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Estimation of Area Under the Curve (AUC) and Standard Deviation of Estimated AUC when Using Destructive Measurement Technique : An Evaluation of Available Methods

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Area under the curve of concentration versus time (AUC) is considered an important indicator of drug availability. It is a common practice to take measurements at various time points for each experimental unit in the study and AUC values can be estimated using trapezoidal rule for each unit. In case that only one measurement for each experimental unit has been recorded as experimental unit has to be sacrificed for collecting sample, there are methods for estimating area under the curve with only one sample per experimental unit. In such cases, multiple experimental units are used for each data point post dose. An evaluation of various methods available for estimating AUC and standard deviation (SD) of estimated AUC is made in this paper when destructive measurement techniques are used. It is found that among other existing methods, resampling method is a powerful tool to predict AUC and standard deviation of estimated AUC even if AUC is a non-linear function of concentrations.

Pharmacokinetic analysis has been found to be valuable component of new drug development program. It is now generally recognized that pharmacokinetic data are of utmost importance to the assessment of safety and efficacy of a drug. Most of the pharmacokinetic parameters, for example, the area under the curve (AUC), the Clearance (Cl), and the volume of distribution at steady state (V_{ss}) are based on sufficiently complete concentration-time curves. The AUC of concentration versus time is an index of drug exposure. To accurately measure AUC, multiple blood samples are required from an individual experimental unit. For the situations where the experimental units have to be destroyed[1], the term destructive sampling has been introduced. Destructive sampling delivers independent observations derived from different animals. The major disadvantage with independent data is that only mean profile can be calculated. Complete profile for each experimental unit is not available. In such cases, estimation of AUC and SD of the estimated AUC ($\hat{AUC}$) is not straight forward. The AUC has to be estimated on the basis of the mean concentration values at the given time points. Even though mean parameters may be estimated, the corresponding standard deviations are difficult to obtain.

There are two approaches that have been widely used to estimate pharmacokinetic parameter AUC and SD of $\hat{AUC}$ when using destructive sampling schemes. (i) Method 1: Randomly combining time points from different animals and then AUC and SD $\hat{AUC}$ are calculated[2], (ii) method 2: Bailer's method[3,4] and method 3: There is another method known as resampling (or bootstrapping) method, first suggested by Pai[5] to estimate AUC in toxicokinetic studies, was later recommended by Mager and Gollier[6] to estimate AUC and SD of $\hat{AUC}$ in the situation described above. Two suggested different resampling techniques were; (iii.a) the pseudoprofile-based bootstrap and (iii.b) pooled data bootstrap and judged on the basis of simulated data. Present study was undertaken to evaluate resampling technique (pooled data bootstrap) using real experimental data and to compare it to other two methods. The drug concentration-

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time data in aqueous humor were taken from efficacy studies in rabbits for seven formulations of an ophthalmic drug. New Zealand albino rabbits were placed in restrainers, lowers eyelid pulled aside to form the *cul-de-sac*. Given formulations were carefully instilled into *cul-de-sac* and the eyelids were kept closed for 30 seconds. Samples of aqueous humor were collected under anaesthetia after thoroughly washing eyes with saline. About 250 µl of aqueous humor was aspirated with a sterile needle. The sample were analysed for the drug by HPTLC[7]. On the basis of the analysis of these experimental data, it is demonstrated that resampling techniques may provide sufficiently accurate and robust estimators of AUC and their standard deviations.

MATERIALS AND METHODS

Let the samples be taken from r_k, k = 1,..., n animals at time $0<t_1<t_2<.....<t_n$ post dose. Let c_{ki}, $i=1,....,r_k$ be the r_k concentrations at time t_k and let c_k and s_k^2 be the sample mean and variance of concentrations at time t_k. Assuming the concentration at time $t_0=0$ is 0, the AUC and SD of AÛC are estimated by the following three different approaches.

Method 1:

Time points from different animals are randomly combined to give several complete profiles. The AUC is estimated by the trapezoidal rule for each of the profile. Finally, the mean AUC and SD of AUC are calculated.

Method 2:

Bailer's method which gives estimate of AUC as

$$A\hat{U}C = \sum_{k-0}^{n} w_k \bar{c}_k \qquad (1)$$

where the trapezoidal weights, w_k, are

$$w_k = \begin{cases} (t_1-0)/2 & k = 0 \\ (t_{k+1}-t_{k-1})/2 & k=1,...,n-1 \\ (t_k-t_{k-1})/2 & k=n \end{cases} \qquad (2)$$

The variance of the AÛC is estimated by

$$s^2(A\hat{U}C) = \sum_{K-0}^{n} w_k^2 s_k^2/r_k \qquad (3)$$

Method 3:

Resampling method. To compute the bootstrap estimate of the AUCs and standard deviations of AÛC from a data set with "r_k" observations at each time point, the following algorithm was used : At each time point, the c_{ki} from each individual animal was assigned a random number. Then, a single c_{ki} was randomly chosen from the possible values, and its concentration values was recorded. The c_{ki} was replaced, the samples randomized again and a second c_{ki} was randomly chosen and its values was also recorded. Thus, at each time point, two c_{ki} values were randomly selected; averaged and the AUC was computed. This process was repeated 200-1400 times, with fresh randomization prior to each sampling to obtain bootstrap estimate of AUCs from which the mean and SD of AÛC are determined. All computations were performed on a desktop computer using a program in Basic language developed at the Centre. The program could be availed free of cost from the authors.

RESULTS AND DISCUSSION

Since efficacy studies are very time consuming and require large number of animals as variation among animals is very high, efficacy study of one formulation is carried out. Other formulations are compared with this formulation on the basis of pharmacokinetic studies in animals. Data collected from such pharmacokinetic studies in rabbits for seven formulations of the same ophthalmic drug were used to evaluate the three methods. Rabbits were divided into five groups (for each formulation) with three animals in each group. In this type of experiment, destructive sampling is necessary. One group of animals was sampled at only one time point and samples of aqueous humor were analysed for drug using HPTLC. At the time point 0 h, drug concentrations in samples of aqueous humor were assumed zero and hence no sampling was done. For illustrative purposes, results for the two formulations are summarized in Table 1.

There will be various possible combination of the data points. Two ways of combining the data are given in tables 2 and 3. The AÛCs are calculated using trapezoidal rule after randomly combining time points from different animals and then mean AÛC and standard deviations of AÛC are obtained for both the formulations. The mean predicted AÛC (± SD) for the two formulations are 1066.48 (±4.93) ng.h/ml, 1237.78 (±31.81) ng.h/ml

TABLE 1 : DRUG CONCENTRATION IN AQUEOUS HUMOR

Time (h)	0	1	2	3	4	6
			Formulation 1 : Drug concentration (ng/ml)			
	Nd	328.4	380.5	262.1	198.2	Nd
	Nd	282.1	381.2	212.8	213.1	Nd
	Nd	340.2	413.2	293.1	200.4	Nd
Mean($\bar{c}_k$)	Nd	316.9	391.6	256.0	203.9	Nd
SD(s_k)	Nd	30.7	18.7	40.5	8.0	Nd
			Formulation 2 : Drug concentration (ng/ml)			
	Nd	173.2	260.4	268.4	194.6	284.5
	Nd	182.9	196.4	389.4	703.2	192.5
	Nd	242.1	185.5	300.8	262.5	Nd
Mean($\bar{c}_k$)	Nd	199.4	214.1	319.5	386.8	159.0
SD(s_k)	Nd	37.3	40.5	62.6	276.1	145.2

Drug levels in nanograms/millilitre were analysed after sampling from the aqueous humor of different rabbits at each time point after the administration of either formulation 1 or formulation 2. Nd indicates that drug levels were not detected

in one combination and 1066.48 (± 89.40) ng.h/ml, 1384.68 (±615.62) ng.h/ml in another combination. Table values reveal that different estimates of AÛC and standard deviation of AÛC are obtained from the same data set using different combinations. This may result in an ambiguity when test of hypothesis is performed. This method of calculating AÛC and SD of AÛC after randomly combining time points from different animals does not have any logical or statistical basis.

On applying Bailer's method for both the formulations, the following equation were obtained.

$AÛC_1$ = 1066.48 ng.h/ml, $s^2(AÛC_1)$ = 982.7186 and SD ($AÛC_1$) = 31.35 for formulation 1,

$AÛC_2$ = 1472.185 ng.h/ml, $s^2(AÛC_2)$ = 66530.5 and SD ($AÛC_2$) = 257.94 for formulation 2,

by using equations (1), (2) and (3). Bailer's method uses linear trapezoidal rule to estimate AUC. However, the use of the log-trapezoidal rule instead of the linear trapezoidal rule on the descending portion of the Cxt curves gives better estimates of AUC[8,9]. The log trapezoidal approximation to calculate area under the curve on the descending portion of the Cxt curves is :

$$\sum \frac{(t_{k+1}-t_k)(\bar{C}_{k+1}-\bar{C}_k)}{\log(\bar{C}_{k+1}/\bar{c}_k)},$$

where the summation is over the time points of descending portion of the curves. Bailer's method to estimate standard deviation of AÛC, is based on the basic rule[10] that variance of the sum of independent random variables is equal to sum of the variances of each random variable (equation 3). Because of the non independence of random variables in log-trapezoidal rule, the standard deviation of the estimated AÛC can not be computed using Bailer's method.

For resampling method, AUC was calculated using linear trapezoidal rule in each iteration. The bootstrap estimates of AUC and SD are, then estimated by calculating the mean and standard deviation of all AÛC's obtained in various iterations. The mean predicted AUC and SD are given in Table 4 for different number of iterations for two formulations. As the Bailer's approach has sound statistical basis, it can be taken as a "gold standard" when linear trapezoidal rule is used to estimate AUC. Table 4 demonstrates a close agreement with Bailer's approach and the resampling method even for as little as 200 bootstrap samples. It is also clear from the table

TABLE 2 : ESTIMATES OF AUC AND SD OF AÛC

Time (h)	0	1	2	3	4	6		AUC (ng.h/ml)
Animal No.	One combination of drug concentrations (ng/ml)							
1	Nd	282.1	380.5	293.1	213.1	Nd		1062.25
2	Nd	340.2	413.2	212.8	198.2	Nd		1065.30
3	Nd	328.4	381.2	262.1	200.4	Nd		1071.90
							Mean	1066.48
							Sd	4.93
	Another combination of drug concentrations (ng/ml)							
1	Nd	340.2	413.2	293.1	213.1	Nd		1153.05
2	Nd	282.1	380.5	212.8	198.2	Nd		974.50
3	Nd	328.4	381.2	262.1	200.4	Nd		1071.90
							Mean	1066.48
							SD	89.4

Two different combinations were generated by randomly combining various time points from actual data shown in Table 1 (formulation 1). AUC and SD of AÛC were calculated from such profiles using method 1. Nd indicates that drug levels were not detected.

TABLE 3 : ESTIMATES OF AUC AND SD OF AÛC

Time (h)	0	1	2	3	4	6		AUC (ng.h/ml)
Animal No.	One combination of drug concentrations (ng/ml)							
1	Nd	182.9	196.4	300.8	262.5	192.5		1266.35
2	Nd	173.2	185.5	268.4	194.6	284.5		1203.50
3	Nd	242.1	260.4	389.4	703.2	Nd		1243.50
							Mean	1237.78
							SD	31.81
	Another combination of drug concentrations (ng/ml)							
1	Nd	182.9	196.4	300.8	262.5	Nd		811.35
2	Nd	173.2	260.4	389.4	194.6	192.5		1307.40
3	Nd	242.1	185.5	268.4	703.2	284.5		2035.30
							Mean	1384.68
							SD	615.62

Two different combinations were generated by randomly combining various time points from actual data shown in Table 1 (formulation 2). AUC and SD of AÛC were calculated from such profiles using method 1. Nd indicates that drug levels were not detected.

TABLE 4 : ESTIMATES OF AUC AND SD OF AÛC

No. of iterations	Formulation 1 AÛC (SD) (ng.h/ml)	Formulations 2 (AUC (SD) (ng.h/ml)
200	1064.29 (28.05)	1436.23 (235.44)
400	1065.7 (29.25)	1498.00 (268.07)
600	1066.15 (28.56)	1485.82 (254.17)
800	1066.43 (28.53)	1460.90 (250.46)
1000	1065.44 (29.19)	1483.428 (256.58)
1200	1067.22 (28.10)	1479.78 (259.21)
1400	1067.31 (28.44)	1480.84 (246.39)
Bailer's estimate	1066.48 (31.35)	1472.19 (257.94)

The estimates are obtained for the data sets given in Table 1 using method 3 (resampling technique) for various number of iterations. Bailer's estimates (method 2) are obtained using equations 1, 2 and 3.

that 400 iterations are enough to get reliable bootstrap estimates of AUC and SD. The AUC can be estimated with any of the rules (either linear or non-linear function of concentrations) and bootstrap estimates of AUC and SD of AÛC, are obtained just by taking mean and SD of the AUC 's obtained in all iterations, therefore, the resampling method can be used even if AUC is calculated using log-linear trapezoidal rule. These results justify the recommendation to use resampling technique as a suitable alternative when linear trapezoidal rule is not used to estimate AUC.

The above conclusions are not only true for the data sets given here for illustrative purposes, but also true for all other data sets tried. It has been shown that an ambiguous inference may be derived using an existing procedure (method 1) if separate profiles are generated by combining data randomly from different animals. Hence this method should not be used to estimate AUC and SD of AÛC. Bailer's method has a sound statistical basis and can be used to estimate AUC and SD of AUC. However, a disadvantage of Bailer's method is its restriction to linear functions of the concentrations. This drawback can be overcome with the resampling technique described in the paper as it can be used when AUC is either linear or non-linear function of concentrations. Hence, it is recommended to use the proposed method as all calculations can be performed on a desktop computer using a simple computer algorithm.

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