ANTIMICROBIAL TREATMENT OF EXPERIMENTAL INFECTION DUE TO AN EXTENDED-SPECTRUM ß-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* STRAIN

Ph.D. Theses

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Introduction

Extended-spectrum ß-lactamase (ESBL) production is one of the main mechanisms of resistance to ß-lactam antibiotics among the strains of the family *Enterobacteriaceae*. Infections due to these strains are difficult to treat, because genes of ESBLs are carried by large plasmids which could be responsible for the resistance to other antimicrobial drugs. The in vitro activity of several antimicrobials was evaluated, however, extremely limited in vivo and clinical data are available.

Carbapenems show excellent in vitro activity, and based on two clinical studies imipenem is the drug of choice in the treatment of infections due to ESBL-producing organisms. But the emergence of carbapenem-resistant ESBL-producing *K. pneumoniae* strains warrants the need to reduce their selective pressure.

The role of the fourth-generation cephalosporins is still debated. ESBLs attack the broad-spectrum cephalosporins, but the MICs of the fourth-generation cephalosporins may not reach the breakpoint value. In vitro susceptibility of ESBL-producing strains to cefepime was found to be 52 - 90% using standard

inoculum size. However, cefepime is subject to a marked inoculum effect when challenges against ESBL-producing strains. The significance of in vitro inoculum effect on clinical outcome remained questionable.
Seventy-five to 100% of the ESBL-producing strains are susceptible to amikacin based on the SENTRY report. Although amikacin has a so good bactericidal effect itself, it was investigated only in a few in vitro studies.
Only very limited in vitro data and clinical cases are available on the combination of imipenem and aminoglycoside against ESBL-producing organisms. There are no in vivo study that would compare the activity of imipenem with amikacin plus imipenem.

The use of animal models has become integral in the evaluation of the efficacies of antimicrobial agents. However, the pharmacology of antimicrobial agents in most animal species is still markedly different from that in humans. The simulation of the human pharmacokinetic profiles in animal models is necessary to assess efficacy, which then can be applied to clinical settings. Human kinetics in animals can be simulated by frequent

injections of decreasing amounts of a drug or by impairing renal function. Renal dysfunction caused by a nephrotoxic agent was shown to be a simple method of simulating the time course of human serum concentration for renally excreted drugs in small animals. Uranyl nitrate was used in more animal studies to induce renal impairment, but cisplatin was not used for this purpose. Cisplatin-induced acute renal failure in rats resulted apparently comparable to renal dysfunction produced by uranyl nitrate as determined by biochemical measurements.

Aims

Our aim was to evaluate the in vitro and in vivo efficacy of different antibiotics and their combinations against an ESBL-producing *K. pneumoniae* strain.

For the further in vivo experiments we needed to create a renal impairment model in mice to simulate human pharmacokinetics of renally excreted drugs. We would determine the highest dose of cisplatin, which ensure significant elongation of half-life of cefepime without significant increase of lethality of mice. We compared the

serum concentrations of cefepime in mice (with and without cisplatin pre-treatment) and in humans.

Than, we examined the in vitro and vivo efficacy of amikacin, cefepime, amikacin plus cefepime and imipenem against our ESBL-producing strain. We searched the possible alternatives of imipenem therapy. We wanted to know whether cefepime can be effective using high inoculum size.

We compared the in vitro and vivo activity of amikacin and imipenem alone and in combination. We searched a possible synergy between the two drugs.

Materials and methods

Bacterial strain

A clinically isolated SHV-5 ESBL-producing *K. pneumoniae* strain was used in the studies. Before inoculation the count of bacteria was determined using a spectrophotometer at 540 nm.

Drugs

Amikacin, cefepime (Bristol-Myers Squibb) and imipenem (Merck Sharp & Dohme) were freshly diluted with saline to the appropriate concentrations,

meanwhile solution of cisplatin (Ebewe Pharma) was given undiluted before each experiment.

Antibiotic susceptibility testing

The minimal inhibitory concentrations (MICs) and the minimal bactericidal concentrations (MBCs) for the tested drugs were determined by the microdilution method using inoculum concentrations of approximately 10^5 and 10^7 colony-forming unit (CFU)/mL.

Time–kill studies

The in vitro bactericidal activity was determined using the time–kill methods. Initial bacterial concentration was 8 $\log_{10}$ CFU/mL. Concentrations of antibiotics were chosen to be close to the mean in vivo serum levels: amikacin 4 or 8 μg/mL, imipenem 16 μg/mL, cefepime 40 μg/mL, amikacin plus cefepime 4 and 40 μg/mL, and amikacin plus imipenem: 8 and 16 μg/mL, respectively. The viable bacterial counts were determined three times in the 24-hours incubation period. The lowest accurate bacterial count was 300 CFU/ml. Bactericidal activity was defined as $\geq$3 $\log_{10}$ decrease in CFU/ml. Synergy was defined as a $\geq$2 $\log_{10}$ decrease in the number of

CFU/ml between the combination and its most active constituent after 24 h.

Chequerboard synergy testing

Dilutions ranging from 64 to 0.03 μg/mL of amikacin and imipenem were tested against two inoculum sizes (10^5 and 10^7 CFU/mL). The fractional inhibitory concentration index (FICI) was calculated using formulae previously published. Synergy was defined as FICI ≤0.5, no interaction was defined as FICI >0.5 to 4.0, and antagonism was defined as FICI >4.0.

Animal model (General conditions)

In each experiment male CD-1 mice, weighing 30-35 grams were used. Drugs and saline were given by intraperitoneal injections. Ten to fifteen randomly selected mice were used in each group.

Renal impairment model

Cisplatin was administered in 10, 14, 18, 22 and 26 mg/kg doses; the control group received saline. Either saline or cisplatin were given three days before the administration of cefepime and the determination of the pharmacokinetic parameters of cefepime. Single dose of 80 mg/kg cefepime was given to each mouse. The groups

were followed up for up to 8 days after cisplatin or saline administration and the survival of mice was recorded.

Animal infection models

Cisplatin (18 mg/kg of body weight) had been administered 3 days before infection in order to cause renal impairment. The mice were infected with 10^7 CFU/g *K. pneumoniae*, the uninfected group received only cisplatin. The treatment started 3 hours after infection and lasted for 24 hours. Blood samples from the tail vein were taken of 5 randomly selected mice in different points for the determination of blood bacterial counts. The lowest accurate bacterial count was 300 CFU/mL. Survival analysis was performed for the groups treated with amikacin, cefepime, imipenem and amikacin plus cefepime.

Statistical analysis

The Kruskal-Wallis test followed by the Mann-Whitney test were used for statistical analysis of the half-lives of cefepime and the blood bacterial counts, $P < 0.05$ was significant. Survival was assessed by estimating the cumulative probability using the Kaplan-Meier survival curve. The relative risk between groups was estimated by

the hazard ratio with the appropriate 95% confidence interval comparing the observed deaths with expected deaths. The log-rank test was used for statistical analysis, accepting a P value of <0.05 as significant.

Pharmacokinetic analysis

Blood samples were taken at 15 and 30 minutes, 1, 2 and 3 hours after amikacin, cefepime and imipenem administration. Antibiotic levels in sera were determined by a paper disk method for cefepime and imipenem with *Escherichia coli* ATCC 25922 and *Bacillus subtilis* ATCC 6633, respectively, as the indicator organism on Antibiotic Medium 1. Contents of the disks were determined from a semi-logarithmic linear regression plot of the inhibition zone diameters versus $\log_{10}$ concentration of the drugs. The amikacin serum levels were detected by a fluorescence polarization immunoassay (Abbott TDx system). Values for pharmacokinetic variables were calculated by a non-compartmental analysis. The peak serum concentrations (C_{max}) were measured and the elimination half-life ($t_{1/2}$), the area under the serum concentration-time curve (AUC) and the area under the first moment of serum

concentration-time curve (AUMC), the mean residence time (MRT) and total body clearance (CL) were calculated.

Results

Renal impairment model

All of the control group and the groups receiving 10 and 14 mg/kg cisplatin survived for 8 days, while in the groups receiving 18, 22 and 26 mg/kg the survival rates were 8/10, 7/10 and 3/10, respectively. The survival of the 26 mg/kg cisplatin group differed significantly from the control, and from the groups receiving 18 and 22 mg/kg cisplatin ($P= 0.001$, 0.034 and 0.04, respectively). The survival of the groups receiving 10, 14, 18 and 22 mg/kg cisplatin did not differ significantly from the control group.

There was a significant difference between the elimination half-lives of cefepime when comparing all groups ($P< 0.001$). Comparing the groups significant differences were found between control and the 10 mg/kg cisplatin group ($P= 0.001$), between the groups receiving 14 mg/kg and 18 mg/kg dose of cisplatin ($P= 0.001$) and

between the groups received 22 and 26 mg/kg cisplatin ($P< 0.001$).

In vitro susceptibility testing and killing curves

The ESBL-producing *K. pneumoniae* showed susceptibility to amikacin and imipenem at both inoculum concentrations, although MICs and MBCs for both imipenem and amikacin were increased by the inoculum. The MIC and MBC of cefepime were 1 μg/ml at 10^5 CFU/ml, and the MIC was > 256 μg/ml at 10^7 CFU/ml.

There was no interaction for the combination of imipenem and amikacin by the chequerboard technique: FICI was 1 at 10^5 CFU/mL and 1.0625 at 10^7 CFU/mL.

In the killing curve studies the initial 8 $\log_{10}$ CFU/ml was reduced below the level of determination after 24 hours in the presence of amikacin, imipenem amikacin plus cefepime and amikacin plus imipenem. The bacterial count increased in the absence of antibiotic and in the presence of cefepime. Synergy was not detected between amikacin and cefepime, or amikacin and imipenem.

Blood bacterial counts

The blood bacterial count increased persistently in the untreated group; cefepime initially decreased it, but an increase occurred after 6 h, while it decreased persistently in the other treated groups. The difference was significant when all groups were compared ($P< 0.02$). There was significant difference between the untreated control and the amikacin, imipenem, amikacin plus cefepime and amikacin plus imipenem treated groups. The cefepime-treated group differed statistically from the groups treated with the other antibiotics. There was no difference between groups receiving amikacin, amikacin plus cefepime, and imipenem ($P> 0.37$ in each pair compared). Amikacin and imipenem alone and in combination similarly decreased the blood bacterial counts (amikacin versus amikacin plus imipenem, $P > 0.38$; and amikacin plus imipenem and imipenem, $P > 0.74$).

Survival analysis

Amikacin, amikacin plus cefepime and imipenem significantly prolonged the survival of mice compared with the infected untreated group and the group treated with cefepime alone ($P<0.001$). The combined effect of

amikacin and cefepime did not differ significantly from the effect of amikacin alone (P>0.2). The 24-hour cumulative probability of survival in the imipenem group and the amikacin group did not differ statistically

Pharmacokinetic parameters

The pharmacokinetic/pharmacodynamic parameters of amikacin, imipenem and cefepime determined at standard inocula exceeded the magnitude required for efficacy (T>MIC for cefepime and imipenem was 105 and 82%, respectively, AUC/MIC and C_{max}/MIC for amikacin was 161.5 and 40.3, respectively). The serum levels of the combinations were not determined after co-administration, because they have not affected significantly the pharmacokinetic parameters of each other.

Conclusions

Human-like pharmacokinetic parameters are important to assess the efficacy of antimicrobial agents in animal models. It can be achieved most simple by the administration of a nephrotoxic agent in the case of renally excreted drugs. Using 18 or 22 mg/kg of cisplatin,

the lethality of mice did not differ significantly from the control group, while the elimination half-life of cefepime was significantly prolonged. Up to 6 hours after dose, the serum concentrations of cefepime were comparable in mice pretreated with 18 mg/kg of cisplatin and in humans. Cisplatin pretreatment has an important role in animal experiments, because the intervals of administration of renally excreted drugs are similar to those in clinical conditions which help the study of several factors affecting antibiotic treatment.

Cefepime was ineffective both in vitro and in vivo using high initial inoculum. The susceptibility and the T>MIC with the standard inoculum were not predictive for the biological effectiveness due to a possible in vivo inoculum effect. Cefepime treatment in the infection due to ESBL-producing strains is not recommended since the bacterial count at the site of infection can not be determined.

Amikacin was active in vitro and in vivo using the standard or high inoculum. Difference was not observed in survival of mice between amikacin and imipenem

treated groups. Based on these results, in case of in vitro susceptibility amikacin can be alternative of imipenem.

Combination of amikacin and imipenem did not show in vitro synergy at low and high inoculum using chequerboard technique and the time-kill studies. Significant difference or trend toward higher efficacy of the combination were not detected in the animal model. Even though imipenem and amikacin had very good activity, their combination did not show any additional advantage in the bactericidal effect.

List of publications

1. Szabó D, **Máthé A**, Filetóth Zs, Anderlik P, Rókusz L, Rozgonyi F. (2001) In vitro and in vivo activities of amikacin, cefepime, amikacin plus cefepime and imipenem against an SHV-5 extended-spectrum ß-lactamase-producing *Klebsiella pneumoniae* strain. Antimicrob Agents Chemother, 45: 1287-1291.

IF: **3.954**

2. Szabó D, **Máthé A**, Lengyel J, Filetóth Zs, Anderlik P, Rókusz L, Rozgonyi F. (2002) In vitro and in vivo activities of ciprofloxacin and levofloxacin against an SHV-5 extended-spectrum ß-lactamase-producing *Klebsiella pneumoniae* strain. Curr Med Chem, 9: 437-442.

IF: **4.909**

3. **Máthé A,** Komka K, Forczig M, Szabó D, Anderlik P, Rozgonyi F. (2006) The effect of different doses of cisplatin on the pharmacokinetic parameters of cefepime in mice. Lab Anim - UK, 40: 296-300.

IF: **1.326**

4. **Máthé A**, Szabó D, Anderlik P, Rozgonyi F, Nagy K. (2007) The effect of amikacin and imipenem alone and in combination against an extended-spectrum ß-lactamase–producing *Klebsiella pneumoniae* strain. Diagn Microbiol Infec Dis, 58: 105–110.
IF: **2.738**

Other publications:

1. **Máthé A**, Tóth K, Kovács G. (2004) A moxifloxacin szerepe a területen szerzett pneumonia kezelésében. Orvosi Hetilap, 145: 2227-2230.
2. **Máthé A**. (2006) Béta-laktámok és aminoglikozidok. Gyógyszerészet, 50: 605-611.
3. Bogos K, Tóth K, **Máthé A.** (2006) COPD és infekció: egyre sokszínűbb összefüggés. Orvostovábbképző Szemle - különszám, 31-36.