

위암환자에서 비선형 최소자승 회귀분석과 베이시안 분석에 의한 토브라마이신의 약물동태에 분석오차의 영향

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The Influence of Assay Error on Tobramycin Pharmacokinetics using the Nonlinear Least Square Regression and Bayesian Analysis in Gastric Cancer Patients

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토브라마이신은 그람음성균 감염에 사용하는 아미노글리코사이드계 항생제로 이독성 및 신독성 등의 부작용과 큰 개인차로 혈중농도 모니터를 통한 투여계획이 필요한 약물이다. 본 연구에서는 16명의 위암환자에서 비선형 최소자승 회귀분석과 베이시안 분석에 의한 토브라마이신의 약물동태에 분석오차의 영향에 대하여 연구하였다. 약물투여는 토브라마이신 1-2 mg/kg을 30분에 걸쳐 8시간 간격으로 등속 주입하였으며, 혈액 채취는 정상상태에 도달되었다고 판단되는 첫 약물투여 72시간 후에, 약물 주입 5분전과 주입이 끝난 뒤 30분과 2시간에서 세차례 채취하였다. 혈청중 약물농도는 형광편광면역법으로 측정 하였다. 분석오차를 위해 0, 1, 2, 4, 8 및 12 µg/mL에 해당하는 토브라마이신 혈중농도(C)를 네차례 측정하여 각 혈중농도의 표준편차 (SD)를 구하였다. 토브라마이신 분석오차를 구하기 위한 다항식이 $SD = 0.0224 + 0.0540C + 0.00173C^2$, $R^2 = 0.935$ 이었다. 이 식에서 구한 SD 값으로 분석시 가중치를 주었을 때, 비선형 최소자승 회귀분석에 의한 토브라마이신의 약물동태학적 파라메타 (V_d , K_{el} , K_{slpoc} , $t_{1/2}$)에 유의성있는 영향을 주었으나, 베이시안 분석에 의한 토브라마이신의 약물동태학적 파라메타에는 영향이 없었다. 이 다항식으로 부터 구한 분석오차를 토브라마이신의 비선형 최소자승 회귀분석을 이용한 약물동태 연구 및 파라메타 분석에 적용하여 좀 더 정확한 투여용량을 결정할 수 있으며, 더 나아가 토브라마이신 약물동태 시뮬레이션 연구에 응용할 수 있다.

□ Key words - Tobramycin, Assay error, Bayesian, Nonlinear least squares regression analysis.

Tobramycin is a semi-synthetic aminoglycoside that exhibits anti-bacterial activity against a wide range of bacterial pathogens. Tobramycin is active against gram-negative bacteria, penicillinase, and non-penicillinase producing streptococci. Tobramycin use has been limited because of its potential ototoxic and nephrotoxic effects. Individualized drug dosage for tobramycin therapy now enables one to reduce toxicity.¹⁾ Furthermore, it has been shown that if used with the appropriate

methodology, therapeutic drug monitoring (TDM) is effective in keeping serum concentrations of tobramycin within desired ranges, in increasing the proportion of patients having effective serum concentrations, and in reducing the length of a hospital stay.^{2,3)}

Monitoring tobramycin therapy can be performed with linear least squares regression,⁴⁾ nonlinear least squares regression,^{5,6)} nonparametric expected maximum algorithm⁷⁾, nonlinear mixed effects model,⁸⁻¹⁰⁾ and Bayesian analysis.¹¹⁾ The method of linear least squares regression, fit to the logs of drug levels, is limited only to data acquired during a single dose interval, thus all previous data is not incorporated into the analy-

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sis. In contrast, both nonlinear least squares regression and the Bayesian analysis utilize all serum concentrations throughout the entire regimen.¹²⁾ In addition, both these methods allow one to fit the model to the actual serum concentrations and each serum concentration is given a weight or importance appropriate to the credibility of each measurement.

The actual assay error is usually ignored for purposes of therapeutic drug monitoring in Korea. The goal of the present study is to examine influences of weight on the assay error in the pharmacokinetics of tobramycin in gastric cancer patients. Our results can be used to improve the precision of fitting pharmacokinetic models, which will optimize the process of model simulation, both for population and for individualized pharmacokinetic models.

MATERIALS AND METHODS

Patient population

Timed serum tobramycin concentrations were obtained from 16 gastric cancer patients in Chosun University Hospital. 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 1). Since patients were a part of a comparative antibiotic trial, each patient gave informed consent to be subjected to the procedures of this study, and the study protocol was approved by the Institutional Review Board.

Dosage regimen and specimens

Tobramycin 1-2 mg/kg was administered intravenously over 0.5 h every 8 h after surgery. Three speci-

Table 1. Characteristics of patient population

Characteristics	Patient population
Number (female)	16 (6)
Age (year)	47.8±11.2
Weight (kg)	60.7±9.7
Height (cm)	169±11
S _{cr} (mg/dl)	0.83±0.17

Values are means±SD of 16 patients.
S_{cr} : Serum creatinine concentration.

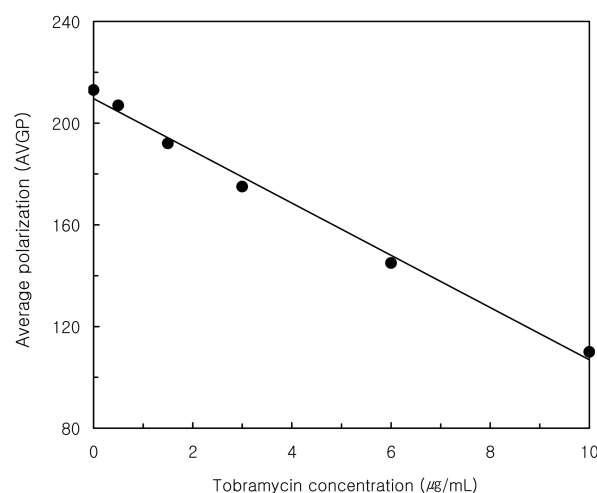


Fig. 1. Calibration curve of tobramycin in serum using fluorescence polarization immunoassay.

mens were collected at 72 h after the first dose from all patients at the following times, 5 min before regularly scheduled infusion, and 0.5 h and 2 h after 0.5 h of infusion.

Tobramycin assay and assay error

Serum tobramycin levels were analyzed by a fluorescence polarization immunoassay technique with TDx-FLx (Abbott laboratories, Irving, TX). Prior to running the assay, the TDxFLx system stored a calibration curve at the tobramycin concentrations of 0, 0.5, 1.5, 3, 6 and 10 µg/mL (Figure 1). Precision was determined using human serum with 1.0, 4.0 and 8.0 µg/mL of tobramycin added. The coefficients of variation were less than 7.2%. The standard deviation (SD) of the assay over its working range was determined at the serum tobramycin concentrations of 0, 1, 2, 4, 8 and 12 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, sub-therapeutic, therapeutic, and toxic levels, is determined. The nonlinear relationship between serum tobramycin concentrations and SD was described in most cases by a second order polynomial equation, which was determined using a StatsDirect statistical program (Version 2.7, StatsDirect Ltd., Cheshire, UK). The second order polynomial had the following 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 this equation, the probable SD was calculated for any subsequent single serum concentration within the defined range.

Nonlinear least squares regression analysis

Nonlinear least squares regression was determined using the MLS program in the USC*PACK Collection.¹³⁾ This program used the entire dosing history, the concentration of tobramycin in serum, and all the estimated creatinine clearance (CL_{cr}) to determine the one-compartment pharmacokinetic values for each patient. The total apparent volume of distribution (V_d) in liters per kilogram of actual body weight, the elimination rate constant (K_{el}), the slope (K_{slope}) of the relationship between K_{el} versus CL_{cr} with the nonrenal intercept (K_{int}), and the biological half-life ($t_{1/2}$) were calculated for each patient. The terms K_{el} and K_{slope} are described by the following equation:

$$K_{el} = K_{slope} \times CL_{cr} + K_{int}$$

The objective function that is minimized when the model is fitted to the patient's data is as follows:

$$\Sigma(C_{obs} - C_{mod})^2 / SD_{Cobs}^2$$

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}) were squared and divided by the variance with which each serum concentration was measured (SD_{Cobs}^2). This expression was then summed and minimized to the smallest number when the model was fit to the data determined for each individual patient.

Bayesian analysis

Bayesian analysis was conducted using the MB program in the USC*PACK Collection.¹⁴⁾ The entire dosing history, the concentration of tobramycin in serum,

all the estimated creatinine clearance, and the *a priori* parameter values of the population were used to arrive at posterior parameter values for each patients. The Bayesian analysis was based on a strategy proposed by Sheiner.¹⁵⁾ The following function was minimized in this fitting procedure:

$$\Sigma(P_{pop} - P_{mod})^2 / SD_{Ppop}^2 + \Sigma(C_{obs} - C_{mod})^2 / SD_{Cobs}^2$$

where the collection of the population parameter values were P_{pop} , and the collection of the revised values of each parameter determined from the fit to the model was P_{mod} . The collection of the patient's observed serum concentrations were C_{obs} , and the collection of the model's estimates based on the fit for these concentrations at the time each was drawn were C_{mod} . The measured variance of each serum concentration was referred to as SD_{Cobs}^2 , and the known variance of each member of P_{pop} was referred to as SD_{Ppop}^2 . The population parameter values (V_d , K_{slope} and K_{int}) for the Bayesian analysis were 0.0393 ± 0.065 L/kg, 0.00139 ± 0.00048 mL/min·h and 0.234 ± 0.022 h⁻¹ respectively.¹⁶⁾

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.

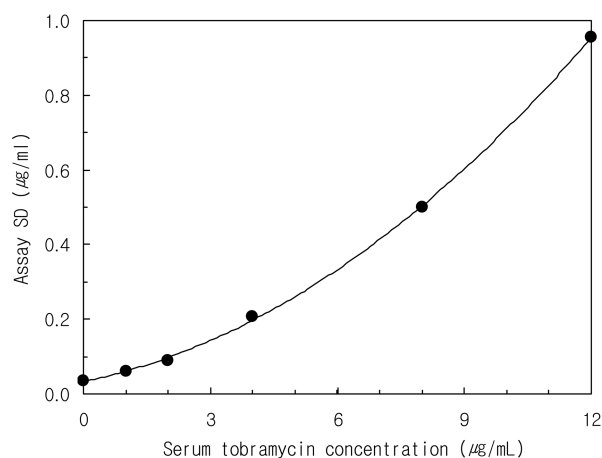


Fig. 2. Assay error of a Abbott TDxFLx assay for tobramycin and its associated polynomial equation. $SD (\mu\text{g/ml}) = 0.0224 + 0.0540C + 0.00173C^2$, $R^2 = 0.935$.

Table 2. Relationship between the serum tobramycin concentrations (C) and standard deviation (SD) of assay error

C ($\mu\text{g/ml}$)	SD ($\mu\text{g/ml}$)	Variance	Weight
0	0.035	0.001	816.33
1	0.062	0.004	260.15
2	0.138	0.019	52.63
4	0.266	0.071	14.08
8	0.571	0.326	3.07
12	0.918	0.843	1.19

Weight = 1 / Variance.

RESULTS

Assay error and weight

The polynomial equation describing the tobramycin assay error was found to be

$$SD (\mu\text{g/ml}) = 0.0224 + 0.0540C + 0.00173C^2, R^2 = 0.935.$$

As shown in Figure 2 and Table 2, this assay had an SD of 0.035 $\mu\text{g/ml}$ at 0 $\mu\text{g/ml}$ (the blank), yielding a variance of 0.001 and weight (1/variance) of 816.33. The SD then increased and the weight progressively dropped to 0.062 $\mu\text{g/ml}$ and 260.15 respectively at a concentration of 1 $\mu\text{g/ml}$, to 0.266 $\mu\text{g/ml}$ and 14.08 at 4 $\mu\text{g/ml}$, and to 0.918 $\mu\text{g/ml}$ and 1.19 respectively at a concentration of 12 $\mu\text{g/ml}$. Note that the weights ranged from a high of 816.33 to a low of 1.19, a factor of 72.5 in the credibility given to the serum concentration data points within this range. The coefficients of the polynomial equation were then stored in the USC*PACK clinical program so that correct weighting of each measured tobramycin serum concentration could be implemented during the Bayesian and nonlinear least squares regression analysis.

Nonlinear least squares regression analysis

Timed serum tobramycin concentrations from 16 gastric cancer patients were showed in Figure 3. Three specimens were collected at 72 h after the first dose from all patients at the following times, 5 min before regularly scheduled infusion, and 0.5 h and 2 h after 0.5 h of infusion. Using the non-weighted nonlinear least squares regression analysis the total apparent volume of distribution, the elimination rate constant, the slope of

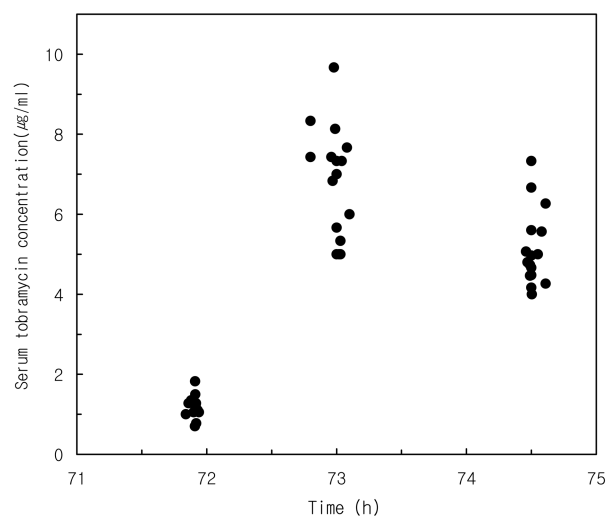


Fig. 3. Timed serum tobramycin concentrations at 5 min before regularly scheduled infusion (72 h after the first dose from all patients), and 0.5 h (73 h) and 2 h (74.5 h) after 0.5 h of infusion.

the relationship between K_{el} versus creatinine clearance, and the biological half-life were determined to be 0.326 ± 0.059 L/kg, 0.399 ± 0.094 h⁻¹, 0.00188 ± 0.00034 min/ml·h and 2.58 ± 0.45 h respectively. When the weighted nonlinear least squares regression analysis was used 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 determined to be 0.399 ± 0.072 L/kg, 0.334 ± 0.059 h⁻¹, 0.00147 ± 0.00039 min/ml·h and 3.05 ± 0.51 h. Thus, when using this analysis statistically significant differences ($p < 0.05$) in the influence of weight on the tobramycin assay error for pharmacokinetic parameters of tobramycin were observed.

Bayesian analysis

Using the non-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 determined to be 0.382 ± 0.081 L/kg, 0.357 ± 0.079 h⁻¹, 0.00172 ± 0.00041 min/ml·h and 2.88 ± 0.47 h respectively. When the weighted Bayesian analysis was used the total apparent volume of distribution, the elimination rate constant, the slope of the relationship between

Table 3. The influence of weight with tobramycin 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)	0.326±0.059	0.399±0.072*	0.382±0.081	0.409±0.093
K_{el} (h^{-1})	0.399±0.094	0.334±0.059*	0.357±0.079	0.361±0.056
K_{slope} (min/ml·h)	0.00188±0.00034	0.00147±0.00039*	0.00172±0.00041	0.00133±0.00032
$t_{1/2}$ (h)	2.58±0.45	3.05±0.51*	2.88±0.47	3.01±0.54
K_{int} (h^{-1})			0.183±0.023	0.172±0.032

Values are means±SD of 16 patients. *Significantly different from the not weighted, $p < 0.05$).

$$K_{el} = K_{slope} \times CL_{cr} + K_{int}$$

K_{el} versus creatinine clearance, and the biological half-life were determined to be 0.409 ± 0.093 L/kg, 0.361 ± 0.056 h^{-1} , 0.00133 ± 0.00032 min/ml·h and 3.01 ± 0.54 h respectively. Thus, in contrast to the nonlinear least squares regression analysis, no statistically significant differences in the influence of weight on the tobramycin assay error for pharmacokinetic parameters of tobramycin were observed by the Bayesian analysis.

DISCUSSION

Laboratory assay error is usually 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 is usually not determined. As a result of this, typically only the measured concentration is reported or used in any practical way. But, the actual assay error is usually ignored for purposes of therapeutic drug monitoring in Korea. The goal of the present study is to examine influences of weight on the assay error in the pharmacokinetics of tobramycin in gastric cancer patients. Our results can be used to improve the precision of fitting pharmacokinetic models, which will optimize the process of model simulation, both for population and for individualized pharmacokinetic models.

The implementation of the Bayesian analysis was introduced into the medical and pharmacokinetic communities by Sheiner,¹⁵⁾ and has since been modified due to previous limitations. The Bayesian analysis balances the relative credibility of the population parameter val-

ues 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 only fits to the logarithms of the serum data.¹³⁾ A specific program¹⁴⁾ available for the Bayesian analysis of serum concentration data provide more cost-effective and precise prediction of future serum concentrations for 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 being fit to any serum data, gave better predictions than the linear least squares regression method. Linear least squares regression has been used in pharmacokinetic program for hand calculators¹⁷⁾ and personal computers.¹⁸⁾

For any data point, an index of its credibility can be given by its Fisher information.¹⁹⁾ This credibility index is the values of the data point multiplied by the reciprocal of the data point's known variance.¹⁹⁾ For the population pharmacokinetic model of a particular drug, this variance is the square of the standard deviation, 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 multiplied by the reciprocal of SD^2 , its variance. When doing Bayesian analysis, one can only give equal weight to various serum concentration when they have the same SD. An assay error pattern with a constant SD over its working range is said to be homoskedastic. Such an assay will have a coefficient of variation that decreases by half as the concentration doubles. None of the assay evaluated in this study displayed this pattern therefore could not be classified as homoskedastic. In contrast, a heteroskedastic 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 heteroskedastic. Under this circumstance, doubling the concentration also doubles the SD and quadruples the variation, thus the weight given to the assay is reduced to one fourth. If one assumes a constant coefficient of variation, a concentration of 1.0 $\mu\text{g/ml}$, for example, has a weight 100 times greater than that of a concentration of 10.0 $\mu\text{g/ml}$, and a concentration of 0.1 $\mu\text{g/ml}$ has a weight 100 times that of the concentration of 1.0 $\mu\text{g/ml}$, and 1000 times that of the concentration of 10.0 $\mu\text{g/ml}$. Because of this, when a constant coefficient of variation is assumed for an assay used in Bayesian analysis, high concentration will generally be ignored compared to lower ones, and the model will not fit the high concentration as closely as one might wish. This is also true for the polynomial equation described above. The difference here is that the polynomial equation is derived from empirically measured SD's over the working range of the assay, and should include the blank concentration as well. Because of this, it is a more accurate estimate of the assay error over its working range, and the fit, while often appearing to ignore the higher concentrations, is actually being correctly done by current standards. One of two following things needs to be improved for more accurate estimates; either the current Bayesian analysis procedure based on the Fisher information of the data points is incorrect, or the assay needs improved precision at the high end to make them more homoschedastic. Discarding the concept of Fisher infor-

mation would overthrow and undermine several decades of carefully acquired and extensively criticized mathematical and statistical knowledge. Thus, improving the precision of the assay at their high end is more likely the solution to this problem. It may even be possible, for example, to alter the ratios of reagents such that the ratio of bound and unbound drug in the assay can be changed to promote an error pattern that is more homoschedastic.

Obviously, errors other than those associated with measuring serum level occur in the clinical environment, 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, it is not yet possible to calculate these types of errors explicitly. Moreover, these error terms would belong 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.

As shown in Table 3, there were statistically significant differences ($p < 0.05$) in the influence of weight on the tobramycin assay error for pharmacokinetic parameters of tobramycin when the nonlinear least squares regression analysis was used. However, there were no statistically significant differences in the influence of weight on the tobramycin assay error for pharmacokinetic parameters of tobramycin when the Bayesian analysis was used.

The coefficients of the polynomial equation were then stored in the clinical pharmacokinetic program so that correct weighting of each measured tobramycin serum concentration could be implemented during the Bayesian and nonlinear least squares regression analysis. The end result would be improved dosage regimens and better, safer care of patients receiving tobramycin.

In summary, the polynomial equation determined in this study can be used to improve the precision of fitting of pharmacokinetic models, which will optimize the process of model simulation, both for population and for individualized pharmacokinetic models.

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