Cultures were diluted at 1 h 100-fold for ceftazidime and gentamicin, and 250-fold for ciprofloxacin.
Controls without antibiotic (——).

a PAE was not observed for the three antibiotics. Exposure of the bacteria during 1 h to 2 times the MBC for ceftazidime, gentamicin and ciprofloxacin or 5 times the MBC for ceftazidime and gentamicin did not result in a substantial delay of regrowth as compared to bacteria not exposed to antibiotic.

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats. The antibacterial activity of ceftazidime, gentamicin and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats after intravenous administration of different doses at 1 h after bacterial inoculation is shown in figure 5.4. Bacterial killing by cefta-

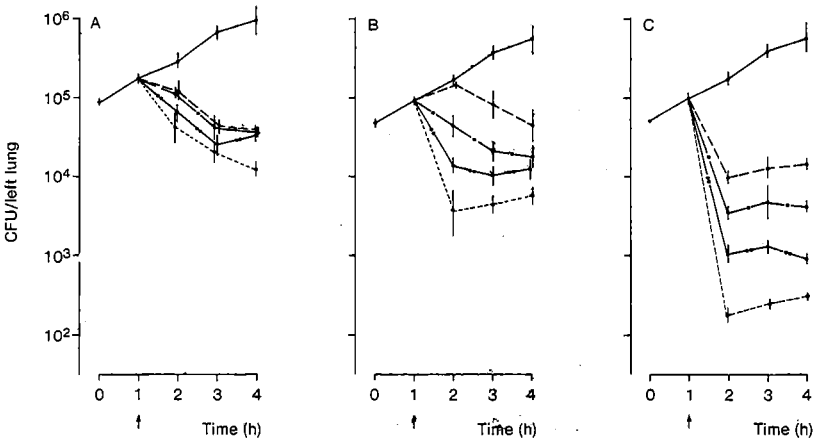


Figure 5.4 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———), and after intravenous administration of ceftazidime (A), gentamicin (B), or ciprofloxacin (C), in doses of 0.3 mg/kg (– – –), 1.0 mg/kg (—·—), 3.0 mg/kg (—··—), or 9.0 mg/kg (------) at 1 h after inoculation of lungs with K.pneumoniae in the logarithmic phase of growth. Each point represents the geometric mean ± SEM for five rats.

zidime was not strongly related to the dose administered although at the different doses used ceftazidime peak concentrations in serum ranged from 1 to 48 µg/ml (figure 5.5) (the numerical data are described in more detail in appendix paper IV). A significant increase in bacterial killing rate was only observed during the first hour after administration of the dosage 3.0 mg/kg compared to 1.0 mg/kg ($P < 0.05$ by Mann-Whitney) (figure 5.4A). A

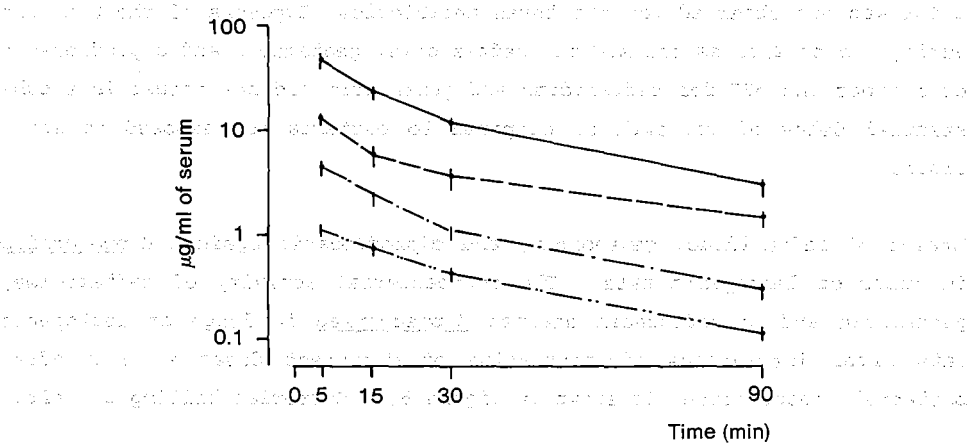


Figure 5.5 Concentrations of ceftazidime in serum of leukopenic rats after intravenous administration in doses of 0.3 mg/kg (—··—), 1.0 mg/kg (—·—), 3.0 mg/kg (— — —), or 9.0 mg/kg (————).
Each point represents the mean ± SEM for five rats.

further increase of the dose up to 9.0 mg/kg did not result in a greater decrease in the numbers of bacteria in the lung ($P > 0.05$). In contrast to ceftazidime killing rates by gentamicin and ciprofloxacin increased with each dose increment from 0.3 up to 9.0 mg/kg ($P < 0.05$) (figures 5.4 B and C). At similar doses bacterial killing by ciprofloxacin was more rapid than that by gentamicin, whereas gentamicin exerted a higher bacterial killing rate compared to ceftazidime from the 3.0 mg/kg dose ($P < 0.05$). Bacterial killing by ceftazidime continued until 2 h after injection ($P < 0.05$ by two-sided analysis of variance), the killing rate between the first and second hour after administration being independent on the dose administered ($P > 0.05$). In contrast, the bacterial killing by gentamicin and ciprofloxacin was completed within 1 h after administration, thereafter no further decrease in bacterial numbers occurred, irrespective the dose administered ($P > 0.05$).

Figure 5.6 shows the numbers of bacteria in the left lung at various intervals until 9 h after intravenous administration of 3.0 mg/kg of ceftazidime, gentamicin, or ciprofloxacin at 1 h after bacterial inoculation. Substantial regrowth of bacteria in the left lung did not occur, although antibiotic concentrations in the lung were below the MBC from 2.5 h after antibiotic administration (data not shown). Bacterial counts started to

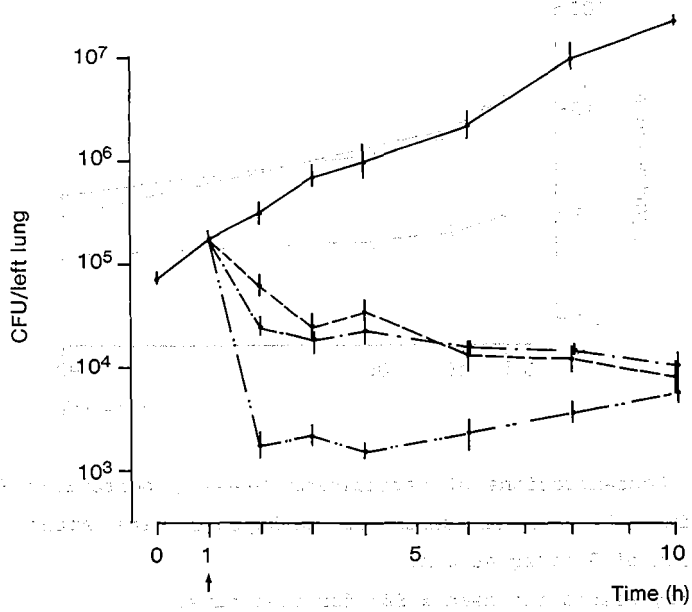


Figure 5.6 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———), and after intravenous administration of ceftazidime (– – –), gentamicin (—·—), or ciprofloxacin (—··—) in doses of 3 mg/kg at 1 h after inoculation of the lungs with K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

increase from the third hour after injection of ciprofloxacin. This increase was significant, although small ($P < 0.05$ by Mann-Whitney).
The concentrations of ceftazidime, gentamicin and ciprofloxacin in serum of rats at various intervals after intravenous administration of 3 mg/kg are shown in figure 5.7 (the numerical data are described in more detail in appendix paper IV). Serum concentrations at 5 min after administration were 13.1, 14.1, and 0.94 µg/ml, respectively. Kinetics of ceftazidime and gentamicin in serum were comparable, with estimated elimination half-lifes ($t_{\frac{1}{2}}\beta$) (measured from 15 min after administration) of 30 min. Ciprofloxacin serum concentrations were about 14-fold lower as compared to those of ceftazidime and gentamicin, within 5 min after administration of a similar dose of 3.0 mg/kg. The estimated $t_{\frac{1}{2}}\beta$ of ciprofloxacin was approximately 100 min.

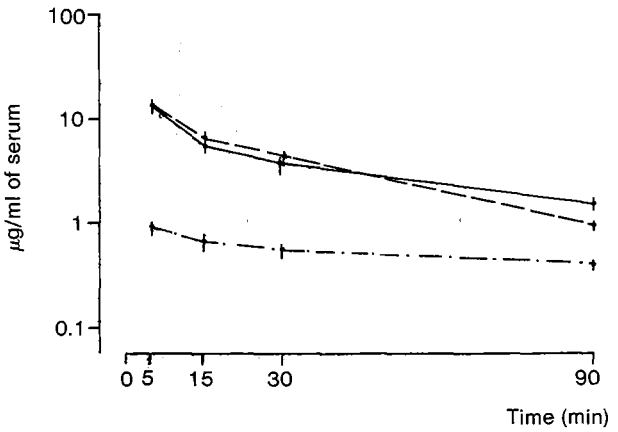


Figure 5.7 Concentrations of ceftazidime (———), gentamicin (– – –), or ciprofloxacin (—·—) in serum of leukopenic rats after intravenous administration of 3 mg/kg at 0 h.
Each point represents the mean ± SEM for five rats.

Therapeutic efficacy of ceftazidime, gentamicin, and ciprofloxacin in K.pneumoniae pneumonia and septicemia in leukopenic rats. Antibiotics were administered in twofold increasing doses to groups of ten rats. A dose range which resulted in survival rates from 0% up to 100% was applied. PD50 values were calculated from the survival rates obtained for the individual doses. These are shown in appendix paper IV together with the mean time to death values. The therapeutic efficacy of ceftazidime, gentamicin, and ciprofloxacin determined in terms of PD_{50} values is shown in table 5.1. The therapeutic effect of ceftazidime was dependent on the dosage regimen, being far more effective when administered by continuous infusion as compared to intermittent treatment at 6-h intervals, resulting in PD_{50} values of 1.52 and 24.37 mg/kg/day, respectively ($P < 0.001$). For gentamicin no significant difference in efficacy could be observed between both modes of administration, PD_{50} values being 3.8 and 2.8 mg/kg/day for continuous and intermittent administration, respectively ($P > 0.05$). The therapeutic efficacy of ciprofloxacin decreased slightly when administered by continuous infusion compared to intermittent administration, as demonstrated by PD_{50} values of 6.5 and 3.3 mg/kg/day, respectively ($P < 0.05$).
The serum concentration-time profiles of ceftazidime, gentamicin and ciprofloxacin observed after administration of PD_{50} doses, either intermittently

Table 5.1 Efficacy of ceftazidime, gentamicin, and ciprofloxacin treatment schedules[a] in leukopenic rats

Antibiotic	PD_{50} (mg/kg/day)	
	intermittent administration	continuous administration
ceftazidime	24.37	1.52
gentamicin	2.84	3.80
ciprofloxacin	3.27	6.53

[a]Antibiotics were administered over a period of 4 days, either as intermittent bolus injections at 6-h intervals or by continuous infusion. Treatment was started at 5 h after inoculation of the left lung with 8×10^4 CFU of K.pneumoniae

or by continuous infusion, are shown in figure 5.8 (mean values ± SD are described in more detail in appendix paper IV). When administered intermittently, the time periods that serum concentrations were above the MBC for the K.pneumoniae strain were about 170 and 75 min for ceftazidime and gentamicin, respectively. For ciprofloxacin serum concentrations were above the MBC for only about 40 min during the 6-h dosing interval. Steady state serum concentrations after administration of PD_{50} doses by continuous infusion were 0.19, 0.29, and 0.14 µg/ml for ceftazidime, gentamicin, and ciprofloxacin, respectively.

DISCUSSION

The impact of the antibiotic dosage schedule on the treatment of serious infections is not clearly established. Particularly in the immunocompromised host, the schedule of drug administration may play an important role in the outcome of antibiotic treatment of infections, as in these patients recovery from infection depends to a high degree on antibiotics, and therapeutic failure still occurs.

During the past few years several investigators have studied the efficacy of different antibiotic dosage schedules in experimental gram-negative infections [18, 49, 52, 57, 64, 67, 79, 96, 101, 120]. In the present study the impact of the antibiotic treatment schedule of ceftazidime, gentamicin, and

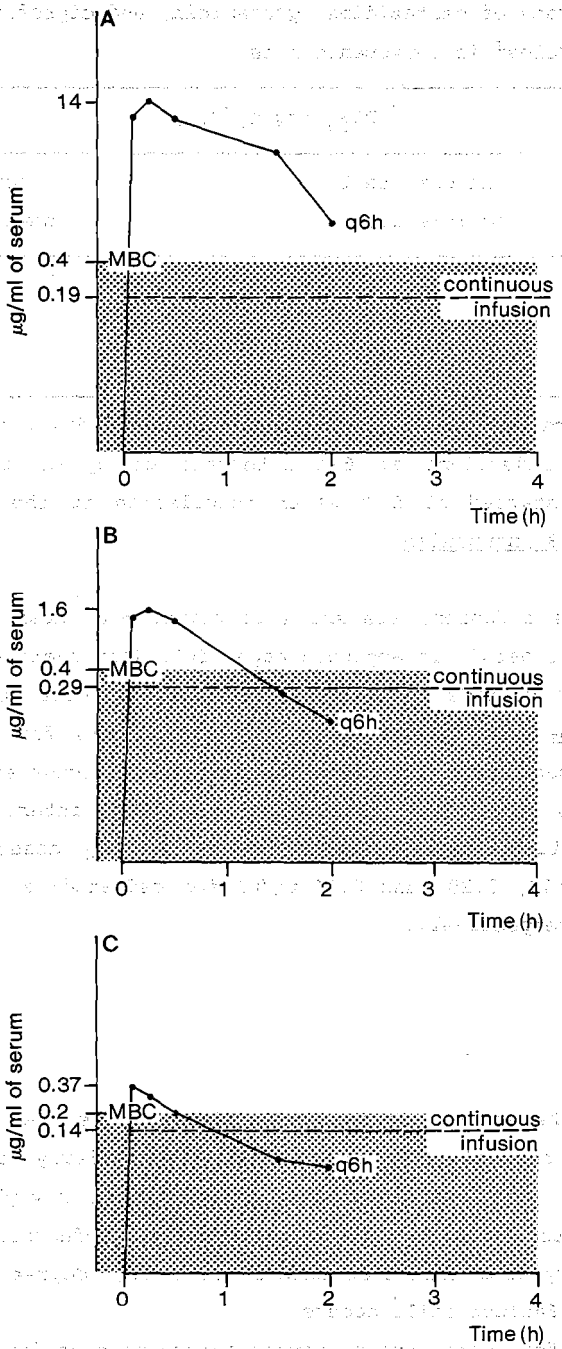


Figure 5.8 Serum concentration-time profiles of ceftazidime (A), gentamicin (B), or ciprofloxacin (C) after administration of PD_{50} doses to infected leukopenic rats.

ciprofloxacin on the therapeutic efficacy was investigated in a model of K.pneumoniae pneumonia and septicemia in leukopenic rats. The in-vitro susceptibility in terms of MBC values for the K.pneumoniae strain used was similar for the three drugs. We observed that ceftazidime was far more effective when administered by continuous infusion compared to treatment at 6-h intervals (see also chapter 4). It appeared that the therapeutic effect of ceftazidime is dependent on the maintenance of antibiotic concentrations in plasma during the entire treatment interval. This observation may be explained by the gradual and relatively dose-independent bactericidal effect of ceftazidime seen in the lungs of leukopenic rats after administration of a single dose. Despite a variation in serum peak concentrations from 1 to 48 µg/ml at the ceftazidime doses used no major differences in bacterial killing rate were observed. This relatively dose-independent antibacterial effect in vivo corresponds to the concentration-independent bacterial killing pattern of ceftazidime in vitro at concentrations around the MBC. In contrast to ceftazidime the therapeutic efficacy of gentamicin was not related to the mode of administration. With gentamicin intermittent treatment resulting in relatively high peak concentrations at relatively long intervals seems to be permitted without loss of efficacy. This was confirmed by the high and dose-dependent bacterial killing rate of gentamicin in lungs of leukopenic rats after intravenous administration of a single dose. Also in vitro, the bacterial killing rate of gentamicin was strongly related to the concentration used. These results show that the activity of gentamicin is strongly dependent on the peak concentration. The therapeutic efficacy of ciprofloxacin, when administered at 6-h intervals, was slightly increased compared to continuous treatment. This confirms the dose-dependent and very rapid killing of K.pneumoniae in lungs of leukopenic rats after a single intravenous injection of ciprofloxacin. At all doses tested ciprofloxacin exerted a high bacterial killing rate which increased with increasing doses. A similar pattern of bactericidal effect was found in vitro.

Besides the bacterial killing rate and the dose-response effect, the rate of bacterial regrowth after exposure to antibiotic may also be an important determinant for the dosage-regimen-dependent therapeutic effect of antibiotic [26, 119]. In this respect we found no major differences between the three antibiotics used. In vitro, a PAE of the K.pneumoniae strain for the antibiotics was not observed. In addition, in vivo patterns of bacterial regrowth in the lung after administration of antibiotic were not substan-

tially different.

Our experimental data with regard to the impact of the antibiotic dosage schedule of ceftazidime and gentamicin on the therapeutic efficacy are in accordance with those of other investigations with respect to treatment of gram-negative infections with ß-lactams or aminoglycosides [18, 49, 52, 57, 79, 101, 120]. Gerber et al. demonstrated that ticarcillin was more effective in reducing the number of Pseudomonas aeruginosa organisms in thighs of leukopenic mice when the interval of administration was reduced from 3 h to 1 h [52]. In contrast the activity of gentamicin was not dependent on the frequency of injection. Using the same infection model, they also demonstrated that the in vivo efficacy of gentamicin and also of netilmicin was mainly dependent on the total dose administered and not on the mode of administration either as a single bolus injection or at very short intervals to mimic human serum kinetics [49]. On the other hand the ß-lactams ticarcillin and ceftazidime were more effective when administered in fractional doses which resulted in longer periods of active drug levels in serum as compared to administration as a single dose. Other investigators using models of thigh muscle infection and pneumonia in mice both caused by K.pneumoniae showed that the efficacy of the ß-lactam cefazolin was related to the time period serum drug levels remained above the MIC of the infecting strain [79, 120]. On the other hand, the efficacy of gentamicin was correlated with the total dose administered irrespective the frequency of administration. The conclusion derived from these experiments that the efficacy of ß-lactam antibiotics increased with increasing dosing frequency, whereas the in-vivo efficacy of aminoglycosides was independent on the dosage interval, was also confirmed in an experimental thigh muscle infection in mice due to K.pneumonia [57] and pneumonia or endocarditis due to P.aeruginosa in rats or rabbits, respectively [101]. In contrast to these findings there are some experimental studies suggesting that the in vivo activity of the aminoglycoside tobramycin is dependent on the antibiotic dosage schedule used [67, 96]. In these experiments, however, long intervals of treatment were used when the short elimination half life of tobramycin in small experimental animals is taken into account. With respect to the quinolones we are not aware of studies concerning the impact of the dosage schedule of quinolones on the therapeutic efficacy in experimental infections. However, one study comparing the efficacy of the ß-lactam BMY-28142 and the quinolone ciprofloxacin in a model of pseudomonas endocarditis, shows that although after administration of single doses the

ratio of concentrations of both antibiotics to the MBC at the site of infection were similar, ciprofloxacin was superior to BMY after a 5 day treatment at 8-h intervals [64]. In order to obtain the same effect as compared with ciprofloxacin BMY had to be administered for a longer period at shorter intervals. This finding is in accordance with our observation that ciprofloxacin was very effective in contrast to ceftazidime when rats were treated at 6-h intervals.

Our results with respect to the kinetics of bactericidal activity of ceftazidime are in accordance with the slow and relatively dose-independent bacterial killing rate as observed generally for ß-lactams in vivo [49, 52, 64, 118] and in vitro [3, 55, 93, 125]. Our observations with respect to gentamicin are also in agreement with the findings of several investigators demonstrating a fast and dose-dependent bactericidal activity of aminoglycosides in vivo [49, 52] as well as a concentration-dependent bacterial killing rate in vitro [3, 10, 125]. Of interest in this respect is the study by Moore et al. who demonstrated a strong correlation between the peaklevel to MIC ratio in serum of a number of aminoglycosides and the clinical outcome of the treatment of gram-negative infections at 8-h intervals [87]. With respect to ciprofloxacin several studies demonstrate a comparable dose-dependent bactericidal activity in vivo [64], a dose response effect which was also observed in vitro [20, 64].

In general, in accordance with our findings in vitro, gram-negative bacteria are found to regrow immediately after ß-lactam concentrations have fallen below active levels. Contrary to our observations their regrowth is suppressed for variable periods of time after exposure to aminoglycosides and quinolones [19, 91]. However, PAEs of aminoglycosides in vitro appeared to be of short duration (exept for pseudomonas) and may vary between the individual strains [19]. There is also strain variation with respect to the presence of a PAE for quinolones against K.pneumoniae [76]. Like in vitro no major differences were found between the individual drugs tested with respect to the bacterial regrowth in lungs of leukopenic rats.

The data derived from the experimental infection models may have implications for the treatment of infections in leukopenic patients. In general, ß-lactams are administered at intervals and, taken into account the elimination half-lives, in relatively high doses resulting in high peak concentrations in order to provide active drug levels for most of the infecting strains until the next dose. The experimental studies show that for successful treatment with ß-lactams it is of great importance that

antibiotic concentrations are maintained at a certain level during the period of treatment. High peak concentrations in plasma do not contribute to therapeutic effect. The data do further suggest that lower daily doses of ß-lactams might even be permitted provided these are administered more frequently or ß-lactams with long half lives are used. This might result in improvement of therapeutic effect, and may be favorable in terms of cost-effectiveness. Bodey et al. showed that patients with persistent granulocytopenia responded better to a regimen including, besides carbenicillin, the constant infusion of cefamandol as compared to intermittent administration of this drug [15]. In a recent clinical study in patients with severe infections Hoepelman et al. demonstrated that a single daily treatment with the highly protein-bound drug ceftriaxone resulting in prolonged plasma concentrations, was more effective than a combination of gentamicin plus cefuroxime [59]. With regard to the aminoglycosides and the quinolones there is no experimental evidence that the dosage schedule is an important determinant for efficacy. In view of the fact that the bactericidal effect of these antibiotics is strongly related to the peak concentration, and their bacterial killing is very fast, it seems worthwile to investigate whether less frequent dosing with increased doses of these agents may be equally effective or even more efficacious in the immunocompromised host. In this respect several studies are of interest demonstrating that animal nephrotoxicity [6, 43, 58, 101, 103, 123] or ototoxicity [123] decreased with decreasing frequency of administration, despite higher individual doses of aminoglycosides. Less frequent dosing was not associated with loss of efficacy [58, 101] or resulted even in increased efficacy [101, 123]. Several human studies did also not reveal nephro- or ototoxicity despite relatively high doses of aminoglycosides given at 24-h intervals, which regimens proved to be effective [21, 40, 101].

CHAPTER 6

THERAPEUTIC EFFECT OF ANTIBIOTICS IN RELATION TO THE DURATION OF INFECTION AND THE BACTERIAL GROWTH RATE

efficacy of ceftazidime, gentamicin, and ciprofloxacin in leukopenic rats with Klebsiella pneumoniae pneumonia and septicemia

INTRODUCTION

In a previous study described in chapter 5 it appeared, using three different classes of antibiotics, that not all bacteria were killed following an intravenous injection at 1 h after inoculation of the lung with Klebsiella pneumoniae. It is not clear which factors are responsible for the survival of bacteria at the site of infection. One explanation may be the absence of active antibiotic concentrations due to rapid drug elimination. Another possible factor is the occurrence of phenotypic resistance, a phenomenon reviewed by Greenwood [56]. Due to phenotypic heterogeneity within a susceptible bacterial population a fraction of the population may be resistant to concentrations of antibiotic that kill most of the bacteria. The role of both factors in survival of K.pneumoniae in the lung was investigated in the present study. Bacterial survival at the site of infection may also be the result of a change in the antibiotic susceptibility of the infecting strain or of changes in the activity of antibiotic due to local environmental factors, which as a consequence is only expressed in vivo. In the present study it was investigated whether the number of persisting bacteria varied with the duration of infection and, in addition whether the persisting bacteria could be eliminated by administration of an antibiotic with a different mode of action.

One factor that probably progressively changes during the course of infection is the bacterial growth rate, which is substantially decreased in established infection [39, 82]. As changes in bacterial growth rate were not observed in the experiments in chapter 5, it was object of the present study to investigate whether changes in bacterial growth rate may have consequences for the antibacterial activity of antibiotic. For ciprofloxacin a considerable bactericidal activity against bacteria in the so-called stationary phase of growth has been observed in vitro [20, 127]. In contrast several ß-lactams, including ceftazidime, appeared not to be bactericidal

against nongrowing bacteria [23]. Therefore, in the present study the bactericidal activity of ciprofloxacin versus ceftazidime was determined in relation to the bacterial growth rate in vitro and in vivo in lungs of leukopenic rats. In addition the effect of delay of antibiotic administration from 1 h until 24 h after bacterial inoculation on the bactericidal effect of ciprofloxacin versus ceftazidime was investigated (data about the impact of the bacterial growth rate on the bactericidal activity of ciprofloxacin versus ceftazidime are also described in appendix paper III). To study the impact of delay of start of treatment on the therapeutic activity of antibiotics, ciprofloxacin or ceftazidime was administered for a period of four days at 6-h intervals, starting at different times after bacterial inoculation with K.pneumoniae.

MATERIALS AND METHODS

Materials and methods that are described in chapter 2: MBC determination, quantitation of numbers of bacteria in the left lung and the blood, antibiotic administration as well as evaluation of in vivo bactericidal activity and therapeutic efficacy. The antibiotics used were ceftazidime, gentamicin, and ciprofloxacin.

For determination of the antibacterial activity in vitro in relation to the bacterial growth rate, bacterial inocula were prepared as follows. A stationary phase culture was diluted to 10^7 CFU/ml in Iso-Sensitest broth and reincubated at 37°C. Logarithmically growing or stationary phase bacteria were obtained at 2 h before or 2 h after the stationary growth phase was achieved, respectively. Bacteria were centrifuged, washed, and suspended to their original number per milliliter in fresh warm (37°C) broth to which antibiotic was added, and reincubated.

For population analysis related to antibiotic susceptibility CFU counts were determined simultaneously on antibiotic-free Iso-Sensitest agar plates and on agar plates containing various concentrations of the antibiotic studied.

The in-vivo activity of the selected antibiotics was examined in leukopenic rats in different ways. Bactericidal activity of antibiotic was examined after intravenous injection at various times after inoculation of the left lung with K.pneumoniae organisms in the logarithmic or stationary growth phase. The numbers of bacteria in the lung were determined at various inter-

vals after administration of antibiotic. The Mann-Whitney test was used for statistical analysis. Therapeutic efficacy of antibiotics was measured in terms of PD_{50} values after administration at 6-h intervals, for a period of 4 days, starting at different times after bacterial inoculation.
For the determination of antibiotic concentrations in lung tissue, the left lung was removed, exsanguinated, and homogenized in saline in a Potter-Elvejhem homogenizer. After centrifugation for 2 min at 10,000 x g, the supernatant was collected and used for the determination of antibiotic concentrations. With the use of diagnostic sensitivity test agar (Oxoid) and an Escherichia coli test strain susceptible to 0.025 µg of ciprofloxacin and 0.2 µg of ceftazidime per ml, all tests were done by a standard large-plate agar diffusion procedure [5]. Standard samples were prepared as follows. Ciprofloxacin and ceftazidime in standard concentrations were added to homogenates of lungs of untreated rats. After incubation for 1 h at 37°C and centrifugation for 2 min at 10,000 x g, supernatants were collected. Samples of 100 µl were assayed. The Mann-Whitney test was used for statistical analysis.

RESULTS

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats. The effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats is shown in figure 6.1. One intravenous injection of 3 mg/kg at 1 h after bacterial inoculation killed a large part of the bacterial population, although not to the same extent for all three antibiotics. Whereas ceftazidime and gentamicin killed about 90% of the bacterial population, ciprofloxacin killed about 99%. A second injection of 3 mg/kg at the time of the maximum bactericidal effect resulted in a slight, but not significant further reduction in numbers of bacteria for the three drugs tested.
Population analysis related to antibiotic susceptibility of the three antibiotics did not reveal the selection of a resistant bacterial subpopulation at the time the maximum bactericidal effect was reached (figure 6.2). In contrast, a shift to a more susceptible population was observed.
Figure 6.3 shows the bactericidal activity of ceftazidime, gentamicin, and ciprofloxacin in lungs of leukopenic rats after administration of 3 mg/kg at different times after inoculation with K.pneumoniae. At the time of

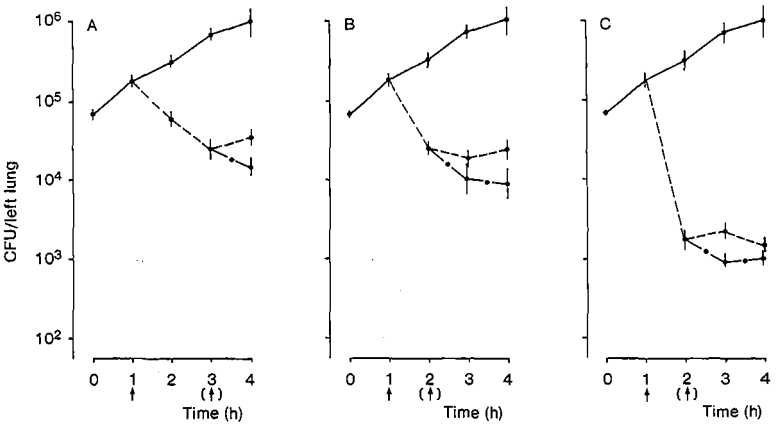


Figure 6.1 Numbers of K.pneumoniae in lungs of untreated leukopenic rats (———) and after intravenous administration of 3 mg/kg of ceftazidime (A), gentamicin (B), or ciprofloxacin (C). Ceftazidime was administered at 1 h (– – –) or at 1 h and 3 h (—·—), and gentamicin or ciprofloxacin at 1 h (– – –) or at 1 h and 2 h (—·—) after inoculation of lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

injection of antibiotic the mean number of bacteria per left lung was similar in all experiments (2.2 x 10^5 CFU). Delay of antibiotic administration resulted in a decrease in bacterial killing for all three drugs tested. Killing by ceftazidime continued during 3 h except when administered at 1 h after inoculation. When injection of ceftazidime was delayed from 0 h until 1 h after inoculation with K.pneumoniae bacterial killing decreased significantly ($P < 0.05$). Further delay of administration of ceftazidime resulted in a slight but not significant decrease of bactericidal activity ($P > 0.05$). For gentamicin delay of administration also resulted in a decrease of bacterial killing in the lung. This decrease was significant for each successive hour injection of gentamicin was postponed ($P < 0.05$). For ciprofloxacin similar results were obtained to those for gentamicin.
Although after administration of 3 mg of ceftazidime/kg at 1 h a second dose of 3 mg of ceftazidime/kg at 2 h was not able to reduce the bacterial numbers in the lung significantly (figure 6.4A), a second dose of 3 mg of ciprofloxacin/kg at 2 h resulted in a significant additional bacterial killing ($P < 0.05$) (figure 6.4B). Similar results were obtained for admini-

stration of ciprofloxacin after gentamicin (figures 6.5A and 6.5B).

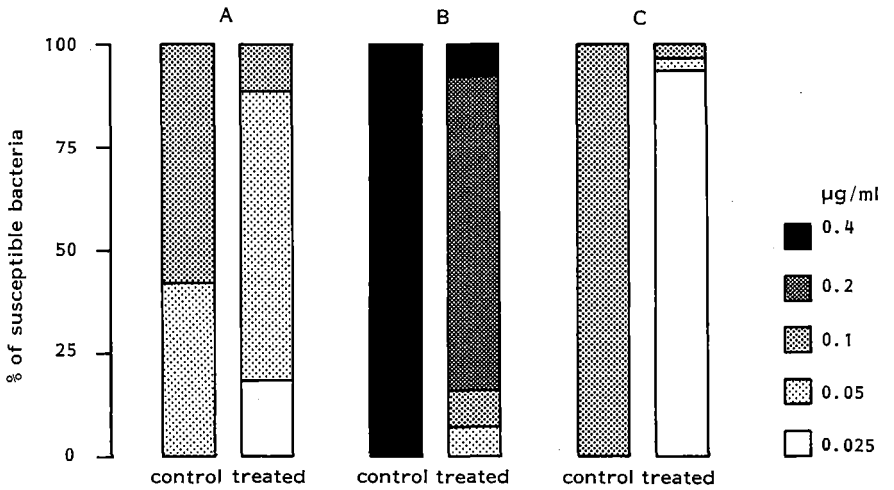


Figure 6.2 Susceptibility distribution of K.pneumoniae. Bacteria were isolated from lungs of leukopenic rats immediately before administration of antibiotic (control). Bacteria from treated rats were isolated 2 h after injection of 3 mg of ceftazidime/kg (A), 1 h after injection of 3 mg of gentamicin/kg (B), or 1 h after injection of 3 mg of ciprofloxacin/kg (C). Antibiotics were administered intravenously at 1 h after inoculation of the lungs with 8 x 10^4 of logarithmically growing K.pneumoniae.

Effect of ceftazidime and ciprofloxacin against K.pneumoniae in broth and in the lungs of leukopenic rats in relation to the bacterial growth rate. The effect of ciprofloxacin and ceftazidime against K.pneumoniae in broth in relation to the bacterial growth rate is shown in figure 6.6. Logarithmically growing bacteria in broth were killed by both ciprofloxacin and ceftazidime dependent on the concentration used (figure 6.6A and 6.6B, respectively). At all concentrations tested, the bacterial killing rate of ciprofloxacin was higher than that of ceftazidime. In addition, administration of ciprofloxacin resulted in immediate killing, whereas bacterial killing by ceftazidime was more gradual and time-dependent. When the K.pneumoniae organisms were in the stationary phase of growth, ceftazidime at the same concentrations was no longer bactericidal (figure 6.6D). On the contrary, ciprofloxacin was still able to kill the bacteria effectively (figure 6.6C), although the bacterial killing was about 10 to 100-fold lower than observed for the logarithmically growing bacteria.

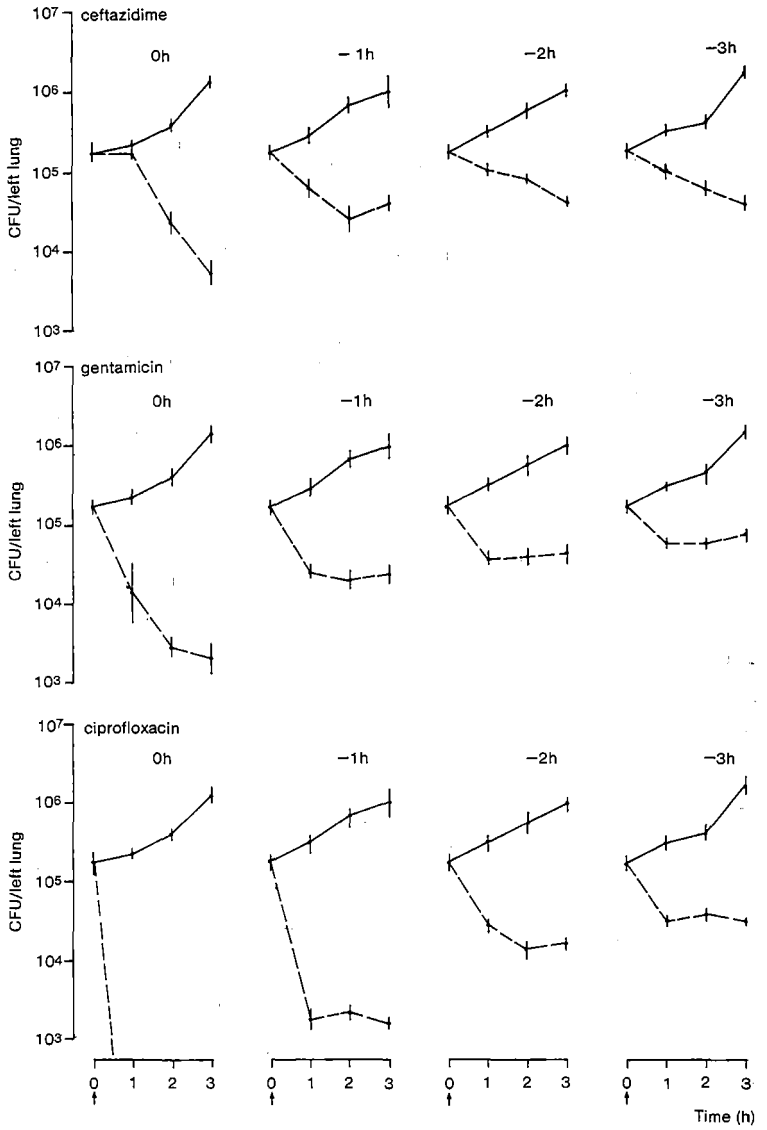


Figure 6.3 Numbers of K.pneumoniae in lungs of untreated leukopenic rats (——) and at various times after intravenous administration of 3 mg/kg of ceftazidime, gentamicin, or ciprofloxacin (— — —). Lungs of leukopenic rats were inoculated with K.pneumoniae in the logarithmic phase of growth at the same time (0 h) or at various times before injection with antibiotic (-1 h, -2 h, or -3 h). Antibiotic was injected at 0 h. At the time of antibiotic administration the mean number of bacteria per left lung was 2.2×10^5 in all experiments. Each point represents the geometric mean ± SEM for five rats.

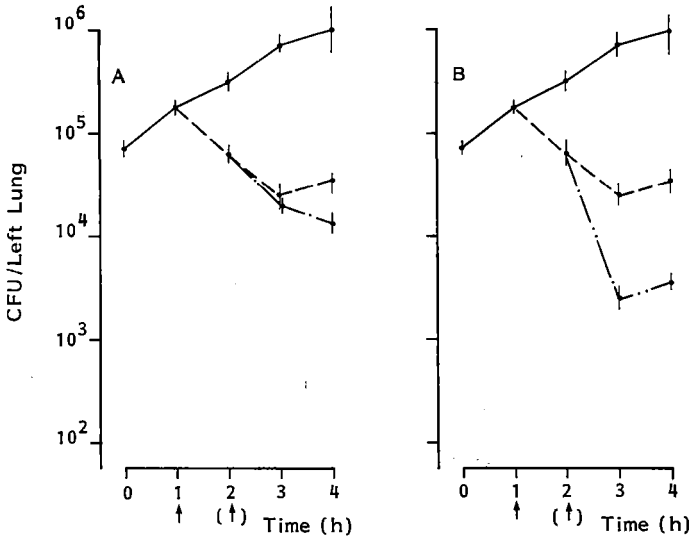


Figure 6.4 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and at different times after administration of ceftazidime at 1 h (— — —) or ceftazidime at 1 h and 2 h (—·—) (A), of ceftazidime at 1 h (— — —) or ceftazidime at 1 h and ciprofloxacin at 2 h (—··—) (B) after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth. Antibiotic was administered in doses of 3 mg/kg.
Each point represents the geometric mean ± SEM for five rats.

The bactericidal effect of ciprofloxacin and ceftazidime against K.pneumoniae in relation to the bacterial growth rate in vivo is shown in figure 6.7. Lungs were inoculated with 8 x 10^4 logarithmically growing K.pneumoniae (figure 6.7A). At 1 h after inoculation, when the bacterial numbers in the lungs had increased about threefold up to 2.2 x 10^5 CFU, ciprofloxacin or ceftazidime was administered at a dose of 3 mg/kg. The actively growing bacteria were killed by both antibiotics, although ciprofloxacin was significantly more effective ($P < 0.05$). Inoculation of the lungs with 2.2 x 10^5 K.pneumoniae in the stationary phase of growth (figure 6.7B) resulted in a 1-h delay of bacterial growth in the lungs. When antibiotic was administered immediately after inoculation, it was observed that ceftazidime at a dose of 3 mg/kg was not able to kill the bacteria during the first hour, whereas ciprofloxacin was highly bactericidal during the first hour after inoculation. From the moment that bacterial numbers in

the lung increased, bacterial killing by ceftazidime was also observed.

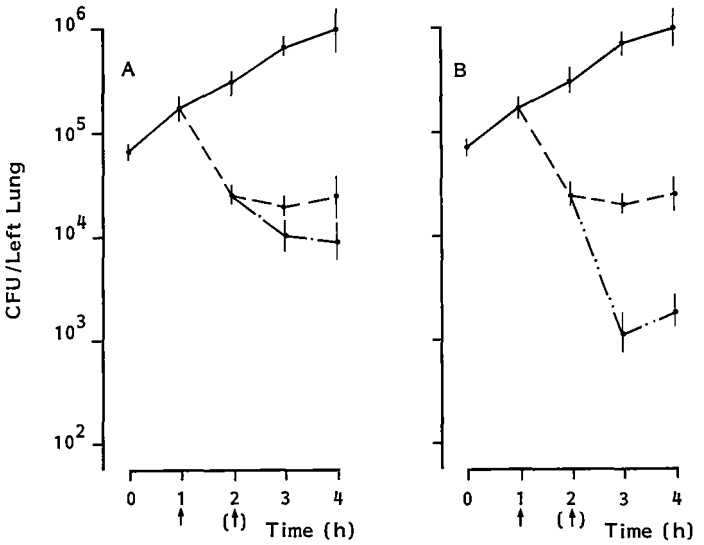


Figure 6.5 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and at different times after administration of gentamicin at 1 h (– – –) or gentamicin at 1 h and 2 h (—·—) (A), of gentamicin at 1 h (– – –) or gentamicin at 1 h and ciprofloxacin at 2 h (—··—) (B) after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth. Antibiotic was administered in doses of 3 mg/kg.
Each point represents the geometric mean ± SEM for five rats.

The effect of delaying antibiotic administration from 1 h up to 24 h after bacterial inoculation upon the antibacterial activity of ciprofloxacin and ceftazidime is shown in figure 6.8. At 1 h after inoculation with logarithmically growing bacteria the bacteria in the lungs had increased 3-fold in number and were killed by both antibiotics (figure 6.8A). At 24 h after inoculation in established lung infection, bacterial numbers had increased 4000-fold and tissue necrosis was observed. All rats had bacteria in the blood. Ciprofloxacin administered at that time still had an antibacterial effect in the lungs (figure 6.8B) and in addition sterilized the blood of all rats. In contrast, ceftazidime was not effective in the lungs and blood cultures remained positive. This difference in efficacy could not be explained by higher concentrations of ciprofloxacin in lungs of leukopenic

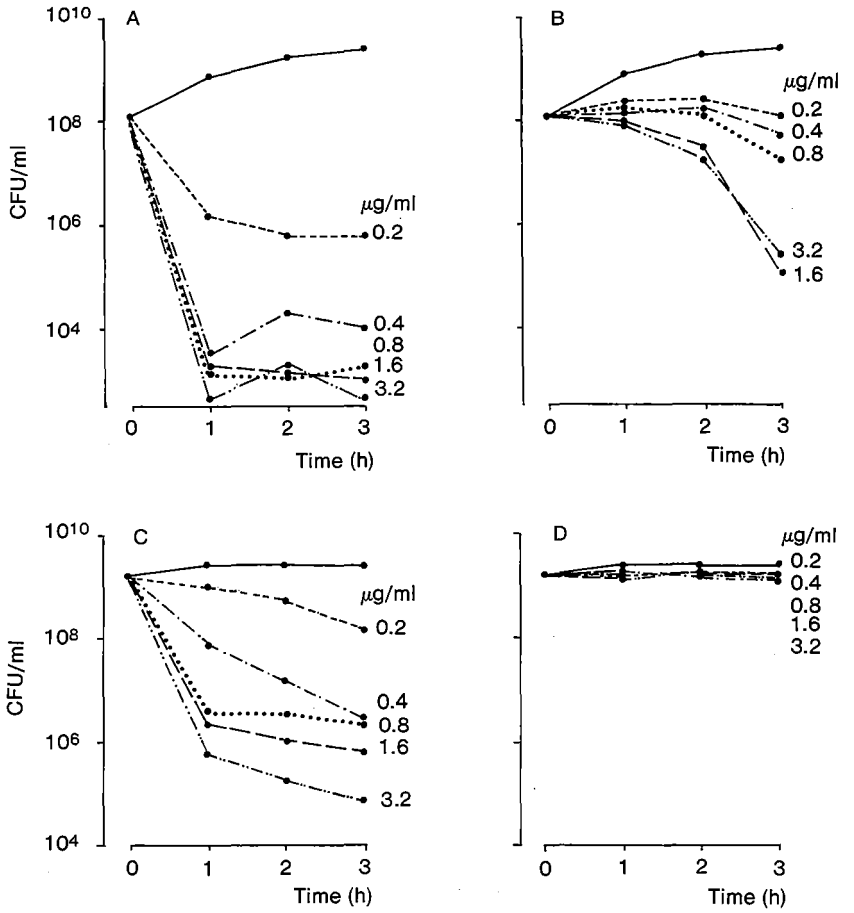


Figure 6.6 Effect of ciprofloxacin (A) or ceftazidime (B) against K.pneumoniae in the logarithmic phase of growth and of ciprofloxacin (C) or ceftazidime (D) against K.pneumoniae in the stationary phase of growth in broth. The solid line represents bacterial growth without antibiotic.

rats as concentrations of ciprofloxacin and ceftazidime in lung tissue at various times after the intravenous administration of 3 mg/kg at 24 h after bacterial inoculation were not significantly different (table 6.1).
Finally the effect of delay of start of treatment on the therapeutic efficacy of ceftazidime and ciprofloxacin is shown in table 6.2. The PD_{50}s of ceftazidime after a four-day treatment at 6-h intervals, when starting at 5, 12 or 24 h after bacterial inoculation increased significantly ($P < 0.05$), and were 24.4, 52.2 and 120 mg/kg/day, respectively. Delay of

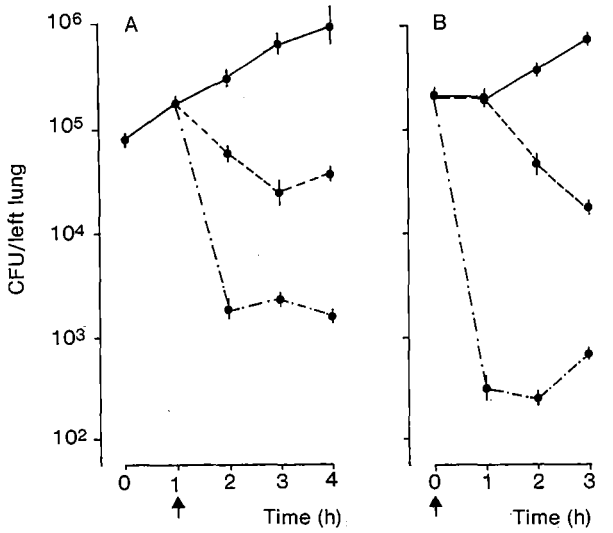


Figure 6.7 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and after intravenous administration of ciprofloxacin (—·—) or ceftazidime (– – –) at 3 mg/kg at 1 h after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth (A), or immediately after inoculation of the lungs with 2.2 x 10^5 K.pneumoniae in the stationary phase of growth (B).
Each point represents the geometric mean ± SEM for five rats.

start of treatment with ciprofloxacin from 5 h to 12 h after inoculation resulted in a significant increase of the PD_{50} value from 3.3 to 12.2 mg/kg/day ($P < 0.05$). Delay up to 24 h resulted in a further, although not significant, reduction in activity as shown by an increase of the PD_{50} up to 21.2 mg/kg/day ($P > 0.05$).

DISCUSSION

The experiments described in chapter 5 show that single doses of ceftazidime, gentamicin, or ciprofloxacin did not kill all K.pneumoniae organisms in the lungs of leukopenic rats. The experiments described in this chapter were performed in order to elucidate some factors that are responsible for this observation. Firstly, it was investigated whether this incapacity of

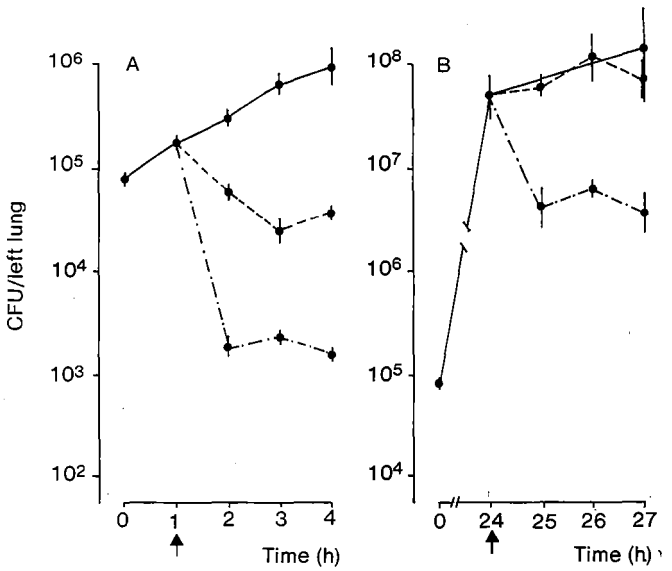


Figure 6.8 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———), and after intravenous administration of ciprofloxacin (—·—) or ceftazidime (– – –) at 3 mg/kg at 1 h (A) (same as figure 6.7A) or at 24 h (B) after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

Table 6.1. Concentrations of ciprofloxacin and ceftazidime in lung tissue at various times after intravenous administration of 3 mg/kg to infected[a] leukopenic rats

Antibiotic	Drug concn (μg/g of lung tissue)[b] at the following times (min) after administration:			
	15	30	60	90
ciprofloxacin	1.33 ± 0.79	1.80 ± 0.89	1.21 ± 0.28	0.56 ± 0.16
ceftazidime	1.72 ± 1.58	2.15 ± 0.60	1.16 ± 0.59	0.94 ± 0.40

[a]Antibiotics were administered 24 h after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae.

[b]Each value represents the mean ± SD for five rats.

Table 6.2 Efficacy of ceftazidime and ciprofloxacin treatment schedules[a] in leukopenic rats

Start of treatment (h)	PD_{50} (mg/kg/day)	
	ceftazidime	ciprofloxacin
5	24.4	3.3
12	52.2	12.2
24	120.0	21.2

[a]Antibiotics were administered at 6-h intervals over a period of four days starting at different times after bacterial inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae.

antibiotic to kill all bacteria in the lung was the result of insufficient concentrations of antibiotic at the site of infection due to rapid elimination of antibiotic. This appeared to be not an important factor as a second injection of the same antibiotic at the time of the maximum bactericidal effect of the first dose resulted only in a slight but not significant additional decrease in bacterial numbers. In addition the persisting bacteria appeared not to represent a less susceptible subpopulation selected after antibiotic administration. On the contrary, a shift to a more susceptible population was observed. Several investigators demonstrated, however, the selection of more resistant bacteria after exposure to antibiotic [10, 11, 51, 53, 88, 89]. It has for instance been shown that exposure of enterobacteriaceae to aminoglycosides [88] as well as Pseudomonas aeruginosa to aminoglycosides or quinolones [10, 11, 51] resulted in the selection of bacteria with increased resistance to the drugs used. These bacterial subpopulations were responsible for the bacterial regrowth in an in vitro kinetic model [10, 11]. Regrowth of bacteria with increased resistance were also found after treatment of a thigh muscle infection due to P.aeruginosa with gentamicin [53]. It has been suggested that these resistant subpopulations may be of clinical relevance [51, 89]. An explanation for the absence of resistant bacteria in our experiments may be that the occurrence of less susceptible bacteria only becomes substantial after multiple injections of antibiotic.

In the experiments described in this study it was investigated by which factors the size of the persisting bacterial population was determined. In chapter 5 it was shown that the class of antibiotic used and in addition

for gentamicin and ciprofloxacin the dose that was used were important determinants in this respect. In this chapter it is shown that bacteria that could not be eliminated by a second injection of ceftazidime or gentamicin were killed to a large extent by ciprofloxacin. The additional killing was similar when ciprofloxacin was administered either after ceftazidime or after gentamicin. The total bactericidal effect, however, did not exceed that of ciprofloxacin alone, so total bacterial killing was determined by the antibiotic with the highest bactericidal activity. It was also demonstrated for the three drugs tested that the number of persisting bacteria increased with the duration of the infection. This effect was most pronounced for ciprofloxacin. The diminished bacterial killing in the lungs of leukopenic rats due to delay of antibiotic administration could not be explained by an inoculum effect because up at the time of administration bacterial numbers were similar in all experiments. Neither was a reduction in bacterial growth rate responsible for the effect. Similar results were obtained by Totsuka et al. [115]. In his experiments intensive treatment with several antibiotics did not result in eradication of K.pneumoniae from thighs of neutropenic mice. Persisting bacteria were fully susceptible to the drugs used. Delay of administration for only 1 h resulted in a substantial decrease of bacterial killing. In a model of pseudomonas endophthalmitis in rabbits it was demonstrated that delay of treatment from 24 up to 48 h after infection resulted in loss of bactericidal activity of ciprofloxacin, gentamicin and imipenem [29]. This appeared not to be related to a decrease of susceptibility of bacteria, as they were fully susceptible to drugs tested after subculture in vitro. Recently, similar observations were reported by Davey for the same drugs not only in vivo in a pseudomonas granuloma-pouch model in rats, but also in in-vitro batch cultures in which the drugs became less bactericidal with progressive incubation [30]. A possible explanation for our observations and those of others may be that changes in the environment induce phenotypic resistance to antibiotic [52], which cannot be demonstrated after bacterial isolation and standard susceptibility testing. The exact nature of these changes is not clear, but one of the important factors may be that of nutrient depletion [17]. This may be responsible for a change of the bacterial phenotype and subsequent altered antibiotic susceptibility. Another responsible factor may be a diminished activity of antibiotic due to local factors such as altered pH or oxygen depletion.

In further experiments the relevance of our observations with regard to the

therapeutic efficacy of ceftazidime and ciprofloxacin was investigated. As mentioned, early injection of a single dose ciprofloxacin killed a greater part of the bacterial population than ceftazidime, however, the activity of both drugs decreased with delay of administration. These findings related to the antibacterial activity of antibiotics in the lung were reflected in the therapeutic activity of both antibiotics. Ciprofloxacin appeared to be more effective than ceftazidime when administered intermittently, and the efficacy of both drugs decreased with delay of start of treatment. These data are in accordance with the observation that institution of empirical antibiotic treatment in granulocytopenic patients starting at the first signs of fever has reduced the morbidity and mortality due to gram-negative infections [121]. This, however, may not apply to gram-positive infections in those patients. In a recent study Rubin et al. demonstrated that waiting for the laboratory results, identifying gram-positive organisms and antibiotic susceptibility, did not affect the effectiveness of treatment with vancomycin [104].

Another factor that probably progressively changes during the course of infection is the bacterial growth rate [39, 82]. As bacterial growth rates did not substantially change in the experiments described above, other studies were performed in order to investigate the relevance of this factor for the activity of antibiotic treatment. Rapid and strong bactericidal activity of ciprofloxacin against nongrowing P.aeruginosa and E.coli has been demonstrated in vitro [20, 127], whereas a lack of bactericidal activity of several ß-lactam antibiotics, including ceftazidime, has been observed against nongrowing enterobacteriaceae [23]. Therefore we studied the bactericidal activity of ciprofloxacin versus ceftazidime against K.pneumoniae in relation to the bacterial growth rate. We demonstrated in vitro that ciprofloxacin was able to kill K.pneumoniae that were not actively growing, whereas ceftazidime was not. However, compared to its activity against logarithmically growing bacteria, the bactericidal effect of ciprofloxacin was reduced. In vivo ciprofloxacin was highly bactericidal against nongrowing bacteria in the lung, whereas bacterial killing by ceftazidime occurred only from the moment of bacterial multiplication.

It has to be realized that the experimental set-up used is not directly related to the clinical situation. However, in an infection that is well established slowly growing or nongrowing bacteria form a substantial part of the total bacterial population. Therefore the activity of ciprofloxacin versus ceftazidime was also compared in K.pneumoniae pneumonia and

septicemia in relation to the time of start of administration. Antibiotics administered 1 h after bacterial inoculation resulted in a decrease in bacterial numbers cultured from the lungs of leukopenic rats by both agents, although ciprofloxacin was more effective than ceftazidime. Administration of the same doses of antibiotic at 24 h, when the infection had further progressed, reduced the activity of both ciprofloxacin and ceftazidime. At that time ciprofloxacin was still bactericidal, but ceftazidime was not. Besides a reduced bacterial growth rate other factors such as inaccessibility of bacteria, local inactivation of antibiotics or changes in the susceptibility of bacteria may also have contributed to the diminished bacterial killing observed. The capacity of ciprofloxacin to kill nongrowing bacteria in contrast to ceftazidime appeared not to be of major importance for its therapeutic efficacy in this experimental pneumonia/septicemia model, as the therapeutic effect of both antibiotics decreased with delay of start of treatment. However, this capacity may be relevant in treating infections in which bacteria grow very slowly or do not multiply at all. This is confirmed by the successful treatment with ciprofloxacin of experimental osteomyelitis, an infection that could not be cured by intensive treatment with ß-lactams or aminoglycosides [94].

In conclusion, the experimental data obtained in our model of K.pneumoniae pneumonia and septicemia in leukopenic rats with antibiotics of three different classes underline the need to start antibiotic treatment of serious infection as early as possible, because the number of bacteria persisting in the lung despite administration of antibiotic increases with the duration of the infection, as shown for three antibiotics with different mode of action. The use of highly bactericidal drugs may be of advantage in this respect. Whereas ciprofloxacin was bactericidal against nongrowing K.pneumoniae in vitro and in vivo in lungs of leukopenic rats, no evidence was found that this property is of major importance for the treatment infection that has been in progress for a certain time.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Clinical experience indicates that infections in patients who are granulocytopenic due to underlying malignant disease or to treatment with anticancer chemotherapeutic agents are often difficult to treat. In these patients antibiotics are given empirically and blindly at the first signs of fever.

New antibiotics of different classes have been developed and introduced for the treatment of serious infections. Their selection for clinical use is partly based on their in vitro antibacterial activity in relation to currently-used antibiotics. MIC/MBC values are frequently used as a parameter for relative in vitro activity [60]. However, the application of recently-developed antibiotics with high bactericidal activity in vitro against frequently isolated pathogens, does not always result in a greater clinical effect in patients. Different strategies for the potentiation of antimicrobial treatment in severe infections were outlined by O'Grady [54]. One approach involves intensification of the antibiotic treatment in terms of modification of the dosage schedule. Because of the complicated, fulminant and life-threatening character of infections in granulocytopenic patients, clinical trials in this respect are only permissible when based on sufficient supporting data derived from studies in experimental infections. Reproducible, well-characterized infectious disease models allow for comparison of antibiotics or antibiotic dosage schedules under similar conditions of intensity and duration of infection. The present experimental study intends to contribute to a better understanding of the role of the antibiotic dosage schedule for its therapeutic efficacy.

An experimental infection model of Klebsiella pneumoniae pneumonia and septicemia in immunocompetent or leukopenic rats was used, and is described in chapter 2. This model was selected because in leukopenic patients septicemia frequently occurs, originating from different sources, for instance a localized infection such as pneumonia. In addition, K.pneumoniae is one of the pathogens that are frequently recovered [68, 74, 99, 116, 122, 124]. This model appears to have a certain clinical relevance, as many characteristics of the infection are also observed in human lung infections caused by K.pneumoniae [80]. In immunocompetent rats after inoculation of

the lung the numbers of bacteria increased. The histological features of the pneumonic lesion are characterized by an outer margin with edema fluid that mainly contained gram-negative bacteria. More to the center of the lesion a cellular infiltrate composed of gram-negative bacilli, polymorphonuclear leukocytes and a few macrophages is observed. In the center of the lesion edema-filled alveoli are packed with leukocytes and tissue necrosis, small lung abscesses containing numerous polymorphonuclear leukocytes and large numbers of gram-negative bacilli are present. Host defenses were impaired by means of injections with cyclophosphamide (CY), which resulted in a substantial reduction of the number of circulating granulocytes and monocytes. The granulocyte plays an important role in the defense against K.pneumoniae infections in the lung in the early phase of infection as demonstrated by Rehm et al. [102]. This correlates with the observations that compared to immunocompetent rats, in leukopenic rats bacterial multiplication in the lung was very rapid, resulting in septicemia at an early stage of infection. A substantial cellular infiltrate in the lung of leukopenic rats was absent despite the presence of large numbers of gram-negative bacilli. Alvolear macrophages also phagocytize encapsulated K.pneumoniae organisms but for optimal function they require the presence of antiserum [117]. CY acts in an aspecific way. It is suggested that CY does not influence the granulocyte function [61]. Other investigators found that alveolar macrophage numbers were not reduced by CY administration [62, 83, 97], whereas their function was reduced only after prolonged exposure [62] but not after a single dose [83, 97].

In this infectious disease model the role of the antibiotic dosage schedule as a determinant of therapeutic activity was investigated. This subject was studied in relation to various factors such as the severity of infection, the presence of host defense factors and the kinetics of antibacterial activity in vitro and in vivo in lungs of leukopenic rats. Antibiotics were chosen from three different classes that are important for the treatment of severe infections in the immunocompromised host, i.e. the ß-lactams, the aminoglycosides and the quinolones. From the respective classes ceftazidime, gentamicin, and ciprofloxacin were chosen because the in vitro susceptibility of the K.pneumoniae strain used in terms of MBC values was similar for these antibiotics allowing a good evaluation of the therapeutic results. In addition, these antibiotics are considered to be clinically relevant on the basis of their in vitro activity and antimicrobial spectrum.

In chapter 3 the effect of different dosage schedules of the ß-lactam antibiotic ceftazidime on the therapeutic efficacy is examined in immunocompetent rats. It appeared in these experiments that continuous administration was more effective than intermittent treatment at relatively long intervals. The difference between both dosage schedules increased with increasing severity of infection. In addition, in order to obtain a substantial therapeutic effect steady state ceftazidime concentrations in serum needed not to exceed the MBC value of ceftazidime for the klebsiella strain used.

In chapter 4 it was demonstrated that the therapeutic efficacy of ceftazidime decreased as a result of impairment of host defenses due to severe leukopenia. This decrease was again strongly dependent on the dosage schedule, in the sense that ceftazidime was moderately less effective when administered by continuous infusion but substantially less effective when given intermittently. In other words the dosage schedule plays an important role especially when host defense mechanisms are impaired. It appeared that under those circumstances the activity of ceftazidime was highly dependent on the maintenance of appropriate antibiotic concentrations in serum during the entire treatment period. Results of other experiments suggest that treatment with ceftazidime has to be continued until the return of circulating leukocytes.

In chapter 5 it is described that the importance of the antibiotic dosage schedule as a determinant of therapeutic activity in leukopenic rats did not necessarily apply to other classes of antibiotics. Whereas for therapeutic activity of ceftazidime continuous antibiotic concentrations in plasma are more important than relatively high peak concentrations at intervals, for gentamicin and ciprofloxacin the schedule of antibiotic administration did not have a major impact on the therapeutic activity. Gentamicin was equally effective with both modes of administration, whereas ciprofloxacin demonstrated a slightly increased efficacy with intermittent dosage. As the efficacy of the different treatment schedules was evaluated in terms of daily doses of antibiotic, the data obtained for gentamicin and ciprofloxacin suggest that a dosage schedule resulting in relatively long periods of low antibiotic concentrations in serum are permitted, provided the serum peak concentrations are sufficiently high. The differences in therapeutic efficacy in relation to the antibiotic dosage schedule correlate with the differences between the three antibiotics with regard to their kinetic of antibacterial activity in vitro and in the infected lungs

of leukopenic rats. The bacterial killing rate of K.pneumoniae in vitro and in lungs of leukopenic rats by ceftazidime was gradual and not strongly dependent on the concentration or the dose administered, despite a large variation in plasma peak levels after injection of the various doses. In contrast, the bacterial killing rate effected by gentamicin or ciprofloxacin was high and dose-dependent. Another difference is that the bactericidal activity of ceftazidime was more dependent on the time of exposure to antibiotic, whereas bacterial killing by gentamicin and ciprofloxacin was complete within a short time period after administration. These observations may explain why the therapeutic efficacy of ceftazidime in our model was mainly dependent on the maintenance of active concentrations during treatment. Relative high concentrations did not substantially contribute to its activity. In contrast, due to their high and dose-dependent bactericidal effect gentamicin and ciprofloxacin could be administered at relative long intervals without loss of efficacy. For the latter two agents therapeutic efficacy was mainly determined by the total dose administered. In various models of experimental gram-negative infections, especially those concerning animals with impaired host defenses, similar results were obtained with respect to the importance of the dosage schedule for the efficacy of antibiotics of different classes for the in vivo activity [18, 49, 52, 57, 64, 79, 101, 120]. Differences in efficacy are found to be in agreement with differences in kinetics of antibacterial activity between several antimicrobial agents belonging to the ß-lactams, the aminoglycosides and quinolones [3, 10, 20, 49, 52, 55, 64, 93, 118, 125]. These findings also correlate with the observation that with respect to ß-lactam antibiotics from a number of pharmacokinetic parameters the time period serum drug levels remained above the MBC correlated the best with in vivo activity [79, 120]. On the contrary, for aminoglycosides it was demonstrated that the area under the concentration time curve mainly determined the in vivo efficacy, irrespective of the frequency of administration. With respect to the quinolones we are not aware of studies showing which pharmacokinetic parameters determine the in vivo activity of these antibiotics. In the present study ciprofloxacin behaved like gentamicin with regard to the bactericidal activity against K.pneumoniae in vitro as well as in vivo, and no major differences were found between both drugs with regard to the impact of the dosage schedule on their therapeutic efficacy.

Similar conclusions with respect to the dosage schedule-dependent efficacy

related to the class of antibiotic may be drawn with respect to infections caused by gram-positive bacteria [2, 24, 44, 45, 113].

Besides the kinetics of antibacterial activity, another factor that may be relevant with respect to the dosage schedule dependent activity of antibiotics is the post-antibiotic effect (PAE) [26]. After the early observations on the PAE of Bigger in 1944 related to the persistent suppression of bacterial growth of staphylococci and streptococci after removal of penicillin G, Parker and Luse also demonstrated that bacterial growth of Staphylococcus aureus in vitro was suppressed for a certain time period after removal of penicillin G [9, 95]. Later on studies on the PAE were extended to various drug-pathogen combinations [19]. These studies allowed to formulate a number of conclusions on the occurrence of the PAE [19, 26]. ß-Lactams induce PAEs of various duration against gram-positive cocci in vitro, whereas no or very short PAEs were found for gram negative bacilli. On the other hand inhibitors of protein synthesis, including aminoglycosides, as well as quinolones [76, 91] induce PAEs of moderate to long duration against both types of micro-organisms. In general PAEs in vitro correlate well with PAEs in vivo, except for streptococci that did not show PAE with ß-lactams in experimental infections [119]. In the present study a PAE in vitro was not found for K.pneumoniae with ceftazidime neither with gentamicin or ciprofloxacin (chapter 5). This is not completely in agreement with what is generally found for gram-negative bacteria. As observed by others, such differences may be explained by strain variations [19, 76]. Like in vitro, also in lungs of leukopenic rats there were no major differences between ceftazidime, gentamicin, and ciprofloxacin with respect to regrowth of K.pneumoniae after a single injection of the respective drugs. The regrowth patterns in lungs may have been influenced by sub-MIC concentrations of antibiotics which may explain that in contrast to the in vitro observations bacteria did not multiply for a relatively long period of time. This suggests that in our model differences in PAE are not contributing to the differences in antibiotic dosage schedule-dependent therapeutic efficacy as observed between ceftazidime, gentamicin and ciprofloxacin.

Although our experimental data on the efficacy of the different treatment schedules are in agreement with the findings of most other investigators there are some discrepancies. With respect to the studies in immunocompetent animals some of them may be attributed to the simultaneous action of the host defense system and antimicrobial drugs in curing the infection

as suggested by results obtained by others for ß-lactams [2, 49] and also found by us (chapter 4). Antibiotic free periods that remain without much consequence in immunocompetent animals resulted in a severe loss in efficacy when host defenses were impaired. Regarding the aminoglycosides the use of long treatment intervals relative to the short half lives of antibiotics in small animals may explain a suggested dosage schedule-dependent activity [67, 96]. Few authors stated that the efficacy of ß-lactams can be optimalized, by injecting these drugs at short intervals [48, 107]. This would bacteria allow to grow, and as a consequence make them more susceptible to the lethal action of cell-wall active antibiotics. However, this result is not necessarily conflicting with that found by others as not all investigators used more than two treatment intervals. A more substantial exception may be the treatment of pneumococcal meningitis with ß-lactam antibiotics. In a rabbit meningitis caused by S.pneumoniae it was demonstrated that the efficacy of ampicillin was not dependent on the frequency of administration, despite drug levels in CSF that were below the MBC for about 50% at the longest dosage interval used [112]. It appeared that the most important determinant of in vivo activity was the peak level of ampicillin in CSF. This correlated with the observations that with several ß-lactams bacterial killing rates of S.pneumoniae in CSF increased with increasing doses to levels up to 30 times the MBC although in vitro no concentration dependent bactericidal activity was found [111]. Also the slow decline of ampicillin levels in CSF may have contributed to the efficacy of intermittent treatment with ampicillin.

The pattern of bacterial killing in vitro and in vivo appeared to be related to the class of antibiotics as studied and discussed for ß-lactams, aminoglycosides and quinolones. There may, however, be also some difference in bacterial killing rates between several ß-lactams [22, 63]. Also ß-lactam kill characteristics may not always be the same in vitro and in vivo as demonstrated in experimental meningitis [111]. However, in general increase of bacterial killing rates occurs over a much larger concentration range with aminoglycosides and quinolones than with ß-lactams, and in addition their maximum killing rates are much higher. These differences in bactericidal kinetics are probably related to differences in mode of action. Whereas ß-lactams inhibit cell-wall synthesis, and aminoglycosides inhibit protein synthesis, the quinolones do interact with the bacterial enzyme DNA-gyrase. An intact gyrase activity is essential for bacterial survival [110].

Another factor that may be important with regard to the impact of the dosage schedule on the in vivo efficacy is the extravascular penetration of antibiotics [78]. This subject has been studied by several investigators [4, 7, 8, 38, 77, 98]. Some experiments revealed higher extravascular antibiotic levels obtained with intermittent dosage compared to continuous infusion [4, 7]. However, the study periods used were relatively short. Other studies show that, at steady state conditions, levels of antibiotics of different classes in Visking chambers implanted in rabbits were similar or even higher after continuous infusion than after administration at intervals [38, 98]. Some investigators have studied the mode of drug administration on the extravascular penetration concurrently with its impact on the bactericidal activity [8, 77]. Whereas continuous infusion of several antibiotics including ß-lactams produced at equilibrium conditions similar mean levels in infected fibrin clots as compared to intermittent dosage, the resulting bactericidal effect was rather poor. This may be due to the slow drug penetration after continuous infusion, resulting in a substantial delay of killing of that part of the bacterial population, that is difficult to reach. This is suggested by the higher bactericidal effect after intermittent dosage and an even better bacterial killing after administration of the same total dose as a single bolus injection. These studies, however, concern relatively short treatment periods and the use of an artificial extravascular compartment which is extremely difficult to penetrate [4].

The studies in chapter 6 show that a single dose or multiple doses of antibiotic were unable to kill all bacteria at the site of infection. The number of bacteria persisting in the lung was dependent on the class of antibiotic used, and when gentamicin and ciprofloxacin were used, also on the dose administered. The incomplete eradication of bacteria was not due to insufficient antibiotic concentrations at the site of infection resulting from rapid drug elimination. In addition, persisting bacteria did not represent a less susceptible subpopulation selected after antibiotic administration as found by others [10, 11, 51, 53, 88]. Probably local factors present in the microenvironment of the infection are responsible for diminished susceptibility of the bacteria or reduced activity of the antibiotics used [56]. These factors can, however, not be analyzed ex vivo. The fact that diminished bacterial killing was due to local factors was confirmed by the observation that the number of persisting bacteria increased with increase of the duration of infection as demonstrated for

antibiotics of three different classes. Corresponding to these observations the therapeutic activity of ceftazidime and ciprofloxacin also decreased after a four-day treatment when start of treatment was delayed.
One factor that may be of major importance for the bactericidal activity of an antibiotic in established infection is the capacity to kill slow growing nongrowing bacteria. Bacterial growth rates are substantially decreased in established infection [39, 82]. Ciprofloxacin and ceftazidime differ extremely with regard to their growth rate-dependent bactericidal activity. Whereas ciprofloxacin is able to kill bacteria that are not actively growing, ceftazidime and almost all other ß-lactams are not [20, 23, 127]. In the present study the difference in bacterial growth rate-dependent bactericidal activity between ceftazidime and ciprofloxacin was also demonstrated in vitro as well as in vivo in lungs of leukopenic rats. These findings are in accordance with data of other investigators [20, 23, 127]. The bactericidal activity of most of the ß-lactams tested in a Chemostat (a continuous culture system in which the bacterial growth rate can be determined by the flow rate at which fresh medium is added) appeared to be proportional to the bacterial growth rate [23]. Ciprofloxacin was able to kill Escherichia coli in the so-called stationary phase of growth [20, 127]. However, in this experimental pneumonia/septicemia model the capacity of ciprofloxacin to kill nongrowing bacteria in contrast to ceftazidime appeared not to be of major importance for its therapeutic efficacy, as the therapeutic effect of both antibiotics decreased in infection that was well established. Substantial bactericidal activity that is not influenced by the bacterial growth rate may become increasingly important when treating infections where bacteria grow extremely slow or do not grow at all, such as osteomyelitis.

Implications for the clinical use of antibiotics

The experimental studies with regard to the impact of the dosage schedule on the therapeutic efficacy of antibiotic in immunocompromised animals, described in this thesis as well as in studies by other investigators come to more or less similar conclusions, although the studies differ in choice of infection model and animal species, of bacterial strain, of antibiotic and dosage schedule and, of parameter to measure efficacy. These animal data may provide a basis for clinical investigations on the treatment of serious infections in leukopenic patients with regard to mode of administration, start of treatment and duration of treatment.

Regarding dosage schedule, ß-lactam antibiotics are in general administered at intervals and, taken into account their elimination half-lives, in relatively high doses resulting in high plasma peak concentrations in order to provide active drug levels for most of the infecting strains until the next dose. The experimental studies show that for successful treatment with ß-lactams high peak concentrations in plasma do not contribute to a therapeutic effect, as long as antibiotic concentrations are maintained at a certain level during treatment. This suggests that lower daily doses of ß-lactams might even be used provided they are administered more frequently or ß-lactams with long half lives, for instance due to high serum protein binding, are used. This might result firstly in improvement of therapeutic effect, and secondly this may be favourable in terms of cost-effectiveness, as the newer ß-lactams are relatively expensive. To date only few clinical data are available on this subject. Bodey et al. showed that patients with persistent granulocytopenia responded better to a regimen including, besides carbenicillin, the constant infusion of cefamandol as compared to intermittent administration of this drug [15]. In a recent clinical study in patients with serious systemic infections Hoepelman et al. demonstrated that a single daily dose of ceftriaxone resulting in prolonged plasma concentrations due to high protein binding, was more effective than a combination of gentamicin plus cefuroxime [59]. Recently two case reports were described in which neutropenic patients were cured from Pseudomonas aeruginosa infection with continuously infused ceftazidime where intermittent treatment with the same antibiotic had failed [27]. Additional clinical data are needed to offer more support for the use of treatment schedules with constant levels of ß-lactams.

Aminoglycosides are in general administered intermittently in doses resulting in non-toxic plasma peak concentrations. There is no experimental evidence that the dosage schedule is an important determinant for efficacy. This is confirmed by clinical studies of Feld et al. who demonstrated no superiority of either continuous or intermittent treatment of febrile granulocytopenic patient with aminoglycosides [41, 42]. In view of the fact that the bactericidal effect of these antibiotics is strongly dependent on the peak concentration, and the bacterial killing is very fast, intermittent administration at relatively long intervals seems permissible without loss of efficacy. The dose-dependent bactericidal effect which is important with intermittent treatment was confirmed by clinical studies described by Moore et al. [87]. They showed a strong correlation between the peak level

to MIC ratio in serum of a number of aminoglycosides and the clinical outcome of the intermittent treatment of gram-negative infections. In relation to the peak concentration-dependent bactericidal effect individualizing aminoglycoside dosing in patients seems to be of major importance not only for the prevention of toxicity, but also for more effective treatment. Another question that needs further investigation is whether less frequent dosing with increased doses, causing infrequent relatively high serum concentrations, may be equally effective or even more efficacious in the immunocompromised host, and in addition may be less toxic. In this respect several studies are of interest, demonstrating that animal nephrotoxicity [6, 43, 58, 101, 103, 123] or ototoxicity [123] decreased with decreasing frequency of administration, despite higher individual doses of aminoglycosides. Less frequent dosing was not associated with loss of efficacy [58, 101] or resulted even in increased efficacy [101, 123]. Several human studies did also not reveal nephro- or ototoxicity despite relative high doses of aminoglycosides given at 24-h intervals, which regimens proved to be effective [21, 40, 101].

The newer and recently introduced quinolones are administered intermittently. From the few experimental studies performed at this moment there are no indications that the dosage schedule is an important determinant for efficacy. No clinical data are available to support the superiority of either continuous or intermittent administration of these drugs. However, their high bactericidal effect which is dependent on the dose suggest that administration at intervals may be the most appropriate treatment schedule.

Regarding start of treatment, the experimental data obtained with K.pneumoniae for the three antibiotics underline the need to start antibiotic treatment as early as possible, because the number of bacteria persisting in the infected tissue in spite of antibiotic administration increases with delay of treatment. The use of highly bactericidal drugs may be of advantage in this respect. The results do not give indications that the capacity of an antibiotic to kill nongrowing or slowly growing bacteria may be of importance for the outcome of treatment. This capacity, however, may be of relevance for the treatment of infections in which bacteria grow extremely slowly or do not multiply at all.

Regarding the duration of antibiotic treatment in relation to the duration of leukopenia, the experimental data obtained for ceftazidime suggest that antibiotic treatment should preferably be continued until the return of circulating leukocytes. However, prolonged antibiotic treatment may cause

an increase in adverse side-effects. This must be outweighed against the risk on development of infections. In addition, the main factor determining the risk on development of serious infection in persistent granulocytopenic patients after stopping antibiotic treatment may be the number of days they are afebrile after initial therapy as suggested by DiNubile [31].

Implications for in vitro testing of antibiotics

Besides the clinical implications for antibiotic treatment of patients, the experimental studies may also be of importance for the methods of determination of antimicrobial susceptibility in vitro. For in vitro determination of antibacterial activity of recently-developed antibiotics in relation to currently-used antibiotics MIC/MBC values are frequently used as a parameter. The present experimental study shows that despite similar MIC/MBC values, antibiotics of different classes may differ extremely with respect to their short-term killing capacity of bacteria. The therapeutic efficacy of antibiotic, administered at different dosage schedules appeared to correlate well with the in vitro short-term killing pattern of the antibiotic, but not with the MIC/MBC value as an in vitro parameter. In other words, determination of short-term bacterial killing patterns seems to be far preferable for comparison of antibacterial activity of antibiotics. The data on the bactericidal kinetics will have to be compared with the pharmacokinetic behaviour of the antibiotics tested as the outcome of treatment depends mainly on these parameters [32]. PAE determinations may give still more information.

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SUMMARY

Clinical experience indicates that infections in patients who are granulocytopenic due to underlying malignant disease or its treatment with anticancer chemotherapeutic agents are often difficult to treat. Despite the application of recently-developed antibiotics with high bactericidal activity in vitro against frequently isolated pathogens, a substantial percentage of treatments fail. One approach to improve therapeutic efficacy involves intensification of the antibiotic treatment in terms of modification of the dosage schedule. Because of the complicated, fulminant and life-threatening character of infections in granulocytopenic patients, clinical trials in this respect are only permitted when based on sufficient supporting data derived from studies in experimental infections. The present experimental study intends to contribute to a better understanding of the role of the antibiotic dosage schedule as a determinant of therapeutic efficacy. This subject was studied in relation to various factors such as the severity of infection, the presence of host defense factors, and the kinetics of antibacterial activity in vitro and in vivo in the lungs of leukopenic rats.

The experimental design is described in chapter 2. An experimental infection model of Klebsiella pneumoniae pneumonia and septicemia in immunocompetent or leukopenic rats was used, which appeared to have a certain clinical relevance. Antibiotics were chosen from three different classes that are important for the treatment of severe infections in the immunocompromised host, i.e. the ß-lactams, the aminoglycosides and the quinolones. From the respective classes ceftazidime, gentamicin, and ciprofloxacin, were chosen because the in vitro susceptibility of the K.pneumoniae strain used in terms of MBC values was similar for these antibiotics allowing a good evaluation of the therapeutic results. In addition, these antibiotics are considered to be clinically relevant on the basis of their in vitro activity and antimicrobial spectrum.

In chapter 3 it is shown that continuous administration of ceftazidime was more effective than intermittent treatment at relatively long intervals. The difference between both dosage schedules increased with increasing severity of infection. In addition, in order to obtain a substantial therapeutic effect steady state ceftazidime concentrations in serum needed not to exceed the MBC value of ceftazidime for the Klebsiella strain used.

In chapter 4 it is demonstrated that the therapeutic efficacy of ceftazidime decreased as a result of impairment of host defenses due to severe leukopenia. This decrease was again strongly dependent on the dosage schedule in the sense that ceftazidime was moderately less effective when administered by continuous infusion but substantially less effective when given intermittently. In other words the dosage schedule plays an important role especially when host defense mechanisms are impaired. It appeared that under those circumstances the activity of ceftazidime was highly dependent on the maintenance of appropriate antibiotic concentrations in serum during the entire treatment period. Results of other experiments suggest that treatment with ceftazidime has to be continued until the return of circulating leukocytes.

Chapter 5 describes that the importance of the antibiotic dosage schedule as a determinant of therapeutic activity of ceftazidime in leukopenic rats did not necessarily apply to other classes of antibiotic. Whereas for its therapeutic activity continuous antibiotic concentrations in serum are more important than relatively high peak concentrations at intervals, for gentamicin and ciprofloxacin the schedule of antibiotic administration did not have a major impact on the therapeutic activity. Gentamicin was equally effective with both modes of administration, whereas ciprofloxacin demonstrated a slightly increased efficacy with intermittent dosage. As the efficacy of the different treatment schedules was evaluated in terms of daily doses of antibiotic, the data obtained for gentamicin and ciprofloxacin suggest that a dosage schedule resulting in relatively long periods of low antibiotic concentrations in serum are permissible , provided the serum peak concentrations are sufficiently high. The differences in therapeutic efficacy in relation to the antibiotic dosage schedule correlate with the differences between the three antibiotics with regard to their kinetic of antibacterial activity in vitro and in the infected lungs of leukopenic rats. The bacterial killing of K.pneumoniae by ceftazidime was gradual and not strongly dependent on the concentration or the dose administered, despite a large variation in serum peak levels after injection of the various doses. In contrast, the rate of bacterial killing achieved by gentamicin or ciprofloxacin was high and dose-dependent. Another difference is that the bactericidal activity of ceftazidime was more dependent on the time of exposure to antibiotic, whereas bacterial killing by gentamicin and ciprofloxacin was complete within a short time period after administration. These observations may explain why the therapeutic efficacy of ceftazidime

in our model was mainly dependent on the maintenance of active concentrations in serum during treatment. Relatively high concentrations did not substantially contribute to its activity. On the contrary, due to their high and dose-dependent bactericidal effect gentamicin and ciprofloxacin could be administered at relative long intervals without loss of efficacy. For the latter two agents therapeutic efficacy was mainly determined by the total dose administered.

Besides the kinetics of antibacterial activity, another factor that may be relevant with respect to the dosage schedule-dependent activity of antibiotics is the post-antibiotic effect (PAE). In the present study a PAE in vitro was not found for K.pneumoniae with ceftazidime nor with gentamicin and ciprofloxacin (chapter 5). As in vitro, in lungs of leukopenic rats there were no major differences between ceftazidime, gentamicin, and ciprofloxacin with respect to regrowth of K.pneumoniae after a single injection of the respective drugs. The regrowth patterns in lungs may have been influenced by sub-MIC concentrations of antibiotics which may explain that in contrast to the in vitro observations bacteria did not multiply for a relatively long period of time. Thus in our model differences in the PAE appeared not to contribute to the differences in antibiotic dosage schedule-dependent therapeutic efficacy as observed for ceftazidime, gentamicin and ciprofloxacin.

The studies in chapter 6 show that a single dose or multiple doses of antibiotic were unable to kill all bacteria at the site of infection. The number of bacteria persisting in the lung was dependent on the class of antibiotic used and in the case of gentamicin and ciprofloxacin also on the dose administered. The incomplete eradication of bacteria was not due to insufficient antibiotic concentrations at the site of infection resulting from rapid drug elimination. In addition, persisting bacteria did not represent a less susceptible subpopulation selected after antibiotic administration. Probably local factors present in the microenvironment of the infection are responsible for diminished susceptibility of the bacteria or reduced activity of the antibiotics used. These factors can, however, not be analyzed ex vivo. The fact that the diminished bacterial killing was due to local factors was confirmed by the observation that the number of persisting bacteria increased with increase in the duration of infection, as demonstrated for antibiotics of three different classes. Corresponding to these observations concerning antibacterial activity in the lung a decrease in therapeutic efficacy due to delay of start of treatment was

shown for ceftazidime and ciprofloxacin.

One factor that may be of major importance for the bactericidal activity of an antibiotic in established infection is the capacity to kill slow growing or nongrowing bacteria. In the present study the difference in the bacterial growth rate-dependent bactericidal activity between ceftazidime and ciprofloxacin was demonstrated in vitro as well as in vivo in lungs of leukopenic rats. Whereas ciprofloxacin was able to kill bacteria that were not actively growing, ceftazidime was not. However, the capacity of ciprofloxacin to kill nongrowing bacteria in contrast to ceftazidime appeared not to be of major importance for its therapeutic efficacy in the experimental pneumonia/septicemia model, as the therapeutic effect of both antibiotics decreased in infection that was well established.

The experimental studies with regard to the impact of the dosage schedule on the therapeutic efficacy of antibiotic in immunocompromised animals, described in this thesis as well as by other investigators, come to more or less similar conclusion, although the studies differ in choice of infection model, of bacterial strain, of antibiotic and dosage schedule, of parameters to measure efficacy. These animal data may provide a basis for clinical investigations on the antimicrobial treatment of serious infections in leukopenic patients with regard to mode of administration, start of treatment and duration of treatment.

Regarding dosage schedule, in clinical practice ß-lactam antibiotics are in general administered at intervals and, taken into account their elimination half-lives, in relatively high doses resulting in high serum peak concentrations in order to provide active drug levels for most infecting strains until the next dose. The experimental studies show that for successful treatment with ß-lactams high peak concentrations in serum do not contribute to a therapeutic effect, as long as antibiotic concentrations are maintained at a certain level during the period of treatment. This suggests that lower daily doses of ß-lactams might even be used provided they are administered more frequently or ß-lactams with long half lives, for instance due to high serum protein binding, are used. This might result firstly in improvement of therapeutic effect and secondly this might be favourable in terms of cost-effectiveness, as the newer ß-lactams are relatively expensive.

Aminoglycosides are in general administered intermittently in doses resulting in non-toxic serum peak concentrations. There is no experimental evidence that the dosage schedule is an important determinant for efficacy. In view of the fact that the bactericidal effect of these antibiotics is

strongly dependent on the peak concentration, and the bacterial killing is very fast, intermittent administration at relatively long intervals seems permissible without loss of efficacy. In relation to the peak concentration-dependent bactericidal effect individualizing aminoglycoside dosing in patients seems to be of major importance, not only for prevention of toxicity, but also for effective treatment. Another question that needs further investigation is whether less frequent dosing with increased doses, causing infrequent relatively high serum concentrations, may be equally effective or even more efficacious in the immunocompromised host, and in addition may be less toxic.

The newer and recently introduced quinolones are administered intermittently. From the few experimental studies performed at this moment there are no indications that the dosage schedule is an important determinant for efficacy. Their high bactericidal effect which is dependent on the dose suggest that administration at intervals may be the most appropriate treatment schedule.

Regarding start of treatment, the experimental data obtained for the three antibiotics underline the need to start antibiotic treatment as early as possible, because the number of bacteria persisting in the infected tissue in spite of antibiotic administration, increases with delay of treatment. The use of highly bactericidal drugs may be of advantage in this respect. The results do not give indications that the capacity of an antibiotic to kill nongrowing or slowly growing bacteria may be of importance for the outcome of treatment.

Regarding the duration of antibiotic treatment in relation to the duration of leukopenia, the experimental data obtained for ceftazidime suggest that antibiotic treatment should be preferably continued until the return of circulating leukocytes.

Besides the clinical implications for antibiotic treatment of patients, the experimental studies may also be of importance for the methods of determination of antimicrobial susceptibility in vitro. The present experimental study shows that despite similar MIC/MBC values, antibiotics of different classes may differ extremely with respect to their short-term killing capacity of bacteria. The therapeutic efficacy of antibiotic, administered at different dosage schedules appeared to correlate well with the in vitro short-term killing pattern of the antibiotic, but not with the MIC/MBC value as an in vitro parameter. In other words, determination of short-term bacterial killing patterns seems to be far preferable for comparison of

antibacterial activity of antibiotics.

SAMENVATTING

Klinische ervaring heeft aangetoond dat infecties bij patienten die granulocytopenisch zijn als gevolg van maligniteiten of door toediening van chemotherapeutica vaak niet met succes zijn te behandelen. Ondanks de toepassing van recent ontwikkelde antibiotica met een goede bactericide werking in vitro ten opzichte van de frequent geïsoleerde bacteriële infectieverwekkers, faalt een aanzienlijk deel van de behandelingen. Een van de benaderingen om het therapeutisch effect te vergroten zou kunnen zijn, intensiveren van de antibioticumbehandeling door verandering van het doseringsschema. Vanwege het gecompliceerde en levensbedreigende karakter van infecties bij granulocytopenische patienten zijn klinische trials op dit punt alleen dan verantwoord wanneer ze gebaseerd zijn op voldoende ondersteunende gegevens verkregen uit dierexperimenteel onderzoek. Deze dierexperimentele studie beoogt bij te dragen tot een beter inzicht in het belang van het doseringsschema voor de therapeutische werkzaamheid van antibiotica. Deze vraagstelling werd bestudeerd in relatie tot verschillende factoren, zoals de ernst van de infectie, de aanwezigheid van afweerfactoren en de kinetiek van de antibacteriële werking in vitro en in vivo in longen van leukopenische ratten.

De experimentele aanpak is beschreven in hoofdstuk 2. Als experimentele infectie werd een pneumonie en sepsis veroorzaakt door Klebsiella pneumoniae in immunocompetente en leukopenische ratten gekozen, een infectie model dat een zekere klinische relevantie heeft. Antibiotica werden gekozen uit drie verschillende klassen die belangrijk zijn voor de behandeling van ernstige infecties bij patienten met verminderde weerstand, nl. de ß-lactams, de aminoglycosiden en de quinolonen. Van deze klassen werden respectievelijk ceftazidime, gentamicine en ciprofloxacin gekozen, omdat de in vitro gevoeligheid van de K.pneumoniae stam in termen van MBC-waarden gelijk was voor de drie antibiotica. Hierdoor werd een goede evaluatie van de behandelingsresultaten mogelijk gemaakt. Bovendien zijn deze antibiotica thans in de kliniek relevant vanwege hun in vitro werkzaamheid en antimicrobieel spectrum.

De studies beschreven in hoofdstuk 3 laten zien dat voor de therapeutische werkzaamheid van het ß-lactam antibioticum ceftazidime in immunocompetente ratten de wijze van doseren een bepalende factor is. Toediening door middel van continu infuus bleek effectiever dan intermitterende behandeling met

relatief lange intervallen. Het verschil in effectiviteit tussen beide doseringsschema's nam toe met toenemende ernst van de infectie. Bovendien bleek dat voor het verkrijgen van een therapeutisch effect het niet nodig was dat steady state concentraties in het serum de MBC-waarde van de Klebsiella stam voor ceftazidime overschreden.

Hoofdstuk 4 laat zien dat de therapeutische werkzaamheid van ceftazidime afnam als gevolg van vermindering van afweermechanismen in de vorm van een ernstige leukopenie. Deze afname in therapeutisch effect was opnieuw sterk afhankelijk van het doseringsschema, in die zin dat de afname slechts gering was wanneer ceftazidime via continu infuus werd toegediend, terwijl de effectiviteit drastisch verminderde bij intermitterende behandeling. Met andere woorden, het doseringsschema bleek in belangrijke mate bepalend te zijn voor de werkzaamheid van ceftazidime, met name wanneer de weerstand van de gastheer verminderd is. De werkzaamheid van ceftazidime bleek onder die omstandigheden voornamelijk afhankelijk van de permanente aanwezigheid van voldoende hoge antibioticumconcentraties in het serum gedurende de behandelingsperiode. Andere experimenten suggereren dat in de leukopenische gastheer behandeling met ceftazidime moet worden voortgezet totdat de circulerende leukocyten terugkeren.

In hoofdstuk 5 wordt beschreven dat het belang van het doseringsschema als bepalende factor voor de therapeutische werkzaamheid van het antibioticum in leukopenische ratten niet noodzakelijk van toepassing is op andere klassen van antibiotica. Terwijl voor het therapeutisch effect van ceftazidime permanent aanwezige antibioticumconcentraties in serum belangrijker waren dan relatief hoge piekconcentraties met intervallen, had voor gentamicine en ciprofloxacin het doseringsschema geen belangrijke invloed op de effectiviteit. Gentamicine was even effectief bij beide doseringswijzen, terwijl ciprofloxacin in geringe mate meer effectief was wanneer intermitterend toegediend. Aangezien de werkzaamheid van de diverse doseringsschema's werd geëvalueerd aan de hand van totale dagdoses van antibiotica, suggereren de gegevens betreffende gentamicine en ciprofloxacin, dat een doseringsschema waarin relatief lange perioden van lage antibioticum serumconcentraties toegestaan is, mits de piekconcentraties voldoende hoog zijn. De verschillen tussen de antibiotica met betrekking tot de invloed van het doseringsschema op de werkzaamheid komen overeen met hun verschillen betreffende de kinetiek van antibacteriële activiteit in vitro en in de geïnfecteerde longen van leukopenische ratten. De bactericide werking van ceftazidime was traag, maar ging door in de tijd en was niet sterk afhankelijk van de

gebruikte concentratie, c.q. dosis, ondanks dat piekconcentraties in serum na toediening van de gebruikte doseringen sterk uiteenliepen. Daarentegen was de bactericide werking van gentamicine en ciprofloxacin sterk, snel en gerelateerd aan de concentratie, c.q. de dosis. Deze waarnemingen kunnen een verklaring zijn voor het feit dat de beste therapeutische werkzaamheid van ceftazidime werd verkregen wanneer antibioticumconcetraties in serum blijvend aanwezig zijn. Relatief hoge concentraties dragen niet wezenlijk bij tot de effectiviteit van dit antibioticum. Daarentegen konden gentamicine en ciprofloxacin als gevolg van hun sterke en dosisafhankelijke werking intermitterend met relatief lange intervallen worden toegediend, zonder verlies aan effectiviteit. De therapeutische werkzaamheid van deze twee middelen werd voornamelijk bepaald door de totale toegediende dosis.
Een andere factor die naast de kinetiek van de antibacteriële werking mogelijk relevant is voor de doseringsschema afhankelijke werking van antibiotica is het post-antibiotic effect (PAE). In deze studie echter werd in vitro geen PAE gevonden voor de gebruikte K.pneumoniae stam met ceftazidime, noch met gentamicine en ciprofloxacin (hoofdstuk 5). Overeenkomstig de in vitro gegevens werden in longen van leukopenische ratten geen verschillen in hergroei van bacteriën gevonden na toediening van enkelvoudige doses van de verschillende antibiotica. De in vergelijking met in vitro tragere hergroei van bacteriën in de long is waarschijnlijk het gevolg van de aanwezigheid van lage antibioticumconcentraties onder de MIC-waarden. In het door ons gebruikte infectiemodel bleken verschillen in PAE niet bij te dragen tot de verschillen in doseringsschema-afhankelijke werkzaamheid gevonden voor ceftazidime, gentamicine en ciprofloxacin.
In hoofdstuk 6 werd aangetoond dat enkelvoudige of meervoudige injecties met antibiotica niet in staat waren alle bacteriën op de plaats van infectie te doden. Het aantal in de long overblijvende bacteriën was afhankelijk van de gebruikte klasse van antibiotica en was voor gentamicine en ciprofloxacin bovendien afhankelijk van de toegediende dosis. Dat niet alle bacteriën konden worden geëlimineerd was niet het gevolg van onvoldoende concentraties van antibiotica op de plaats van infectie als gevolg van een snelle eliminatie. Bovendien bleken de overblijvende bacteriën ook geen selectie van minder gevoelige bacteriën te vertegenwoordigen. Waarschijnlijk zijn factoren op de plaats van de infectie verantwoordelijk voor hetzij de verminderde gevoeligheid van de bacteriën, hetzij de verminderde activiteit van de antibiotica, factoren die echter ex vivo niet onderzocht kunnen worden. Het feit dat verminderde bacteriële killing het gevolg was van locale

factoren werd bevestigd door de waarneming dat het aantal overblijvende bacteriën toenam met toename van de duur van de infectie zoals aangetoond voor de 3 antibiotica van de 3 verschillende klassen. Overeenkomstig de waarnemingen met betrekking tot de antibacteriële activiteit in de long, nam de therapeutische werkzaamheid van ceftazidime en ciprofloxacin af als gevolg van uitstel van start van de behandeling.

Een factor die van groot belang kan zijn voor de bactericide werking van een antibioticum in een gevestigde infectie is het vermogen om langzaam groeiende of niet-groeiende bacteriën te doden. In de huidige studie werd het verschil in bacteriële groeisnelheid afhankelijke werking tussen ceftazidime en ciprofloxacin aangetoond zowel in vitro als in vivo in longen van leukopenische ratten. Terwijl ciprofloxacin in staat was bacteriën te doden die zich niet actief vermenigvuldigen, was ceftazidime hiertoe niet in staat. Echter het vermogen van ciprofloxacin om in tegenstelling tot ceftazidime niet-delende bacteriën te doden, bleek niet van groot belang te zijn voor de therapeutische werkzaamheid in het experimentele pneumonie/sepsis model, aangezien het therapeutisch effect van beide middelen in ongeveer dezelfde mate afnam in een gevestigde infectie.

De experimentele studies met betrekking tot de invloed van het doseringsschema op de therapeutische werkzaamheid van antibiotica in dieren met verminderde afweer, zoals beschreven in dit proefschrift en de studies van andere onderzoekers, komen min of meer tot gelijkluidende conclusies, hoewel deze verschillen in o.a. keuze van infectiemodel en diersoort, van bacteriestam, van antibioticum, doseringsschema en parameter voor effectiviteit. Deze dierexperimentele gegevens zouden een basis kunnen vormen voor klinische onderzoek aangaande de antibioticum behandeling van ernstige infecties bij leukopenische patienten met betrekking tot de wijze van toediening, start en duur van de behandeling.

In de kliniek worden ß-lactam antibiotica over het algemeen intermitterend toegediend en, rekening houdend met hun eliminatie halfwaardetijd, in relatief hoge doseringen, resulterend in hoge piekconcentraties in serum zodat de spiegels niet onder de werkzame concentratie voor de meeste infectieverwekkers komen vóór de volgende dosis. De experimentele studies tonen aan dat voor succesvolle behandeling van infecties met ß-lactam antibiotica, hoge piekconcentraties in het serum niet bijdragen tot het therapeutisch effect, zolang antibioticum concentraties op een bepaald niveau gehandhaafd blijven gedurende de behandelingsperiode. Deze resultaten suggereren dat lagere dagdoses van ß-lactam antibiotica gepermitteerd zijn,

mits ze meer frequent worden toegediend of ß-lactams met een langere halfwaarde tijd, als gevolg van b.v. hoge eiwitbinding, worden gebruikt. Dit zou kunnen resulteren enerzijds in een verbeterde therapeutische werkzaamheid en anderzijds in een verlaging van de kosten van antimicrobiële behandeling, aangezien de nieuwere ß-lactam antibiotica relatief duur zijn.

Aminoglycosiden worden in het algemeen intermitterend toegediend in doses, waarbij serum piekconcentraties geen toxiciteit tot gevolg hebben. Uit experimentele studies zijn geen aanwijzingen gevonden dat het doseringsschema een bepalende factor is voor de werkzaamheid van deze antibiotica. Gezien het feit dat de bactericide werking van deze middelen sterk afhankelijk is van piekconcentraties en bovendien erg snel is, lijkt intermitterende toediening met relatief lange intervallen verantwoord, zonder dat de effectiviteit afneemt. In relatie tot de piekconcentratie-afhankelijke werking van deze middelen lijkt het bepalen van individuele doseringen van aminoglycosiden voor de patient van groot belang, niet alleen om toxiciteit te vermijden, maar ook uit het oogpunt van effectiviteit. Daarnaast zou moeten worden onderzocht of minder frequente toediening van hogere doses resulterend in relatief hoge serumconcentraties met relatief grote intervallen even werkzaam of zelfs beter werkzaam is in de gastheer met verminderde weerstand, en daarnaast leidt tot verlaging van toxiciteit.

De nieuwere en recent geïntroduceerde quinolonen worden intermitterend toegediend. Van de weinige thans beschikbare experimentele studies betreffende deze middelen, zijn er geen die aantonen dat het doseringsschema een belangrijke determinant is voor de therapeutische werkzaamheid. De sterke en dosisafhankelijke bactericide werking van deze middelen suggereert dat intermitterende toediening ervan de meest geschikte is.

Met betrekking tot de start van de antimicrobiële behandeling onderstrepen de experimentele gegevens van deze studie verkregen voor de drie antibiotica het belang om antibioticumbehandeling zo vroeg mogelijk te beginnen, ongeacht de klasse van het antibioticum. Reden hiervan is dat het aantal bacteriën dat overblijft in het geïnfecteerde weefsel na de toediening van antibioticum toeneemt met toename van de duur van de infectie. Het gebruik van sterk bactericide middelen kan hierbij een voordeel zijn. Uit de resultaten worden geen aanwijzingen verkregen dat het vermogen van een antibioticum om niet- of langzaam groeiende bacteriën te doden belangrijk is voor het resultaat van de behandeling.

Met betrekking tot de duur van de antibioticumbehandeling in relatie tot de

duur van de leukopenie, suggereren de experimentele gegevens verkregen voor ceftazidime dat de behandeling moet worden voortgezet totdat de circulerende leukocyten terugkeren.

Naast de klinische implicaties met betrekking tot de behandeling van patienten, kunnen deze experimentele studies ook van belang zijn voor de bepalingsmethoden van gevoeligheid van bacteriën voor antibiotica in vitro. De huidige experimentele studie laat zien dat ondanks gelijke MIC/MBC-waarden, antibiotica van verschillende klassen aanzienlijk kunnen verschillen ten aanzien van hun bactericide werking op korte termijn. De therapeutische werkzaamheid van antibiotica op verschillende wijze gedoseerd, bleek goed te correleren met de bactericide werking op korte termijn, maar niet met de MIC/MBC-waarden als in vitro parameter. Met andere woorden het bepalen van de bactericide werking op korte termijn verdient de voorkeur bij het vergelijken van de antimicrobiële activiteit van verschillende antibiotica.

DANKWOORD

Graag wil ik iedereen bedanken die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift, met name:

mijn co-promotor Dr. Irma Bakker-Woudenberg voor haar grote inzet en enthousiasme bij de begeleiding van het onderzoek, voor het stimuleren om alle resultaten zo veel mogelijk op wetenschappelijke bijeenkomsten te presenteren en vooral ook voor het in mij gestelde vertrouwen,

mijn promotor Prof.dr. M.F. Michel voor het belangstellend en kritisch volgen van mijn onderzoek,

de commissieleden Prof.dr. J.W.M. van der Meer, Prof.dr. H.J. Neijens en Prof.dr. D. van der Waaij voor de beoordeling van het manuscript en hun waardevolle suggesties,

Marion van den Berghe-van Raffe voor haar grote inzet bij het uitvoeren van de vele experimenten, ook op ongemakkelijke tijden,

Ada Beukelman voor haar toewijding en nauwgezetheid waarmee ze het manuscript heeft vervaardigd en ook voor alle secretariaatswerkzaamheden tijdens mijn onderzoek,

Joke Vink-van den Berg, Yvonne Steinvoort, August Lokerse en Peter Fontijne voor hun onmisbare hulp bij uiteenlopende werkzaamheden, vaak ook op zeer vroege of late uren,

Tannie Mourik-Zijderveld, Bertus van der Klift en Victor Garritsen voor het verzorgen van alle materialen,

Wim van Vianen voor het verrichten van alle "koeriersdiensten",

de secretaresses Linda Harkes, Paula Jansen-Bogaars en Margreet Boeren-den Boer,

de stagiair Erik Colijn,

de statisticus Ir. Wim Hop,

Ed Lansbergen en medewerkers van het Centraal Proefdieren Bedrijf,

Cor van Dijk en medewerkers van het Audio Visueel Centrum,

Drs. Jan Branolte en Rob Zikkenheimer voor de prettige contacten tijdens mijn onderzoek,

Bayer Nederland B.V., Beecham Research Laboratories (Nederland) en Glaxo Pharmaceuticals Ltd. (Engeland) voor hun financiële ondersteuning van een deel van het onderzoek,

tot slot mijn ouders die mij de gelegenheid hebben geboden om een academische opleiding te volgen en Annet voor haar onmisbare steun bij alles.

CURRICULUM VITAE

De schrijver van dit proefschrift is geboren op 24 oktober 1954 te Roermond.

1973	Eindexamen gymnasium ß aan het Bisschoppelijk College te Roermond
1978	Kandidaatsexamen Biologie (B4)
1981	Doctoraalexamen Biologie Hoofdrichtingen: Moleculaire Biologie Experimentele Immunologie Bijvak : Electronenmicroscopische Structuuranalyse
mei 1981-september 1982	Militaire Dienst; Detachering bij de afdeling Levensmiddelenmicrobiologie en Zoönosen van het Rijksinstituut voor Volksgezondheid en Milieuhygiëne te Bilthoven.
december 1982-januari 1988	Aanstelling als wetenschappelijk assistent bij de afdeling Klinische Microbiologie en Antimicrobiële Therapie van de Erasmus Universiteit Rotterdam, alwaar het in dit proefschrift beschreven onderzoek werd verricht.
februari 1988-heden	Aanstelling als universitair docent bij de afdeling Klinische Microbiologie en Ziekenhuishygiëne van het Academisch Ziekenhuis Vrije Universiteit Amsterdam.

APPENDIX PAPER I

THERAPEUTIC EFFICACY OF CONTINUOUS VERSUS INTERMITTENT ADMINISTRATION OF CEFTAZIDIME IN AN EXPERIMENTAL KLEBSIELLA PNEUMONIAE PNEUMONIA IN RATS*

Robert Roosendaal, I.A.J.M. Bakker-Woudenberg, J.C. van den Berg, and M.F. Michel

ABSTRACT

An experimental Klebsiella pneumoniae pneumonia in rats was used to study the influence of continuous or of intermittent (8-hr intervals) administration of ceftazidime on therapeutic efficacy. Antimicrobial response was evaluated with respect to the calculated total daily dose that protected 50% of the animals from death (PD_{50}) until 16 days after termination of a four-day treatment. When antibiotic treatment was started 5 hr after bacterial inoculation, the PD_{50} values after continuous and after intermittent administration of ceftazidime were 0.36 and 1.42 mg/kg per day, respectively ($P < .001$). With a delay in the administration of the antibiotic to 34 hr after inoculation, the respective PD_{50} values were 1.08 and 13.06 mg of ceftazidime/kg/per day ($P < .001$). These studies show an improved therapeutic efficacy that increased with a delay in treatment when ceftazidime was administered by continuous infusion as compared with administration at 8-hr intervals. Continuous administration of PD_{50} doses of ceftazidime resulted in serum levels that were constantly below the MIC of the infecting Klebsiella strain.

INTRODUCTION

Various aspects of the use of continuous vs. intermittent administration of antibiotics have been studied by a number of investigators [1-7]. Most antibiotic dose schedules are empirical [8-9]. Antibiotic serum levels

*The Journal of Infectious Diseases (1985) **152**:373-378. Reprinted with permission. [Data related to the dosage of ceftazidime at 6-h intervals have been added to tables 1 and 2].

obtained after administration of the drug in relation to the MIC of the infecting strain form the basis for the development of dose regimens in most cases. In the past few years more attention has been directed to pharmacokinetic parameters of antimicrobial agents. Some studies deal with the effect of the mode of drug administration on the penetration of the extravascular compartment [2, 3, 5]. Only a few experimental infections and clinical trials deal with the efficacy of continuous or intermittent administration of antibiotics. These studies do not show a better therapeutic efficacy with either mode of administration [1, 4, 6, 7].
The present study was undertaken to investigate the in vivo activity of ceftazidime when administered intermittently i.m. or when administered by continuous infusion against experimentally induced Klebsiella pneumoniae pneumonia in rats. Treatment was started either 5 hr or 34 hr after bacterial inoculation in order to study whether the severity of infection resulting in tissue necrosis had an effect on the outcome of antibiotic treatment. The experimental model was selected because pneumonia and septicemia caused by K.pneumoniae and by other gram-negative bacilli are a major problem in patients undergoing anticancer chemotherapy [10-12].

MATERIALS AND METHODS

Animals. Female R strain albino rats (specific-pathogen free; bred at the Laboratory Animals Centre of Erasmus University Rotterdam) were used in all experiments. These rats were 14-18 weeks old and weighed 185-215 g.

Bacteria. A K.pneumoniae strain (capsular serotype 2) was used in these experiments. Inocula were prepared as previously described [13].

Pneumonia. Experimental pneumonia was produced as previously described by Bakker-Woudenberg [13]. In brief, rats were anesthetized with fluanisone (Hypnorm^{R}; Duphar, Amsterdam, The Netherlands) and pentobarbital (Abbott Laboratories; Ceva, Paris). The left main-stem bronchus was intubated and the left lung was inoculated with 0.02 ml of a saline suspension of K.pneumoniae containing 8×10^4 (6×10^4-10×10^4) cfu. Determination of the number of viable organisms in the left lung and in the blood, the method of obtaining pleural fluid for culture, and the course of untreated infection have been described earlier [13]. In brief, infection developed

within 24 hr and increased in severity afterwards. Animals died spontaneously, a mean of 5.1 ± 1.3 days (mean ± SD; group of 10 rats) after inoculation. The number of cfu in the left lung increased and on day 5 an average of 2.10^{10} cfu was reached. With the progression of the infection, an increasing number of animals had bacteria in blood and in pleural fluid.

Histology. The histological features of the pneumonic lesions were studied at 5 hr and 34 hr after infection by the following method. Lungs were fixed by injecting the trachea with 10% formalin under constant pressure (to reexpand the lungs). Segments of the left lung were then dehydrated in ethanol and toluol, embedded in paraffin, sectioned, and stained with hematoxylin-eosin or by the gram-stain technique.

Antimicrobial susceptibility test. Ceftazidime (Glaxo Laboratories, Greenford, Middlesex, England) was used in the experiments. The MIC of the drug defined as the lowest concentration that suppressed visible growth after inoculation of an inoculum of 10^5 cfu/ml for 18 hr at 37°C in tubes containing 4 ml of Todd-Hewitt broth (Oxoid Basingstoke, Hants, England) [14], was 0.2 µg/ml. The MBC, defined as the lowest concentration that reduced the number of organisms in the 18-hr culture to fewer than 50 cfu/ml [14], was 0.2 µg/ml.

Antimicrobial treatment. Ceftazidime was administered either as intermittent bolus injections or as continuous infusions over a period of four days. Treatment was started 5 hr or 34 hr after bacterial inoculation. Twofold increasing doses were administered, each dose to a group of ten rats. Intermittent injections were administered into the thigh muscles of the rear legs at 8-hr intervals. Because iv-infusion techniques did not function properly over such a long period of time, an alternative technique developed by Thonus et al. [15] was used. In brief, six weeks before its use, a tissue cage constructed of perforated teflon (length, 40 mm; outer diameter, 7 mm; inner diameter, 5 mm; 30% perforation) and connected with a 50-mm polyethylene tube, was implanted sc. All junctions were sealed with silicone adhesive. Before the start of antibiotic treatment, animals were anesthetized with fluanisone and pentobarbital. The distal portion of the polyethylene tube was recovered through an incision in the neck of the animal and was connected, by means of a polyethylene tube and a swivel, to a 12-ml syringe. This syringe was placed on a Varifusor pump (Breda

Scientific, Breda, The Netherlands). Ceftazidime, at the required concentration, was infused at a constant rate of 0.116 ml/hr. With this technique, prolonged, steady state levels were reached within 3 hr after the start of the infusion. These levels were 0.031 μg/ml (range, 0.024-0.043) and 0.073 μg/ml (range, 0.059-0.086) after administration of PD_{50} doses obtained after the start of treatment that was begun 5 hr and 34 hr after infection.

Therapeutic results. Therapeutic activity was evaluated with respect to the calculated daily dose that protected 50% of the animals from death (median protective dose, PD_{50}). PD_{50} values were calculated according to the method of Spearman-Kärber (see Sachs [16]). Deaths were recorded daily for 16 days after the termination of antimicrobial treatment. After that time no change in death rate occurred. The left lung from each rat died was cultured to check for the presence of only K.pneumoniae. Only K.pneumoniae organisms were recovered from the lungs of both treated and untreated animals.

Measurement of antibiotic concentrations in serum. Blood specimens obtained by orbital puncture under light ether anesthesia were collected from each rat and serum was separated. With use of a diagnostic-sensitivity agar (Oxoid) and an Escherichia coli test strain susceptible to 0.2 μg of ceftazidime/ml, all tests were done according to a standard large-plate agar diffusion procedure [17]. For determination of ceftazidime concentrations above 0.2 μg/ml, 100-μl samples were assayed. For determination of concentrations below 0.2 μg/ml, some modifications were introduced in order to increase the sensitivity of the test. We applied 225-μl samples to metal rings (internal diameter, 6 mm) placed on the agar. To nine parts of each sample and of each standard concentration, one part of a solution of 0.8 μg of ceftazidime/ml of serum was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. Determination of samples with known concentrations of ceftazidime in serum with both the modified and the original method yielded similar results.

RESULTS

Course of the untreated infection. At 5 hr after inoculation, the number

of organisms in the left lung had increased up to sevenfold. Blood and pleural fluid were sterile. Histological studies of the pulmonary lesion at that time revealed no signs of tissue necrosis. A moderate number of PMNLs and a few macrophages were observed at the site of infection.

Thirty-four hours after bacterial inoculation, an average of 2×10^8 organisms were found in the left lung. One of 10 rats had bacteria in the blood (80 cfu/ml) and in the pleural fluid. The histological features of the pneumonic lesion were described earlier [13]. In brief, large numbers of PMNLs and gram-negative bacilli and a few macrophages were observed in the lesion. Tissue necrosis had occurred to some extent.

Response to ceftazidime treatment. After both intermittent and continuous administration of ceftazidime, the number of surviving animals increased with increasing total daily doses. In a similar way the mean time of death of the animals increased (table 1).

When ceftazidime treatments were started 5 hr after bacterial inoculation, the PD_{50} values obtained for continuous and intermittent administration of the antibiotic were 0.36 and 1.42 mg/kg per day, respectively ($P < .001$).

With a delay in administration of the drug to 34 hr after inoculation, the difference in efficacy of continuous and of intermittent administration increased. PD_{50} values were 1.08 and 13.06 mg of ceftazidime/kg per day, respectively ($P < .001$).

The mean time to death recorded with almost all continuous treatment schedules (started either 5 hr or at 34 hr) was prolonged, although not significantly, when compared with the mean time to death obtained with the same total daily doses given at 8-hr intervals.

Multiple colonies of K.pneumoniae isolates were recovered from the lungs of rats that died of survived after antibiotic treatment. The MBC values of these isolates for ceftazidime were not different from the MBC value of the parent strain.

Concentration of ceftazidime in serum. The concentrations of ceftazidime in the sera of uninfected rats after administration of PD_{50} doses obtained for the intermittent and the continuous treatment schedules are shown in tables 2 and 3. Serum concentrations of ceftazidime in infected rats are not significantly different from those in uninfected rats treated with similar doses. The serum concentrations at various intervals after administration of 0.47 or 4.35 mg of ceftazidime/kg (doses corresponding to

Table 1 The efficacy of ceftazidime treatment schedules started 5 h or 34 h after bacterial inoculation.

Dosage (mg/kg per day)	Start of treatment (hr after bacterial inoculation)											
	5						34					
	Intermittent administration				Continuous administration		Intermittent administration				Continuous administration	
	8-h intervals		6-h intervals				8-h intervals		6-h intervals			
	Survival rate*	Time to death (days)†	Survival rate*	Time to death (days)†	Survival rate*	Time to death (days)†	Survival rate*	Time to death (days)†	Survival rate*	Time to death (days)†	Survival rate*	Time to death (days)†
0.06	...	...	0/10	8.9±2.0	0/10	5.5±0.7	...	...	...	...	...	...
0.12	0/10	7.1±2.5	1/10	9.4±2.5	2/10	5.8±0.7	...	...	...	...	...	...
0.23	1/10	6.0±0.7	4/10	8.2±3.3	1/10	6.8±1.6	...	...	0/10	7.6±2.5	...	...
0.47	2/10	7.3±2.0	6/10	8.8±1.5	6/10	9.0±1.4	...	...	1/10	6.8±2.7	0/10	8.8±2.0
0.94	2/10	7.6±1.3	9/10	8	10/10	...	...	...	1/10	8.4±2.3	7/10	8.0±4.4
1.88	7/10	11.0±1.7	9/10	8	...	...	0/10	5.6±1.9	1/10	9.2±1.6	9/10	11.0
3.75	8/10	10.5±2.1	10/10	...	...	...	1/10	6.4±1.2	6/10	14.3±1.9	8/10	9.5±6.4
7.50	9/10	12.0	...	...	...	...	2/10	8.0±2.1	8/10	13.0±7.1	9/10	13.0
15.00	10/10	...	...	...	...	...	5/10	9.2±1.3	9/10	16	10/10	...
30.00	...	...	...	...	...	...	9/10	15	10/10	...	...	...
60.00	...	...	...	...	...	...	10/10	...	...	...	...	...

NOTE. PD_{50} doses (mg/kg per day; 99.9% confidence limits) of ceftazidime were; after start of administration at 5 hr, for administration at 8-h intervals 1.42 (0.70-2.89), for administration at 6-h intervals 0.35 (0.18-0.69) and for continuous infusion 0.36 (0.21-0.69); after start of administration at 34 hr, for administration at 8-h intervals 13.06 (7.28-23.42), for administration at 6-h intervals 3.50 (1.80-6.79), and for continuous infusion 1.08 (0.61-1.90).

*No. of survivors/total no. of animals

†Mean ± SD.

the PD_{50} daily doses of 1.42 and 13.06 mg of ceftazidime/kg administered at 8-hr intervals) are shown in table 2. Peak concentrations were 1.32 µg/ml (for the 0.47-mg dose) and 14.17 µg/ml (for the 4.35-mg dose). Ceftazidime concentrations in excess of 0.2 µg/ml (the MBC for the Klebsiella strain used) were present for 75 and 120 min at the respective doses. The concentrations in serum at various intervals after starting continuous infusion of the PD_{50} doses of 0.36 and 1.08 mg of ceftazidime/kg per day are shown in table 3. Steady-state levels were reached within 3 hr after the start of the infusion. These levels corresponded to 0.032 µg/ml (range, 0.024-0.043) and 0.073 µg/ml (range, 0.059-0.086) at the respective doses.

DISCUSSION

Few clinical data are available about the influence of continuous or intermittent dosage of antibiotics on the therapeutic efficacy in the treatment of serious infections. Bodey [4] showed that carbenicillin plus continuously infused cefamandole was more effective in the treatment of neutropenic cancer patients with gram-negative bacillary infections than was carbenicillin plus the same dose of cefamandole injected intermittently.

Some investigators studied the influence of continuous or intermittent administration of antibiotics on the therapeutic efficacy in experimental infections. Sande et al. [7] showed that the time needed to sterilize the CSF in a rabbit model of pneumococcal meningitis was the same for both intermittent and continuous administration of penicillin. The conclusion derived from this study should be considered with caution, however, because both treatment schedules were compared at only one equivalent dose. A previous study in our laboratory [1] using rats with pneumonia caused by Streptococcus pneumoniae demonstrated that sterilization of lungs with penicillin administered either on a 12-hr schedule or continuously infused was equally effective, provided that host defenses were intact. Contrary to our earlier study, this study in rats with pneumonia caused by K.pneumoniae demonstrates that in terms of PD_{50} doses, continuous infusion of ceftazidime resulted in greater efficacy than did intermittent injections. Powell et al. [6] used a model of acute pneumonia due to Pseudomonas aeruginosa in guinea pigs and observed a better response to a once-daily dosing regimen of tobramycin when compared with continuous infusion of this

Table 2 Concentrations of ceftazidime in serum at various intervals after im administration of different doses for intermittent treatment at 8-hr or 6-hr intervals.

Dose (mg/kg)	Drug concentration (μg/ml of serum ± SD) at the following times (min) after dosage					
	5	15	30	60	90	120
0.09	0.15±0.04	0.14±0.02	0.07±0.01	ND	0.02±0.02	0.01±0.01
0.88	2.28±0.17	2.56±0.11	1.58±0.06	ND	0.23±0.07	0.19±0.02
0.47	0.96±0.20	1.32±0.18	1.01±0.07	0.37±0.09	0.12±0.03	0.05±0.01
4.35	9.48±2.58	14.17±1.43	9.81±0.56	2.61±0.35	0.88±0.22	0.27±0.05

NOTE. Doses corresponded to the PD_{50} daily doses of 0.35 and 3.50 mg/kg administered at 6-h intervals of 1.42 and 13.06 mg/kg administered at 8-h intervals. Each value represents the mean of five rats ± SD. ND = not done.

Table 3 Concentrations of ceftazidime in serum at various intervals after starting administration of different PD_{50} doses for continuous treatment.

Dose (mg/kg)	Drug concentration (μg/ml of serum ± SD) at the following times (hr) after dosage					
	3	5	24	48	72	96
0.36	0.031±0.009	0.043±0.026	0.036±0.006	0.024±0.005	0.027±0.006	0.030±0.005
1.08	0.059±0.014	0.061±0.034	0.075±0.015	0.078±0.007	0.076±0.009	0.086±0.006

NOTE. Each value represents the mean of five rats ± SD.

drug. Chronic pneumonia in rats and endocarditis in rabbits due to the same organism responded equally to both regimens [6]. The discrepancies between these in vivo studies may be explained by the postantibiotic effect that was demonstrated in vitro by Bundtzen et al. [18]. They demonstrated that the effect of ß-lactam antibiotics on several gram-negative organisms in vitro persisted for a much shorter time than did the effect on a number of gram-positive organisms [18]. In addition the action of an aminoglycoside on P.aeruginosa showed a clear postantibiotic effect that may have contributed to the therapeutic efficacy of the widely spaced doses in the experiments of Powell et al. [6].

The effect of the mode of antibiotic administration on tissue penetration may provide a further explanation for the difference in efficacy between intermittent and continuous treatment. Investigations dealing with this subject produce somewhat contradictory results [2, 3, 5]. Experiments of Peterson et al. [5] showed that with a given amount of antibiotic, intermittent administration is advantageous at an early stage but that eventually, in equilibrium conditions at 26 hr, the constant drug infusion led to higher extravascular levels. These experiments were performed in uninfected animals. Nevertheless, the experiments of Peterson et al. are in agreement with our observations that continuous administration of ceftazidime was more effective than was intermittent treatment.

We found that administration of PD_{50} doses by continuous infusion resulted in serum levels of antibiotic that were constantly below the MIC of the infecting Klebsiella strain. Several authors studied the in vivo efficacy of sub-MIC concentrations in experimental infections. Zak et al. [19], Comber et al. [20], and Merrik and Rolinson [21] found that treatment of experimental infections due to lethal numbers of different gram-negative organisms with ß-lactam antibiotics and aminoglycosides resulted in substantial survival rates, despite antibiotic serum levels that never reached the MIC of the infecting strain. Also, in our study, steady-state serum levels of ceftazidime below the MIC of the Klebsiella strain used were therapeutically active. Ceftazidime concentrations of 0.032 µg/ml or 0.073 µg/ml in serum protected 50% of the animals from death when treatment was started 5 hr and 34 hr after inoculation, respectively. The MIC of the Klebsiella strain was 0.2 µg of ceftazidime/ml.

A possible explanation for the therapeutic efficacy of sub-MIC concentrations of antibiotics in vivo may be found in data derived from in vitro experiments. Effects of sub-MIC concentrations of ß-lactam antibiotics on

growth rate and morphology (including filamentation of gram-negative organisms) were demonstrated [22-24]. The main effect of antibiotic concentrations below the MIC may be reduction of bacterial growth, as suggested by Atkinson and Amaral [22] and Lorian [25]. In addition, the effects of low antibiotic concentrations on the susceptibility of bacteria to host defense mechanisms - in particular the phagocytosis process - have been widely investigated. The effects of low antibiotic concentrations of the action of host defense mechanisms have also been investigated. The results are to a large extent, however, controversial [22, 26].

From our studies we conclude that in normal rats ceftazidime given by continuous infusion resulted in a better therapeutic response to K.pneumoniae pneumonia than did administration of the antibiotic at 8-hr intervals.

Although the clinical relevance of our findings in animals is highly speculative, one might at least envisage the possibility that certain patients, suffering from severe infections due to gram-negative bacteria, may get additional benefit from an antibiotic like ceftazidime when it is administered continuously. Moreover, the ratio of serum level to MIC required for curing the infection may be less important for determining dosage if the levels are maintained at a steady state rather than fluctuating.

In further experiments the role of the dose and the administration schedule of antibiotics will be studied in neutropenic rats.

ACKNOWLEDGEMENTS

This work was supported in part by research grants from Glaxo Research Ltd. (United Kingdom).

We thank Ada L. Beukelman and Linda P.C. Harkes for secretarial help.

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APPENDIX PAPER II

CONTINUOUS VERSUS INTERMITTENT ADMINISTRATION OF CEFTAZIDIME IN EXPERIMENTAL KLEBSIELLA PNEUMONIAE PNEUMONIA IN NORMAL AND LEUKOPENIC RATS*

Robert Roosendaal, Irma A.J.M. Bakker-Woudenberg, Marion van den Berghe-van Raffe, and Marc F. Michel

ABSTRACT

Experimental Klebsiella pneumoniae pneumonia was used to study the influence of cyclophosphamide-induced leukopenia on the relative therapeutic efficacy of continuous and intermittent (6-h intervals) administration of ceftazidime. The antimicrobial response was evaluated with respect to the calculated daily dose that protected 50% of the animals from death (PD_{50}) until 16 days after the termination of a 4-day treatment. When ceftazidime was administered intermittently to leukopenic rats, the PD_{50} was 24.37 mg/kg per day, 70 times ($P < 0.001$) the PD_{50} of 0.35 mg/kg per day for normal rats. Continuous administration of ceftazidime to leukopenic rats resulted in a PD_{50} of 1.5 mg/kg per day, four times ($P < 0.001$) the PD_{50} of 0.36 mg/kg per day for normal rats. Continuous administration of ceftazidime in daily doses that protected 100% of normal and leukopenic rats from death resulted in serum levels of 0.06 and 0.38 µg/ml, respectively, whereas the MIC for the infecting K.pneumoniae strain was 0.2 µg of ceftazidime per ml. The effect of the duration of ceftazidime treatment by continuous infusion on the therapeutic efficacy in relation to the persistence of leukopenia was then investigated in leukopenic rats. The administration of 3.75 mg of ceftazidime/kg per day for 4 days protected all leukopenic rats from death, provided the circulating leukocytes returned at the end of antibiotic treatment. When leukopenia persisted for 8 days this ceftazidime treatment schedule resulted in the mortality of rats ($P < 0.05$). However, when ceftazidime treatment was continued for 8 days, until the return of the leukocytes, there was no significant mortality ($P > 0.05$).

*Antimicrobial Agents and Chemotherapy (1986) 30:403-408. Reprinted with permission. [Data with respect to the concentrations of ceftazidime in serum after administration of PD_{50} doses (tables 6 and 7) have been added.]

INTRODUCTION

Infections caused by gram-negative bacilli constitute a serious problem in patients with underlying malignant disease [9]. Few clinical data are available about the therapeutic efficacy of continuous or intermittent administration of ß-lactam antibiotics in the treatment of serious infections. Bodey et al. demonstrated that carbenicillin plus continuously infused cefamandole was more effective in the treatment of neutropenic cancer patients with gram-negative bacillary infections than was carbenicillin plus the same dose of cefamandole injected intermittently [4]. There is limited experimental work on the effect of antibiotic drug schedules on therapeutic efficacy in animal infections. As will be discussed later, these studies have yielded somewhat contradictory results.

The present study was undertaken first to evaluate the activity of ceftazidime administered intermittently at 6-h intervals or by continuous infusion against experimentally induced Klebsiella pneumoniae pneumonia in normal and leukopenic rats. This model was selected because pneumonia and septicemia caused by K.pneumoniae and other gram-negative bacilli are often difficult to treat in patients undergoing intensive anticancer chemotherapy [9, 21]. Second, we studied whether antibiotic treatment should be continued during the persistence of leukopenia in leukopenic rats with K.pneumoniae pneumonia.

MATERIALS AND METHODS

Animals. Female R strain albino rats (specific pathogen free; 14 to 18 weeks old; weight, 185 to 215 g; bred at REPGO-TNO, Rijswijk, The Netherlands) were used in al experiments.

Bacteria. A capsular serotype 2 K.pneumoniae strain was used in these experiments. Inocula were prepared as described previously [2].

Antimicrobial susceptibility test. Ceftazidime (Glaxo Pharmaceuticals, Ltd., Greenford, England) was used in the experiments. The MIC of the drug was defined as the lowest concentration that suppressed visible growth after incubation of an inoculum of 5×10^5 CFU/ml for 18 h at 37°C in tubes containing 4 ml of Todd-Hewitt broth (Oxoid Ltd., London, England). The MBC

was defined as the lowest concentration that killed 99.9% of the original inoculum. The MBC was determined by spreading subculture volumes of 0.01 ml onto Iso-Sensitest Agar (Oxoid) plates. The concentrations of the serial dilutions decreased by steps of 0.2 µg/ml. The MIC and MBC of ceftazidime for the K.pneumoniae strain were both 0.2 µg/ml.

Induction of leukopenia. Leukopenia was induced by intraperitoneal injections of cyclophosphamide (CY) (Koch-Light Limited, Haverhill, England) in two doses of 90 and 60 mg/kg at 5 days and 1 day before bacterial inoculation, respectively. In some experiments, a third dose of 60 mg/kg was administered at 4 days after bacterial inoculation.

Quantitation of blood leukocytes. Blood samples obtained by orbital puncture under light CO_2 anesthesia from at least five rats were collected in plastic vials containing 1 mg of disodium EDTA per ml. For total leukocyte counts, blood was diluted 1:10 with Türk solution (0.1% crystal violet in 1% acetic acid), and numbers of leukocytes were determined in duplicate in a Bürkers hemacytometer. The total numbers of granulocytes and monocytes were calculated from the total number of leukocytes and differential counts of 400 leukocytes in cytocentrifuge preparations of buffy coats obtained by centrifugation of blood samples for 10 min at 1,500 x g in hematocrit tubes.

Effect of CY on the growth of K.pneumoniae in vitro. Blood specimens were collected from rats before treatment with CY and at 15 min and 1 day after the second CY injection, and serum was separated. Pooled serum from three rats was used. The effect of rat serum on the in vitro growth of K.pneumoniae was determined as follows. A stationary-phase culture which had been incubated for 16 h at 37°C was diluted in Iso-Sensitest broth (Oxoid) to a concentration of 2×10^6 CFU/ml and reincubated for 2 h at 37°C. An inoculum of 5×10^5 CFU/ml was incubated in Hanks balanced salt solution (Oxoid) with 90% serum at 37°C. The numbers of viable organisms were then determined at regular intervals over a 3-h period by plate counts on Iso-Sensitest agar.

Pneumonia. Experimental pneumonia was produced as described previously by Bakker-Woudenberg et al. [2]. In brief, rats were anesthetized with fluanisone (Hypnorm; Duphar B.V., Amsterdam, The Netherlands) and pentobarbital

(Abbott Laboratories, North Chicago, Ill.). The left-main-stem bronchus was intubated, and the left lung was inoculated with 0.02 ml of a saline suspension of K.pneumoniae containing 8 x 10^4 (6 x 10^4 to 10 x 10^4) CFU. After bacterial inoculation, the narcotic antagonists nalorphine bromide and pentetrazolum (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands) were injected. The numbers of viable organisms in the left lung, blood, and pleural fluid were determined as described earlier [2].

Histology. The histological features of the pneumonic lesions were studied in groups of two rats each at 2 days after bacterial inoculation of normal rats and CY-treated rats. Lungs were fixed by injecting the trachea with 10% Formalin under constant pressure to reexpand the lungs. Segments of the left lung were then dehydrated in ethanol and toluol, embedded in paraffin, sectioned, and stained with haematoxylin-eosin or by the Gram stain technique.

Antimicrobial treatment. Ceftazidime was administered either as intermittent bolus injections over 4 days or as continuous infusions over either 4 or 8 days. Treatment was started 5 h after bacterial inoculation. Twofold increasing doses of ceftazidime were administered, each dose to a group of 10 rats. Intermittent injections were administered into the thigh muscles of the rear legs at 6-h intervals. Because intravenous infusion techniques did not function properly over such a long period, an alternative technique developed by Thonus et al. [22] was used. In brief, 6 weeks prior to its use, a tissue cage constructed from perforated Teflon (length, 40 mm; outer diameter, 7 mm; inner diameter, 5 mm; 30% perforation) connected to 50 mm of polyethylene tubing was implanted subcutaneously. Rats were anesthetized prior to treatment with antibiotics. The distal portion of the polyethylene tube was recovered via an incision in the neck of the animal and connected via a polyethylene tube and a swivel to a 12-ml syringe. This syringe was placed on a Varifusor pump (Breda Scientific, Breda, The Netherlands). Ceftazidime at the required concentration was infused at a constant rate of 0.113 ml/h. By this technique, prolonged steady-state serum levels were reached within 5 h after the start of infusion.

Therapeutic results. The response to antimicrobial treatment was evaluated with respect to the calculated daily dose that protected 50% of the animals from death (median protective dose, PD_{50}). PD_{50}s were calculated by the

method of Spearman-Kärber as described by Sachs [18]. The effect of the duration of antimicrobial treatment on therapeutic efficacy was evaluated by comparing mortality rates obtained for the different treatment groups by the Fisher test. In all experiment, deaths of rats were recorded daily until 16 days after the termination of antimicrobial treatment. After that time, no changes in death rates occurred. The left lung of each rat that died was cultured to check for the presence of K.pneumoniae. Only K.pneumoniae was recovered from lungs of both treated and untreated animals.

Measurement of antibiotic concentrations in serum. Blood specimens obtained by orbital puncture under light ether anesthesia were collected from each rat, and serum was separated. All tests were done by a standard large-plate agar diffusion procedure with diagnostic sensitivity test agar (Oxoid) and an Escherichia coli test strain susceptible to 0.2 μg of ceftazidime per ml (3). For the determination of ceftazidime concentrations above 0.2 μg/ml, 100-μl samples were assayed, for the determination of concentrations below 0.2 μg/ml, some modifications were introduced to increase the sensitivity of the test. We applied 225-μl samples to metal rings (internal diameter, 6 mm) placed on the agar. To nine parts of each sample and of each standard concentration of ceftazidime, one part of a solution of 1 μg of ceftazidime per ml of serum was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. The determination of drug concentrations of samples with known concentrations of ceftazidime in serum by both the modified and original methods yielded similar results.

RESULTS

Effect of CY on the number of peripheral blood leukocytes. CY treatment at two doses of 90 and 60 mg/kg with a 4-day interval resulted in a substantial reduction in the number of blood granulocytes and monocytes to less than 100 and 30/mm^3, respectively, 5 days after the first CY injection (Fig. 1). At that time rats were inoculated with K.pneumoniae. Granulocytopenia and monocytopenia continued for 4 days, with a return of circulating granulocytes and monocytes between 4 and 6. A third CY injection of 60 mg/kg given at day 4 resulted in a prolongation of granulocytopenia and monocytopenia until day 8 (Fig. 2). The numbers of circulating granulocytes and monocytes in CY-treated, infected rats 1 and 4 days after bacterial inocula-

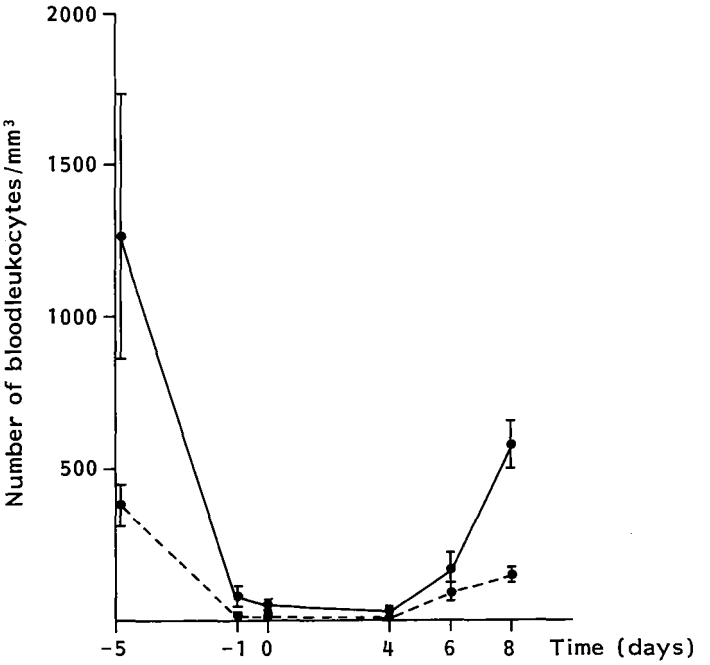

Figure 1 Total granulocyte (———) and monocyte (– – –) counts following CY injections given intraperitoneally 5 days (90 mg/kg) and 1 day (60 mg/kg) before infection on day 0.
Each value represents the mean ± SD for five rats.

tion were not significantly different from those in CY-treated uninfected rats.

Effect of CY on the growth of K.pneumoniae in vitro. Rat sera from normal rats at a concentration of 90% had no effect on the growth of K.pneumoniae in broth. Rat sera obtained from CY-treated rats either 15 min or 1 day after the second CY injection did not affect bacterial growth either.

Effect of CY treatment on the course of K.pneumoniae pneumonia. The course of K.pneumoniae pneumonia in CY-treated rats was more severe than that in normal rats (table 1). The increase in the numbers of K.pneumoniae in the left lung during the first 5 h after inoculation was similar to both CY-

Table 1 Course of K.pneumoniae pneumonia in normal and leukopenic rats[a]

h after inoculation	Normal rats (n = 5)				Leukopenic rats (n = 5)			
	Log CFU in left lung (range)	blood		No. of rats with bacteria in pleural fluid	Log CFU in left lung (range)	blood		No. of rats with bacteria in pleural fluid
		No. of rats positive	Log CFU/ml[b]			No. of rats positive	Log CFU/ml[b]	
5	5.7 (5.3-6.0)	0		0	5.7 (5.7-5.9)	0		0
10	5.8 (4.8-6.3)	0		0	6.5 (6.2-6.7)	0		0
18	5.6 (3.6-5.8)	0		0	7.5 (7.0-7.6)	1	1.5	0
24	6.5 (5.7-7.4)	0		0	8.5 (7.7-8.8)	5	2.3 (1.6-2.2)	0
29	6.3 (5.7-7.3)	1	1.9	0	8.7 (8.2-8.9)	5	3.0 (1.4-3.5)	2
34	8.3 (7.4-9.2)	1	1.9	0	8.9 (8.7-9.4)	5	3.3 (3.1-4.7)	3

[a]Groups of five rats each were studied after inoculation of the left lung with 8×10^4 CFU. The values given are median values. The mean times to death ± SDs were 5.1 ± 1.3 and 2.3 ± 0.3 days for normal and leukopenic rats, respectively.

[b]Calculated for positive cultures only.

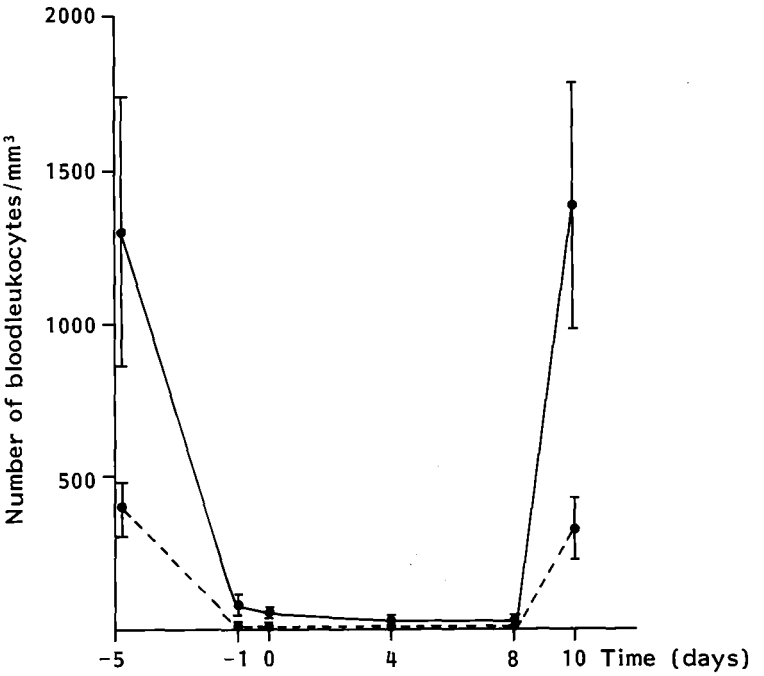

Figure 2 Total granulocyte (———) and monocyte (– – –) counts following CY injections given intraperitoneally 5 days before (90 mg/kg), 1 day before (60 mg/kg), and 4 days after (60 mg/kg) infection on day 0. Each value represents the mean ± SD for five rats.

treated and untreated rats. From that time the numbers of K.pneumoniae in the left lung increased more rapidly in leukopenic rats than in normal rats. By 34 h an average number of 5 x 10^8 CFU was cultured from the left lung of both leukopenic and normal rats. At 24 h after inoculation K.pneumoniae was cultured from the blood of all leukopenic rats, whereas the blood of all normal rats was sterile. At 29 and 34 h after inoculation about half of the leukopenic rats had bacteria in the pleural fluid, whereas the pleural fluid of all normal rats was sterile. The mean time to death ± standard deviation (SD) recorded for CY-treated rats was 2.3 ± 0.3 days, compared with 5.1 ± 1.3 days for normal rats. Histological studies at 2 days after bacterial inoculation revealed that large numbers of gram-negative bacteria, polymorphonuclear leukocytes, and a few macrophages were

present in the left lung of normal rats. Lungs of leukopenic animals did not show any substantial cellular infiltrate at the site of infection, whereas large numbers of gram-negative bacteria were present.

Therapeutic efficacy of ceftazidime in normal and CY-treated rats. After both intermittent and continuous administration of ceftazidime, the number of surviving animals (both normal and CY-treated rats) increased with increasing total daily doses. In normal and leukopenic rats treated by continuous infusion, the mean time to death increased in a similar way (table 2). In normal rats intermittent administration and continuous administration of ceftazidime were equally effective, in terms of PD_{50}s being 0.35 and 0.36 mg/kg per day for the respective treatment schedules ($P > 0.05$). On the contrary, in leukopenic rats continuous administration of ceftazidime was significantly superior to administration at 6-h intervals, PD_{50}s being 1.52 and 24.37 mg/kg per day, respectively ($P < 0.01$). Compared to the PD_{50}s in normal rats, the PD_{50}s in leukopenic rats had to be increased 70-fold when ceftazidime was administered at 6-h intervals and 4-fold when ceftazidime was administered by continuous infusion.

Efficacy of ceftazidime treatment of various durations in relation to the persistence of leukopenia. A total daily dose of 3.75 mg of ceftazidime per kg administered by continuous infusion over a period of 4 days cured all rats that were leukopenic for 4 days, with a return of circulating leukocytes at the end of antibiotic treatment (day 4) (table 2). This ceftazidime treatment schedule was not successful in rats with leukopenic persisting for 8 days, resulting in the mortality of rats ($P < 0.05$) (table 3). However, the survival rate increased, although not significantly, when ceftazidime treatment was continued until the return of circulating leukocytes (day 8). The survival rates of rats treated with ceftazidime during a leukopenic period of either 4 or 8 days were not significantly ($P > 0.05$) different.

Concentrations of ceftazidime in serum. The concentrations of ceftazidime in sera of infected rats at various intervals after administration of 0.94 and 15 mg/kg (doses corresponding to the PD_{100}s [daily doses that protected 100% of the animals from death] of 3.75 and 60 mg/kg administered at 6-h intervals to normal and leukopenic rats, respectively) are shown in table 4. Peak concentrations were 2.16 and 23.81 μg/ml, respectively. Ceftazidime

Table 2 Efficacy of ceftazidime treatment schedules[a] in normal and leukopenic[b] rats

Dosage (mg/kg per day)	Normal rats (n = 10)				Leukopenic rats (n = 10)			
	Intermittent administration		Continuous administration		Intermittent administration		Continuous administration	
	No. of survivors	Time to death (days)[c]	No. of survivors	Time of death (days)[c]	No. of survivors	Time to death (days)[c]	No. of survivors	Time to death (days)[c]
0.06	0	8.9 ± 2.0	0	5.5 ± 0.7				
0.12	1	9.5 ± 3.1	2	5.8 ± 0.7				
0.23	4	8.2 ± 3.3	1	6.8 ± 1.6				
0.47	6	8.8 ± 1.5	6	9.0 ± 1.4			0	2.8 ± 0.4
0.94	9	8	10				1	5.2 ± 1.9
1.88	9	8					7	9.3 ± 3.5
3.75	10						10	
7.50					0	3.4 ± 1.2		
15.00					1	3.5 ± 0.7		
30.00					7	5.3 ± 2.1		
60.00					10			

[a] To groups of 10 rats each ceftazidime was administered either as intermittent bolus injections at 6-h intervals or as a continuous infusion (infusion rate 0.113 ml/h) over a period of 4 days. Treatment was started 5 h after inoculation of the left lung with 8 x 10^4 CFU of *K.pneumoniae*. The PD_{50}s (milligrams per kilogram per day) for intermittent administration and continuous administration for normal rats were 0.35 and 0.36, respectively (99.9% confidence limits, 0.19 to 0.67 and 0.21 to 0.61, respectively); the corresponding values for leukopenic rats were 24.37 and 1.52 (16.07 to 36.97 and 1.00 to 2.31).

[b] CY was administered intraperitoneally in two doses of 90 and 60 mg/kg at 5 days and 1 day before bacterial inoculation, respectively.

[c] Mean ± SD; based on the time of bacterial inoculation (day 0).

Table 3 Efficacy of ceftazidime treatment[a] of various durations in relation to the persistence of leukopenia

Persistence of leukopenia (days)	Duration of treatment (days)	No. of survivors	Time to death (days)[b]
4[c]	4	10	
8[d]	4	3	7.3 ± 1.0
8[d]	8	7	8.0 ± 1.0

[a]To groups of 10 rats each 3.75 mg of ceftazidime per kg per day was administered by continuous infusion (infusion rate, 0.113 ml/h). Treatment was started 5 h after inoculation of the left lung with 8 x 10^4 CFU of K.pneumoniae.

[b]Mean ± SD; based on the time of bacterial inoculation (day 0).

[c]CY was administered intraperitoneally in two doses of 90 and 60 mg/kg at 5 days and 1 day before bacterial inoculation, respectively.

[d]CY was administered intraperitoneally in three doses of 90, 60 and 60 mg/kg at 5 days before, 1 day before, and 4 days after bacterial inoculation, respectively.

concentrations in excess of 0.2 µg/ml, the MBC for the K.pneumoniae strain used, were present for about 90 and 190 min at the respective doses. The concentrations of ceftazidime in sera of infected rats at various intervals after the start of continuous infusions of 0.94 and 3.75 mg/kg per day (the PD_{100}s obtained for normal and leukopenic rats, respectively) are shown in table 5. Steady-state levels in serum were 0.06 (SD, ± 0.03) and 0.38 (SD, ± 0.12) µg of ceftazidime per ml, respectively.

DISCUSSION

Gram-negative bacillary infections are often difficult to treat in granulocytopenic cancer patients [21]. Most antibiotic dose schedules are empiric. Dose regimens are mostly based on achievable antibiotic levels in serum in relation to the MIC for the infecting strain [10, 13]. The number of clinical studies dealing with the effect of antibiotic dose schedules on therapeutic efficacy in the treatment of infections in immunosuppressed

Table 4 Concentrations of ceftazidime in serum at various intervals after intramuscular administration at 6-h intervals of PD_{100}s to infected normal and leukopenic rats

Dose (mg/kg)[a]	Drug concn (μg/ml of serum)[b] at the following times (min) after dosage:					
	5	15	30	60	90	120
0.94	1.97 ± 0.60	2.16 ± 0.16	1.35 ± 0.04	0.50 ± 0.16	0.19 ± 0.05	0.08 ± 0.08
15.00	15.35 ± 4.44	23.81 ± 2.45	18.65 ± 2.48	8.34 ± 2.71	3.18 ± 1.87	1.39 ± 0.96

[a]Doses correspond to the PD_{100}s of 3.75 and 60.00 mg/kg administered at 6-h intervals to normal and leukopenic rats, respectively. Sera were taken from rats at various intervals after injection 12 of ceftazidime.

[b]Each value represents the mean ± SD for five rats.

Table 5 Concentrations of ceftazidime in serum at various intervals after continuous administration (infusion rate, 0.113 ml/h) of PD_{100}s to infected normal and leukopenic rats

Dose (mg/kg per day)	Drug concn (μg/ml of serum)[a] at the following times (h) after dosage:		
	5	24	48
0.94	0.08 ± 0.05	0.06 ± 0.02	0.05 ± 0.02
3.75	0.37 ± 0.10	0.38 ± 0.15	0.40 ± 0.07

[a]Each value represents the mean ± SD for five rats.

Table 6 Concentrations of ceftazidime in serum at various intervals after intramuscular administration at 6-h intervals of PD_{50}s to infected normal and leukopenic rats

Dose (mg/kg)	Drug concn (μg/ml of serum)[b] at the following times (min) after dosage:				
	5	15	30	90	120
0.09	0.15 ± 0.04	0.14 ± 0.02	0.07 ± 0.01	0.02 ± 0.002	0.01 ± 0.01
6.10	10.60 ± 2.23	14.30 ± 0.70	9.58 ± 1.18	4.88 ± 2.55	0.98 ± 0.08

[a]Doses correspond to the PD_{50}s of 0.35 and 24.4 mg/kg/d administered at 6-h intervals to normal and leukopenic rats, respectively. Sera were taken from rats at various intervals after injection 12 of ceftazidime.

[b]Each value represents the mean ± SD for five rats.

Table 7 Concentrations of ceftazidime in serum at various intervals after continuous administration (infusion rate, 0.113 ml/h) of PD_{50}s to infected normal and leukopenic rats

Dose (mg/kg per day)	Drug concn (μg/ml of serum)[a] at the following times (h) after dosage:		
	5	24	48
0.36	0.04 ± 0.03	0.04 ± 0.01	0.03 ± 0.01
1.52	0.17 ± 0.09	0.17 ± 0.05	0.22 ± 0.05

[a]Each value represents the mean ± SD for five rats.

patients is small [4]. Therefore, animal experiments have been performed with the object of comparing the efficacy of intermittent and continuous administration of antibiotics [1, 17, 19, 20]. For infections in immunocompromised animals, it has, however, not been clearly established whether repeated of high serum drug concentrations of short duration are superior to continuously maintained lower concentrations.

This study was undertaken to compare the efficacy of ceftazidime administered intermittently at 6-h intervals or by continuous infusion against experimental K.pneumoniae pneumonia in normal and leukopenic rats. CY was used to induce granulocytopenia and monocytopenia in rats. Circulating granulocytes play an important role in the defense against K.pneumoniae infections in the lung in the early phase of the infection, as demonstrated by Rehm et al. in normal and granulocytopenic mice [16]. In vitro experiments demonstrated that macrophages also phagocytize encapsulated K.pneumoniae organisms in the presence of antiserum [7, 23]. The course of infection in CY-treated rats was more severe than in untreated rats, as reflected by increased numbers of K.pneumoniae cultured from the left lung and blood, as well as a shortened survival period. These changes were the result of CY-induced leukopenia, as CY itself did not influence the survival of K.pneumoniae in serum. We demonstrated that in rats with intact host defenses continuous infusion of ceftazidime was as effective intramuscular administration at 6-h intervals. However, in leukopenic rats continuous administration of ceftazidime was far more effective than intermittent administration. Our data are in agreement with those of a study by Bakker-Woudenberg et al. demonstrating an equal therapeutic efficacy of penicillin G in experimental pneumococcal pneumonia in rats with intact host defense mechanisms given either as intramuscular injections at 12-h intervals or by continuous infusion [1]. When host defenses were impaired by cobra venom factor treatment, continuous infusion of penicillin G was superior to administration at 8-h intervals. These data are not in agreement with those of a study by Schmidt et al. indicating that penicillin G was more effective in rats with pneumococcal pneumonia when administered at 8-h intervals than when given in more frequent doses [20]. In a rabbit model of pneumococcal meningitis Sande et al. found that duration of penicillin treatment required for sterilization of the cerebrospinal fluid was not dependent on the mode of drug administration [19].

We demonstrated that in rats with impaired host defenses continuous administration of ceftazidime was far more effective than intermittent

administration. Compared with the PD_{50}s in normal rats, the PD_{50}s in leukopenic rats had to be increased 70-fold for the intermittent treatment schedule and 4-fold for continuous infusion. The superiority of the continuously infused antibiotic in rats with impaired host defenses may be partially explained by increased tissue penetration as a result of maintained antibiotic concentrations in serum [11, 14]. Peterson et al. showed that, with a given amount of antibiotic, intermittent administration was advantageous at an early stage, but eventually, in equilibrium conditions at 26 h, the constant drug infusion led to higher extravascular levels [14]. However, Lavoie et al. did not find higher extravascular concentrations of antibiotic at 24 h with either mode of drug administration [11]. Van Etta et al. demonstrated that, for three of four antimicrobial agents tested, including two ß-lactam and two aminoglycoside antibiotics, continuous infusion led to the same tissue levels under steady-state conditions as did intermittent administration [24]. The discrepancy among these results may be explained by differences in the antibiotics and dosage schedules used. The differences in tissue levels after intermittent and continuous dosing found in the studies by the above-mentioned investigators are relatively small and therefore may not be the only explanation for the superiority of the continuous infusion regimen in our leukopenic rats. Another important factor contributing to the superiority of the continuous administration of antibiotic in leukopenic rats may be the prevention of bacterial regrowth, owing to the maintenance of active ceftazidime concentrations at the site of infection. Intermittent dosing results in fluctuating levels of ceftazidime, and drug levels in serum above the MIC are only present for a limited period of time during the dosing interval, with a maximum of about 190 min for leukopenic rats at the PD_{100}. Regrowth may occur as soon as ceftazidime levels have dropped below the MIC. As demonstrated in vitro by Bundtzen et al. [5], the effect of different ß-lactam antibiotics on several gram-negative organisms only persisted for a very short period after the removal of the drug. These data, together with our observation that the PD_{50} of ceftazidime was about 16-fold lower in leukopenic rats with continuous dosing than with intermittent dosing, suggest that maintaining an antibiotic at active concentration is of critical importance.

The steady-state level in serum obtained for continuous administration of ceftazidime at a dose that protected all rats with intact host defenses mechanisms from death (PD_{100}) was 0.06 µg/ml, below the MIC for the

K.pneumoniae strain used 0.2 µg/ml). Also, other experimental studies, in which antibiotic concentrations in serum were related to therapeutic effect and antibacterial activity in vitro, showed that substantial survival rates could be obtained by treatment with concentrations of antibiotics in serum below the MIC's [6, 23, 26]. However, to cure leukopenic rats with impaired host defenses, a steady-state level of 0.38 µg/ml of serum was needed.

It is not clearly established whether antimicrobial treatment of neutropenic patients should be continued when neutropenia persists [8, 15, 25]. Pizzo et al. randomized a group of 33 granulocytopenic cancer patients who became afebrile during initial antimicrobial treatment although remaining granulocytopenic to either continue of discontinue antibiotic treatment [15]. Only in the group in which antimicrobial treatment was discontinued did infectious sequelae develop. They suggested that granulocytopenic cancer patients may profit from the prolongation of antimicrobial treatment when granulocytopenic persists. Our data on the efficacy of ceftazidime treatment of various durations, administered by continuous infusion, in relation to the persistence of leukopenia also suggest that treatment should be continued until the return of circulating leukocytes, although the differences in mortality between rats with prolonged or discontinued treatment were not statistically significant.

From this study it can be concluded first that in rats with intact host defense mechanisms, a given amount of ceftazidime is equally effective when administered continuously or at 6-h intervals, whereas in rats with impaired host defense mechanisms, continuous administration of ceftazidime is superior to intermittent administration. Second, since a significant reduction in the therapeutic activity of continuously administered ceftazidime was observed when leukopenia persisted at the end of antibiotic treatment, antibiotic treatment should be continued until the return of circulating leukocytes.

ACKNOWLEGDEMENTS

This work was supported in part by reasearch grants from Beecham Research Laboratories (The Netherlands) and Glaxo Pharmaceuticals, Ltd. (United Kingdom).

We thank Linda P.C. Harkes for secretarial help.

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APPENDIX PAPER III

COMPARATIVE ACTIVITIES OF CIPROFLOXACIN AND CEFTAZIDIME AGAINST KLEBSIELLA PNEUMONIAE IN VITRO AND IN EXPERIMENTAL PNEUMONIA IN LEUKOPENIC RATS*

Robert Roosendaal, Irma A.J.M. Bakker-Woudenberg, Marion van den Berghe-van Raffe, Joke C. Vink-van den Berg, and Marc F. Michel

ABSTRACT

The antibacterial activities of ciprofloxacin and ceftazidime against Klebsiella pneumoniae in vitro and in vivo were compared. Although there was only a minor difference between MBCs of both drugs, the bacterial killing rate of ciprofloxacin in vitro was very fast in comparison with that of ceftazidime. Similarly, the intravenous administration of ciprofloxacin at 1 h after bacterial inoculation resulted in effective bacterial killing in the lungs of leukopenic rats. This killing was dose-dependent, in contrast to the dose-independent bactericidal effect of ceftazidime. The high antibacterial activity of ciprofloxacin in the lungs as compared with that of ceftazidime was also reflected in its therapeutic efficacy in K.pneumoniae pneumonia and septicemia in leukopenic rats when these infections were treated at 6-h intervals during 4 days, starting at 5 h after bacterial inoculation. Concentrations of ciprofloxacin and ceftazidime in lung tissue were not significantly different. Regarding the antibacterial activity of both drugs against K.pneumoniae in relation to the bacterial growth rate in vitro and in the lungs of leukopenic rats, ciprofloxacin killed K.pneumoniae organisms that were not actively growing, whereas ceftazidime did not. In addition, it was demonstrated that when the intravenous administration of antibiotic was delayed from 1 h up to 24 h after bacterial inoculation, ceftazidime lost its antibacterial activity in the lungs and blood of leukopenic rats,whereas ciprofloxacin was still very effective. These data suggest that the capacity of an antibiotic to kill bacteria at a slow growth rate may be relevant for its therapeutic effect in established infections, in which slowly growing bacteria form a substantial part of the total population.

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INTRODUCTION

There is an ongoing search for antimicrobial agents which are more effective in treating infections than are currently used drugs. In the past few years, a group of quinolone carboxylic acid derivatives known as the fluoroquinolones has been developed [11, 27]. One of its representatives is ciprofloxacin. This drug has some specific characteristics, such as a high bactericidal activity in vitro [7, 25, 27, 28], a large volume of distribution [11, 26], and a high capacity for tissue penetration [11, 21, 23, 26]. In addition, ciprofloxacin has a substantial bactericidal activity against bacteria in the stationary phase of growth [7, 28], which is probably related to its specific mode of action [24, 27]. The significance of these new antimicrobial agents in relation to currently used drugs has been investigated in clinical trials [1, 4]. However, the relative importance of these characteristics of the quinolones with respect to their antimicrobial activity in vivo can only be investigated in experimental infection models, which allow comparison of antibiotics under similar conditions of intensity and duration of infection.

In this study, a model of K.pneumoniae pneumonia and septicemia in leukopenic rats was used to compare the antibacterial activities of ciprofloxacin and ceftazidime. Leukopenic rats were chosen because infections in granulocytopenic cancer patients are often difficult to treat [13, 14, 22]. Although there was a minor difference between the MBCs of both antibiotics for the K.pneumoniae strain, ciprofloxacin and ceftazidime differed considerably with respect to their rates of bacterial killing. We investigated first whether the difference between the bacterial killing rates in vitro of both drugs was reflected in their antibacterial activities in the lungs of leukopenic rats and second what the consequences were with regard to their efficacies in the treatment of pneumonia and septicemia in leukopenic rats. In addition, we compared the antibacterial activities in vitro and in vivo of both drugs against the K.pneumoniae strain in relation to the bacterial growth rate.

MATERIALS AND METHODS

Animals. Female R strain albino rats (specific pathogen free; 14 to 18 weeks old; weight, 185 to 215 g; bred at REPGO-TNO, Rijswijk, The

Netherlands) were used in all experiments.

Bacteria. A K.pneumoniae strain (capsular serotype 2) was used in these experiments. Stationary-phase cultures were obtained by incubation for 16 h at 37°C in Iso-Sensitest broth (Oxoid Ltd., London, England). After proper dilution and reincubation for 2 h at 37°C, suspensions of logarithmically growing bacteria were prepared.

Antibiotics. Ciprofloxacin (Bayer AG, Leverkusen, Federal Republic of Germany) was supplied as powder or in ampoules of 100 mg of drug in 10 ml of diluent manufactured by Bayer. Dilutions were made in distilled water. Solutions were stored at 4°C and protected from light. Ceftazidime (Glaxo Pharmaceuticals, Ltd., Greenford, England) was supplied as powder. Solutions were made in accordance with the enclosed instructions. Dilutions were made in distilled water, and solutions were stored at -80°C.

Antimicrobial susceptibility tests. The MICs of the drugs were defined as the lowest concentrations that suppressed visible growth after incubation of 5×10^5 CFU/ml for 18 h at 37°C in tubes containing 4 ml of Iso-Sensitest broth. The MBCs were defined as the lowest concentrations that killed 99.9% of the original inoculum. MBCs were determined by spreading subculture volumes of 200 µl onto Iso-Sensitest agar (Oxoid) plates. The concentrations of the serial dilutions decreased by steps of 0.2 µg/ml. The effect of the two drugs on the short-term growth of K.pneumoniae was studied in both Iso-Sensitest broth and in Hanks balanced salt solution (HBSS) (Oxoid) with 90% normal rat serum at 37°C. A stationary-phase culture was diluted in Iso-Sensitest broth to a concentration of 2×10^6 CFU/ml. After reincubation for 2 h at 37°C, the number of CFU was 4×10^7, and antibiotic was added (zero time). Killing experiments were started with an inoculum of 5×10^5 CFU per ml. In studies of the antibacterial activity in vitro in relation to the bacterial growth rate, bacterial inocula were prepared as follows. At 2 h before or 2 h after the stationary growth phase in broth was achieved, bacteria were centrifuged, washed, suspended to their original number per milliliter in fresh, warm (37°C) Iso-Sensitest broth to which antibiotic had been added, and reincubated. During incubation, the numbers of viable organisms were determined at regular intervals by plate counts on Iso-Sensitest agar. Before plating, the concentrations of ciprofloxacin and ceftazidime were reduced to an inactive level by

centrifugation of 1-ml samples for 2 min at 10,000 x g, followed by replacement of the drug- containing medium by physiological saline.

Pneumonia. Experimental pneumonia was produced as described previously by Bakker-Woudenberg et al. [2]. In brief, rats were anesthetized with fluanisone (Hypnorm; Duphar B.V., Amsterdam, The Netherlands) and pentobarbital (Abbott Laboratories, North Chicago, Ill.). The left main-stem bronchus was intubated, and the left lung was inoculated with 0.02 ml of a saline suspension of K.pneumoniae. The number of CFU used to inoculate the left lung in the different experiments was confirmed by plate counts on blood agar. After inoculation, the narcotic antagonists nalorphine bromide and Pentetrazolum (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands) were injected.

Induction of leukopenia. Leukopenia was induced by intraperitoneal injections of cyclophosphamide (Koch-Light Limited, Haverhill, Suffolk, England) in two doses of 90 and 60 mg/kg at 5 days and 1 day before bacterial inoculation, respectively. Cyclophosphamide-induced leukopenia resulted in a more severe course of the untreated infection than that seen in immunocompetent rats [17].

Antimicrobial treatment. Ciprofloxacin and ceftazidime were administered in two different schedules. In some experiments, antibiotic at various doses was administered intravenously as a single injection into the tail vein at different times after bacterial inoculation. The response to antimicrobial treatment was evaluated with respect to the numbers of bacteria in the left lung at different times after administration. Rats were sacrificed, and the left lung was removed and homogenized in 20 ml of physiological saline for 30 s at 10,000 rpm in a VirTis homogenizer (The Virtis Co., Inc., Gardiner, N.Y.). Serial 10-fold dilutions of homogenates in saline were prepared. Volumes of 0.2 ml of each dilution and 1-ml volumes of the undiluted lung homogenate were spread on blood agar plates. The Mann-Whitney test was used for statistical analysis. In other experiments, antibiotic was administered intramuscularly into the thigh muscles of the rear legs at 6-h intervals over a period of 4 days, in twofold-increasing doses, each dose to a group of 10 rats, starting at 5 h after bacterial inoculation. The response to antimicrobial treatment was evaluated with respect to the daily dose that protected 50% of the animals

from death (median protective dose, PD_{50}). PD_{50} values were calculated by the method of Spearman-Kärber as described by Sachs [19]. The deaths of rats were recorded daily until 16 days after the termination of treatment. After that time, no changes in the death rate occurred. The left lung of each rat that died was cultured to check for the presence of K.pneumoniae. Only K.pneumoniae organisms were recovered from lungs of both treated and untreated animals.

Measurement of antibiotic concentrations in serum and in lungs of infected rats. Blood specimens were collected from each rat by orbital puncture under light CO_2 anesthesia, and serum was separated. For the determination of antibiotic concentrations in lung tissue, the left lung was removed, exsanguinated, and homogenized in saline in a Potter-Elvejhem homogenizer. After centrifugation for 2 min at 10,000 x g, the supernatant was collected and used for the determination of antibiotic concentrations. With the use of diagnostic sensitivity test agar (Oxoid) and an Escherichia coli test strain susceptible to 0.025 μg of ciprofloxacin and 0.2 μg of ceftazidime per ml, all tests were done by a standard large-plate agar diffusion procedure [6]. Standard samples for the determination of antibiotic concentrations in serum were prepared from pooled normal rat serum. Standard samples for the determination of drug concentrations in lung tissue were prepared as follows. Ciprofloxacin and ceftazidime in standard concentrations were added to homogenates of lungs of untreated rats. After incubation for 1 h at 37°C and centrifugation for 2 min at 10,000 x g, supernatants were collected. Samples of 100 μl were assayed. For the determination of concentrations in serum below 0.2 μg of ceftazidime per ml, some modifications were introduced to increase the sensitivity of the test. We applied 225-μl samples to metal rings (internal diameter, 6 mm) placed on the agar. To nine parts of each sample and of each standard concentration, one part of a solution of 1 μg of ceftazidime per ml of serum was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. Determination of samples with known concentrations of ceftazidime in serum by both the original method and the modified method yielded similar results. The Mann-Whitney test was used for statistical analysis.

Protein binding. Protein binding of ciprofloxacin in serum was measured by the ultrafiltration technique with the Micropartition System (MPS1; model

4010; Amicon Corp., Lexington, Mass.) and YMT membranes (Amicon) by centrifugation at 2,000 x g for 30 min at 25°C. Concentrations of ciprofloxacin were measured by high-pressure liquid chromatography. Separation was carried out by reverse-phase partitioning on a column (internal diameter, 4.6 mm; length, 25 cm) packed with Spherisorb 5-ODS (Chrompack, Middelburg, The Netherlands). The eluent contained 150 ml of acetonitrile, 150 ml of N,N-dimethylformamide, and 113 g of phosphoric acid plus 0.38 g of tetramethyl ammonium chloride diluted to 1 liter of water. Column elution was carried out at a flow rate of 1.5 ml/min and a pressure of 230 atm (ca. 23.3 MPa). The eluent was monitored at 278 nm. Protein binding of ceftazidime was measured as described earlier [3].

RESULTS

Effect of ciprofloxacin and ceftazidime against K.pneumoniae in vitro. The MIC and the MBC of ciprofloxacin were both 0.2 µg/ml, and the MIC and MBC of ceftazidime were both 0.4 µg/ml. The rates of bacterial killing in broth at concentrations around the MBC differed considerably for both antibiotics (Fig. 1). Bacterial killing by ciprofloxacin was very fast as compared with

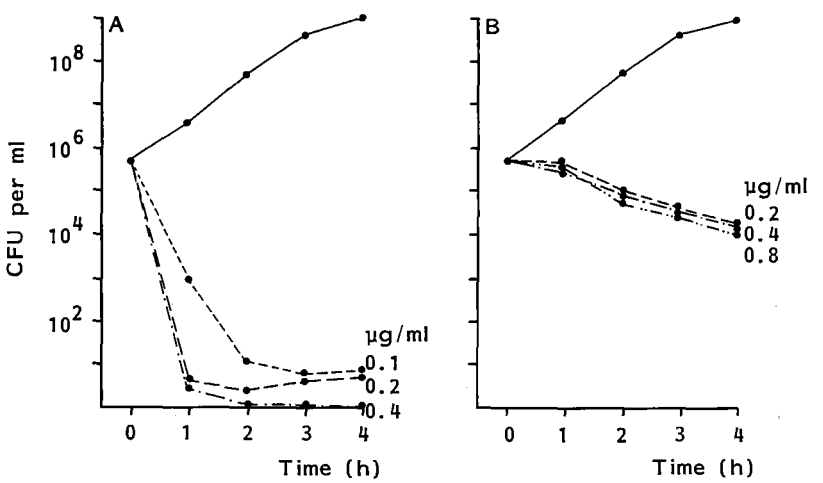

Figure 1 Effect of ciprofloxacin (A) or ceftazidime (B) at concentrations indicated against K.pneumoniae in the logarithmic phase of growth in broth. The solid line represents bacterial growth without antibiotic.

that by ceftazidime. At the concentration of 0.2 µg/ml, ciprofloxacin reduced the numbers of bacteria 10^5-fold within 1 h of incubation, whereas ceftazidime led to only a 10-fold reduction in bacterial numbers within 3 h. Although the bactericidal activities of ciprofloxacin and ceftazidime in HBSS with 90% serum were not identical to those in broth, no major differences were found between the killing rates in both media. In HBSS with 90% serum, ciprofloxacin again demonstrated an immediate high bacterial killing rate, whereas the bactericidal activity of ceftazidime was more time dependent (Fig. 2).

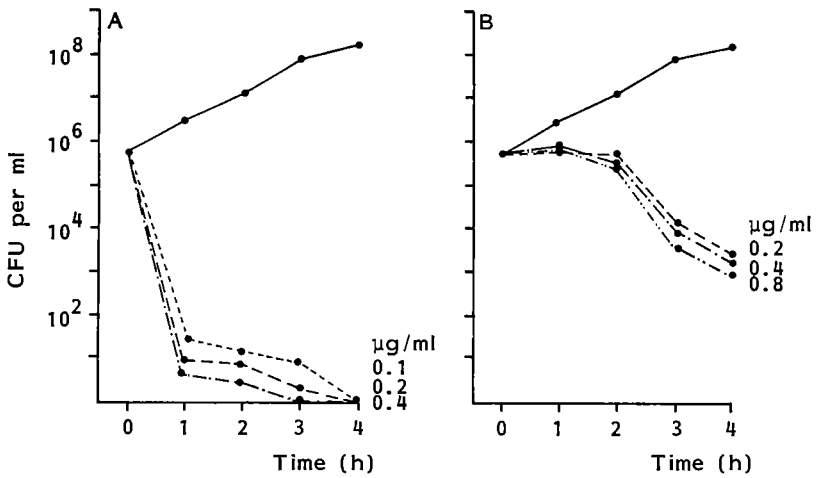

Figure 2 Effect of ciprofloxacin (A) and ceftazidime (B) at the concentrations indicated against K.pneumoniae in the logarithmic phase of growth in HBSS with 90% serum. The solid line represents bacterial growth without antibiotic.

Effect of ciprofloxacin and ceftazidime against K.pneumoniae in lungs of leukopenic rats. The antibacterial activities of ciprofloxacin and ceftazidime after intravenous administration of different doses at 1 h after inoculation of the left lung with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth are shown in Fig. 3. As compared with ceftazidime, ciprofloxacin was more effective in bacterial killing. Whereas the bactericidal effect of ciprofloxacin was dependent on the dose administered and was complete within 1 h after administration of the antibiotic, the antibacterial activity of ceftazidime was not dose dependent but more time-dependent. The concentra-

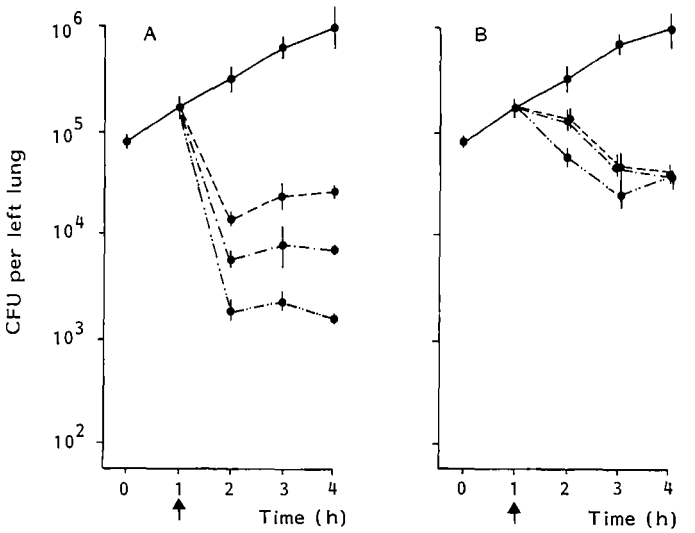

Figure 3 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and after intravenous administration of ciprofloxacin (A) or ceftazidime (B) at 0.3 mg/kg (– – –), 1 mg/kg (—·—), or 3 mg/kg (—··—), 1 h after inoculation of lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

tions of ciprofloxacin and ceftazidime in the serum of rats at various times after intravenous administration of 3 mg/kg are shown in Table 1. Ciprofloxacin concentrations in serum were about 14-fold lower than ceftazidime concentrations in serum within 5 min after dosing. The elimination half-lives for ciprofloxacin and ceftazidime were 98 and 31 min, respectively. Protein binding percentages were 41 and 14%, respectively.

Therapeutic efficacy of ciprofloxacin versus ceftazidime in K.pneumoniae pneumonia and septicemia in leukopenic rats. The therapeutic efficacy of ciprofloxacin versus ceftazidime was determined in terms of PD_{50} values after administration at 6-h intervals for 4 days starting 5 h after bacterial inoculation with 8 x 10^4 K.pneumoniae (Table 2). For both ciprofloxacin and ceftazidime, the numbers of surviving rats increased with increasing total daily doses. Ciprofloxacin treatment was significantly superior to ceftazidime treatment, PD_{50}s being 3.3 and 24.4 mg/kg per day

Table 1. Concentrations of ciprofloxacin and ceftazidime in serum at various times after intravenous administration of 3 mg/kg to leukopenic rats

Drug	Drug concn (μg/ml of serum)[a] at the following times (min) after administration:				
	5	15	30	60	90
Ciprofloxacin	0.94 ± 0.13	0.67 ± 0.15	0.55 ± 0.08	0.36 ± 0.04	0.41 ± 0.08
Ceftazidime	13.11 ± 0.76	5.67 ± 1.74	3.87 ± 1.44	3.77 ± 1.07	1.51 ± 0.42

[a]Each value represents the mean ± standard deviation for five rats.

Table 2. Therapeutic efficacy of ciprofloxacin and ceftazidime in leukopenic rats

Dosage (mg/kg per day)[a]	Ciprofloxacin		Ceftazidime	
	No. of survivors	Time to death (days)[b]	No. of survivors	Time to death (days)[b]
0.94	0	3.1 ± 0.7		
1.88	1	4.9 ± 0.6		
3.75	8	7.5 ± 2.1		
7.50	9	17	0	3.4 ± 1.2
15.00	9	10	1	3.5 ± 0.7
30.00	10		7	5.3 ± 2.1
60.00			10	

[a]Each dose was administered to a group of 10 rats at 6-h intervals. Treatment was started 5 h after inoculation of rats with 8 x 10^4 CFU of *K. pneumoniae*. The PD_{50}s (milligrams per kilogram per day) for ciprofloxacin and ceftazidime were 3.3 and 24.4, respectively (99.9% confidence limits, 1.98 to 5.38 and 16.07 to 36.97, respectively).

[b]Mean ± standard deviation; based on the time of bacterial inoculation (day zero).

for the respective drugs ($P < 0.001$). Intramuscular injection of 0.82 mg of ciprofloxacin and 6.1 mg of ceftazidime per kg (doses corresponding to the daily PD_{50}s of 3.3 and 24.4 mg/kg for the respective drugs administered at 6-h intervals) into infected rats resulted in relatively low concentrations of ciprofloxacin in serum (Table 3). Concentrations in serum above the MBC of 0.4 μg/ml after the administration of PD_{50}s were present for about 220 min for ceftazidime, whereas the concentrations of ciprofloxacin in serum did not reach the MBC during the 6-h dosing interval.

Table 3. Concentrations of ciprofloxacin and ceftazidime in serum at various times after intramuscular administration of PD_{50}s at 6-h intervals to infected leukopenic rats

Drug	Dose (mg/kg)[a]	Drug concn (μg/ml of serum)[b] at the following times (min) after administration:		
		15	30	90
Ciprofloxacin	0.82	0.29 ± 0.07	0.20 ± 0.05	0.07 ± 0.02
Ceftazidime	6.09	14.29 ± 0.70	9.58 ± 1.18	4.88 ± 2.55

[a]Doses correspond to the PD_{50}s of 3.3 and 24.4 mg of ciprofloxacin and ceftazidime per kg per day, respectively, administered at 6-h intervals. Sera were taken from rats at various times after injection eight of the respective drugs.

[b]Each value represents the mean ± standard deviation for five rats.

Concentrations of ciprofloxacin and ceftazidime in the lungs of infected rats. Concentrations of ciprofloxacin and ceftazidime at various times after the intravenous administration of 3 mg/kg at 24 h after bacterial inoculation are shown in Table 4. At the various times after administration there was no significant difference between the concentrations of ciprofloxacin and ceftazidime in lung tissue. Concentrations of ciprofloxacin in lung tissue were higher than those in serum until 1 h after administration.

Effect of ciprofloxacin and ceftazidime against K.pneumoniae in broth and in the lungs of leukopenic rats in relation to bacterial growth rate. Logarithmically growing bacteria in broth were killed by both ciprofloxacin and ceftazidime (Fig. 4A and 4B, respectively). At all concentrations tested, the bacterial killing rate of ciprofloxacin was higher than that

Table 4. Concentrations of ciprofloxacin and ceftazidime in lung tissue at various times after intravenous administration of 3 mg/kg to infected[a] leukopenic rats

Drug	Drug concn (μg/g of lung tissue)[b] at the following times (min) after administration:			
	15	30	60	90
Ciprofloxacin	1.33 ± 0.79	1.80 ± 0.89	1.21 ± 0.28	0.56 ± 0.16
Ceftazidime	1.72 ± 1.58	2.15 ± 0.60	1.16 ± 0.59	0.94 ± 0.40

[a]Drugs were administered 24 h after inoculation of the left lung with 8×10^4 CFU of K.pneumoniae

[b]Each value represents the mean ± standard deviation for five rats.

of ceftazidime. In addition, the administration of ciprofloxacin resulted in immediate killing, whereas bacterial killing by ceftazidime was more gradual and time dependent. When the K.pneumoniae organisms were in the stationary phase of growth, ceftazidime at the same concentrations was no longer bactericidal (Fig. 4D). On the contrary, ciprofloxacin was still able to kill the bacteria effectively (Fig. 4C), although the bacterial killing rates were lower than those observed for the logarithmically growing bacteria.

The bactericidal effect of ciprofloxacin and ceftazidime against K.pneumoniae in relation to the bacterial growth rate in vivo was demonstrated by the following experiments. Lungs were inoculated with 8×10^4 logarithmically growing K.pneumoniae (Fig. 5A). At 1 h after inoculation, when the bacterial numbers in the lungs had increased about threefold up to 2.2×10^5 CFU, ciprofloxacin or ceftazidime was administered at a dose of 3 mg/kg. The actively growing bacteria were killed by both antibiotics, although ciprofloxacin was significantly more effective ($P \leq 0.05$). Inoculation of the lungs with 2.2×10^5 K.pneumoniae in the stationary phase of growth (Fig. 5B) resulted in a 1-h delay of bacterial growth in the lungs. When antibiotic was administered immediately after inoculation, it was observed that ceftazidime at a dose of 3 mg/kg was not able to kill the bacteria during the first hour, whereas ciprofloxacin was highly bactericidal during that period after inoculation. When bacterial numbers in the lung increased, bacterial killing by ceftazidime was also observed.

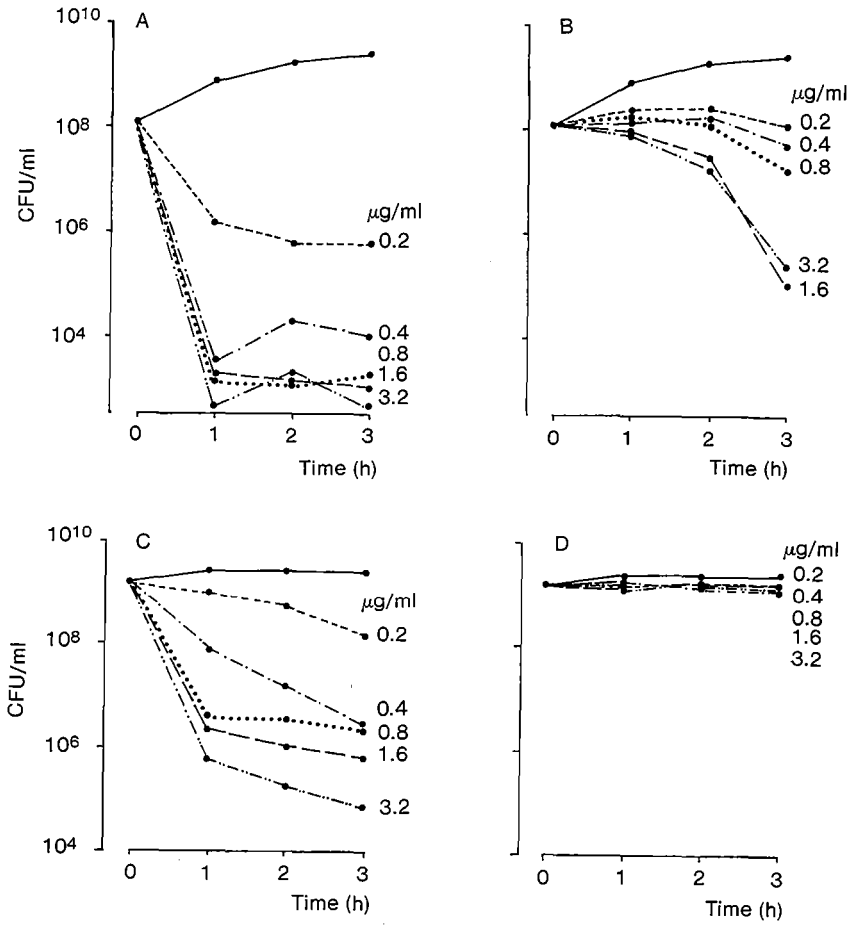

Figure 4 Effect of ciprofloxacin (A) or ceftazidime (B) against K.pneumoniae in the logarithmic phase of growth and of ciprofloxacin (C) or ceftazidime (D) against K.pneumoniae in the stationary phase of growth in broth. The solid line represents bacterial growth without antibiotic.

Finally, we investigated the effect of delaying antibiotic administration from 1 h up to 24 h after bacterial inoculation upon the antibacterial activities of ciprofloxacin and ceftazidime. Whereas at 1 h after inoculation with logarithmically growing bacteria (Fig. 5A) the bacteria in the lungs had increased 3-fold in number and were killed by both antibiotics, at 24 h after inoculation (Fig. 6), in an established lung infection, bacterial numbers had increased 4000-fold and had resulted in tissue necrosis. All rats had bacteria in the blood. Ciprofloxacin still had an anti-

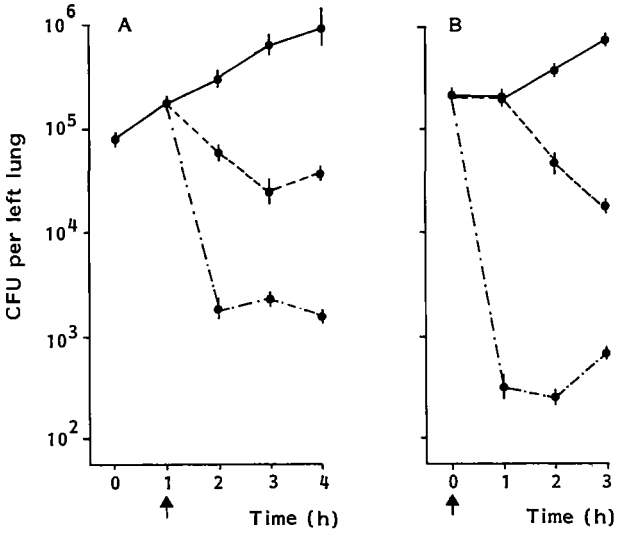

Figure 5 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and after intravenous administration of ciprofloxacin (—·—) or ceftazidime (– – –) at 3 mg/kg 1 h after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth (A), or immediately after inoculation of the lungs with 2.2 x 10^5 K.pneumoniae in the stationary phase of growth (B).
Each point represents the geometric mean ± SEM for five rats.

bacterial effect in the lungs and sterilized the blood of all rats. In contrast, ceftazidime was not effective in the lungs and blood cultures remained positive.

DISCUSSION

Several specific characteristics of ciprofloxacin may contribute to a high therapeutic efficacy. Ciprofloxacin has a high bactericidal activity against gram-negative bacteria [7, 25, 27, 28]. Another important characteristic of ciprofloxacin is a large volume of distribution and a high capacity of tissue penetration [21, 23, 26]. Wise et al. found that ciprofloxacin was very rapidly eliminated from serum after intravenous administration and had a volume of distribution of about two times the

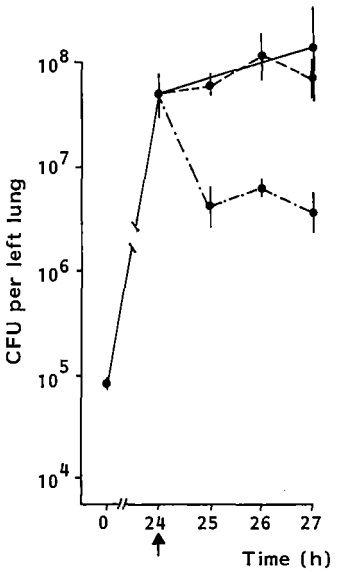

Figure 6 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———), and after intravenous administration of ciprofloxacin (—·—) or ceftazidime (— — —) at 3 mg/kg 24 h after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

body weight, as demonstrated in healthy volunteers [26]. In addition, penetration of ciprofloxacin into blister fluid was rapid, with extravascular concentrations exceeding those in serum from 30 min after intravenous administration. Siefert et al. also demonstrated that concentrations of ciprofloxacin in several tissues in rats exceeded those in plasma 4 h after intravenous administration and from 1 h after oral dose of 5 mg/kg [23]. As shown by Schlenkhoff et al., ciprofloxacin concentrations in human lung tissue were higher than those in serum up to 4 h after intravenous administration of 100 mg [21]. Another factor, the mode of action of ciprofloxacin, may also contribute to its high therapeutic activity. Quinolones inhibit DNA gyrase, the enzyme that maintains the DNA in the supercoiled state and is essential for survival of the bacteria [24]. Thus, they are expected to act upon a greater part of the bacterial population than are many other classes of antibiotics whose activity depends on the bacterial growth rate. Rapid and strong bactericidal activity of ciprofloxacin against nongrowing Pseudomonas aeruginosa and E.coli has been demonstrated

in vitro [7, 28], whereas a lack of bactericidal activity of several ß-lactam antibiotics, including ceftazidime, has been observed against nongrowing members of the family enterobacteriaceae [8]. Few studies have dealt with the measurement of bacterial multiplication at the site of infection [9, 15]. Maw and Meynell demonstrated that the generation time of Salmonella typhimurium in the spleens of mice was 10 h, as compared with a generation time of 30 min in broth [15]. Eudy and Burrows demonstrated that Proteus mirabilis and E.coli multiplied in the kidneys of rats, with generation times exceeding those in broth substantially and increasing with the duration of the infection [9]. These data indicate that growth rates in vivo are much lower than those in vitro. It is suggested that slowly or nongrowing bacteria form a substantial part of the total bacterial population in established infections. In this respect, the bactericidal activity of ciprofloxacin against nongrowing bacteria may be of major importance.

The relative importance of the specific characteristics of the quinolones in their therapeutic efficacy can be established only in experimental infections. The therapeutic efficacy of ciprofloxacin was investigated in several infection models [5, 10, 12, 16, 20]. Bayer et al. showed that ciprofloxacin was more effective in reducing bacterial counts and preventing mortality or relapse in experimental left-sided P.aeruginosa endocarditis in rabbits than was a combination of azlocillin plus netilmicin [5]. Another study revealed that acute P.aeruginosa pneumonia was more effectively treated with ciprofloxacin or tobramycin than with ticarcillin with respect to the number of CFU cultured from the infected tissue, whereas, using the same parameter, in a chronic pneumonia caused by the same microorganism, ciprofloxacin was superior to tobramycin or ticarcillin [20]. Gordin et al. and Rusnak et al. demonstrated retrospectively that ciprofloxacin was as effective as several combinations of ß-lactams and aminoglycosides and superior to monotherapies with the same drugs in reducing bacterial counts in the lungs of neutropenic guinea pigs with P.aeruginosa pneumonia [10, 18]. Although in the above-mentioned studies a high therapeutic activity of ciprofloxacin was demonstrated, the relative efficacy of this quinolone cannot be directly related to the efficacy of the ß-lactam antibiotics and aminoglycosides used. This is due to the fact that different dose schedules were applied and the bacterial strains used differed in their susceptibility to the respective drugs. Norden and Shinners used a P.aeruginosa strain which was equally susceptible to ciprofloxacin and tobramycin in terms of MBCs in a model of osteomyelitis in

rabbits; they demonstrated that the therapeutic efficacy of ciprofloxacin was superior to that of the aminoglycosides [16]. However, it must be realized that different dose schedules were used. Ingerman et al. treated P.aeruginosa endocarditis in rats with ciprofloxacin and the ß-lactam BMY 28142 at doses that resulted in comparable peak serum concentration/MBC ratios and found a superior efficacy of the quinolone [12].

In the present study, the antibacterial activities of ciprofloxacin and ceftazidime against K.pneumoniae in the lungs of leukopenic rats were compared in relation to their activities in vitro. It was demonstrated that although there was a slight difference in the MICs and MBCs of both antibiotics for the K.pneumoniae strain, the agents differed extremely with respect to their rate of bacterial killing. The difference in bacterial killing rates between ciprofloxacin and ceftazidime in vitro was also observed in vivo in terms of a decrease in numbers of K.pneumoniae in the lungs of leukopenic rats after administration of the antibiotic intravenously at 1 h after bacterial inoculation. The high bactericidal activity of ciprofloxacin in the lungs of leukopenic rats as compared with that of ceftazidime was also reflected in the increased survival (lowered the PD_{50}) of leukopenic rats after antibiotic administration at 6-h intervals for 4 days.

The high efficacy of ciprofloxacin may be explained by its high bactericidal effect, as demonstrated in vitro in broth as well as in vivo in lungs of leukopenic rats. Also, the relative long half-live of ciprofloxacin as compared with that of ceftazidime may have contributed to its high efficacy. The superior bactericidal activity of ciprofloxacin in the lungs as compared with that of ceftazidime cannot be explained by higher ciprofloxacin concentrations at the site of infection. Although until 1 h after administration the concentrations of ciprofloxacin in lung tissue were higher than those in serum, the concentrations of ciprofloxacin in lung tissue were comparable to those of ceftazidime. Siefert et al. [23] and Schlenkhoff et al. [21] also demonstrated relatively high concentrations of ciprofloxacin in lung tissue as compared to plasma and serum, respectively.

In addition to a high killing rate, the mode of action may also be relevant to the therapeutic efficacy of ciprofloxacin. We demonstrated in vitro that ciprofloxacin was able to kill bacteria that were not actively growing, whereas ceftazidime was not. Also in the lungs of leukopenic rats the bactericidal effect of ceftazidime appeared to be dependent on bacterial

growth. This was demonstrated in rats that were inoculated with nongrowing bacteria. It must be realized that this experimental set-up is not directly related to the clinical situation. However, in established infections, non-growing or slowly growing bacteria undoubtedly form a substantial part of the total bacterial population [9, 15]. Therefore, it may be expected that the success of antibiotic treatment is at least in part dependent on the capacity of the antibiotic to kill nongrowing bacteria. Because the failure of antibiotic treatment in established infections may be partly related to the fact that bacteria are inaccessible to antibiotics or have a slow growth rate, we studied the effect of delaying antibiotic administration on the antibacterial activities of ciprofloxacin and ceftazidime. While both antibiotics administered 1 h after bacterial inoculation resulted in a decrease in bacterial numbers cultured from the lungs of leukopenic rats, when administered 24 h after inoculation, ciprofloxacin was still very effective, whereas ceftazidime was not. The success of ciprofloxacin in the treatment of chronic P.aeruginosa pneumonia in guinea pigs or P.aeruginosa osteomyelitis in rabbits [16, 20] may also be related to its bacterial activity against at slowly growing bacteria.

In summary, from animal infection models there is substantial evidence that the therapeutic efficacy of an antibiotic is related not only to the bacterial killing rate and to the capacity for tissue penetration but also to the bactericidal activity against slowly growing bacteria. The relevance of these antibiotic characteristics to therapeutic efficacy in infections in immunocompromised hosts needs to be established.

ACKNOWLEDGEMENTS

This work was supported by research grants from Bayer Nederland B.V., Mijdrecht, The Netherlands and Glaxo Pharmaceuticals Ltd., Greenford, England.

We thank Ada Beukelman for secretarial help.

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APPENDIX PAPER IV

IMPACT OF THE DOSAGE SCHEDULE ON EFFICACY OF CEFTAZIDIME, GENTAMICIN, AND CIPROFLOXACIN IN KLEBSIELLA PNEUMONIAE PNEUMONIA AND SEPTICEMIA IN LEUKOPENIC RATS*

R. Roosendaal, I.A.J.M. Bakker-Woudenberg, M. van den Berghe-van Raffe, J.C. Vink-van den Berg, M.F. Michel

Leukopenic rats with Klebsiella pneumoniae pneumonia and septicemia were treated with antibiotic during 4 days either intermittently at 6-h intervals or by way of continuous infusion. Daily doses (mg/kg) that protected 50% of the animals from death obtained for intermittent or continuous administration were: ceftazidime 24.4 and 1.5 ($P < 0.001$), gentamicin 2.8 and 3.8 ($P > 0.05$), and ciprofloxacin 3.3 and 6.5 ($P < 0.05$), respectively. The therapeutic results in relation to the dosage schedule correlate with the different patterns of short-term bacterial killing in vitro as well as in the lungs of leukopenic rats for the three antibiotics. Killing by ceftazidime was slow, but continuing, and relatively independent on the concentration c.q. dosage. This correlates with the highest therapeutic effect obtained when ceftazidime was administered continuously resulting in permanent concentrations in plasma during the entire treatment interval. In contrast, killing by gentamicin or ciprofloxacin was rapid, and related to the concentration c.q. dosage, which correlates with a good therapeutic effect even when administered intermittently resulting in high peak concentrations at relatively long intervals.

INTRODUCTION

Most antibiotic dose schedules are empiric and based upon achievable serum concentrations in relation to the MIC of the infecting strain [1, 2]. It is, however, not clearly established whether or not transient relative high antibiotic peak concentrations in serum are more effective than sustained drug concentrations at a lower level for the treatment of serious infec-

*(Submitted for publication)

tions. Optimal antimicrobial treatment is especially required with regard to infections in the compromised host, whose cure depends mainly on antibiotics. Only few clinical trials deal with the effect of the mode of administration, either intermittently or continuously, on the therapeutic efficacy of antibiotics [3, 4, 5]. During the past few years more attention has been directed to the impact of the antibiotic dosage schedule on the in vivo efficacy in experimental infections [6-15]. Data derived from these investigations suggest that the importance of the antibiotic dosage schedule as a determinant of therapeutic efficacy is related to the class of antibiotic. However, some of these studies were performed in infection models with limited clinical relevance. Other data were derived from therapy experiments in which only one class of antibiotic was studied. In the present study the impact of the dosage schedule on the therapeutic efficacy of antibiotics of three different classes was investigated in a Klebsiella pneumoniae pneumonia and septicemia in leukopenic rats. This experimental model was chosen because in leukopenic patients septicemia is a real threat and K.pneumoniae is one of the pathogens that are frequently recovered [16, 17]. The in-vitro susceptibility in terms of MBC values of the K.pneumoniae strain used for the selected drugs ceftazidime, gentamicin and ciprofloxacin was similar. The efficacy of the drugs was evaluated in terms of PD_{50} doses obtained after a four day treatment, starting 5 h after bacterial inoculation of the lung. Drugs were administered either intermittently at 6-h intervals or by way of continuous infusion. The therapeutic results obtained after a four day treatment were compared with the kinetics of antibacterial activity of the antibiotics against the K.pneumoniae strain in vitro as well as in lungs of leukopenic rats.

MATERIALS AND METHODS

Animals. Female R strain albino rats (specific pathogen free; 14 to 18 weeks old; weight, 185 to 215 g; bred at REPGO-TNO, Rijswijk, The Netherlands) were used in all experiments.

Bacteria. A K.pneumoniae strain (capsular serotype 2) was used in these experiments. Cultures were obtained by incubation for 16 h at 37°C in Iso-Sensitest broth (Oxoid Ltd., London, England). After proper dilution and reincubation for 2 h at 37°C suspensions of logarithmically growing

bacteria were prepared.

Antibiotics. Ceftazidime (Glaxo Pharmaceuticals, Ltd., Greenford, England), and gentamicin (Schering Corporation Kenilsworth, N.Y., United States) were supplied as powder. Solutions were made in distilled water and stored at minus 80°C. Ciprofloxacin (Bayer AG, Leverkusen, Federal Republic of Germany) was supplied as powder or in ampoules of 100 mg of drug in 10 ml diluent manufactured by Bayer. Solutions were made in distilled water, stored at 4°C and protected from light.

Antimicrobial susceptibility tests. The MICs of the antibiotics were defined as the lowest concentrations that suppressed visible growth after incubation of 5 x 10^5 CFU/ml for 18 h at 37°C in tubes containing 4 ml of Iso-Sensitest broth (Oxoid Ltd., London, England). The MBCs were defined as the lowest concentrations that killed 99.9% of the original inoculum. MBC was determined by spreading subculture volumes of 200 µl onto Iso-Sensitest agar (Oxoid) plates. The concentrations of the serial dilutions decreased by steps of 0.2 µg/ml.

The effect of the three drugs on the short-term growth of K.pneumoniae at 37°C was studied both in Iso-Sensitest broth and in Hanks balanced salt solution (HBSS) (Oxoid) with 90% normal rat serum. A stationary phase culture was diluted in Iso-Sensitest broth to a concentration of 2 x 10^6 CFU/ml. After reincubation for 2 h at 37°C the number of CFU was 4 x 10^7/ml, and antibiotic was added (zero time). Killing experiments were started with an inoculum of 5 x 10^5 CFU/ml. For PAE (postantibiotic effect) determinations a stationary phase culture was diluted to 5 x 10^6 CFU/ml. After reincubation for 2 h at 37°C the number of CFU was 10^8/ml and antibiotic was added (zero time). PAE experiments were started at an inoculum of 5 x 10^7 CFU/ml. After 1 h of incubation with antibiotic bacterial cultures containing ceftazidime and gentamicin were diluted 100-fold in fresh prewarmed medium and cultures containing ciprofloxacin 250-fold. Cultures without antibiotic were treated simularly. Control experiments revealed that at the dilutions used the highest residual antibiotic concentrations obtained for all experiments did not affect the growth of logarithmically growing K.pneumoniae. PAE was quantitated as described by Craig and Gudmundsson for PAE measurement by viable counts [18]. In both the killing experiments and the PAE determinations the numbers of viable bacteria were determined at regular intervals by plate

counts on Iso-Sensitest agar. Before plating the concentrations of ceftazidime, gentamicin, and ciprofloxacin were reduced to an inactive level by centrifugation of 1 ml samples for two minutes at 10,000 g followed by replacement of the drug containing medium by physiological saline.

Pneumonia. Experimental pneumonia was produced as described previously by Bakker-Woudenberg et al. [19]. In brief, rats were anesthetized with fluanisone (Hypnorm; Duphar B.V., Amsterdam, The Netherlands) and pentobarbital (Abbott Laboratories, North Chicago, Ill.). The left main stem bronchus was intubated and the left lung was inoculated with 0.02 ml of a saline suspension of 8 x 10^4 CFU of K.pneumoniae. The number of CFU used to inoculate the left lung in the different experiments was confirmed by plate counts on blood agar. After inoculation the narcotic antagonists Nalorphine bromide and Pentetrazolum (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands) were injected.

Induction of leukopenia. Leukopenia was induced by intraperitoneal injections of cyclophosphamide (CY) (Koch-Light Limited, Haverhill, Suffolk, England) in two doses of 90 mg/kg and 60 mg/kg at five days and one day before bacterial inoculation, respectively. Compared to the course of infection in non-leukopenic rats, CY-induced leukopenia resulted in a rapid bacterial multiplication in the lung followed by septicemia at an early stage of the infection [20].

Antimicrobial treatment. Antibiotics were administered in different ways. In experiments in which the kinetics of antibacterial activity of the antibiotics in lungs of leukopenic rats was studied antibiotic at various doses was administered intravenously as a single injection into the tail vein, at 1 h after bacterial inoculation. Response to antimicrobial treatment was evaluated with respect to the numbers of bacteria in the left lung at different intervals after administration. Rats were sacrificed, the left lung was removed and homogenized in 20 ml of physiological saline in a VirTis homogenizer (The VirTis Co., Inc., Gardiner N.Y.) during 30 s at 10,000 rpm. Serial ten-fold dilutions of homogenates in saline were prepared. Volumes of 0.2 ml of each dilution, and 1 ml volumes of the undiluted lung homogenate were spread on blood agar plates. Statistical analysis was performed by use of two-sided analysis of variance and the Mann-Whitney test. In experiments in which the therapeutic efficacy of the

antibiotics was studied, antibiotic was administered in twofold increasing doses, each dose to a group of ten rats, during a period of four days, starting at 5 h after bacterial inoculation, either intermittently at 6-h intervals into the thigh muscles of the rear legs or by way of continuous infusion. Continuous infusion of antibiotic was achieved according to the method described by Thonus et. al [21]. Response to antimicrobial treatment was evaluated with respect to the daily dose that protected 50% of the animals from death (median protective dose, PD_{50}). PD_{50} values were calculated according to the method of Spearman-Kärber as described by Sachs [22]. Deaths of rats were recorded daily until 16 days after the termination of treatment. After that time no changes in death rate occurred. The left lung of each rat that died was cultured to check for the presence of K.pneumoniae. Only K.pneumoniae organisms were recovered from lungs of both treated and untreated animals. The MBC values of the recovered strains were unchanged as compared to the parent strain.

Measurement of antibiotic concentrations in serum. Blood specimens, obtained by puncture of the retroorbital plexus under light CO_2 anesthesia, were collected from each rat, and serum was separated. With use of a diagnostic sensitivity agar (Oxoid) and an Escherichia coli test strain susceptible to 0.2 µg of ceftazidime/ml, and 0.025 µg of ciprofloxacin/ml, and a Staphylococcus epidermidis strain susceptible to 0.2 µg of gentamicin/ml, all tests were done according to a standard large-plate agar diffusion procedure [23]. Standard samples for determination of antibiotic concentrations in serum were prepared in pooled normal rat serum. Samples of 100 µl were assayed. For determination of concentrations in serum below 0.2 µg ceftazidime/ml some modifications were introduced in order to increase the sensitivity of the test. We applied 225 µl samples into metal rings (internal diameter, 6 mm) placed on the agar. To nine parts of each sample and of each standard concentration, one part of a solution of 1 µg of ceftazidime/ml of serum was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. Control determination of samples with known concentrations of ceftazidime in serum with both the original and the modified method yielded similar results.

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae **in vitro.** The MICs as well as the MBCs of ceftazidime and gentamicin for the Klebsiella strain used were both 0.4 µg/ml. The MIC and MBC of ciprofloxacin were both 0.2 µg/ml. Kinetics of bactericidal activity of the three drugs at concentrations of ½, 1, and 2 times the MBC against K.pneumoniae in broth is shown in figure 1. Bacterial killing by ceftazi-

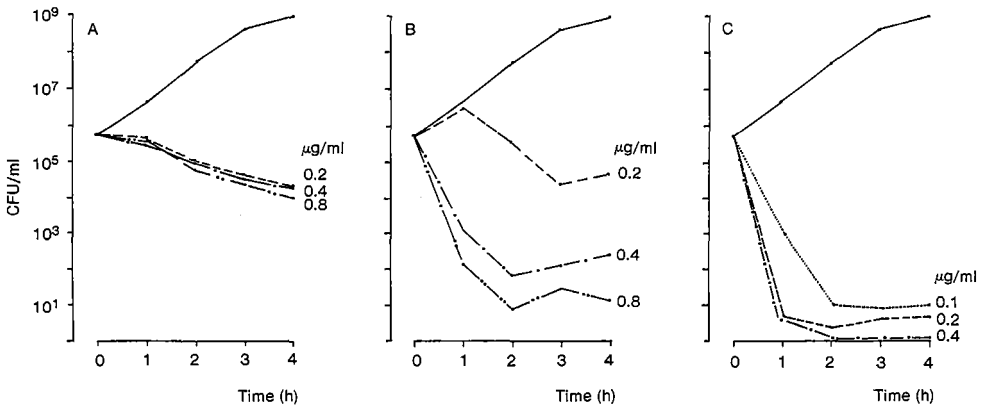

Figure 1 Effect of ceftazidime (A), gentamicin (B), or ciprofloxacin (C) at concentrations indicated against K.pneumoniae in broth.
Bacterial growth without antibiotic (———).

dime was slow but continued during the 4 h incubation period and relatively independent on the concentration. On the contrary both gentamicin and ciprofloxacin demonstrated a rapid bacterial killing that was related to the concentration of antibiotic. The killing rate by ciprofloxacin was extremely high. Although the bactericidal activities of ceftazidime, gentamicin and ciprofloxacin in HBSS with 90% serum were not identical as compared to those in broth (figure 2), no major differences were observed between the killing patterns in both media. As shown in figure 3 a PAE was not observed for the three antibiotics: exposure of the bacteria during 1 h to 5 times the MBC for ceftazidime and gentamicin or 2 times the MBC for ceftazidime, gentamicin and ciprofloxacin did not result in a substantial delay of regrowth as compared to bacteria not exposed to antibiotic.

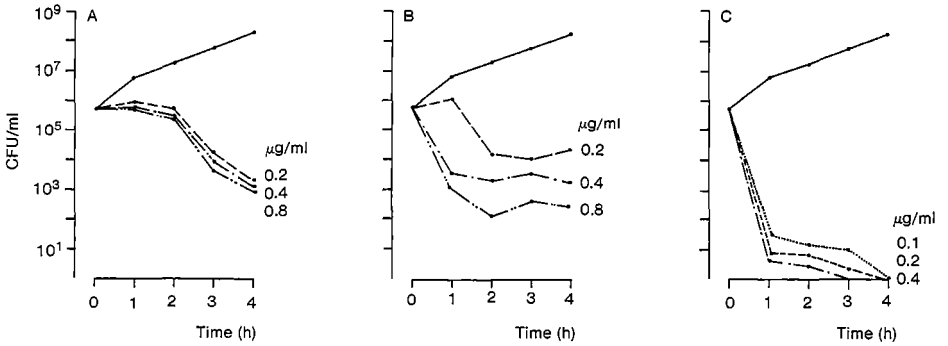

Figure 2 Effect of ceftazidime (A), gentamicin (B), or ciprofloxacin (C) at concentrations indicated against K.pneumoniae in HBSS with 90% serum. Bacterial growth without antibiotic (———).

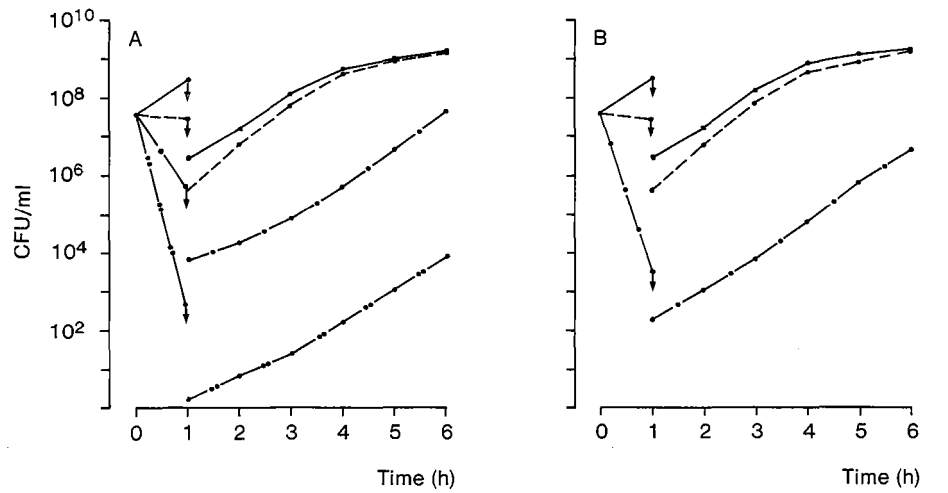

Figure 3 PAE determinations of ceftazidime (– – –), gentamicin (—·—), or ciprofloxacin (—··—) after 1 h exposure of K.pneumoniae to concentrations of 2 times the MBC (A) or 5 times the MBC (B). At 1 h cultures were diluted 100-fold for ceftazidime and gentamicin, and 250-fold for ciprofloxacin. Bacterial growth without antibiotic (———).

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats. The antibacterial activity of ceftazidime, gentamicin and ciprofloxacin against K.pneumoniae in lungs of leukopenic rats after intravenous administration of different doses at 1 h after bacterial inoculation is shown in figure 4. Bacterial killing by ceftazi-

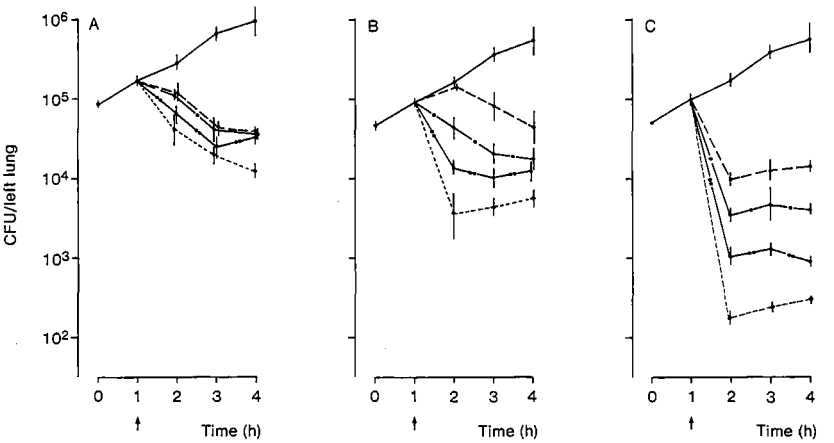

Figure 4 Numbers of K.pneumoniae in lungs of untreated leukopenic rats (———), and after intravenous administration of ceftazidime (A), gentamicin (B), or ciprofloxacin (C), in doses of 0.3 mg/kg (– – –), 1.0 mg/kg (—·—), 3.0 mg/kg (—··—), or 9.0 mg/kg (------) at 1 h after inoculation of lungs with K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

dime was not strongly related to the dosage administered although at the different doses used ceftazidime peak concentrations in serum ranged from 1 to 48 µg/ml (table 1). A significant increase in bacterial killing rate was only observed during the first hour after administration of the dosage 3.0 mg/kg compared to 1.0 mg/kg ($P < 0.05$ by Mann-Whitney). A further increase of the dose up to 9.0 mg/kg did not result in a stronger decrease of the numbers of bacteria in the lung ($P > 0.05$). In contrast to ceftazidime killing rates by gentamicin and ciprofloxacin increased with each dose increment from 0.3 up to 9.0 mg/kg. ($P < 0.05$). At similar doses bacterial killing by ciprofloxacin was more rapid compared to gentamicin, whereas gentamicin exerted a higher bacterial killing rate compared to ceftazidime from the 3.0 mg/kg dose ($P < 0.05$). Bacterial killing by ceftazidime continued until 2 h after injection ($P < 0.05$ by two-sided analysis

Table 1 Concentrations of ceftazidime, gentamicin, and ciprofloxacin in serum at various intervals after intravenous administration to leukopenic rats

antibiotic	dose (mg/kg)	drug concentration (µg/ml of serum) at the following times (min) after dosage			
		5	15	30	90
ceftazidime	0.3	1.1 ±0.19	0.76±0.13	0.42±0.08	0.12±0.03
	1.0	4.4 ±0.75	2.3 ±0.15	1.1 ±0.04	0.30±0.10
	3.0	13.1 ±0.76	5.7 ±1.74	3.8 ±1.44	1.5 ±0.42
	9.0	48.2 ±1.89	23.9 ±1.32	12.2 ±0.58	3.10±0.65
gentamicin	3.0	14.1 ±1.04	6.8 ±0.86	4.5 ±0.31	0.95±0.06
ciprofloxacin	3.0	0.94±0.13	0.67±0.15	0.55±0.08	0.41±0.08

NOTE. Each value represents the mean ± SD for five rats.

of variance), the killing rate between the first and second hour after administration being independent on the dose administered ($P > 0.05$). On the contrary the bacterial killing by gentamicin and ciprofloxacin was completed within 1 h after administration, thereafter no further decrease in bacterial numbers occurred, irrespective the dose administered ($P < 0.05$).

Figure 5 shows the numbers of bacteria in the left lung at various intervals until 9 h after intravenous administration of 3.0 mg/kg of ceftazidime, gentamicin, or ciprofloxacin at 1 h after bacterial inoculation. Substantial regrowth of bacteria in the left lung did not occur, although antibiotic concentrations in the lung were below the MBC from about 2.5 h after antibiotic administration (data not shown). Although bacterial counts started to increase from the third hour after injection of ciprofloxacin, this increase was only slightly significant.

The concentrations of ceftazidime, gentamicin and ciprofloxacin in serum of rats at various intervals after intravenous administration of different doses are shown in table 1. Kinetics of ceftazidime and gentamicin in serum were comparable, with estimated elimination half-lifes ($t_{\frac{1}{2}\beta}$) of 30 minutes. Ciprofloxacin serum concentrations were about 14-fold lower as compared to those of ceftazidime and gentamicin, within 5 minutes after administration of a similar dose of 3.0 mg/kg. The estimated $t_{\frac{1}{2}\beta}$ of ciprofloxacin was

approximately 100 minutes.

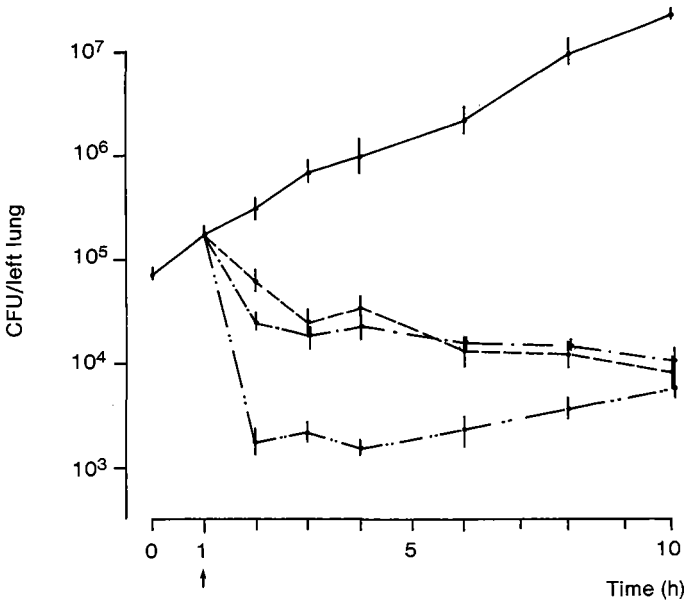

Figure 5 Numbers of K.pneumoniae in lungs of untreated leukopenic rats (———), and after intravenous administration of 3.0 mg/kg of ceftazidime (– – –), gentamicin (—·—) or ciprofloxacin (—··—) at 1 h after inoculation of lungs with K.pneumoniae in the logarithmic phase of growth. Each point represents the geometric mean ± SEM for five rats.

Therapeutic efficacy of ceftazidime, gentamicin, and ciprofloxacin in K.pneumoniae pneumonia and septicemia in leukopenic rats. The therapeutic efficacy of ceftazidime, gentamicin, and ciprofloxacin determined in terms of PD_{50} values is shown in table 2. The therapeutic effect of ceftazidime was dependent on the dosage regimen, being far more effective when administered by continuous infusion as compared to intermittent treatment at 6-h intervals, resulting in PD_{50} values of 1.5 and 24.4 mg/kg per day, respectively ($P < 0.001$). For gentamicin no significant difference in efficacy could be observed between both modes of administration: PD_{50} values were 3.8 and 2.8 mg/kg per day ($P > 0.05$) for continuous and intermittent administration, respectively. The therapeutic efficacy of ciprofloxacin was slightly increased when administered intermittently

Table 2 Efficacy of ceftazidime, gentamicin and ciprofloxacin treatment schedules in leukopenic rats

dose (mg/kg/day)	Antibiotic											
	ceftazidime				gentamicin				ciprofloxacin			
	intermittent administration		continuous administration		intermittent administration		continuous administration		intermittent administration		continuous administration	
	No. of surv.	Time to* death (days)	No. of surv.	Time to* death (days)	No. of surv.	Time to* death (days)	No. of surv.	Time to* death (days)	No. of surv.	Time to* death (days)	No. of surv.	Time to* death (days)
0.47			0	2.8±0.4	0	3.7±0.5						
0.94			1	5.2±1.9	2	6.5±1.4	0	4.1±1.2	0	3.1±0.7		
1.88			7	9.3±3.5	7	8.3±2.5	3	7.9±3.1	1	4.9±0.6	0	5.6±1.5
3.75			10		5	9.2±3.4	7	7.3±3.2	8	7.5±2.1	4	7.3±1.4
7.50	0	3.4±1.2			6	14.3±5.4	5	10.4±3.4	9	17	4	7.0±2.0
15.00	1	3.5±0.7			9	7	10		9	10	9	7.0
30.00	7	5.3±2.1			10				10		10	
60.00	10											

NOTE. To groups of 10 rats each antibiotics were administered either intermittently at 6-h intervals or by way of continuous infusion during a period of 4 days. Treatment was started 5 h after inoculation of the left lung with $8x10^4$ CFU of K.pneumoniae. PD_{50} doses in mg/kg per day (95% confidence limits) obtained after intermittent and continuous administration, respectively were for ceftazidime 24.4 (19.07-31.24) and 1.5 (1.19-1.95), for gentamicin 2.8 (1.83-4.42) and 3.8 (2.59-5.43), and for ciprofloxacin 3.3 (2.42-4.40) and 6.5 (4.64-9.19).

* Mean ± SD; based on the time of bacterial inoculation (day 0).

compared to continuous infusion, demonstrated by PD_{50} values of 3.3 and 6.5 mg/kg per day, respectively ($P < 0.05$).

The concentrations of ceftazidime, gentamicin and ciprofloxacin in serum after administration of PD_{50} doses intermittently or by continuous infusion, are shown in tables 3 and table 4, respectively. When administered intermittently, the time periods that serum concentrations were above the

Table 3 Concentrations of ceftazidime, gentamicin and ciprofloxacin in serum at various intervals after intramuscular administration at 6-h intervals of PD_{50}s to infected leukopenic rats

antibiotic	dose (mg/kg)	drug concentration (μg/ml of serum) at the following times (min) after dosage		
		15	30	90
ceftazidime	6.1	14.3 ±0.70	9.6 ±1.18	4.9 ±2.55
gentamicin	0.7	1.6 ±0.31	1.4 ±0.58	0.28±0.05
ciprofloxacin	0.8	0.29±0.07	0.20±0.05	0.07±0.02

NOTE. Doses correspond to the PD_{50} daily doses of 24.4, 2.8, and 3.3 mg/kg obtained for ceftazidime, gentamicin, and ciprofloxacin, respectively. Sera were taken at various intervals after the 8^{th} injection.
Each value represents the mean ± SD for five rats.

Table 4 Concentrations of ceftazidime, gentamicin and ciprofloxacin in serum at various intervals after continuous administration of PD_{50}s to infected leukopenic rats

antibiotic	dose (mg/kg/day)	drug concentration (μg/ml of serum) at the following times (h) after dosage		
		5	24	48
ceftazidime	1.5	0.17±0.09	0.17±0.05	0.22±0.05
gentamicin	3.5	0.29±0.05	0.28±0.03	0.26±0.01
ciprofloxacin	6.5	0.15±0.03	0.16±0.03	0.12±0.02

NOTE. Each value represents the mean ± SD for five rats.

MBC of the K.pneumoniae strain were about 170 and 75 minutes for ceftazidime and gentamicin, respectively. For ciprofloxacin serum concentrations were above the MBC for omly 40 minutes during the 6-h dosing interval. Steady state serum concentrations after administration of PD_{50} doses by continuous infusion were 0.19, 0.28, and 0.14 µg/ml for ceftazidime, gentamicin, and ciprofloxacin, respectively.

DISCUSSION

The impact of the antibiotic dosage schedule for the treatment of serious infections is not clearly established. Particularly in the immunocompromised host, the schedule of drug administration may play an important role in the outcome of antibiotic treatment of infections, as in these patients recovery from infection depends to a high degree on antibiotics, and therapeutic failure still occurs. During the past few years several investigators studied the efficacy of different antibiotic dosage schedules in experimental gram-negative infections [6-15]. In the present study the impact of the antibiotic treatment schedule of ceftazidime, gentamicin, and ciprofloxacin on the therapeutic efficacy was investigated in a model of K.pneumoniae pneumonia and septicemia in leukopenic rats. The in-vitro susceptibility in terms of MBC values of the K.pneumoniae strain used was similar for the three drugs. We observed that ceftazidime was far more effective when administered by continuous infusion compared to treatment at 6-h intervals. It appeared that the therapeutic effect of ceftazidime is dependent on the maintenance of antibiotic concentrations in plasma during the entire treatment interval. This observation is in accordance with the gradual and relatively dose-independent bactericidal effect of ceftazidime seen in the lungs of leukopenic rats after administration of a single dose. Despite a variation in serum peak concentrations from 1 to 48 µg/ml at the ceftazidime doses used no major differences in bacterial killing rate were observed. This relatively dose-independent antibacteridal effect in vivo is also corresponding to the concentration-independent bacterial killing pattern of ceftazidime in vitro at concentrations around the MBC. In contrast to ceftazidime the therapeutic efficacy of gentamicin was not related to the mode of administration. With gentamicin intermittent treatment resulting in relatively high peak concentrations at relatively long intervals seems to be permitted without loss of efficacy. This was

confirmed by the high and dose-dependent bacterial killing rate of gentamicin in lungs of leukopenic rats after intravenous administration of a single dose. Also in vitro the bacterial killing rate of gentamicin was related to the concentration used. These results show that the activity of gentamicin is strongly dependent on the peak concentration. The therapeutic efficacy of ciprofloxacin when administered at 6-h intervals was slightly increased compared to continuous treatment. This confirms the dose-dependent and very rapid killing of K.pneumoniae in lungs of leukopenic rats after a single intravenous injection. At all doses tested ciprofloxacin exerted a high bacterial killing rate which increased with increasing doses. A similar pattern of bactericidal effect was found in vitro. Differences in bactericidal kinetics between ceftazidime, gentamicin, and ciprofloxacin are probably related to differences in mode of action.

Besides the bacterial killing rate and the dose-response effect, the rate of bacterial regrowth after exposure to antibiotic may also be an important determinant for the dosage-regimen-dependent therapeutic effect of antibiotic [25, 26]. In this respect we found no major differences between the three antibiotics used. In vitro a PAE of the K.pneumoniae strain for the antibiotics was not observed. In addition, in vivo patterns of bacterial regrowth in the lung after administration of antibiotic were not substantially different.

Our experimental data with regard to the impact of the antibiotic dosage schedule of ceftazidime and gentamicin on the therapeutic efficacy are in accordance with those of other investigations with respect to gram-negative infections [6-9, 12, 14, 15]. Gerber et al. demonstrated that ticarcillin was more effective in reducing the number of Pseudomonas aeruginosa organisms in thighs of leukopenic mice when the interval of administration was reduced from 3 hours to 1 hour [7]. In contrast, the activity of gentamicin was not dependent on the frequency of injection. Using the same infection model, they also demonstrated that the in-vivo efficacy of gentamicin and also of netilmicin was mainly dependent on the total dose administered and not on the mode of administration either as a single bolus injection or at very short intervals to mimic human serum kinetics [8]. On the other hand the ß-lactams ticarcillin and ceftazidime were more effective when administered in fractional doses which resulted in longer periods of active drug levels in serum as compared to administration as a single dose. Other investigators using models of pneumonia and thigh muscle

infection in mice both caused by K.pneumoniae showed that the efficacy of the ß-lactam cefazolin was related to the time period serum drug levels remained above the MIC of the infecting strain [12, 15]. On the contrary the efficacy of gentamicin was correlated with the total dose administered irrespective the frequency of administration. The conclusion derived from these experiments that the efficacy of ß-lactam antibiotics increased with increasing dosing frequency, whereas the in vivo efficacy of aminoglycosides was independent on the dosage interval, was also confirmed in an experimental high muscle infection in mice due to K.pneumonia [9] and pneumonia or endocarditis caused by P.aeruginosa in rats, or rabbits, respectively [14]. In contrast to these findings there are some experimental studies suggesting that the in-vivo activity of the aminoglycoside tobramycin is dependent on the antibiotic dosage schedule used [11, 13]. In these experiments, however, long treatment intervals in relation to the short elimination half life of tobramycin in small experimental animals as compared to that in humans were used. We are not aware of studies concerning the impact of the dosage schedule of quinolones on the therapeutic efficacy in experimental infections. However, one study comparing the efficacy of the ß-lactam BMY-28142 and the quinolone ciprofloxacin in a model of pseudomonas endocarditis, shows that although after administration of single doses the ratio of concentrations of both antibiotics to the MBC at the site of infection were similar, ciprofloxacin was superior to BMY after a 5 day treatment at 8-h intervals [10]. In order to obtain the same effect as compared to ciprofloxacin BMY had to be administered for a longer period at shorter intervals. This observation is in accordance with our observation that ciprofloxacin was very effective in contrast to ceftazidime, when rats were treated at 6-h intervals.

Our results with respect to the kinetics of bactericidal activity of ceftazidime are in accordance with the slow and relatively dose-independent bacterial killing rate as observed generally for ß-lactams in vivo [7, 8, 10, 26] and in vitro [27-30]. Our observations with respect to gentamicin are also in agreement with the findings of several investigators demonstrating a fast and dose-dependent bactericidal activity of aminoglycosides in vivo [7, 8] as well as a concentration-dependent bacterial killing rate in vitro [27, 30, 31]. Of interest in this respect is the study by Moore et al. who demonstrated a strong correlation between the peak level to MIC ratio in serum of a number of aminoglycosides and the clinical outcome of the treatment of gram-negative infections at 8-h

intervals [32]. With respect to ciprofloxacin several studies demonstrate a comparable dose-dependent bactericidal activity in vivo [10], a dose response effect which was also observed in vitro [10, 33].

In general, in accordance with our findings in vitro, gram-negative bacteria are found to regrow immediately after ß-lactam concentrations have fallen below active levels. Contrary to our observations their regrowth is suppressed for variable periods of time after exposure to aminoglycosides and quinolones [34, 35]. However, PAEs of aminoglycosides in vitro appeared to be of short duration (with the exception of Pseudomonas) and may vary between the individual strains [34]. There is also strain variation with respect to the presence of a PAE for quinolones against K.pneumoniae [36]. Like in vitro no major differences were found between the individual drugs tested with respect to the bacterial regrowth in the lungs of leukopenic rats.

The data derived from the experimental infection models may have implications for the treatment of infections in leukopenic patients. In general, ß-lactams are administered at intervals and, taken into account their elimination half-lives, in relatively high doses resulting in high peak concentrations in order to provide active drug levels for most infecting strains until the next dose. The experimental studies show that for successful treatment with ß-lactams it is of great importance that antibiotic concentrations are maintained at a certain level during the period of treatment. High peak concentrations in plasma do not contribute to therapeutic effect. These data suggest that lower daily doses of ß-lactams may be permitted provided they are administered more frequently or ß-lactams with long half lives are used. This might firstly result in improvement of therapeutic effect, and secondly this might be favorable in terms of cost-effectiveness. Bodey et al. showed that patients with persistent granulocytopenica responded better to a regimen including besides carbenicillin the constant infusion of cefamandol as compared to intermittent administration of this drug [3]. In a recent clinical study in patients with serious systemic infections Hoepelman et al. demonstrated that a single daily treatment with the highly protein-bound drug ceftriaxone resulting in prolonged plasma concentrations, was more effective than a combination of gentamicin plus cefuroxime [37]. With regard to the aminoglycosides and the quinolones there is no experimental evidence that the dosage schedule is an important determinant for efficacy. In view of the fact that the bactericidal effect of these antibiotics is strongly

related to the peak concentration, and the bacterial killing is very fast, further studies are needed to demonstrate whether less frequent dosing with increased doses may be equally effective or even more efficacious in the immunocompromised host. In this respect several studies are of interest demonstrating that animal nephrotoxicity [14, 38-42] or ototoxicity [42] decreased with decreasing frequency of administration, despite higher individual doses of aminoglycosides. Less frequent dosing was not associated with loss of efficacy [14, 41] or resulted even in increased efficacy [14, 42]. Several human studies did also not reveal nepro- or ototoxicity despite relatieve high doses of aminoglycosides given at 24-h intervals, which regimens proved to be effective [14, 43, 44].

ACKNOWLEDGEMENTS

This work was supported in part by research grants from Bayer Nederland B.V. (The Netherlands), Glaxo Pharmaceuticals Ltd. (United Kingdom) and Beecham Research Laboratories (The Netherlands).
We thank Caroline van Bakel for secretarial help.

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APPENDIX PAPER V

IMPACT OF THE DURATION OF INFECTION ON THE ACTIVITY OF CEFTAZIDIME, GENTAMICIN, AND CIPROFLOXACIN IN KLEBSIELLA PNEUMONIAE PNEUMONIA AND SEPTICEMIA IN LEUKOPENIC RATS*

R.Roosendaal, I.A.J.M. Bakker-Woudenberg, M. van den Berghe-van Raffe, J.C. Vink-van den Berg, M.F. Michel

ABSTRACT

An experimental Klebsiella pneumoniae pneumonia and septicemia in leukopenic rats was used to study the impact of the duration of infection on the efficacy of ceftazidime, gentamicin, and ciprofloxacin. It appeared that a single dose of each of the respective drugs administered intravenously at 1 h after bacterial inoculation did not kill all the K.pneumoniae organisms in the lung. This was not due to the rapid elimination of antibiotic. Persisting bacteria did also not represent a less susceptible subpopulation selected after antibiotic administration. The size of this subpopulation was determined by several factors. The class of antibiotic and in addition for gentamicin and ciprofloxacin the dose administered were determinants in this respect. It was also demonstrated for the three drugs tested that the number of persisting bacteria increased with increase of the duration of the infection. This effect was most pronounced for ciprofloxacin. An inoculum effect could not explain this diminished bacterial killing. In further experiments the relevance of this observation for the therapeutic efficacy of ceftazidime and ciprofloxacin was investigated. Rats were treated for four days at 6-h intervals, starting at different times after bacterial inoculation. Ciprofloxacin appeared to be more effective than ceftazidime, which correlated with its higher bacterial activity. However the therapeutic efficacy of both drugs decreased as a result of delay of start of treatment. Our data underline the need to start antimicrobial treatment as soon as possible, because the number of bacteria persisting after antibiotic administration increases with the duration of the infection.

*(Submitted for publication)

INTRODUCTION

Granulocytopenic patients are treated with antibiotics empirically and blind at the first signs of fever. It appeared that early start of treatment of gram-negative infections, instead of waiting for the laboratory results regarding identity and susceptibility of the causative organism, reduced mortality rates substantially [19]. It is, however, not clearly established which factors are responsible for the reduction of the activity of antibiotics as a result of delay of treatment. We used an experimental K.pneumoniae pneumonia and septicemia in leukopenic rats in order to investigate this phenomenon. This model was selected because in leukopenic patients septicemia is a real threat and K.pneumoniae is one of the pathogenes that is recovered [11,17].

In our model we observed that single doses of ceftazidime, gentamicin, or ciprofloxacin did not kill all K.pneumoniae organisms in the lungs of leukopenic rats. It is not clear which factors are responsible for the survival of bacteria at the site of infection. One explanation may be the absence of active antibiotic concentrations due to rapid drug elimination. Another possible factor is the occurrence of phenotypic resistance, a phenomenon reviewed by Greenwood [10]. Due to phenotypic heterogeneity within a susceptible bacterial population a fraction of the population may be resistant to concentrations of antibiotic that kill most of the bacteria. The role of both factors in survival of K.pneumoniae in the lung was investigated in the present study. Bacterial survival at the site of infection may also be the result of a change in the antibiotic susceptibility of the infecting strain or of changes in the activity of antibiotic due to local environmental factors, which as a consequence is only expressed in vivo. In the present study it was investigated whether the number of persisting bacteria varied with the duration of infection and, in addition whether the persisting bacteria could be eliminated by administration of an antibiotic with a different mode of action.

To study the impact of delay of start of treatment on the therapeutic activity of antibiotic ciprofloxacin or ceftazidime were administered for a period of four days at 6-h intervals, starting at times after bacterial inoculation with K.pneumoniae.

MATERIALS AND METHODS

Animals. Female R strain albino rats (specific pathogen free; 14 to 18 weeks old; weight, 185 to 215 g; bred at REPGO-TNO, Rijswijk, The Netherlands) were used in all experiments.

Bacteria. A K.pneumoniae strain (capsular serotype 2) was used in these experiments. Cultures were obtained by incubation for 16 h at 37°C in Iso-Sensitest broth (Oxoid Ltd., London, England). After proper dilution and reincubation for 2 h at 37°C suspensions of logarithmically growing bacteria were prepared.

Antibiotics. Ceftazidime (Glaxo Pharmaceuticals, Ltd., Greenford, England), and gentamicin (Schering Corporation Kenilsworth, N.Y., United States) were supplied as powder. Solutions were made in distilled water and stored at -80°C. Ciprofloxacin (Bayer AG, Leverkusen, Federal Republic of Germany) was supplied as powder or in ampoules of 100 mg of drug in 10 ml diluent manufactured by Bayer. Solutions were made in distilled water, stored at 4°C and protected from light.

Antimicrobial susceptibility tests. The MICs of the antibiotics were defined as the lowest concentrations that suppressed visible growth after incubation of 5 x 10^5 CFU/ml for 18 h at 37°C in tubes containing 4 ml of Iso-Sensitest broth (Oxoid Ltd., London, England). The MBCs were defined as the lowest concentrations that killed 99.9% of the original inoculum. MBC was determined by spreading subculture volumes of 200 µl onto Iso-Sensitest agar (Oxoid) plates. The concentrations of the serial dilutions decreased by steps of 0.2 µg/ml.
For population analysis related to antibiotic susceptibility CFU counts were determined simultaneously on antibiotic-free ISO-sensitest agar plates and on agar plats containing various concentrations of the antibiotic studied.

Pneumonia. Experimental pneumonia was produced as described previously by Bakker-Woudenberg et al. [1]. In brief, rats were anesthetized with fluanisone (Hypnorm; Duphar B.V., Amsterdam, The Netherlands) and pento-barbital (Abbott Laboratories, North Chicago, Ill.). The left main stem bronchus was intubated and the left lung was inoculated with 0.02 ml of a

saline suspension of 8 x 10^4 CFU of K.pneumoniae. The number of CFU used to inoculate the left lung in the different experiments was confirmed by plate counts on blood agar. After inoculation the narcotic antagonists Nalorphine bromide and Pentetrazolum (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands) were injected.

Induction of leukopenia. Leukopenia was induced by intraperitoneal injections of cyclophosphamide (CY) (Koch-Light Limited, Haverhill, Suffolk, England) in two doses of 90 mg/kg and 60 mg/kg at five days and one day before bacterial inoculation, respectively. Compared to the course of infection in non-leukopenic rats, CY-induced leukopenia resulted in a rapid bacterial multiplication in the lung followed by septicemia at an early stage of the infection [14].

Antimicrobial treatment. The in vivo activity of the selected antibiotics was examined in leukopenic rats in different ways. Bactericidal activity of antibiotic was examined after intravenous injection at various times after inoculation of the left lung with K.pneumoniae organisms in the logarithmic growth phase. The numbers of bacteria in the lung were determined at various intervals after administration of antibiotic. The Mann-Whitney test was used for statistical analysis. Therapeutic efficacy was determined after treatment at 6-h intervals, for a period of 4 days, starting at different times after bacterial inoculation.
Response to antimicrobial treatment was evaluated with respect to the daily dose that protected 50% of the animals from death. PD50 values were calculated according to the method of Spearman-Kärher as described by Sachs [16].

Measurement of antibiotic concentrations in serum. Blood specimens, obtained by puncture of the retro-orbital plexus under light CO_2 anesthesia, were collected from each rat, and serum was separated. With use of a diagnostic-sensitivity agar (Oxoid) and an Escherichia coli test strain susceptible to 0.2 µg of ceftazidime/ml, and 0.025 µg of ciprofloxacin/ml, and a Staphylococcus epidermidis strain susceptible to 0.2 µg of gentamicin/ml, all tests were done according to a standard large-plate agar diffusion procedure [2]. Standard samples for determination of antibiotic concentrations in serum were prepared in pooled normal rat serum. Samples of 100 µl were assayed. For determination of concentrations in serum below

0.2 µg ceftazidime/ml some modifications were introduced in order to increase the sensitivity of the test. We applied 225 µl samples into metal rings (internal diameter, 6 mm) placed on the agar. To nine parts of each sample and of each standard concentration, one part of a solution of 1 µg of ceftazidime/ml of serum was added. Correlation coefficients of the calculated regression lines were >0.99 in all determinations. Control determination of samples with known concentrations of ceftazidime in serum with both the original and the modified method yielded similar results.

RESULTS

Effect of ceftazidime, gentamicin, and ciprofloxacin against K.pneumoniae **in lungs of leukopenic rats.** The MICs as well as the MBCs of ceftazidime and gentamicin for the Klebsiella strain used were both 0.4 µg/ml. The MIC and MBC of ciprofloxacin were both 0.2 µg/ml.
The effect of ceftazidime, gentamicin, and ciprofloxacin is shown in fig 1.

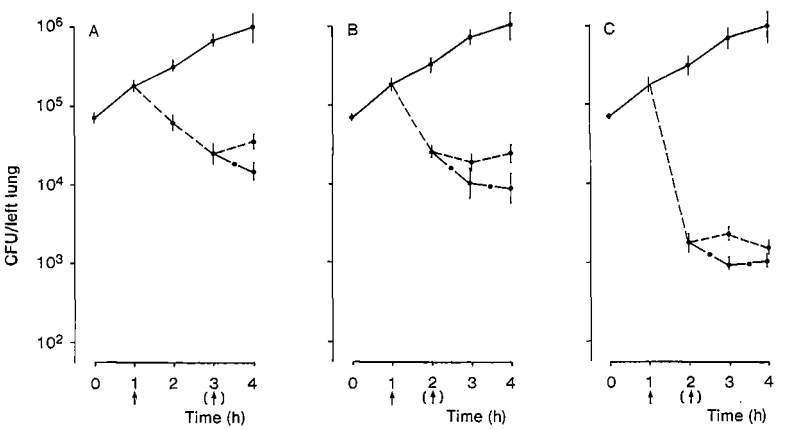

Figure 1 Numbers of K.pneumoniae in lungs of untreaed leukopenic rats (——) and after intravenous administration of 3 mg/kg of ceftazidime (A), gentamicin (B), or ciprofloxacin (C). Ceftazidime was administered at 1 h (— — —) or at 1 h and 3 h (—·—), and gentamicin or ciprofloxacin at 1 h (— — —) or at 1 h and 2 h (—·—) after inoculation of lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth.
Each point represents the geometric mean ± SEM for five rats.

One intravenous injection of 3 mg/kg at 1 h after bacterial inoculation killed a large fraction of the bacterial population, although not to the same extent for all three antibiotics. Whereas ceftazidime and gentamicin killed about 90% of the bacterial population, ciprofloxacin killed about 99%. A second injection of 3 mg/kg at the time the maximum bactericidal effect was reached resulted in a slight, but not significant further reduction in numbers of bacteria for the three drugs tested.
Population analysis related to antibiotic susceptibility of the three antibiotics did not reveal the selection of a resistant bacterial subpopulation at the time the maximum bactericidal effect was reached (fig 2). In contrast, a shift to a more susceptible population was observed.

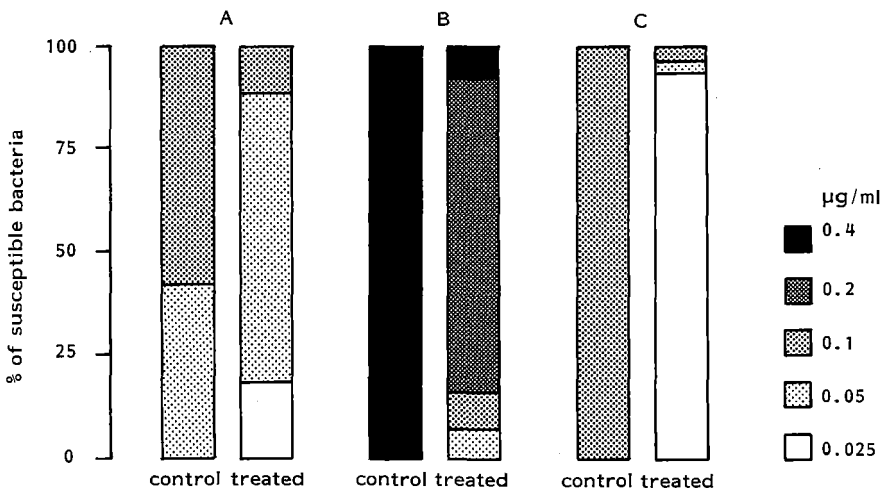

Figure 2 Susceptibility distribution of K.pneumoniae. Bacteria were isolated from lungs of leukopenic rats immediately before administration of antibiotic (control), 2 h after injection of 3 mg of ceftazidime/kg (A), 1 h after injection of 3 mg of gentamicin/kg (B), or 1 h after injection of 3 mg of ciprofloxacin/kg (C). Antibiotics were administered intravenously at 1 h after inoculation of the lungs with 8 x 10^4 of logarithmically growing K.pneumoniae.

Fig 3 shows the bactericidal activity of ceftazidime, gentamicin, and ciprofloxacin in lungs of leukopenic rats after administration of 3 mg/kg at different times after inoculation with K.pneumoniae. At the time of injection of antibiotic the mean number of bacteria per left lung was similar in

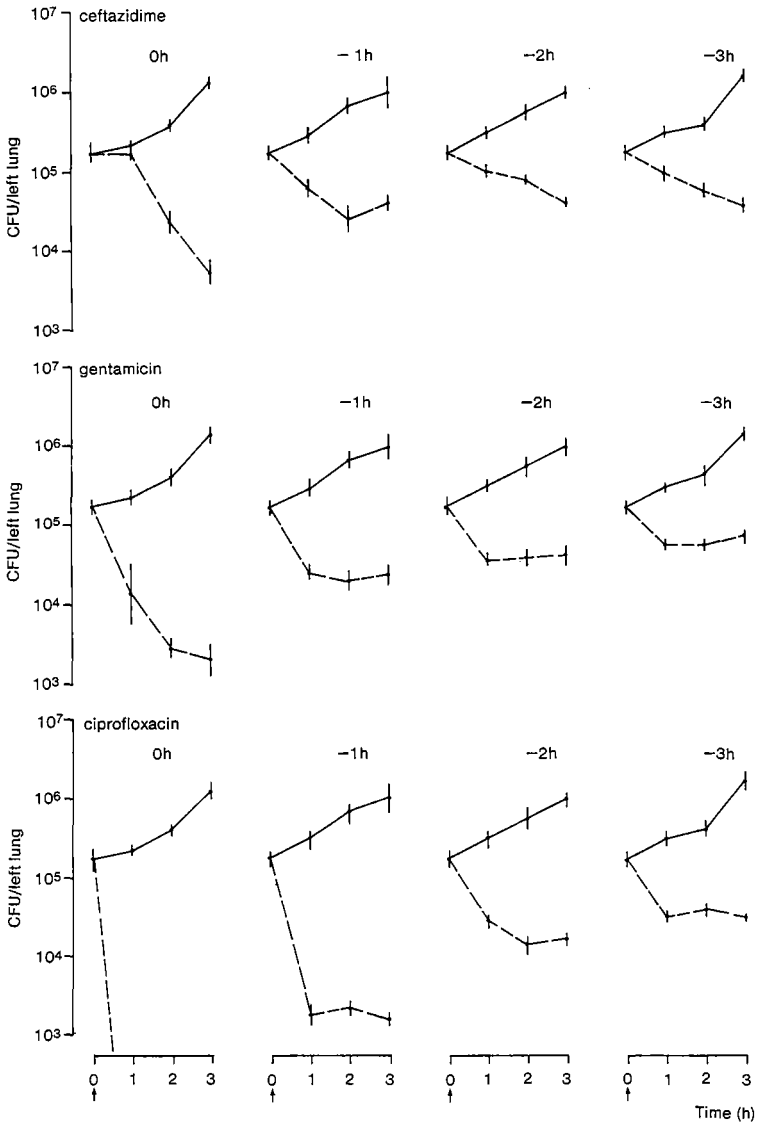

Figure 3 Numbers of K.pneumoniae in lungs of untreated leukopenic rats (——) and at various times after intravenous administration of 3 mg/kg of ceftazidime, gentamicin, or ciprofloxacin (– – –). Lungs of leukopenic rats were inoculated with K.pneumoniae in the logarithmic phase of growth at the same time (0 h) or at various times before injection with antibiotic (-1 h, -2 h, or -3 h). Antibiotic was injected at 0 h. At the time of antibiotic administration the mean number of bacteria per left lung was 2.2×10^5 in all experiments. Each point represents the geometric mean ± SEM for five rats.

all experiments (2.2 x 10^5 CFU). Delay of antibiotic administration resulted in a decrease in bacterial killing for all three drugs tested. Killing by ceftazidime continued during 3 h except when administered at 1 h after inoculation. When injection of ceftazidime was delayed from 0 h until 1 h after inoculation with K.pneumoniae bacterial killing decreased significantly ($P < 0.05$). Further delay of administration of ceftazidime resulted in a slight but not significant decrease of bactericidal activity ($P > 0.05$). For gentamicin delay of administration also resulted in a decrease of bacterial killing in the lung. This decrease was significant for each successive hour injection of gentamicin was postponed ($P < 0.05$). For ciprofloxacin similar results were obtained.

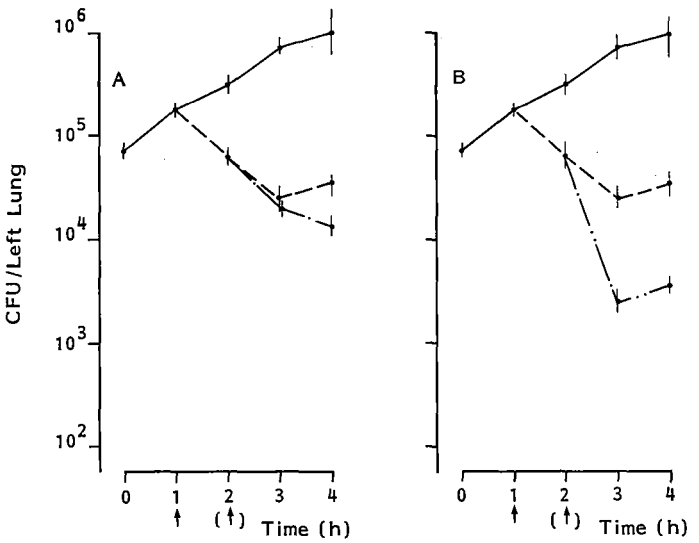

Figure 4 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and at different times after administration of ceftazidime at 1 h (– – –) or ceftazidime at 1 h and 2 h (—·—) (A), of ceftazidime at 1 h (— – —) or ceftazidime at 1 h and ciprofloxacin at 2 h (—··—) (B) after inoculation of the lungs with 8 x 10^4 K.pneumoniae in the logarithmic phase of growth. Antibiotic was administered in doses of 3 mg/kg.
Each point represents the geometric mean ± SEM for five rats.

Although after administration of 3 mg of ceftazidime/kg at 1 h a second dose of 3 mg of ceftazidime/kg at 2 h was not able to reduce the bacterial

numbers in the lung significantly (fig 4A), a second dose of 3 mg of ciprofloxacin/kg at 2 h resulted in a significant additional bacterial killing ($P < 0.05$) (fig 4B). Similar results were obtained for administration of ciprofloxacin after gentamicin (fig 5A and 5B).

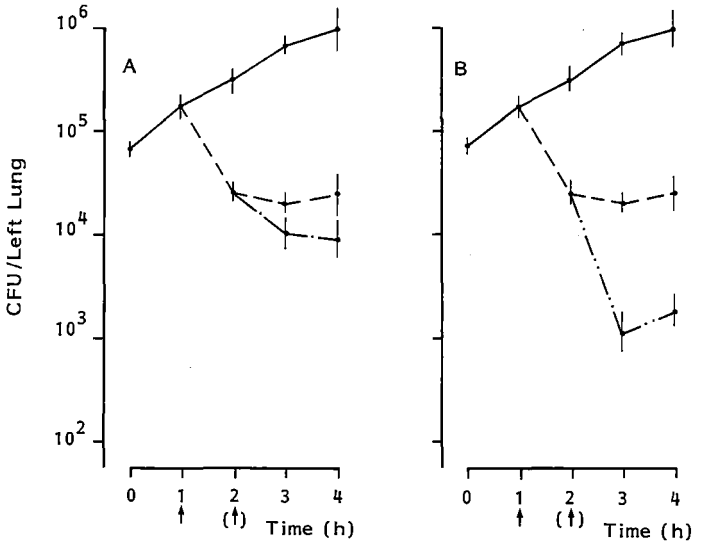

Figure 5 Numbers of K.pneumoniae in the lungs of untreated leukopenic rats (———) and at different times after administration of gentamicin at 1 h (– – –) or gentamicin at 1 h and 2 h (—·—) (A), of gentamicin at 1 h (– – –) or gentamicin at 1 h and ciprofloxacin at 2 h (—··—) (B) after inoculation of the lungs with 8×10^4 K.pneumoniae in the logarithmic phase of growth. Antibiotic was administered in doses of 3 mg/kg.
Each point represents the geometric mean ± SEM for five rats.

Finally the effect of delay of start of treatment on the therapeutic efficacy of ceftazidime and ciprofloxacin is shown in table 1. The PD_{50}s of ceftazidime after a four-day treatment at 6-h intervals, when starting at 5, 12 or 24 h after bacterial inoculation increased significantly ($P < 0.05$), and were 24.4, 52.2 and 120 mg/kg/day, respectively. Delay of start of treatment with ciprofloxacin from 5 h to 12 h after inoculation resulted in a significant increase of the PD_{50} value from 3.3 to 12.2 mg/kg/day ($P < 0.05$). Delay up to 24 h resulted in a further, although not significant, reduction in activity as shown by an increase of the PD_{50} up to

21.2 mg/kg/day (P >0.05).

Table 1 Efficacy of ceftazidime and ciprofloxacin treatment schedules[a]

start of treatment (h)	PD_{50} (mg/kg/day)	
	ceftazidime	ciprofloxacin
5	24.4	3.3
12	52.2	12.2
24	120.0	21.2

[a]Antibiotics were administered at 6-h intervals over a period of four days starting at different times after bacterial inoculation of the left lung with 8×10^4 CFU of K.pneumoniae.

DISCUSSION

It was observed that single doses of ceftazidime, gentamicin, or ciprofloxacin did not kill all K.pneumoniae organisms in the lungs of leukopenic rats. The experiments described in this study were performed in order to elucidate some factors that are responsible for this observation. Firstly, it was investigated whether this incapacity of antibiotic to kill all bacteria in the lung was the result of insufficient concentrations of antibiotic at the site of infection due to rapid elimination of antibiotic. This appeared to be not an important factor as a second injection of the same antibiotic at the time of the maximum bactericidal effect of the first dose resulted only in a slight but not significant additional decrease in bacterial numbers. In addition the persisting bacteria appeared not to represent a less susceptible subpopulation selected after antibiotic administration. In contrast to our observation the selection of more resistant bacteria after exposure to antibiotic has been demonstrated by several investigators [3, 4, 8, 9, 12, 13]. It has for instance been shown that exposure of enterobacteriaceae to aminoglycosides [12] as well as Pseudomonas aeruginosa to aminoglycosides or quinolones [3, 4 ,8] resulted in the selection of bacteria with increased resistance to the drugs used. These bacterial subpopulations were responsible for the bacterial regrowth in an in vitro kinetic model [3, 4]. Regrowth of bacteria with increased

resistance was also found after treatment of a thigh muscle infection due to P.aeruginosa with gentamicin [9]. It has been suggested that these resistant subpopulations may be of clinical relevance [8, 13]. An explanation for the absence of resistant bacteria in our experiments may be that the occurrence of less susceptible bacteria only becomes substantial after multiple injections of antibiotic.

In the experiments described in this study it was investigated by which factors the size of the persisting bacterial population was determined. It was shown that the class of antibiotic used and in addition for gentamicin and ciprofloxacin the dose that was used were important determinants in this respect. In this chapter it is shown that bacteria that could not be eliminated by a second injection of ceftazidime or gentamicin were killed to a large extent by ciprofloxacin. The additional killing was similar when ciprofloxacin was administered either after ceftazidime or after gentamicin. The total bactericidal effect, however, did not exceed that of ciprofloxacin alone, so total bacterial killing was determined by the antibiotic with the highest bactericidal activity. It was also demonstrated for the three drugs tested that the number of persisting bacteria increased with increase of the duration of the infection. This effect was most pronounced for ciprofloxacin. The diminished bacterial killing in the lungs of leukopenic rats due to delay of antibiotic administration could not be explained by an inoculum effect because at the time of administration bacterial numbers were similar in all experiments. Neither was a reduction in bacterial growth rate responsible for the effect. Similar results were obtained by Totsuka et al. [18]. In his experiments intensive treatment with several antibiotics did not result in eradication of K.pneumoniae from thighs of neutropenic mice. Persisting bacteria were fully susceptible to the drugs used. Delay of administration for only 1 h resulted in a substantial decrease of bacterial killing. In a model of pseudomonas endophtalmitis in rabbits it was demonstrated that a delay of treatment from 24 up to 48 h after infection resulted in loss of bactericidal activity of ciprofloxacin, gentamicin and imipenem [6]. This appeared not to be related to a decrease of the susceptiblity of bacteria as they were fully susceptible to the drugs tested after subculture in vitro. Recently similar observations were reoprted by Davey for the same drugs not only in vivo in a pseudomonas granuloma-pouch model in rats, but also in in-vitro batch cultures in which the drugs became less bactericidal with progressive incubation [7]. A possible explanation for our observations and those of

others may be that changes in the environment induce phenotypic resistance to antibiotic [10], which cannot be demonstrated after bacterial isolation and standard susceptibility testing. The exact nature of these changes is not clear, but one of the important factors may be that of nutrient depletion [5]. This may be responsible for a change of the bacterial phenotype and subsequent altered antibiotic susceptibility. Another responsible factor may be a diminished activity of antibiotic due to local factors such as altered pH or oxygen depletion.

In further experiments the relevance of our observations with regard to the therapeutic efficacy of ceftazidime and ciprofloxacin was investigated. As mentioned, after early injection of a single dose ciprofloxacin killed a greater part of the bacterial population than ceftazidime, however, the activity of both drugs decreased with delay of administration. These findings related to the antibacterial activity of antibiotics in the lung were related to the therapeutic activity of both antibiotics. Ciprofloxacin appeared to be more effective than ceftazidime when administered intermittently, and the efficacy of both drugs decreased with delay of start of treatment. These data are in accordance with the observation that institution of empirical antibiotic treatment in granulocytopenic patients starting at the first signs of fever has reduced the morbidity and mortality due to gram-negative infections [19]. This, however, may not apply to gram-positive infections in those patients. In a recent study Rubin et al. demonstrated that waiting for the laboratory results, identifying gram-positive organisms and antibiotic susceptibility, did not affect the effectiveness of treatment with vancomycin [15].

In conclusion, the experimental data obtained with antibiotics of three different classes underline the need to start antibiotic treatment of serious infections as early as possible because the number of bacteria persisting in the lung despite administration of antibiotic increases with the duration of the infection. The use of highly bactericidal drugs may be of advantage in this respect.

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