Arch Pharm Res Vol 28, No 5, 598-603, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

The Influence of Assay Error Weight on Gentamicin Pharmacokinetics Using the Bayesian and Nonlinear Least Square Regression Analysis in Appendicitis Patients

Jin Pil Burm
Chosun Nursing College, Gwangju 501-825, Korea

(Received September 6, 2004)

The purpose of this study was to determine the influence of weight with gentamicin assay error on the Bayesian and nonlinear least squares regression analysis in 12 Korean appendicitis patients. Gentamicin was administered intravenously over 0.5 h every 8 h. Three specimens were collected at 48 h after the first dose from all patients at the following times, just before regularly scheduled infusion, at 0.5 h and 2 h after the end of 0.5 h infusion. Serum gentamicin levels were analyzed by fluorescence polarization immunoassay technique with TDxFLx. The standard deviation (SD) of the assay over its working range had been determined at the serum gentamicin concentrations of 0, 2, 4, 8, 12, and 16 μg/mL in quadruplicate. The polynominal equation of gentamicin assay error was found to be SD (μg/mL) = 0.0246 - (0.0495C) + ($0.00203C^2$). There were differences in the influence of weight with gentamicin assay error on pharmacokinetic parameters of gentamicin using the nonlinear least squares regression analysis but there were no differences on the Bayesian analysis. This polynominal equation can be used to improve the precision of fitting of pharmacokinetic models to optimize the process of model simulation both for population and for individualized pharmacokinetic models. The result would be improved dosage regimens and better, safer care of patients receiving gentamicin.

Key words: Weight, Gentamicin, Assay error, Bayesian, Nonlinear least squares regression analysis, Appendicitis patients

INTRODUCTION

Gentamicin is still recognized as first line therapeutic agent in the management of severe Gram-negative sepsis (Bisno *et al.*, 1989; Garrison *et al.*, 1990). Toxicity of gentamicin is determined primarily by the accumulation of the drug in the body. Wide intra- and inter-individual variability of the pharmacokinetic parameter values of gentamicin make the design of optimal dosage regimens difficult for patients with normal or impaired renal function. Gentamicin use has been limited by its potential ototoxicity and nephrotoxicity (Dahlgren *et al.*, 1975; Federspil *et al.*, 1978; Moore *et al.*, 1984; Powell *et al.*, 1983). Individualized drug dosage for gentamicin therapy now enables one to reduce toxicity. Furthermore, it has been shown that if used with the appropriate methodology (Burm *et al.*, 1995; Choi *et al.*, 1996) therapeutic drug monitoring (TDM) is effective in keeping serum concentrations of gentamicin within desired ranges, increasing the proportion of patients having effective serum concentrations, and in reducing length of hospital stay with a potential cost-saving per patient (Noone *et al.*, 1974).

Monitoring of gentamicin therapy can be performed with linear least squares regression (Sawchuk *et al.*, 1976), nonlinear least squares regression (Jelliffe *et al.*, 1988a), and maximum a posteriori probability Bayesian analysis (Chrystn *et al.*, 1988; Jelliffe *et al.*, 1991; Schumacher *et al.*, 1984; Sheiner *et al.*, 1982). Linear least squares fitted to the logs of drug levels is the method is limited to analysis of data acquired during a single dose interval, discarding all previous data. In contract, both nonlinear least squares regression and the Bayesian analysis can conserve all data serum concentrations during the current regimen. Both methods use the data intelligently when a pharmacokinetic model is employed that has a slope relatioship between its parameters and various clinical descriptors. In addition, both these methods can fit the model to the actual serum concentrations (not just their

Correspondence to: Jin Pil Burm, Chosun Nursing College, Gwangju 501-825, Korea
Tel: 82-62-231-7361, Fax: 82-62-232-9072
E-mail: jpburm@venus.cnc.ac.kr

logarithms), and each serum concentrations is given a weight or importance appropriate to the credibility of each measurement.

In Korea, the actual assay error is usually ignored for purposes of therapeutic drug monitoring. The goal of the present study examines influences of weight with assay error on the pharmacokinetics of gentamicin in appendicitis patients. We can improve the precision of fitting of pharmacokinetic model to optimize the process of model simulation, both for population and for individualized pharmacokinetic models.

MATERIALS AND METHODS

Patient population

Timed serum gentamicin concentrations were obtained from 12 appendicitis patients in Chosun University hospital. Patients were all part of comparative antibiotic trials receiving gentamicin for the diagnosis of appendicitis. All patients had normal renal function (serum creatinine < 2.5 mg/dL), were not grossly underweight (40 kg or less), and were free of other infections including sepsis (Table I). As patients were a part of comparative antibiotic trial, each patient gave informed consent for the procedures of the study, and the study protocol was approved by the institutional review board.

Dosage regimen and specimens

Gentamicin was administered intravenously over 0.5 h every 8 h. Loading dose was determined by the following equations.

Loading dose = desired C_{pmax} (V_d)
V_d = 0.25 L/kg × IBW
IBW = 50 kg + 2.3 kg/inch greater 5ft (male)
IBW = 45 kg + 2.3 kg/inch greater 5ft (female)

Maintenance dose was determined by the following equations.

$$\text{Maintenance dose} = \frac{\text{Desired } C_{pmax}(K_{el})(V_d)(t)(1-e^{-KT})}{1-e^{-Kt}}$$

$$K_{el} = 0.0034\ CL_{cr} + 0.01$$

Table I. Characteristics of patient population

Characteristics	Patient population
Number (female)	12 (3)
Age (year)	30.0 ± 6.11
Weight (kg)	62.0 ± 10.8
Height (cm)	161 ± 7.6
S_{cr} (mg/mL)	0.99 ± 0.16

Values are means ± S.D. of 12 patients.
S_{cr} : Serum creatinine concentration.

$$CL_{cr} = \frac{(140-age)\times IBW}{72(S_{cr})} \times 0.85(female)$$

in which IBW is the ideal body weight (kg), t is the infusion time (h), T is the dosing interval (8 h), CL_{cr} is the creatinine clearance (mL/min), and S_{cr} is the serum creatinine concentration (mg/dL). Three specimens were collected at 48 h after the first dose from all patients at the following times, just before regularly scheduled infusion, at 0.5 h and 2 h after the end of 0.5 h infusion.

Gentamicin assay

Serum gentamicin levels were analyzed by fluorescence polarization immunoassay technique with TDxFLx (Abbott lab, Irving, TX). The following described the step-by-step procedure for performing a random-acess assay run. Seleted a sample carousel and loaded sample cartridges and cuvetts for each sample to be assayed. Pipetted at least 50 of patient serum into sample wells for each position being used. Sanpped the gentamicin reagent packs onto the reagent carousel and measured the fluorescence intensity. Prior to running an assay, the TDxFLx system stored a calibration curve at the gentamicin concentrations of 0, 0.5, 1.5, 3.0, 6.0, and 10.0 μg/mL (Fig. 1).

Assay error

The standard deviation (SD) of the assay over its working range had been determined at the serum gentamicin concentrations of 0, 2, 4, 8, 12, and 16 μg/mL in quadruplicate. This can be done, for example, on a blank sample, a low sample, two intermediate ones, a high one, and a very high one, so that the entire assay range, subtherapeutic, therapeutic, and toxic, is covered. The nonlinear relatioship between serum gentamicin concentrations and SD was described the polynominal

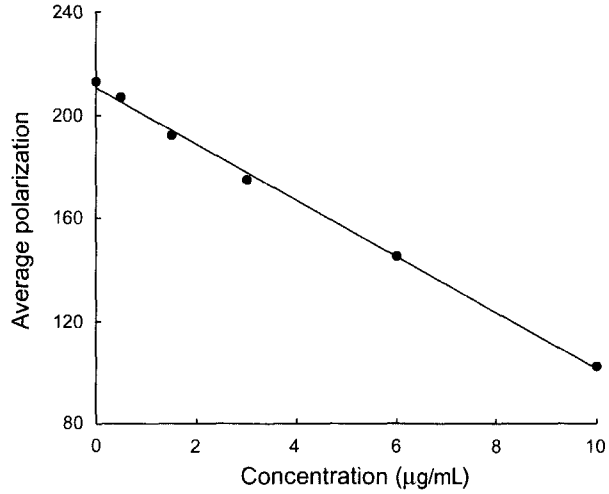

Fig. 1. Calibration curve of vancomycin in serum by using fluorescence polarization immunoassay with TDxFLx

equation by using a PCSTAT program (The University of Georgia, Athens, Geogia), usually of second order. Usually only a second-order polynominal is required. It has the form.

$$SD = A_0C^0 + A_1C^1 + A_2C^2$$

where A_0, A_1, and A_2 are the various coefficients, C^0 is concentration raised to the zero power (C^0=1), C^1 is concentration raised to the first power (or itself), and C^2 is the squares of the concentration. Using the equation, the probable SD was calculated with any subsequent single serum concentration within that range.

Nonlinear least squares regression analysis

Nonlinear least squares regression was analyzed by using the MLS program in the USC*PACK Collection (Jelliffe *et al.*, 1988b). It used the entire dosing history, the concentration of gentamicin in serum, and all the estimated creatinine clearance to determine the parameter values calculated for each patients, as follows: the total apparent volume of distribution (V_d), the elimination rate constant (K_{el}), the slope of the relationship between K_{el} versus creatinine clearance (K_{slope}, $K_{el} = K_{slope} \times CL_{cr} + K_{int}$), the nonrenal intercept (K_{int}), and the biological half-life ($t_{1/2}$). The function that was minimized in this fitting procedure was

$$\sum \frac{(C_{obs}-C_{mod})^2}{SD^2_{Cobs}}$$

In this procedure, the difference between the collection of the patient's observed serum concentrations (C_{obs}) and the collection of the fitted model's estimates of these concentrations at the time each was drawn (C_{mod}) are squared and divided by the variance with which each serum concentrations was measured (SD^2_{Cobs}). This expression is then summed and minimized to the smallest number when model is fitted to the data for the individual patients.

Bayesian analysis

Bayesian anlysis used the MB program in the USC*PACK Collection (Jelliffe *et al.*, 1988b). It evaluated the entire dosing history, the concentration of gentamicin in serum, all the estimated creatinine clearance, and the a priori parameter values of the population to arrive at posterior parameter values for each patients. The Bayesian anlysis was based on a strategy proposed by Sheiner *et al* (Sheiner *et al.*, 1979). The function that was minimized in this fitting procedure was

$$\sum \frac{(C_{obs}-C_{mod})^2}{SD^2_{Cobs}} + \sum \frac{(P_{pop}-P_{mod})^2}{SD^2_{Ppop}}$$

where the collection of the population parameter values are P_{pop}, and the collection of the revised values of each parameter as the model is fitted are P_{mod}. The collection of the patient's observed serum concentrations are C_{obs}, and the collection of the fitted model's estimates of these concentrations at the time each was drawn are C_{mod}. The variances with which each serum concentrations was measured are SD^2_{Cobs}, and the variances by which each member of P_{pop} is known are SD^2_{Ppop}.

Statistics

Student's t-test was used to compare the means for the weighted and not weighted parameters. Statistical significance was set at 0.05 and estimates of p values were reported.

RESULTS

Assay error and weight

The polynominal equation of gentamicin assay error was found to be

$$SD\ (\mu g/mL) = 0.0246 - (0.0495C) + (0.00203C^2)$$

As shown in Table II and Fig. 2, this assay had an SD of 0.025 μg/mL at 0 μg/mL (the blank), yielding a variance of 0.001 and weight (1/variance) of 1652.4. The SD then

Table II. Relatioship between the serum gentamicin concentrations (C) and standard deviation (SD) of assay error

C (μg/mL)	SD	Variance	Weight
0	0.025	0.001	1652.4
2	0.132	0.017	57.64
4	0.255	0.065	15.37
8	0.551	0.303	3.30
12	0.911	0.830	1.21
16	1.336	1.789	0.56

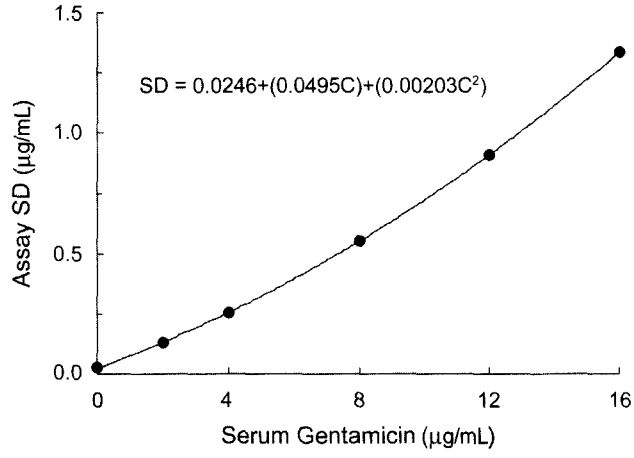

Fig. 2. Assay error of a Abbott TDxFLx assay for gentamicin and its assoiated polymoninal equation

rised and the weight progressively falled to 0.132 μg/mL and 57.64 respectively at a concentration of 2 μg/mL, to 0.551 μg/mL and 3.30 at 8 μg/mL, and to 1.336 μg/mL and 0.56 respectively at a concentration of 16 μg/mL. Note that the weights range from a heigh of 1652.4 to a low of 0.56, a factor of 2950 in the credibility given to the serum concentration data points within this range. The coefficients of the polynominal equation were then stored in the USC*PACK clinical program so that correct weighting of each measured gentamicin serum concentration can be implemented during the Bayesian and nonlinear least squares regression analysis.

Nonlinear least squares regression analysis

The total apparent volume of distribution, the elimination rate constant, the slope of the relationship between K_{el} versus creatinine clearance, and the biological half-life were 0.200 ± 0.056 L/kg, 0.417 ± 0.099 h^{-1}, 0.00432 ± 0.00096 min/mL·h and 1.75 ± 0.427 h respectively by using not weighted nonlinear least squares regression analysis. The total apparent volume of distribution, the elimination rate constant, the slope of the relationship between K_{el} versus creatinine clearance, and the biological half-life were 0.238 ± 0.070 L/kg, 0.334 ± 0.047 h^{-1}, 0.00343 ± 0.00038 min/mL·h and 2.11 ± 0.284 h respectively by using weighted nonlinear least squares regression analysis. There were diferences ($p<0.05$) in the influence of weight with gentamicin assay error on pharmacokinetic parameters of gentamicin using the nonlinear least squares regression analysis.

Bayesian analysis

The total apparent volume of distribution, the elimination rate constant, the slope of the relationship between K_{el} versus creatinine clearance, and the biological half-life were 0.217 ± 0.033 L/kg, 0.346 ± 0.034 h^{-1}, 0.00357 ± 0.00042 min/mL·h and 2.02 ± 0.201 h respectively by using not weighted Bayesian analysis. The total apparent volume of distribution, the elimination rate constant, the slope of the relationship between K_{el} versus creatinine clearance, and the biological half-life were 0.232 ± 0.057 L/kg, 0.337 ± 0.040 h^{-1}, 0.00346 ± 0.00026 min/mL·h and 2.09 ± 0.236 h respectively by using weighted Bayesian analysis. There were no diferences in the influence of weight with gentamicin assay error on pharmacokinetic parameters of gentamicin using Bayesian analysis.

DICUSSION

Laboratory assay error usually have been analyzed by determining control sample values and keeping their variation within certain specified limits. Once this has been done, however, specific and explicit characterization of the analytic error associated with each measured serum drug concentration usually has not been done. Usually only the measured concentration has been reported or used in any practical way. The implementation of the Bayesian analysis was introduced into the medical and pharmacokinetic communities by Sheiner *et al* (Sheiner *et al.*, 1979), and has changed previous limitations. The Bayesian analysis can balance the relative credibility of the population parameter values for the pharmacokinetic model of a drug's behavior against the relative credibility of the serum level data acquired as an individual patient receives therapy. It thus predicts future serum concentrations slightly more precisely than weighted nonlinear least squares regression, and significantly more so than linear least squares regression, which fits only to the logarithms of the serum data (Jelliffe *et al.*, 1988c). Specific program (Jelliffe *et al.*, 1988b) now available for the Bayesian analysis of serum concentration data provide more cost-effective and precise prediction of future serum concentrations of many drugs having linear kinetic behavior. When evaluated against the methods of weighted nonlinear least squares and linear least squares regression, the Bayesian program has been shown to give better prediction of future serum level. Even the population pharmacokinetic model, without fitting to any serum data, gave better predictions than the linear least squares regression method. Linear least squares regression has been used in pharmacokinetic

Table III. The influence of weight with gentamicin assay error on the nonlinear least square regression and Bayesian analysis

Parameters	Nonlinear Least Square Regression		Bayesian Analysis	
	not weighted	weighted	not weighted	weighted
V_d (L/kg)	0.200 ± 0.056	0.238 ± 0.070*	0.217 ± 0.033	0.232 ± 0.057
K_{el} (h^{-1})	0.417 ± 0.099	0.334 ± 0.047*	0.346 ± 0.034	0.337 ± 0.040
K_{slope} (min/mL·h)	0.00432 ± 0.00096	0.00343 ± 0.00038*	0.00357 ± 0.00042	0.00346 ± 0.00026
$t_{1/2}$ (h)	1.75 ± 0.427	2.11 ± 0.284*	2.02 ± 0.201	2.09 ± 0.236
K_{int} (h^{-1})			0.00694 ± 0.000010	0.00692 ± 0.000045

Values are means ± S.D. of 12 patients. Significantly different from the not weighted (* $p<0.05$).
$K_{el} = K_{slope} \times CL_{cr} + K_{int}$

program for hand calculators (Zaske *et al.*, 1983) and personal computers (Stevens *et al.*, 1987).

For any data point, an index of its credibility can be given by its Fisher infoemation (DeGroot *et al.*, 1975). This credibility index is the values of a data point multiplied by the reciprocal of the variance by which that point is known (DeGroot *et al.*, 1975). For the population pharmacokinetic model of a particular drug, these variances are the squares of the standard deviations, which represent the uncertainties surrounding each pharmacokinetic parameter value. Thus the credibility of a population drug model can be expressed as the collection of all its parameter values, each divided by its variance. In exactly the same way, the credibility of a collection of measured serum concentrations can be expressed as each measured concentration is multiplied by the reciprocal of SD_2, its variance. When doing Bayesian analysis, one can only given equal weight to various serum concentration when they have the same SD. An assay with a constant SD over its working range is said to be homoschedastic. Such an assay will have a coefficient of variation that decreases by half as the concentration doubles. None of the assay evaluated here are homoschedastic. In contrast, a heteroschedastic assay error pattern is one in which the assay SD changes over its working range. Even an assay with a constant coefficient of variation is very heteroschedastic. As the concentration doubles, the SD also doubles, the variation quadruples, and the weight given to the assay is reduced to one fourth. If one assumes a constant coefficient of variation, a concentration of 1.0 μg/mL, for example, has a weight 100 times greater than that of a concentration of 10.0 μg/mL, and a concentration of 0.1 μg/mL has a 100 times that of the concentration of 1.0 μg/mL, and 1000 times that of the concentration of 10.0 μg/mL. Because of this, when a constant coefficient of variation is assumed for an assay used in Bayesian analysis, high concentration will be relatively ignored compared to lower ones, and the fitted model will not approach the high concentration as closely as one might wish. This is also true for the polynominal equation described above. The differene here is that the polynominal equation is derived from empirically measured SD's over the working range of the assay, and should include blank concentration as well. Because of this, it is a more correct estimate of the assay error over its working range, and the fit, while often appearing to ignore the high concentration, is actually being correctly done by current standards. One of two things needs to be improved. Either the current Bayesian analysis procedure based on the Fisher information of the data points is incorrect, or the assay need to have their precision improved at the high end to make them more homoschedastic. To discard the concept of Fisher information would be to overthrow several decades of carefully acquired and searchingly criticized mathematical and statistical knowledge. To improve the precision of assay at their high end is probably the most constructive thing to do. It may even be possible, for example, to alter the ratios of reagents so that the ratio of bound and unbound drug in the assay can be changed, with a resultant change in the error pattern toward homoschedasticity.

Obviously, error occure in the clinical environment other than associated with measuring serum level, such as: specimen labeling errors, dosage preparation errors, and errors in dosage times and start and stop times of infusion. Such errors have important consequences; however, they are not yet capable of being calculated explicitly as are assay errors, and their error term belongs in the dynamic equations for the pharmacokinetic model, not in its output equations where the assay error term resides. Proper consideration of these other factors requires the use of stochastic differential equations for making pharmacokinetic models.

In summary, this polynominal equation can be used to improve the precision of fitting of pharmacokinetic models to optimize the process of model simulation both for population and for individualized pharmacokinetic models. The end result would be improved dosage regimens and better, safer care of patients receiving gentamicin.

ACKNOWLEDGEMENT

This work was supported by a grant from Chosun Nursing College in 2004.

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