SHORT COMMUNICATION

A Note on Testing the Equality of areas under the curve when using destructive Measurement Technique in animal Pharmacokinetics

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Area under the curve (AUC) of concentration versus time is considered in many toxicological, pharmacological and medical investigations. In case that only one measurement for each experimental unit has been recorded, Bailer developed a method for estimating and comparing AUCs with only one sample per experimental unit but with multiple experimental units at each of several time points post dose. In this paper, a modification of Bailer's[1] method is reported to account for estimation of the variance for small sample sizes.

Area under the curve (AUC) is a well known pharmacokinetic parameter that has been widely used to evaluate bioavailability and bioequivalence of different formulations. It is often the case that measurements at each time point of concentration-time (*c.t*) profile are made for each experimental unit in the study and AUC values can be estimated using trapezoidal rule for each unit. These values can be compared using t-test or analysis of variance (ANOVA) techniques.

Here we shall be concerned with studies where only one measurement for each experimental unit is recorded as animal is sacrificed. By sampling from different animals at different times of *c.t* profile, a mean concentration-time profile may be generated. The AUC has to be estimated on the basis of the mean concentration values at the time of measurement. Suppose samples are taken from r_k, k=1,...,n animals at time $0<t_1<t_2<...<t_n$ post dose. Let c_k be the sample average of the r_k concentrations at time t_k, and let s^2_k be the sample variance. Suppose the concentration at time t_o is 0, then the mean AUC from o to t_n is estimated using the trapezoidal rule[1]

$$\hat{AUC} = \sum_{k=0}^{n} w_k \bar{c}_k, \qquad (1)$$

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 $\hat{AUC}$ is estimated by[1]

$$s^2\hat{AUC} = \sum_{k=0}^{n} w^2_k s^2_k / r_k \qquad (3)$$

To test the null hypothesis of equality among two AUCs i.e.,

$H_o: AUC_1=AUC_2$,

Bailer[1] gave the following test statistic

$$Z_{obs} = \frac{\hat{AUC}_1 - \hat{AUC}_2}{\sqrt{s^2(\hat{AUC}_1) + s^2(\hat{AUC}_2)}} \qquad (4)$$

and tested the hypothesis with a critical value, Z_{crit}, from a standard normal distribution. Generally, substituting sample variances for true variance is safe when sample sizes are large enough. But in most of the studies, samples are not large enough and therefore, a modification of Bailer's method is reported to account for estimation of the variances when sample sizes are small.

As the distribution of $s^2(\hat{AUC}_1)+s^2(\hat{AUC}_2)$ can be

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Table I - Drug levels in aqueous humor

Time (h)	0	1	2	3	4	6	0	1	2	3	4	6
	Formulation 1						Formulation 2					
	Nd	328.4	380.5	262.1	198.2	Nd	Nd	173.2	260.4	268.4	194.6	284.5
	Nd	282.1	381.2	212.8	213.1	Nd	Nd	182.9	196.4	389.4	703.2	192.5
	Nd	340.2	413.2	293.1	200.4	Nd	Nd	242.1	185.5	300.8	262.5	259.4
Mean($\overline{C_k}$)	Nd	316.9	391.6	256.0	203.9	Nd	Nd	199.4	214.1	319.5	386.8	245.47
sd(s_k)	Nd	30.7	18.7	40.5	8.0	Nd	Nd	37.3	40.5	62.6	276.1	47.56

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

approximated by Chi-square distribution with degrees of freedom v given by Satterthwaite's approximation[2]

$$v = (\sum_{j=1}^{2}\sum_{k=0}^{n} w^2_k s^2_{jk} / r_k)^2 / \sum_{j=1}^{2}\sum_{k=0}^{n} [w_k^4 s^4_{jk} / \{r_k^2 (r_k - 1)\}], \quad (5)$$

where s^2_{jk} is the sample variance of the r_k concentrations at time t_k of j^{th} (j=1,2) formulation, it is appropriate to test the hypothesis with t_{crit}, from a t-distribution as our samples are not very large. Since a t_{crit} would be larger than Z_{crit}, there may be a difference in conclusion drawn using t distribution or standard normal distribution. This is illustrated by way of an example.

Efficacy studies are very time consuming and require large number of animals as variation among animals is very high. Sometimes efficacy study of one formulation is carried out and other formulations are compared with this formulation on the basis of pharmacokinetic profiles in animals. Data collected from such an efficacy study in rabbits for an ophthalmic drug have been presented here. Rabbits were divided into five groups (for each formulation) of three animals each (r_k=3 $\forall$k=1,...5). One group of animals was sampled at only one time point and sample were analysed separately. Results have been shown in Table 1.

On applying equations (1), (2) and (3), we get

$A\hat{U}C_1$ = 1066.48, $A\hat{U}C_2$ = 1558.65,

s^2 ($A\hat{U}C_1$)=982.7186, s^2 ($A\hat{U}C_2$) = 60258.78

Hypothesis of interest is

H_0:AUC_1=AUC_2 vs H_1:$AUC_1 \neq AUC_2$.

Using equations (4) and (5), we get Z_{obs} = 1.9888 and v = 2, which result in accepting the hypothesis of equality of AUCs at 5% level of significance using $t_{crit,0.05}$ = 4.303. But if $Z_{crit,0.05}$ = 1.96 is used, null hypothesis of equality will be rejected. Hence, it is appropriate to use the more accurate proposed method when the sample sizes are not very large.

REFERENCES

1. Bailer A.J., **J. Pharm. Biopharm.**, 1988, 16, 303.
2. Satterthwaite F.E., **Biometrics Bulletin,** 1946, 2, 110.