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CLINICAL THERAPEUTICS

Amikacin Pharmacokinetic-Pharmacodynamic Analysis in Pediatric Cancer Patients

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ABSTRACT We performed pharmacokinetic-pharmacodynamic (PK-PD) and simulation analyses to evaluate a standard amikacin dose of 15 mg/kg once daily in children with cancer and to determine an optimal dosing strategy. A population pharmacokinetic model was developed from clinical data collected in 34 pediatric patients and used in a simulation study to predict the population probability of various dosing regimens to achieve accepted safety (steady-state unbound trough plasma concentration [fC_{min}] of $<$10 mg/liter)- and efficacy (free, unbound plasma concentration-to-MIC ratio [fC_{max}/MIC] of $\geq$8)-linked targets. In addition, an adaptive resistance PD (ARPD) model of *Pseudomonas aeruginosa* was built based on literature time-kill curve data and linked to the PK model to perform PK-ARPD simulations and compare results with those of the probability approach. Using the probability approach, an amikacin dose of 60 mg/kg administered once daily is expected to achieve the target fC_{max}/MIC in 80% of pediatric patients weighing 8 to 70 kg with a 97.5% probability, and almost all patients were predicted to have fC_{min} of $<$10 mg/liter. However, PK-ARPD simulation predicted that 60 mg/kg/day is unlikely to suppress bacterial resistance with repeated dosing. Furthermore, PK-ARPD simulation suggested that amikacin at 90 mg/kg, given in two divided doses (45 mg/kg twice a day), is expected to hit safety and efficacy targets and is associated with a lower rate of bacterial resistance. The disagreement between the two methods is due to the inability of the probability approach to predict development of drug resistance with repeated dosing. This originates from the use of PK-PD indices based on the MIC that neglects measurement errors, ignores the time course dynamic nature of bacterial growth and killing, and incorrectly assumes the MIC to be constant during treatment.

KEYWORDS pharmacokinetics, pharmacodynamics, pediatrics, cancer

Bacterial resistance to currently available antibiotics is a growing health problem worldwide, as it compromises the efficacy of modern antibiotic therapies and endangers millions of human lives (1). According to the U.S. Centers for Disease Control and Prevention (CDC), antibiotic-resistant bacteria infect 2 million people in the United States annually and directly cause 23,000 deaths every year (2). Appropriate dosing is a major determinant of successful drug therapy, and for anti-infective medications, suboptimal dosing can lead to greater harm than just therapeutic failure. Considerable evidence from *in vitro* and *in vivo* work links antibiotic suboptimal dosing to the emergence of bacterial resistance (3, 4). An inverted U-shaped function is found to describe the relationship between drug exposure and selection of resistant bacteria that initially rises and then declines with increasing exposure until reaching a threshold that prevents amplification of resistant bacteria (4).

The optimization of antibiotic dosing could prevent or at least delay the emergence of resistance. To do so, an understanding of pharmacokinetic-pharmacodynamic

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(PK-PD) relationships of a drug against a bacterial pathogen is key (5). For example, the licensure of daptomycin, a lipopeptide antibiotic active against Gram-positive bacteria, failed in the 1970s because of poor PK-PD understanding. In 2003, the same drug was approved by the Food and Drug Administration (FDA) for clinical uses after switching from a fixed dose three times a day to weight-based once-daily dosing (3).

Likewise, amikacin, an aminoglycoside antibiotic used either alone or in combination with β-lactams to treat serious Gram-negative infections, was first approved at 15 mg/kg of body weight in 1 to 3 divided doses a day (5–7). Over time, as amikacin PK-PD became better understood, dosing changed to larger once-daily doses in order to maximize efficacy and limit toxicity (7–10). Amikacin is an antibiotic that exhibits a concentration-dependent bactericidal activity, and better clinical outcomes were associated with a high free, unbound plasma concentration-to-MIC ratio (fC_{max}/MIC). Because a bacterial population is not homogeneous but a mixture of distinct populations having their own MIC levels, a population MIC value, defined as the drug concentration that inhibits the growth of 90% of bacteria (MIC_{90}), is usually used in the fC_{max}/MIC. Generally, an fC_{max}/MIC ratio of 8 to 10 has been shown to result in at least a 90% therapeutic success rate and is believed to suppress bacterial resistance (11–14). In addition, amikacin safety was linked to the steady-state unbound trough plasma concentration (fC_{min}), being below 10 and ideally less than 5 mg/liter (14). Nephrotoxicity is also lower with once-daily administration because it limits repeated exposure of amikacin and provides more time for kidneys to eliminate the drug. There is no doubt that dose optimization of antibiotics is of paramount importance and a promising approach to improve therapeutic outcomes, limit adverse events, and combat bacterial resistance.

Two of several methods are widely used for the selection of optimal dosing: probability of target attainment (PTA) and PK-PD simulation. PTA was first proposed by Drusano's team, in which drug exposure in a virtual patient population of interest was simulated based on a population PK model developed from similar patients (15). The simulated time course PK profiles were then used to compute a PK-PD index of interest (fC_{max}/MIC; $fAUC_{24}$/MIC is the ratio of the area under the unbound concentration-time curve over 24 h to MIC, and $\%fT_{>MIC}$ is the cumulative percentage of the dosing interval that the unbound concentration is above MIC) and the PTA of achieving the selected PK-PD target within the population for a given dosing regimen. The acceptable PTA level is still subjective and debatable. PTA of 90% or higher is advocated, since it is associated with higher probability to achieve desired clinical outcomes. PK-PD simulation is similar to PTA, except that time-concentration drug profile is linked to a dynamic PD model based on *in vitro* or *in vivo* time-kill curve data of a drug-bacterium combination of interest to select a dose that is likely to eradicate bacteria.

The aim of this study was to use a modeling and simulation approach to suggest an optimal dosing regimen of amikacin in pediatric cancer patients through the application of pharmacometric principles. A population pharmacokinetic model was developed from data collected in pediatric cancer patients and used in a simulation study to predict the PTA of various dosing regimens to achieve accepted PK-PD targets for efficacy and safety of an fC_{max}/MIC of $\geq$8 and fC_{min} of $<$10 mg/liter, respectively. In addition, an adaptive resistance PD (ARPD) model of *Pseudomonas aeruginosa* was built based on literature time-kill curve data of amikacin and linked to the PK model to perform PK-ARPD simulations of the same dosing regimens explored in the PTA simulations and suggest a regimen associated with maximum bacterial killing and minimum development of resistance.

RESULTS

Pharmacokinetic model. The PK analysis included 34 Egyptian pediatric patients whose demographics and clinical characteristics are summarized in Table 1. A total of 68 amikacin plasma concentrations (2 samples per subject) were collected for modeling. The data were best fit by a two-compartment PK model with first-order elimination

TABLE 1 Summary of patient demographic and clinical characteristics

Characteristic	Value (n = 34)
Age, *n*, median (range)	34, 9 (1–18) yr
Sex, *n* (%)	
Male	14 (41)
Female	20 (59)
Weight, *n*, median (range)	34, 25 (8–70) kg
Height, *n*, median (range)	34, 127.5 (75–169) cm
Body surface area, *n*, median (range)	34, 0.94 (0.41–1.74) m^2
CrCL,[a] *n*, median (range)	34, 207.85 (58.06–418) ml/min
BUN, *n*, median (range)	31, 21 (7–93) mg/dl
Hemoglobin b, *n*, median (range)	34, 8.60 (6.23–11.70) mg/dl
Albumin, *n*, median (range)	19, 3.55 (200–4.30) g/liter
ALT, *n*, median (range)	17, 22 (5–135)
AST, *n*, median (range)	17, 23 (9–176)
Bilirubin, *n*, median (range)	33, 0.44 (0.15–1.50)
Concomitant nephrotoxic medication, *n* (%)	
Vancomycin	12 (35)
Amphotericin B	20 (50)
Fever, *n* (%)	6 (18)
Malignancy, *n* (%)	
Hematological	26 (76)
Solid	7 (21)

[a]CrCL is creatinine clearance and is calculated by the Schwartz equation.

and between-subject variability (BSV) on clearance (CL) and volume distribution of the central compartment (V_1). All parameters were allometrically scaled to a 70-kg subject using the following equations:

$$\mathrm{CL} = \theta_1 \times \left(\frac{\mathrm{weight}}{70}\right)^{0.75} \times e^{\eta_1} \quad (1)$$

$$Q = \theta_3 \times \left(\frac{\mathrm{weight}}{70}\right)^{0.75} \quad (2)$$

$$V_1 = \theta_2 \times \left(\frac{\mathrm{weight}}{70}\right) \times e^{\eta_2} \quad (3)$$

$$V_2 = \theta_4 \times \left(\frac{\mathrm{weight}}{70}\right) \quad (4)$$

Compared to a one-compartment model, the two-compartment model was associated with better diagnostic plots and a reduction in OFV by 19.74 points. No other covariates were found to influence the PK parameters. The adequacy of the final model was supported by diagnostics plots and prediction-corrected visual predictive check (pcVPC) (Fig. 1 and 2). Parameter estimates of the final PK model and their sampling importance resampling (SIR)-based 95% confidence intervals (CI) are presented in Table 2.

PTA. The single-dose median and range of fC_{max} and fC_{min} in Egyptian patients, estimated by empirical Bayesian estimates (EBEs), were 23.7 (8.69 to 27.7) mg/liter and 0.044 (0.0075 to 2.70) mg/liter, respectively. The fC_{max} was the estimated concentration at the end of infusion, and fC_{min} was the estimated concentration at 24 h from the start of infusion. The calculated PTA of an fC_{max}/MIC of ≥8 was zero for MIC_{90} of 4 and 8 mg/liter (16, 17). All patients had an estimated unbound trough concentration substantially below the target concentration of 10 mg/liter.

The median and range of simulated weights were 25.5 (8 to 70) kg, and they are similar to the observed values reported in Table 1. The predicted PTA was plotted against MIC values stratified by total daily dose and dosing interval (Fig. 3). PTA of an fC_{max}/MIC of ≥8 is higher for higher doses of amikacin for any given MIC level and

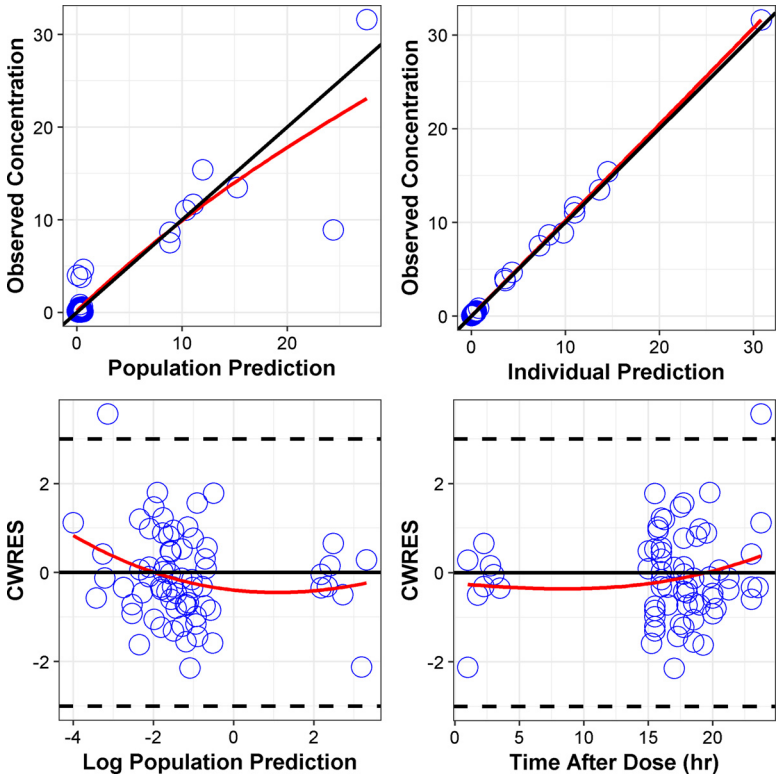

FIG 1 Diagnostic plots for the final PK model. CWRES, conditional weighted residuals.

dosing interval. A total daily dose of amikacin given in divided doses resulted in significantly lower PTA than when given once a day. The width of 95% CI around PTA increased with amikacin doses when given at the same frequency. The 95% CI was the widest for 90 mg/kg and the narrowest for 15 mg/kg when given once a day. On the contrary, the width of 95% CI increased as the frequency of dosing (number of doses per day) decreased. The 95% CI of PTA was wider for 90 mg/kg when administered once compared to twice or thrice daily. The overall probability of fC_{min} being below 10 mg/liter ranged from 95 to 100% for all simulated dosing regimens.

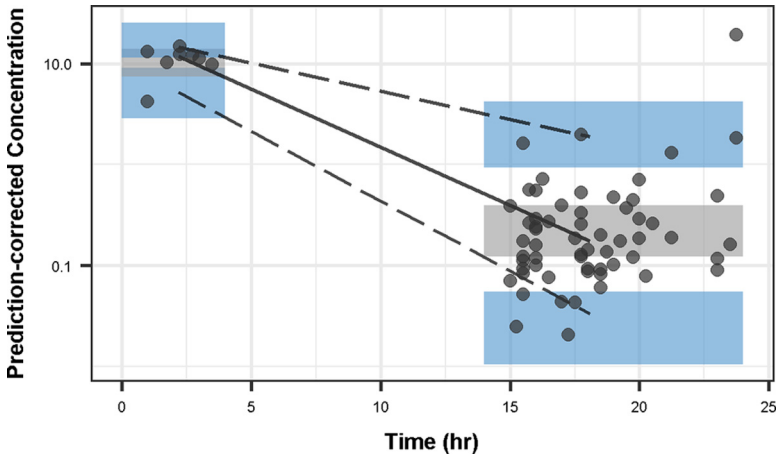

FIG 2 Prediction-corrected visual predictive check for the final PK model. The solid circles represent the prediction-corrected concentrations, and solid and dashed lines represent the median and the 5th and 95th percentiles, respectively. Shaded areas are the simulated 95% CI of each percentile.

TABLE 2 Parameter estimates for the final PK model

Parameter (unit)	Definition	Estimate (RSE[b], %)	SIR median (95% CI)
CL[a] (liter/h/70 kg)	Clearance	11.1 (10)	11.1 (9.28–13.2)
V_1[a] (liter/70 kg)	Central vol of distribution	30.2 (21)	30.70 (16.3–43.0)
Q[a] (liter/h/70 kg)	Intercompartmental clearance	4.26 (42)	4.48 (2.52–8.81)
V_2[a] (liter/70 kg)	Peripheral vol of distribution	14.9 (13)	15.41 (12.37–19.80)
BSV CL (% CV)	Between subject variability in CL	33.0 (22)	33.6 (25.1–46.6)
BSV V_1 (% CV)	Between subject variability in V_1	63.1 (22)	67.7 (35.6–106)
RE (% CV)	Proportional residual error	2.28 (28)	2.39 (1.37–4.83)

[a]Parameters are allometrically scaled to a 70-kg person.
[b]RSE, relative standard errors.

***In vitro* pharmacodynamic model.** The PubMed search identified 10 studies in which *in vitro* time-kill curve data of amikacin against *Pseudomonas aeruginosa* were available. Eight studies were included in the development of the *in vitro* PD model after excluding 2 studies (5, 18–24). One excluded study was an *in vivo* experiment, and the other study had a design quite different from the others. One included study was static, while the rest were dynamic experiments of single and multiple dosing up to 48 h (Table 3).

Overall, there were 302 data points in which 21 observations (6.9%) were at or below the limit of detection (LOD). The final analysis included 281 data points after excluding the below-LOD observations. The final ARPD model included additive interstudy variability (ISV) and inter-arm variability (IAV) on N_o (initial bacterial count), exponential ISV on K_g (first-order bacterial growth rate constant) and maximal killing effect (E_{max}), and additive residual unexplained variability (RUV) for static and dynamic experiments separately. Goodness-of-fit plots did not show any model deficiency (Fig. 4). The parameter estimates of the ARPD model and their uncertainty distributions were successfully computed by SIR and are presented in Table 4.

PK-ARPD simulations. Amikacin unbound plasma concentration-time profiles and fC_{max} and fC_{min} distributions were similar to those obtained in PTA simulations. In general, the probability of an fC_{min} of $<$10 mg/liter decreased when higher amikacin doses were given more frequently, as shown in the top panels of Fig. 5. Significant reduction of bacterial counts occurred with the first dose of amikacin, followed by bacterial regrowth. The magnitude of initial bacterial reduction and time to regrowth were larger and longer with higher doses of amikacin for a given dosing interval. This pattern is clearly evident with the daily dosing, as shown in the bottom right plot of Fig. 5. Administering the same amikacin amount in divided doses was also associated with lower initial bacterial reduction. A reduction in bacterial killing with subsequent doses of the same amount was also observed, indicating development of bacterial resistance. This is a documented phenomenon for aminoglycoside antibiotics, and it is well captured by the ARPD model (3, 25). The loss of bacterial killing is the highest for the dose of 15 mg/kg, and the rate of resistance was faster when the same total daily dose was given more frequently in divided doses. Predicted bacterial counts below the LOD

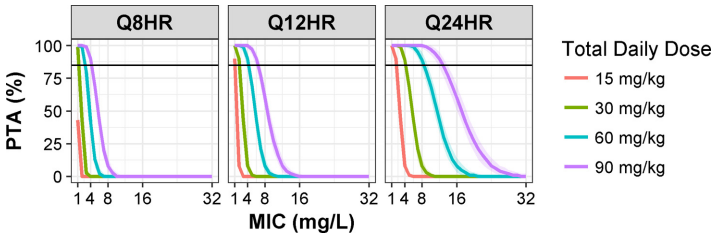

FIG 3 PTA of achieving an fC_{max}/MIC of ≥8 versus MIC for amikacin dosing of 15, 30, 60, and 90 mg/kg when given in one (Q24HR) or divided doses (Q8HR or Q12HR). Each PTA line is the median of 500 Monte Carlo simulations from 100 patients with different weights randomly sampled from a truncated log-normal distribution to match the observed weights of patients included in the study. Shaded areas around each PTA line represent the 95% CI. The horizontal line indicates 80% PTA.

TABLE 3 Summary of *in vitro* time-kill curve studies included in the final analysis

Study	Expt	Strain	MIC (mg/liter)	LOD (log_{10} CFU/ml)	C_{max} (mg/liter)	$t_{1/2}$ (h)	Reference
1	Static	27853	4	NR[f]	0, 2, 8, 32, 128		23
2[b,e]	Dynamic	27853/16690	2/2	2	0, 80	2.1	22
3[c,e]	Dynamic	27853/27853R	2/2	2	0, 40, 80	2.3	21
4[c]	Dynamic	27853	3.13	2	0, 80	2.35	20
5[a,b,e]	Dynamic	64/244	8/16	NR	24, 72	2.2	19
6[a,c,d]	Dynamic	27853	8	1	0, 24, 72	2	18
7[b]	Dynamic	27853	3.7	NR	10	2.4	24
8	Dynamic	99063	NR	NR	0		5

[a]Drug was infused over 1 h instead of being given as a bolus.
[b]Single-dose experiment over 24 h.
[c]Multiple-dose experiment up to 48 h.
[d]One-compartment infection model used instead of 2-comparment model.
[e]Studies included more than one strain of *Pseudomonas aeruginosa*.
[f]NR, not reported.

(2 log_{10} or 10^2 CFU/ml) were achieved by 90 mg/kg administered either at once or in divided doses. The 60-mg/kg dose also resulted in a bacterial count below the LOD but only when administered daily and not in divided doses. Lower doses (15 to 30 kg/mg) failed to drive bacterial counts to the LOD level.

DISCUSSION

The simulation results suggest that 15 mg/kg/day is a safe but suboptimal dose of amikacin in our pediatric cancer patients. Using the EBEs and percent protein binding, not a single patient in the study was calculated to achieve the target fC_{max}/MIC of ≥8. The ramification of a low dose is not only limited to high probability of therapeutic failure in a patient but also associated with significant collateral damage that affects our societies through the selection of drug-resistant bacteria (26). Our PTA simulation study suggests that once-daily administration of larger amikacin doses are expected to be associated with higher PTA and lower risk of toxicities overall. The rationale for once-daily dosing is to maximize bactericidal effects driven by fC_{max}/MIC, prolong

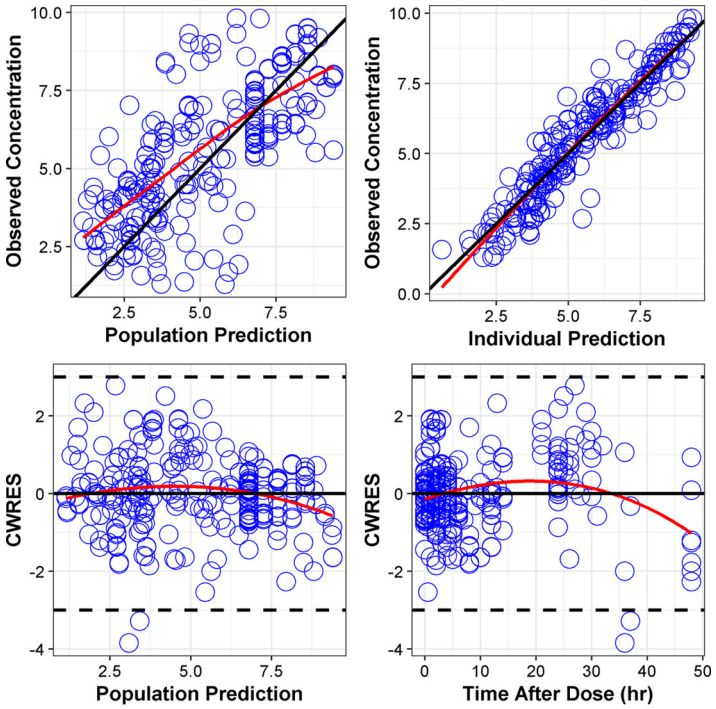

FIG 4 Diagnostic plots for the final PD model.

TABLE 4 Parameter estimates for the final PD model

Parameter (unit)	Definition	Estimate	SIR median (95% CI)
N_0 ($10^{CFU/ml}$)[a]	Initial bacterial count	6.80	6.79 (6.45–7.10)
K_g (h^{-1})	Bacterial growth rate constant	1.04	1.03 (0.89–1.13)
N_{max} ($10^{CFU/ml}$)[a]	Maximum bacterial count	9.41	9.61 (8.96–10.29)
E_{max} (h^{-1})	Maximum killing rate constant	9.38	9.38 (7.36–11.91)
EC_{50} (mg/liter)	Plasma drug concn to achieve 50% maximum killing rate	3.49	3.46 (2.52–4.79)
α (liter/mg · h)	Rate of bacterial adaptation constant	0.0143	0.0143 (0.013–0.016)
β	Maximum bacterial adaptation	29.2	30.4 (23.8–38.3)
ISV N_0 (SD)	Interstudy variability in N_0	0.91	0.914 (0.769–01.018)
ISV K_g (% CV)	Interstudy variability in K_g	23	23.0 (217–24.8)
ISV E_{max} (% CV)	Interstudy variability in E_{max}	33.9	33.9 (31.8–36.2)
IAV N_0 (SD)	Inter-arm variability in N_0	0.14	0.14 (0.12–0.16)
RUV_{static}	Log_{10} residual error for static studies	0.86	0.85 (0.61–1.02)
$RUV_{dynamic}$	Log_{10} residual error for dynamic studies	0.57	0.58 (0.47–0.67)

[a]$10^{CFU/ml}$, 10^estimated parameter (CFU/ml).

postantibiotic effects, and allow a longer time to excrete the drug from the body, leading to lower fC_{min}. Based on the PTA simulation, a minimum amikacin dose of 60 mg/kg administered once daily is expected to achieve the efficacy target of an fC_{max}/MIC of ≥8 in 80% of pediatric patients weighing 8 to 70 kg, with a 97.5% probability and a safety target of <10 mg/liter in almost all patients.

This dosing recommendation is consistent with other studies that suggest higher single daily doses of amikacin of ≥40 mg/kg in different patient populations, including pediatric cancer patients (7, 19, 27–29). In 1998, the amikacin dose of 20 mg/kg administered once a day was recommended for immunocompromised pediatric patients, including those with cancer (10). A recent study presented at the 2017 American Society for Clinical Pharmacology and Therapeutics (ASCPT) conference suggested a high dose of 40 mg/kg for a 5-year-old patient weighing 20 kg and having creatinine clearance (CrCL) of 120 ml/min per 1.73 m^2 of body surface area (29). The increase in recommended dose from 20 to 40 mg/kg daily is likely due to the documented increase in amikacin MIC_{90} from 4 to 8 mg/liter against *Pseudomonas aeruginosa* over the past decades (17, 30). Craig has shown that the magnitude of PK-PD index does not change for resistant bacteria when one corrects for the increase in MIC (31). Higher doses are needed to produce higher exposure and reach the effective PK-PD target as a compensation for the increase in MIC. According to a recent study, the amikacin MIC_{90} is currently at 16 mg/liter (32). The consistent increase in MIC_{90} over time is caused at

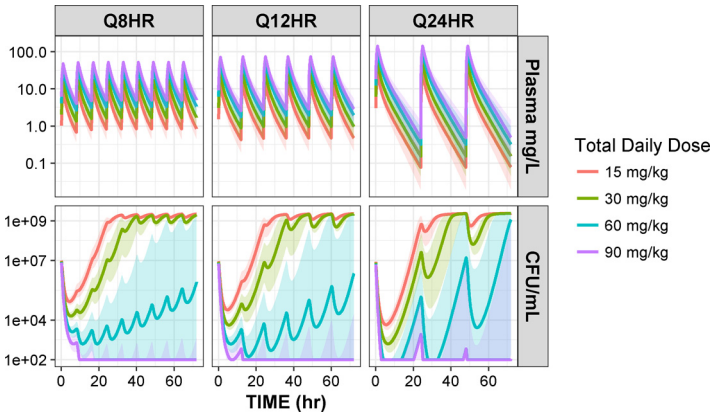

FIG 5 PK-ARPD simulations of 1,000 patients for amikacin dosing of 15, 30, 60, and 90 mg/kg when given in one (Q24HR) or divided doses (Q8HR or Q12HR). Weights of patients were sampled from a truncated log-normal distribution to match the observed weights of patients in the study. (Top) Predicted unbound amikacin plasma concentration-time profiles from the PK model after 1-h infusion. (Bottom) Bacterial time-kill curves predicted by the PD model given the predicted unbound amikacin plasma concentration-time profiles. Lines are the medians and shaded areas are 95% CI.

least partly if not entirely by the repeated exposure of suboptimal concentrations of amikacin that selects for the preexisting less-susceptible bacterial populations.

There are several possible explanations for the study findings. Our PK model was based on limited data (64 observations, 2 data points per subject) from Egyptian patients and did not find CrCL and age to be significant covariates, as has been shown previously (29). Despite that, the estimated CL (4.33 liters/h), V_1 (8.63 liters), Q (intercompartmental clearance, 1.66 liters/h), V_2 (4.26 liters), and half-life ($t_{1/2}$; 3 h) for a typical patient of 20 kg are similar to reported literature values (10, 29, 33, 34). It is documented that oncology patients do have altered amikacin kinetics characterized by increased clearance and volume of distribution (34–37). This is attributed to the disease condition, severity, and drugs used for treatment. However, the kinetic changes are not uniform and cannot be predicted by a single or combined variables, such as malignancy type, degree of neutropenia, disease state, total exposure, or type of chemotherapy (37). This is in agreement with our results that did not find any influential clinical covariates. It is also important to note that the majority of our patients had hematological malignancy. With that, our results may not apply to children with different types of cancer. The generalization of study findings to other populations is also limited due to the heterogeneity in renal functions.

Another justification for our results is that uncertainty of typical PK parameters, BSV, and variability of body weights were accounted for in the Monte Carlo simulations (MCS) when estimating the mean and 95% CI of PTA to achieve an fC_{max}/MIC of $\geq$8 and an fC_{min} of $<$10 mg/liter. It is noted that higher uncertainty of and variability in PK parameters generally leads to higher uncertainty of PTA, resulting in higher optimal dose prediction (38). However, this approach is strongly advised by Colin et al. in the support of quality and proper clinical decision-making for anti-infective optimal dosing (38). They stated that the lower boundary of PTA 95% CI can be conservatively used to select an appropriate dosing regimen for a given MIC where there is a 97.5% probability that the unknown PTA exceeds a certain cutoff (e.g., 80% in our case) in a patient population. The high uncertainty in PK parameter estimates caused by the sparse nature of our data, high BSV, high level of confidence (95% versus 90%), and random selection of weight together can explain the higher predicted optimal dose of 60 instead of 40 mg/kg/day, as suggested by Liu et al. (29).

Despite the merits of PTA with the parameter uncertainty approach, it lacks the ability to account for the adaptive resistance of bacteria upon exposure to amikacin (3, 25). The PTA approach is based on MIC, which provides no information about the kinetics of drug action and the persistent activity when drug concentrations are below the MIC, as it is determined at a single time point (39). This is a major limitation that can lead to incorrect dosing recommendation. PK-ARPD simulations show that the 60-mg/kg/day dose suggested by PTA is unlikely to suppress bacterial resistance. Upon repeated dosing, bacterial resistance is expected to dominate over the killing effect that is initially predicted. This is well demonstrated in Fig. 5: after the initial reduction, the predicted median bacterial count at the end of every day increases in a linear fashion. The steepness of slope decreases as the dosing frequency increases, suggesting lower rates of resistance. The predicted increase in bacterial count is driven by both the drop in amikacin concentration with time during long dosing intervals and the loss of amikacin efficacy with repeated exposure. The PK-ARPD simulation shows that safety and development of resistance goes hand in hand, creating a double-edged sword situation. Large single doses are safer for patients with greater initial killing, but they are likely to be selective for the emergence of resistant bacterial populations. When complete bacterial eradication (bacteriological cure) is not achieved, less susceptible bacterial subpopulations with increased MIC will grow and predominate as the amikacin unbound concentration falls below the MIC threshold. A compromise between efficacy on one hand and acceptable safety and less chance of bacterial resistance on the other hand is achieved with twice-daily dosing, where there is enough time between doses for kidneys to excrete amikacin while limiting the amount of time when drug concentrations are below the MIC and maintaining an acceptable fC_{max}/MIC ratio.

PK-ARPD simulations predicted 90 mg/kg/day given in two divided doses is needed to sustain median bacterial counts below the LOD until the end of day 3. Predicted median bacterial counts remained below the LOD until day 7 (data not shown), but we only presented the results up to day 3, since this is the time it takes to get an informative microbiological report back to adjust therapy accordingly. Taking safety into account, 90 mg/kg administered in two divided doses (45 mg/kg twice a day [BID]) is likely safer for empirical treatment than when given in three divided doses (30 mg/kg three times a day [TID]), which is predicted to have an fC_{min} of $>$10 mg/liter. With twice-daily dosing, fC_{min} is predicted to be $<$10 mg/liter and provides sufficient time for drug excretion before the next dose to avoid drug accumulation. Furthermore, our stimulation shows that the probability of resistance is lower with twice-daily dosing (45 mg/kg BID) than with a single dose (90 mg/kg four times a day).

PK-ARPD simulation also shows the administration of amikacin in divided doses is generally expected to facilitate bacterial resistance more than killing, unless the divided dose is high enough ($\geq$20 mg/kg TID or $\geq$40 mg/kg BID) (data not shown). The simulation results (Fig. 5) also show that the bacterial regrowth rate is the same irrespective of dose and peaks at the time of the next dose. This is consistent with amikacin affecting only growing bacteria while leaving less susceptible bacteria in the stationary phase unaffected (40). This may explain the adaptive resistance seen with aminoglycosides despite their bactericidal effect. A limitation of our PK-ARPD simulation is that the *in vitro* ARPD model does not take into account the role of the immune system of the host. In the antimicrobial world, the observed effect *in vivo* is a combination of both drug effect and host immune response. With that in mind, it is very possible that even lower doses than what our simulations suggested could be effective.

The disagreement in dose recommendation between PTA and PK-ARPD approaches in our simulations highlights the inability of PTA to predict development of drug resistance. Nielsen and Friberg highlighted several limitations associated with the PTA approach in details which are beyond the scope of the manuscript (41). Importantly, they pointed out that PTA limitations originate from the use of MIC-based PK-PD indices as targets for safety and efficacy. All PK-PD indices rely on the MIC, which is a threshold concentration that neglects measurement errors and ignores the dynamic nature of bacterial growth and killing rates occurring over the incubation period. They also mentioned that MIC is incorrectly assumed to stay constant during the treatment period. In addition, the MIC-based PK-PD indices are surrogate endpoints of the true clinical outcome to achieve the complete eradication of pathogenic bacteria, i.e., bacteriological cure. With that, PK-ARPD modeling and simulation is preferred over the MIC-based PTA. It is a better approach to describe the time course relationships among dose, concentration, bacterial killing, and resistance that happen simultaneously. The PK-ARPD models can also be used to predict the efficacy-linked PK-PD indices (42). However, although PK-ARPD modeling and simulation shows superiority over the MIC-based PTA approach, it remains underused in the development and improvement of antibiotic dosing regimens despite its promising potential (41). When adapted, the PK-ARPD approach could result in more appropriate dosing of antibiotics, which is likely to help in battling growing bacterial resistance.

In conclusion, oncology pediatric patients do have altered amikacin pharmacokinetics which probably necessitate higher doses to achieve therapeutic targets. Based on our simulation findings, daily monodose administration of 15 mg/kg may not be optimal in child patients with cancer. In addition, a dose recommendation suggested by PTA is likely incorrect because it uses surrogate indices of clinical outcomes that are not sufficiently sensitive for determining optimal dosage regimens for total bacteriological cure. We find PK-ARPD modeling and simulation to be superior on a theoretical basis, because it provides deeper insight about the intricate time course relationship among dosing regimens, plasma concentrations, and wanted as well as unwanted outcomes. PK-ARPD simulations suggest that 45 mg/kg BID can achieve safety and efficacy targets and is expected to be associated with lower rates of bacterial resistance.

Study results can be used to justify a controlled clinical trial to evaluate suggested doses against current doses used in clinical settings.

MATERIALS AND METHODS

Patients. This study included children with cancer and suspected or documented Gram-negative bacterial infection admitted to the inpatient ward of the National Cancer Institute (NCI), Cairo, Egypt, during the period of June 2009 to December 2009. Patients were diagnosed with different types of malignancies, broadly categorized into hematological and solid cancers, that were treated with different chemotherapy protocols accordingly. All patients were treated empirically with amikacin at 15 mg/kg once daily infused intravenously over 1 h, which was the standard-of-care dose. Patient demographics and pertinent clinical characteristics were collected and recorded. The study was reviewed and approved by the Hospital Ethical Committee. Parental informed consent and child informed assent, when appropriate, were obtained prior to study inclusion.

Sample collection and analytical assay. Two blood samples were drawn from a peripheral vein: one was taken at the end of infusion (1 h), while the second sample was drawn at least 2 h after the first sample. Amikacin serum concentrations were measured by amikacin enzyme multiple immunoassay technique (Emit 2008), supplied by Syva Company Co., Dade Behring, Inc. (Cupertino, CA, USA). The assay was linear from 0 to 50 μg/ml, with reported between-run and within-run coefficients of variation (CV) of 3.9% and 5.8%, respectively. Two controls were assayed in every 24-h period, and amikacin serum concentrations were calculated automatically by the Emit 2008 analyzer.

Pharmacokinetic analysis. Amikacin concentrations from all patients were fitted simultaneously to one- and two-compartment PK models with linear elimination using nonlinear mixed-effects regression analysis. An exponential random-effect model and proportional error model were used to estimate between-subject variability (BSV) and residual unexplained variability (RUV), respectively. To explain variability in model parameters, the effect of patient demographics and clinical characteristics were screened visually as potential covariates, with particular concern for the effects of male versus female, hematological versus solid malignancies, afebrile versus febrile (defined as an oral temperature of >38.5°C or nonoral temperature [axillary or temporal] of 38°C on two separate readings at least 1 h apart) (43), and normal albumin level versus hypoalbuminemia, defined as serum albumin of <3.5 g/dl. The statistical significance of a covariate was tested using a likelihood ratio test (χ^2; $\alpha = 0.01$, df = 1) in which a reduction in objective function value (OFV; a measure of goodness of fits similar to a sum of squares) of 6.63 or greater is observed after the inclusion of a covariate. The final PK model was selected based on the OFV, biological plausibility of parameter estimates, and diagnostics plots. The parameter standard errors and uncertainty distributions were computed by sampling importance resampling (SIR) (44), and model performance was qualified using a prediction-corrected visual predictive check (pcVPC) (45).

PTA calculation. The empirical Bayesian estimates (EBEs) from the final PK model and amikacin protein binding were used to calculate fC_{max} and fC_{min} for a single dose. They consequently were used to estimate the PTA of achieving the efficacy and safety targets of 15-mg/kg/day dosing in study patients.

In addition, the population PK model was used to estimate the population PTA to achieve an fC_{max}/MIC ratio of ≥8 and an fC_{min} of less than 10 mg/liter for different amikacin dosing regimens. Monte Carlo simulations (MCS) were used to simulate 500 trials of 100 patients for each combination of amikacin weight-based total daily dose (15, 30, 60, and 90 mg/kg) and dosing interval (every 8 h [q8h], q12h, and q24h). Weights of pediatric patients were simulated from a truncated log-normal distribution (means, 3.23; standard deviations [SD], 0.55) to match the observed weights of patients in the study. To account for parameter uncertainty, BSV, and the extent of correlation among the estimated values of parameters, the full variance-covariance matrix obtained by SIR was incorporated into the MCS. Taking into consideration a fixed 10% protein binding of amikacin, the PTA (expressed as a percentage) of an fC_{max}/MIC of ≥8 and an fC_{min} of <10 mg/liter for each trial was calculated for a range of assumed MIC values: 1, 2, 4, 8, 16, and 32 mg/liter. For each of the 500 simulated trials for each dose and dosing interval combination, the median and the 5th and 95th percentiles of PTAs were calculated.

***In vitro* pharmacodynamic analysis.** A PubMed literature search was performed and identified time-kill curve studies of amikacin against *Pseudomonas aeruginosa* in which figures were digitized using WebPlotDigitizer (version 3.11) (46). The extracted bacterial counts, measured as CFU per milliliter of medium, from static and dynamic studies were fitted together to a published bacterial dynamic growth model with adaptive resistance (equations 5 to 7) (3).

$$\frac{dN}{dt} = K_g \times \left(1 - \frac{N}{N_{max}}\right) \times N - \mathrm{DE} \times N \tag{5}$$

$$\mathrm{DE} = \frac{E_{max} C_p}{\mathrm{AD} \times \mathrm{EC}_{50} + C_p} \tag{6}$$

$$\mathrm{AD} = 1 + \beta\left[1 - e^{(-\alpha C_p t)}\right] \tag{7}$$

Bacterial growth (equation 5) follows a logistic model in which N is total bacterial counts at time t, K_g is the first-order bacterial growth rate constant, N_{max} is the maximum bacterial count, and DE is the drug effect (it equals 0 when there is no drug). In the presence of amikacin (equation 6), DE was characterized by a maximal killing effect (E_{max}) and EC_{50}, which is the unbound amikacin plasma concentration (C_p) needed to produce 50% of the E_{max}. C_p was simulated from a 1-compartment PK model with half-lives ($t_{1/2}$) and peak concentrations identical to those reported in the *in vitro* studies. To allow for the development of adaptive resistance, EC_{50} was multiplied by an adaptation factor (AD) that causes the EC_{50} to increase with time and C_p. The adaptation factor (equation 7) is a fractional multiplier

in which β represents the maximum fractional increase in EC_{50} when unbound concentration and time become large, and α is the adaptation rate constant that allows adaptation to transition from 1 when drug concentration is zero to $1 + \beta$.

In this meta-analysis of literature data, random-effect terms for interstudy variability (ISV), inter-arm variability (IAV), and $\log_{10}$-based additive RUV were included in the model. The ISV random effect was approximated in NONMEM using $Level and R matrix in the $COVARIANCE record. Below-LOD observations were ignored in the modeling process. The criteria and methods used to develop the PK model were used to select and assess the final ARPD model, except that VPC was not performed due to the sparse nature of extracted literature data.

PK-ARPD simulations. The literature-based *in vitro* ARPD model was linked to the final PK model to perform PK-ARPD simulations for the same dosing regimens explored in PTA simulations. A virtual pediatric patient population of 1,000 subjects with different weights sampled from the same truncated log-normal distribution as that mentioned above was generated for each dose and dosing interval combination. In PK-ARPD simulations, parameter uncertainty and BSV were included only in the PK parameters, while typical values were used for the PD model because of the unsuccessful covariance step.

Software. All nonlinear mixed-effects modeling analyses were performed in NONMEM 7.3 (ICON Development Solutions, Ellicott City, MD) using ADVAN13 and the first-order conditional estimation method with interaction (FOCEI). Data manipulation and plotting were done in R (version 3.2.5). Perl-speaks-NONMEM (PsN) was utilized to perform the VPC and SIR analyses. MCS were performed with the R-based mrg*solve* package (version 0.7.1). The Pirana interface was used to maintain and compare NONMEM and PsN runs.

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