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Original article

THERAPEUTIC DRUG MONITORING OF AMIKACIN: METHODS AND RESULTS IN PATIENTS WITH CYSTIC FIBROSIS

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Abstract

Introduction: Therapeutic drug monitoring (TDM) is essential to optimize amikacin therapy, especially in patients with cystic fibrosis (CF), due to a small therapeutic index and significant interpatient variability. This study presents the methods and results of TDM in CF patients treated with amikacin.

Methods: A prospective, open-label clinical study was conducted in CF patients receiving amikacin. Amikacin serum concentrations were measured using a fluorescence polarization immunoassay (FPIA). Peak and trough concentrations were recorded to maintain therapeutic levels and minimize toxicity. Data on patients' demographics, renal function, and clinical outcome were also collected.

Results: Amikacin serum concentrations were determined in a total of 165 blood samples taken from 33 patients who received amikacin at a dose of 30 mg/kg, but not more than 1.5 g, in the form of an intravenous infusion over 30 minutes once a day. Maximum concentrations of amikacin in patients ranged from 29.6-197.7 μg/ml, while minimum values were always < 2 μg/ml in all patients in the study. No cases of nephrotoxicity and ototoxicity were observed.

Conclusion: According to literature, TDM of amikacin in CF patients is crucial to achieve optimal therapeutic results. Because no dose adjustment was required in any patient in our study, determination of amikacin concentrations in our population is justified only in patients with decreased therapeutic efficacy of the drug or in the case of adverse effects.

Keywords: Amikacin, therapeutic drug monitoring, fluorescence polarization immunoassays, cystic fibrosis,safety

Introduction

Therapeutic drug monitoring (TDM) is a clinical practice that measures specific drug levels in a patient's bloodstream at designated intervals to ensure a constant therapeutic concentration is maintained[1]. This process is essential for optimizing individual drug dosage

regimens, maximizing therapeutic effects while minimizing toxicity[2]. TDM is particularly important for drugs with a narrow therapeutic index, significant inter-individual variability in pharmacokinetics, or potential for severe side effects.

Amikacin, an aminoglycoside antibiotic, is commonly used in treatment of severe bacterial infections, including those caused by Gram-negative bacteria[3]. Due to its potent antibacterial properties and resistance to many bacterial enzymes that degrade other aminoglycosides, amikacin is often reserved for multidrug-resistant infections. [4] However, like other aminoglycosides, amikacin has a narrow therapeutic window and can cause serious side effects such as nephrotoxicity and ototoxicity if not properly monitored.

Given the potential for toxicity, TDM is crucial in the administration of amikacin. The process involves measuring peak and trough serum levels to ensure the drug remains within the therapeutic range[5]. Peak levels, typically measured 30 minutes after an intravenous infusion or one hour after an intramuscular injection, indicate the highest concentration of the drug in the bloodstream, ensuring effective bacterial eradication. Trough levels, measured just before the next dose, indicate the lowest concentration, helping to prevent toxicity.

Effective TDM requires consideration of several factors, including patient-specific characteristics such as age, weight, renal function, and the severity of the infection. Renal function is particularly important as aminoglycosides are primarily excreted by the kidneys, and impaired renal function can lead to drug accumulation and increased risk of toxicity.

In clinical practice, TDM of amikacin not only helps in achieving optimal therapeutic outcomes but also contributes to antimicrobial stewardship by minimizing the emergence of resistant bacterial strains. By ensuring appropriate dosage, TDM helps in maintaining the delicate balance between efficacy and safety, ultimately improving patient outcomes in the treatment of severe infections.

Measuring amikacin plasma concentrations accurately is essential for therapeutic drug monitoring to ensure efficacy and avoid toxicity. Here are the main methods used for this purpose:

High-Performance Liquid Chromatography (HPLC):

Chromatographic technique is used in analytical chemistry for separating, identifying, and quantifying compounds from a given mixture. Liquid chromatography uses smaller columns, smaller media in the column, and higher pressure of the mobile phase. The LC pump uses a pressure of 400 atmospheres to drive the mobile phase through the densely packed column. The increased density of the column enables separation of the smallest particles from the analyzed mixture.

Advantages: High sensitivity and specificity; can be used for a variety of samples.

Limitations: Requires complex and expensive equipment, and skilled technicians.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS):

This method represents a combination of two techniques: liquid chromatography and mass spectrometry. After separation through the LC column, the molecules from the analyzed sample are converted into an ionized state. The fragmented ions are separated based on their mass-to-charge ratio.

Advantages: Very high sensitivity; capable of detecting low concentrations; highly specific.

Limitations: Expensive equipment; requires technical expertise.

Gas Chromatography-Mass Spectrometry (GC-MS):

This method combines gas chromatography and mass spectrometry. By heating the liquid sample, vapor is produced, which moves through the GC column and separates into

smaller particles. These particles are then ionized and differentiated by the spectrophotometer based on their charge and mass.

Advantages: High sensitivity and specificity.

Limitations: Not typically used for amikacin due to the need of derivatization and its polar nature.

Enzyme-Linked Immunosorbent Assay (ELISA):

The method represents an analytical biochemical assay where the analyzed sample is immobilized onto a solid phase along with a binding reagent, forming an antigen-antibody complex. An enzyme is then added to this complex, generating a signal that can be accurately quantified.

Advantages: High sensitivity and specificity; can be used in various settings.

Limitations: Requires preparation of specific antibodies; can be time-consuming.

Radioimmunoassay (RIA):

The method is based on competition between the antigen from the sample and a radioactively labeled antigen for the binding sites on the antibody. RIA combines the specificity of the antigen-antibody reaction with the sensitivity of measuring radioactivity.

Advantages: Very sensitive and specific.

Limitations: Requires handling of radioactive materials, which poses health and safety risks.

Fluorescence Polarization Immunoassay (FPIA):

The method is based on the competition between a fluorescein-labeled antigen and an unlabeled antigen for binding to an antibody. In the procedure, polarized light excites the sample, and the fluorescein emits polarized light, which is detected. The unbound antigen-fluorescein complex emits more light than the bound version of the complex. The greater the amount of antigen in the sample, the less fluorescein-labeled antigen will bind to the antibody, resulting in an increased emission of polarized light.

Advantages: Quick and easy to perform, suitable for routine clinical use.

Limitations: May have cross-reactivity with other compounds; less sensitive compared to LC-MS/MS.

Capillary Electrophoresis (CE)

CE separates ionic species based on their size-to-charge ratio under the influence of an electric field.

Advantages: High resolution and efficiency; requires small sample volumes.

Limitations: Less commonly used in routine clinical practice; requires specialized equipment.

Material and methods

Serum samples in our study were analyzed using the ARCHITECT C4000 analyzer. The method employed was a homogeneous particle-enhanced turbidimetric inhibition immunoassay. The analysis is based on the competition between the drug in the sample and the drug coated on the microparticle for the binding sites of the anti-amikacin antibody reagent. The reagent with amikacin-coated microparticles quickly agglutinates in the presence of an anti-amikacin antibody reagent and in the absence of any competing substance in the sample. The rate of change in absorption is measured photometrically and is directly proportional to the concentration of amikacin produced by Abbott and intended for the

Architect C4000. The reagent is in a liquid form, requires no prior preparation, and is a kit consisting of two reagents that contain:

R1 - Anti-amikacin monoclonal antibody (mouse) < 1.0%;

R2 - Amikacin-coated microparticles ≤0.5%.

Before reading the serum samples, a six-level calibration was performed using the

TDM Multiconstituent Calibrator (TDM MCC) set. The calibration is stable for 54 days. The linearity of the analysis ranges from 2 to 50 μg/mL. The concentrations of the calibrators were as follows: 0.00; 2.99; 9.75; 19.37; 34.38; 50.00 μg/mL. To confirm the calibration curve, amikacin controls were read before each determination of amikacin concentrations in the serum. When using the method, Multichem S plus, Technopath controls were used at three levels:

Control 1 - with an average concentration of 4.76 μg/mL and a range of 3.81-5.72 μg/mL;
Control 2 - with an average concentration of 12.35 μg/mL and a range of 9.90-14.80 μg/mL;
Control 3 - with an average concentration of 33.05 μg/mL and a range of 26.40-39.70 μg/mL.

Before determining the unknown concentration of amikacin in serum, three control samples were analyzed. If these samples were within the predetermined deviation limits, i.e., deviations from the concentration in the sample ±2 SD, the measurement of the unknown concentration of amikacin in patients' serum was carried out. If the samples exceeded the measuring range for amikacin (>50 μg/mL), they were diluted with physiological solution, and the dilution factor was entered into the system when registering a patient for automatic correction. To determine the concentration of amikacin in the serum of patients, 150 μl of serum was required.

Blood Sample Collection

The blood samples in which the concentration of amikacin in serum was determined were taken from patients with cystic fibrosis who received intravenous infusion of amikacin in duration of 30 minutes and at a dose of 30 mg/kg, but not exceeding 1500 mg, once daily. The open, uncontrolled clinical trial included 33 patients with cystic fibrosis aged between 4 and 65 years (≤4-≤65 years) who required amikacin therapy at the specified doses and who signed informed consent. For patients under the age of 18, informed consent was signed by their parent or guardian. The study did not include: patients with a positive history of allergic reactions to amikacin, other aminoglycoside antibiotics, and/or any of the drug's components, patients with myasthenia gravis, patients with creatinine clearance ≤30 ml/min, patients with a history of drug and/or alcohol abuse in the past 12 months, and patients who participated in other clinical studies in the previous two months. Patients requiring hemodialysis, patients requiring therapy with drugs that interfere with creatinine clearance and lead to increased concentrations of amikacin, patients requiring therapy with drugs that have nephrotoxic, , neurotoxic, and ototoxic effects (bacitracin, cisplatin, amphotericin B, cyclosporine, tacrolimus cephaloridine, paromomycin, viomycin, polymyxin B, colistin, vancomycin, other aminoglycoside antibiotics), patients with significant clinical deterioration, patients who developed a condition not present at the time of signing the informed consent that could affect the evaluation of the therapeutic efficacy and safety of amikacin, and patients who did not cooperate during the study were excluded. Blood samples were taken from all patients in a maximum quantity of 1.5 ml at 1 hour before administration of the third dose of the drug and 30 minutes, 6 hours, and 12 hours after the end of the infusion. Blood samples were also taken 0-30 minutes before the administration of the fourth and fifth doses of the drug. Blood samples were collected in serum tubes. After centrifugation (6000 rpm, 10 min), the serum was separated and stored in a refrigerator at -20°C until the determination of amikacin concentration.

Pharmacokinetic Calculations and Statistical Analysis

Based on the obtained concentrations, using formulas for an open one-compartment model, the following pharmacokinetic parameters were calculated: Cssmin (minimum serum amikacin concentrations at steady-state), Cssmax (maximum serum amikacin concentrations at steady-state), Kel (elimination constant), t/2el (elimination half-life), Vd (volume of distribution), and Cl (total clearance).

Descriptive statistical analysis is presented for the demographic data of patients who participated in the study and for the pharmacokinetic parameters of amikacin.

Results

Amikacin serum concentrations were determined in a total of 165 blood samples taken from 33 patients who received amikacin at a dose of 30 mg/kg, but not more than 1.5 g, in the form of an intravenous infusion over 30 minutes, once a day.

A total of 33 patients of both sexes (24 males and 9 females) participated in the open, uncontrolled clinical study.

The demographic characteristics of subjects are shown in Table 1.

Table 1. Demographic characteristics of the subjects

	Age (years)	Body weight (kg)	Height (cm)	Body Mass Index (BMI)
Average	17.197	49.6	156.333	19.278
±SD	5.491	13.943	22.657	3.676
Minimum	6	20	71	14.8
Maximum	37	96	176	33.2

The most significant parameters for assessing renal function (serum creatinine concentration, serum urea concentration, and creatinine clearance) were within normal values throughout the study. The results obtained for these parameters are shown in Tables 2, 3 and 4.

Table 2. Serum Creatinine Concentration During the Study

	Before the first dose of amikacin	After 3 days of amikacin	After 6 days of amikacin	After 9 days of amikacin	After 12 days of amikacin
Average	56.861	53.979	57.506	55.273	58.118
±SD	9.940	9.168	13.578	10.635	11.113
Minimum	41	40,8	41	39	41
Maximum	76	71	116	82.2	80.4

Table 3. Serum Urea Concentration During the Study

	Before the first dose of amikacin	After 3 days of amikacin	After 6 days of amikacin	After 9 days of amikacin	After 12 days of amikacin
Average	4.285	4.151	4.970	4.888	5.378
±SD	1.284	1.104	1.519	1.032	1.194
Minimum	2	2.1	1.2	2.5	2.7
Maximum	7	6.6	9.3	6.5	7.2

Table 4. Serum Creatinine Clearance During the Study

	Before the first dose of amikacin	After 3 days of amikacin	After 6 days of amikacin	After 9 days of amikacin	After 12 days of amikacin
Average	125.242	131	125.273	131.424	130.556
±SD	28.059	24.739	30.319	28.916	28.563
Minimum	68	65	57	67	68
Maximum	208	202	218	202	208

The results obtained for the amikacin serum concentrations are shown in Table 5.

Table 5. Amikacin Serum Concentrations in Treated Patients (μg/ml)

	Before the third dose of amikacin	30 minutes after the end of infusion	6 h after the end of infusion	Before the fourth dose of amikacin	Before the fifth dose of amikacin
Average	<2	82.903	10.479	<2	<2
±SD	/	35.281	5.489	/	/
Minimum	/	29.6	2.2	/	/
Maximum	/	197.7	23	/	/

The values for the pharmacokinetic parameters calculated using the formulas for pharmacokinetic parameters according to the one-compartment open model are shown in Table 6.

Table 6. Pharmacokinetic Parameters in Patients Treated with Amikacin

	Elimination Constant - Kel (h^-1)	Elimination Half-Life (t/2 el) (h)	Volume of Distribution (L)	Total Clearance (ml/min)
Average	0.3961	1.9911	14.3680	5.6917
±SD	0.133023	0.8157	8.3559	1.1115
Minimum	0.1474	1.0978	4.9490	2.7053
Maximum	0.6313	4.7002	44.1260	9.4701

Discussion

The results presented in the paper show that immunoassay of turbidimetric inhibition enhanced by homogeneous particles determined on the ARCHITECT C4000 analyzer is a fast, simple, and precise method for determining amikacin concentrations in serum. This method allows accurate measurement of amikacin serum concentrations within the calibration curve range of 2 to 50 μg/mL. Sample dilution enables measurement of much higher amikacin concentrations in the sample. The highest concentration obtained in samples from our patients was 197.7 μg/mL. Only in 2 patients (6.1%) with amikacin concentrations higher than 50 μg/mL in the sample, the exact concentrations of amikacin were not determined by the dilution method.

In therapeutic monitoring of amikacin, the lowest and highest concentrations of the antibiotic are tracked, and based on these results, the drug dose is individually adjusted[1-3,6-9]. Amikacin therapy monitoring is performed after reaching the steady-state condition of drug concentrations in a patient's blood, which is achieved after 4-5 half-lives of antibiotic elimination. The lowest concentrations achieved during amikacin administration influence the success of the applied therapy. These concentrations are measured within a period of up to 30 minutes before the next dose administration. Monitoring the highest blood concentration in a steady-state condition is important not only for ensuring the drug's efficacy but also because the occurrence of toxic effects during amikacin therapy depends on the magnitude of the achieved concentrations (ototoxicity).

Concentrations of amikacin after administering the drug in the form of an intravenous infusion lasting 30 minutes at a dose of 30 mg/kg, but not more than 1.5g, in all samples taken from our patients before administering the third and fourth doses of the drug were <2 μg/ml. The study protocol provided for a reduction in the amikacin dose if the drug concentrations before administering the third or fourth dose were higher than 3 μg/ml. For these reasons, all patients continued therapy with the same drug dose. In blood samples taken 30 minutes after the end of the infusion, the average amikacin serum concentrations of 82.9±35.28 μg/ml were achieved. Only 4 patients (12.9%) out of 31 in whom amikacin serum concentrations were determined at 30 minutes post-infusion achieved amikacin concentrations

lower than 50 µg/ml. The lowest amikacin concentration obtained 30 minutes before the end of the infusion was 29.6 µg/ml, and the highest was 197.7 µg/ml. In 3 patients (9.68%), amikacin concentrations higher than 100 µg/ml were achieved.

Maximum amikacin concentrations in serum in various clinical studies ranged from 15 to 40 µg/ml and were achieved 20-30 minutes after the start of intravenous infusion[6-9]. Some patients had low maximum plasma amikacin concentrations (Cmax) of 12 µg/ml, while others had maximum plasma amikacin concentrations of 127 µg/ml. It has been found that low amikacin concentrations are due to underdosing of the drug, resulting in insufficient therapeutic efficacy and increased risk of rapid resistance development. In patients with high amikacin concentrations, especially if maintained for a prolonged period, the risk of drug toxicity increases. The necessary dose calculation is performed by entering the obtained amikacin concentration data into a special computer program that calculates the appropriate dose for the patient. The required dose can also be obtained as the quotient of the product of the given dose and the theoretically desired concentration with the obtained drug concentration. Based on these parameters, in our study, no dose adjustment of amikacin was required for any of the patients participating in the study.

In clinical studies, nephrotoxicity is particularly pronounced during treatment with amikacin lasting longer than 10 days[10-12]. Like other aminoglycoside antibiotics, amikacin exhibits a bactericidal effect on sensitive bacterial strains that is dose- or concentration-dependent. Regarding nephrotoxicity, it has been determined that it is not related to the maximum amikacin plasma (serum) concentrations but to maintaining higher drug concentrations for a longer period, during repeated therapy, as well as in patients with pre-existing renal function impairment. It has been proven that adequate hydration of patients during therapy reduces the toxic effects on the kidneys.

The results obtained for objective parameters indicative of renal function (serum creatinine concentration, serum urea concentration, and creatinine clearance) in our study showed no significant differences ($p>0.05$) between the values measured before the start of treatment and 3, 6, 9, and 12 days after amikacin dose administration.

During the entire study period, no adverse effects of amikacin were observed that would suggest its nephrotoxicity, ototoxicity, or vestibular toxicity.

Conclusion

Determining amikacin serum concentrations using the ARCHITECT C4000 analyzer is a fast, straightforward and precise method that can be used in routine practice.

The obtained amikacin serum concentrations and calculated pharmacokinetic parameters show that intravenous infusion of amikacin at a dose of 30 mg/kg over 30 minutes is effective and safe for use in patients with cystic fibrosis. In our population, there was no need to adjust the drug dose for any patient participating in the study. Based on this data, it can be concluded that determining amikacin concentrations in our population is justified only in patients who have reduced therapeutic efficacy of the drug or in the case of adverse effects.

Conflict of interest statement. None declared.

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