RESEARCH PAPER

Accepted 20 May 2003
Revised 11 March 2003
Received 12 February 2003
Indian J. Pharm. Sci., 2003, 65(4): 371-377

Evaluation of Truncated Area under the Curve and Truncated Area under the Curve with Limited Blood Samples in Bioequivalence Studies

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The use of truncated area under the curve has been encouraging in evaluation of bioequivalence trials. The objective of the investigation was to compare the applicability and accuracy of truncated area under the curve from 0 h to time t h versus area under the curve from 0 h to infinity and also to determine whether limiting the number of blood samples would influence its effectiveness. The evaluation was based on the retrospective analysis of five bioequivalence studies. All drugs administered were immediate-release formulations with half lives in the range of 3.66-32.4 h. In all the trials considered, point estimators and 90% confidence intervals of the ratio of the mean areas under the curve of test and reference formulations, were comparable in the case of truncated and untruncated trials. However, the duration of truncation was not equal for all the drugs and was influenced by the measurability of concentration at truncation time point, variability of the concentration-time data and frequency of blood sampling. The results of this study also demonstrated that a limited number of blood samples taken at appropriate times, can suffice in bioequivalence studies when the trial is truncated.

The idea of bioequivalence of different formulations of a given drug is of significant importance. Bioequivalence studies are conducted to determine the therapeutic equivalency of drug products. A direct demonstration of the same therapeutic effect of different formulations of a drug requires a full-scale clinical trial. Bioequivalence trials are accepted because the time and costs required for bioequivalence trials are very much less compared to clinical trials. However, bioequivalence studies may become more complicated for drugs with longer half-life, which leads to a prolonged study and hence increased chances of dropouts. In cases when the analytical method is not very sensitive to assay lower concentration that would pose problems to calculate extrapolated area under the curve (AUC) and for highly variable drugs which require replicate study designs. The use of truncated AUC in bioequivalence evaluation has been shown to be promising by many researchers[1-4]. Many investigators evaluated the truncated AUCs in bioequivalence trials using computer simulation or retrospective analysis of bioequivalence studies and found encouraging results[5-8].

There is general, internationally harmonized consensus on regulatory criteria for the use of common metrics area under the curve (AUC) and the maximum plasma concentration (C_{max}) in bioequivalence trials. In order to measure AUC and C_{max} accurately, multiple blood samples are generally required. Frequent blood sampling from volunteers is time-consuming, expensive and problematic for long half-life drugs or for highly variable drugs, which may require replicate designs. Reducing the frequency of sampling can approach this problem. There are two sampling strategies which have been used to estimate pharmacokinetic parameters from a limited number of samples: (i) the optimum sampling[9,10] and (ii) the limited sampling model (LSM)[11-14]. However, these techniques are not being applied in bioequivalence trials. Recently, Mahmood and Mahayni[15] have proposed to use the LSM approach in bioequivalence

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studies and Jackson[16] showed severely limited utility of the LSM.

Thus the dual objectives of this study were to assess the hypothesis that limiting the duration of bioequivalence trials would not effect the conclusion of the study and to determine the minimum number of sampling time points in truncated trials needed to estimate 90% confidence interval of the ratio of the test and reference formulations mean truncated AUCs, so that it should not alter the conclusion of bioequivalence trial. The investigation was done by retrospective analysis of real experimental data.

MATERIALS AND METHODS

The trials considered in this paper were carried out on 10 to 11 healthy male volunteers, according to a two period, two formulation, two sequence, single-dose crossover design. All drugs administered were immediate-release oral formulations. The five drugs were considered for the evaluation. The considered drugs (half-life) with long half-life values were: meloxicam (30.5 h), olanzepine (32.4 h), celecoxib (18.3 h) and with short half-life values were amoxycillin (3.66 h) and ethambutol (5.03 h). The area under the curve from 0 h to time t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The AUC from 0 h to infinity ($AUC_{0-\infty}$) was calculated by summing the area from 0 to time (t) of last measurable concentration (AUC_{0-t}) and t to infinity ($AUC_{t-\infty}$), where $AUC_{t-\infty}=C_t/kel$, with 'C_t' defined as the last measured plasma concentration at time t, and 'kel' the slope of the terminal portion of the *Ln* plasma concentration versus time curve, obtained by linear regression. For truncated AUC, the trials were curtailed at indicated time points listed in Tables 1-5. Blood sampling schedule with limited number of sampling time points was selected based on the full concentration-time profile of the reference drug in healthy human volunteers. A bioequivalence study usually comes in the later

TABLE 1: A COMPARISON OF POINT ESTIMATORS AND 90% CONFIDENCE INTERVALS OF THE RATIO BASED ON VARIOUS AUCS FOR MELOXICAM (30.5 h*).

Various AUCs	Number of time points used in the calculation of AUC	Time points used in the calculation of AUC (h)	Point estimator of the ratio (%CV)	% Bias of the Point estimator of the ratio	90% confidence interval	
					Lower limit	Upper limit
$AUC_{0-\infty}$	16	0, .5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72, 96	1.01 (24.86)		85.6	112.8
AUC_{0-t}	16	0, .5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72, 96	1.01 (25.97)	0.13	84.2	104.1
AUC_{0-48}	14	0, .5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48	1.06 (25.98)	5.09	88.8	118.8
AUC_{0-24}	13	0, .5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24	0.98 (17.09)	2.85	88.0	107.2
AUC_{0-24}	7	0, 1, 2, 3, 4, 5, 24	1.09 (23.87)	8.06	95.0	119.0
AUC_{0-24}	6	0, .5, 2, 3, 4, 24	1.02 (20.36)	1.13	90.4	111.8
AUC_{0-24}	6	0, .5, 3, 4, 5, 24	1.07 (21.69)	6.08	94.7	116.5
AUC_{0-24}	5	0, .5, 1, 2, 24	1.16 (61.77)	15.00	79.6	132.4

Where $AUC_{0-\infty}$ - area under the curve from time 0 h to infinity; AUC_{0-t} - area under the curve from time 0 h to time of last measurable concentration; AUC_{0-48} - area under the curve from time 0 h to 48 h; AUC_{0-24} - area under the curve from time 0 h to 24 h. *Elimination half life. T_{max} (Mean±SD)=2.91±2.02 for reference formulation and 2.73±1.27 for test formulation.

stages of drug development, hence getting the full concentration-time profile of the drug is not difficult. Bioequivalence was assessed using 90% confidence interval of the ratio of test to reference formulations mean AUCs calculated using log transformed data for all the drugs. To conclude bioequivalence, 90% confidence intervals should lie within the range of 80-1.25[17]. For evaluation of truncated AUC and truncated AUC with limited data, $AUC_{0-\infty}$ was taken as the reference. The parameters considered for comparison purposes, were point estimator and 90% confidence interval of the ratio of test and reference formulations mean AUCs.

RESULTS

Tables 1-5 show point estimators and 90% confidence intervals for the ratio of the test and reference formulations' means obtained with $AUC_{0-\infty}$, AUC_{0-t}, AUCs truncated at various time points and truncated AUCs with limited data points for meloxicam, olanzepine, celecoxib, amoxycillin and ethambutol, respectively. The time points used for the calculation of various AUCs are given in the 3rd column of the Tables 1-5.

Truncated AUC:

For meloxicam and amoxycillin, bioequivalence results obtained from truncated AUCs were invariably the same as those obtained from $AUC_{0-\infty}$. The assessment of bioequivalence of these two drugs had undiminished effectiveness and efficiency even when the duration of the investigation was curtailed to one half life following drug administration. The situation was different for olanzepine. The point estimator and 90% confidence interval of the ratio of test to reference formulations' mean truncated AUCs were not very close to the same for $AUC_{0-\infty}$ even when the concentration-time profiles up to two half lives were considered. The possible explanation for this observation may be the unavailability of 48 h data point for two volunteers (in the case of two half lives truncation) and unavailability of 22 h data point for one volunteer (in the case of one half life trun-

TABLE 2: A COMPARISON OF POINT ESTIMATORS AND 90% CONFIDENCE INTERVALS OF THE RATIO BASED ON VARIOUS AUCS FOR OLANZEPINE (32.4 h').

Various AUCs	Number of time points used in the calculation of AUC	Time points used in the calculation of AUC (h)	Point estimator of the ratio (%CV)	% Bias of the Point estimator of the ratio	90% confidence interval	
					Lower limit	Upper limit
$AUC_{0-\infty}$	15	0, 1, 2, 3, 4, 5, 6, 8, 10, 16, 22, 48, 72, 96, 120	1.12 (16.40)		98.8	112.1
AUC_{0-t}	15	0, 1, 2, 3, 4, 5, 6, 8, 10, 16, 22, 48, 72, 96, 120	1.07 (17.90)	-4.46	96.8	114.6
AUC_{0-48}	12	0, 1, 2, 3, 4, 5, 6, 8, 10, 16, 22, 48	1.07 (14.29)*	-4.46	98.5	115.4
AUC_{0-22}	11	0, 1, 2, 3, 4, 5, 6, 8, 10, 16, 22	1.11 (16.65)**	0.0	104.0	115.2
AUC_{0-22}	8	0, 1, 2, 3, 4, 5, 6, 22	1.04 (15.57)**	-7.14	95.0	115.7
AUC_{0-22}	6	0, 1, 4, 5, 6, 22	1.03 (12.83)**	-8.04	94.9	110.7
AUC_{0-22}	6	0, 1, 3, 4, 5, 22	1.06 (13.52)**	-5.36	96.3	113.7

Where $AUC_{0-\infty}$ - area under the curve from time 0 h to infinity; AUC_{0-t} - area under the curve from time 0 h to time of last measurable concentration; AUC_{0-48} - area under the curve from time 0 h to 48 h; AUC_{0-22} - area under the curve from time 0 h to 22 h. *Excluding two volunteers data. **Excluding one volunteer data. 'Elimination half life. T_{max} (Mean ± SD)=4.91±0.54 for reference formulation and 4.64±1.80 for test formulation.

cation). However, on excluding those volunteers, point estimator and 90% confidence interval were close enough for $AUC_{0-\infty}$ even when the data was curtailed at one half life. Hence it can be inferred that the point estimator and 90% confidence interval using truncated areas were comparable to $AUC_{0-\infty}$ when all the subjects had measurable plasma concentration at last blood collection time, resulting in a proportional loss of data from each subject[18].

In the case of ethambutol, the estimates based on truncated AUCs were comparable to those based on $AUC_{0-\infty}$ when data was taken up to two half lives instead of one half life. As the blood sampling was not intensive, only six (6) blood samples were available if the data was truncated at one half-life. This may be the reason of above observation. For celecoxib, the inter subject variation was very high in plasma concentration and consequently, higher variation was observed in the elimination half-life. The extrapolation in $AUC_{0-\infty}$, was also very high, hence AUC_{0-t} was taken as the reference for comparison instead of $AUC_{0-\infty}$. The estimates based on truncated AUCs were comparable to those based on AUC_{0-t} when data was taken up to two half lives. It is well known that more than 20% can be added in extrapolating the AUC when the bioassays used have problems of sensitivity. Also the extrapolation of AUC to infinity can not be performed when the beta slope can not be correctly calculated, for example, with extended release formulations, it is very difficult to find beta slope. This analysis suggests that even in these types of situations, the truncated AUCs can be used and it performs as good as AUC_{0-t}.

Truncated AUC with limited data:

Subsequently, to determine the minimum number of sampling time points needed to estimate point estimator and 90% confidence intervals of the ratio of test and reference formulations mean truncated AUCs accurately and precisely,

TABLE 3: A COMPARISON OF POINT ESTIMATORS AND 90% CONFIDENCE INTERVALS OF THE RATIO BASED ON VARIOUS AUCS FOR CELECOXIB (18.3 h*).

Various AUCs	Number of time points used in the calculation of AUC	Time points used in the calculation of AUC (h)	Point estimator of the ratio (%CV)	% Bias of the Point estimator of the ratio	90% confidence interval	
					Lower limit	Upper limit
$AUC_{0-\infty}$	15	0, .5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48	0.89 (55.89)		51.2	108.8
AUC_{0-t}	15	0, .5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48	0.58 (36.29)		44.9	65.7
AUC_{0-36}	14	0, .5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36	0.56 (40.14)	-3.45	41.0	64.4
AUC_{0-36}	6	0, 3, 6, 10, 16, 36	0.61 (23.00)	5.17	46.1	71.5
AUC_{0-16}	12	0, .5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16	0.49 (47.31)	-15.58	34.2	58.1
AUC_{0-16}	8	0, .5, 2, 3, 4, 6, 8, 16	0.48 (44.87)	-17.24	35.4	59.8
AUC_{0-16}	7	0, .5, 2, 3, 4, 6, 16	0.49 (42.57)	-10.34	35.1	60.2
AUC_{0-16}	6	0, .5, 1.5, 2, 3, 16	0.53 (45.32)	-8.62	32.3	59.8

Where $AUC_{0-\infty}$ - area under the curve from time 0 h to infinity; AUC_{0-t} - area under the curve from time 0 h to time of last measurable concentration; AUC_{0-36} - area under the curve from time 0 h to 36 h; AUC_{0-16} - area under the curve from time 0 h to 16 h. *Elimination half life. T_{max} (Mean±SD) =2.15±0.88 for reference formulation and 3.20±1.75 for test formulation.

several combination of limited number of data points were examined. To accomplish this, first the appropriate point of truncation was chosen and then the number of sampling points in that duration was reduced. The situation, however, was not similar to the one discussed earlier (truncation without limited data). Table 1 shows that AUC based on only 5 time points (including 0 h data point) was not a good choice. Confidence interval was not within the 0.80-1.25 range whereas for other AUCs it was within the 0.80-1.25 range. Point estimator of the ratio of the test and reference formulations' mean truncated AUCs was also higher (14%) compared to the same for $AUC_{0-\infty}$. As 5 time point sampling design could not give comparable estimates for meloxicam, this design was not considered for other drugs. The AUC based on 6 time points gave good estimates of desired parameters. However, the sampling time points 0,.5, 2, 3, 4, 24 h gave better results compared to 0,.5, 3, 4, 5, 24 h. Similarly, truncated AUC_{0-24} with 6 data points was better than truncated AUC_{0-24} with 7 data points. The reason may be that 0.5 h data point was included in 6 time point sampling design whereas it was not included in 7 time point sampling design. This shows that the number of time points for sampling as well as selection of time points is also very important. For olanzepine, truncated AUCs with 6 time points performed well (Table 2). Analogously, in the case of celecoxib, truncated AUC with limited data did equally good as truncated AUC with all data points. For amoxycillin and ethambutol, truncated AUC with 6 time points could not give

TABLE 4: A COMPARISON OF POINT ESTIMATORS AND 90% CONFIDENCE INTERVALS OF THE RATIO BASED ON VARIOUS AUCS FOR AMOXYCILLIN (3.66 h*).

Various AUCs	Number of time points used in the calculation of AUC	Time points used in the calculation of AUC (h)	Point estimator of the ratio (%CV)	% Bias of the Point estimator of the ratio	90% confidence interval	
					Lower limit	Upper limit
$AUC_{0-\infty}$	14	0, .25, .5, .75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10	0.93 (31.79)		78.3	100.5
AUC_{0-t}	14	0, .25, .5, .75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10	0.94 (33.0)	1.08	78.6	102.3
AUC_{0-8}	13	0, .25, .5, .75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8	0.93 (31.5)	0.00	78.4	100.5
AUC_{0-8}	11	0, .25, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8	0.93 (31.38)	0.00	78.8	100.7
AUC_{0-4}	11	0, .25, .5, .75, 1, 1.5, 2, 2.5, 3, 3.5, 4	0.91 (27.67)	-2.16	79.2	98.4
AUC_{0-4}	9	0, .25, 1, 1.5, 2, 2.5, 3, 3.5, 4	0.92 (30.16)	-1.08	78.7	100.0
AUC_{0-4}	8	0, .25, 1, 2, 2.5, 3, 3.5, 4	0.90 (30.75)	3.23	77.7	96.2
AUC_{0-4}	7	0, .25, 1, 2, 2.5, 3, 4	0.87 (26.87)	6.45	76.3	92.2

Where $AUC_{0-\infty}$ - area under the curve from time 0 h to infinity; AUC_{0-t} - area under the curve from time 0 h to time of last measurable concentration; AUC_{0-8} - area under the curve from time 0 h to 8 h; AUC_{0-4} - area under the curve from time 0 h to 4 h. *Elimination half life. T_{max} (Mean±SD)=1.94±0.75 for reference formulation and 2.42±1.04 for test formulation.

comparable estimates, however, they performed well with 7 time points sampling design.

DISCUSSION

The study period required for drugs with long or very long $t_{1/2}$ values should be at least 3.3 times the $t_{1/2}$ of the drug for blood sampling and at least seven times the $t_{1/2}$ for wash-out. This often complicates the bioequivalence trials. Many researchers showed that the use of truncated AUC appears feasible for long half-life drugs. The results of this investigation infer that truncated AUCs offer advantage in bioequivalence studies for long as well as short half-life drugs.

Although these findings might not come as a complete surprise, they do provide evidence of advantages of truncated AUC in bioequivalence trials. The results also indicate about the selection of truncation point that is based on various conditions such as inter individual variability in plasma concentration, measurability of concentrations at truncation time point etc. The recent USFDA guidelines[19] also state that AUC truncated at 72 h may be used in place of AUC_{0-t} or $AUC_{0-\infty}$ for drugs that demonstrate low intra-subject variability in distribution and clearance.

These results, furthermore, demonstrated that the point estimate and 90% confidence interval of the ratio of the test and reference formulations' mean truncated AUCs can be predicted accurately using limited sampling taken at the appropriate times, hence less blood volume from the volun-

TABLE 5: A COMPARISON OF POINT ESTIMATORS AND 90% CONFIDENCE INTERVALS OF THE RATIO BASED ON VARIOUS AUCS FOR ETHAMBUTOL (5.03 h*).

Various AUCs	Number of time points used in the calculation of AUC	Time points used in the calculation of AUC (h)	Point estimator of the ratio (%CV)	% Bias of the Point estimator of the ratio	90% confidence interval	
					Lower limit	Upper limit
$AUC_{0-\infty}$	12	0, .5, 1.5, 2.5, 3, 4, 6, 8, 10, 11, 12, 16	0.90 (19.68)		82.1	94.7
AUC_{0-t}	12	0, .5, 1.5, 2.5, 3, 4, 6, 8, 10, 11, 12, 16	0.89 (16.9)	-1.11	81.4	94.7
AUC_{0-10}	9	0, .5, 1.5, 2.5, 3, 4, 6, 8, 10	0.93 (15.16)	3.33	84.7	99.4
AUC_{0-10}	8	0, 1.5, 2.5, 3, 4, 6, 8, 10	0.91 (16.4)	1.11	82.8	98.6
AUC_{0-10}	7	0, 1.5, 2.5, 3, 4, 6, 10	0.89 (14.12)	-1.11	81.9	95.9
AUC_{0-10}	6	0, 1.5, 2.5, 3, 4, 10	0.93 (29.69)	3.33	75.7	104.8
AUC_{0-8}	8	0, .5, 1.5, 2.5, 3, 4, 6, 8	0.94 (17.49)	4.44	84.5	101.2
AUC_{0-6}	7	0, .5, 1.5, 2.5, 3, 4, 6	0.97 (22.31)	7.78	84.2	106.2

Where $AUC_{0-\infty}$ - area under the curve from time 0 h to infinity; AUC_{0-t} - area under the curve from time 0 h to time of last measurable concentration; AUC_{0-10} - area under the curve from time 0 h to 10 h; AUC_{0-8} - area under the curve from time 0 h to 8 h; AUC_{0-6} - area under the curve from time 0 h to 6 h. *Elimination half life. T_{max} (Mean±SD)=2.96±0.54 for reference formulation and 2.75±0.94 for test formulation.

teer, reduced cost of sample analysis and may be an increase in the sample size in bioequivalence studies to increase the power of the study without adding any cost to the study. Moreover, comparatively more data points were needed for drugs with shorter half life compared to the drugs with longer half life to accurately measure the point estimate and 90% confidence interval of the ratio of the test and reference formulations' mean AUCs in truncated trials.

Tables 1-5 also illustrate the effect of shortening the duration of a bioequivalence trial on the variation of ratios of truncated AUCs. This variation is given in brackets (4^{th} column) as %CV. The variation of ratios of truncated AUCs initially decreased as the duration of a bioequivalence trial was shortened and then gradually it started increasing on further limiting the length of a bioequivalence trial except for olanzepine for which %CV was more or less same for all cases. Similar findings were shown by Endrenyi and Tothfalusi[7].

The principal conclusion from the present investigation of actual cross over trials is that the effectiveness of bioequivalence studies is not affected adversely by the sensible and substantial truncation of AUCs as well as the limited data points in truncated trials. It appears to be safe to limit the duration of a study to one half life if there is not high inter individual variability in plasma concentration, concentrations are measurable at truncation point for all volunteers and analytical procedures are sensitive to measure the analyte. The beneficial consequences often include better compliance and an improvement in ethical and economical considerations.

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