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A NEW APPROACH TO CALCULATE AUC OF DELAYED RELEASE FORMULATIONS

NASIR M. IDKAIDEK[1,3*] AND NAJİ M. NAJİB[2]

[1] College of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
[2] International Pharmaceutical Research Center, Amman, Jordan

Area under the time-concentration curve (AUC is one of the most important parameters in pharmacokinetics. It is currently being calculated by non-comparmental analysis for regulatory purposes. In this approach, AUC of delayed release formulations is calculated after accounting for the percent of AUC from zero time to first time of drug appearance in plasma (T_{lag}). Two enteric-coated diclofenac tablet formulations (test and reference) were taken as a representative example to show the validity of this new approach. Experiments were performed on 24 healthy humans. The AUC was calculated both ways. The 90% confidence intervals of AUC after logarithmic transformation using the direct and the new approaches ranged from 92-129% and 81-111 % respectively thus changing the final decision from bio-inequivalence to bio-equivalence. Hence, if the computer program used does not correct the error in AUC calculation for delayed release formulations, this approach is suggested to be used for further human bioavailability and bioequivalence studies in order to obtain more accurate and unbiased decisions.

Keywords: *AUC; Bioequivalence; Drug Regulations; Delayed Release*

Introduction and Theory

Area under the time-concentration curve (AUC) is one of the most important parameters in pharmacokinetics that reflects extent of drug absorption after administration by nonparenteral routes. It is currently being calculated by non-comparmental analysis, using linear trapezoidal rule, for regulatory purposes (1, 2). This method is acceptable and accurate for all formulations that essentially have no lag time before appearance in plasma. However, in the case of delayed release formulations such as enteric coated formulations that exhibit a fairly long lag time before appearance in plasma, the calculated AUC will be biased. This bias results from the calculation of the first segment in AUC from time zero to the lag time which in turn over estimates the total $AUC_{0-\infty}$. In this new approach, the AUC is recalculated after accounting for the percent of AUC from zero time to first time of drug appearance in plasma (T_{lag}).

Correspondence: E-mail: dekaidek@just.edujo

* The manuscript represents the personal opinion of the author and does not necessarily represent the views and policies of the center.

Experimental Application

Two enteric-coated diclofenac tablet formulations (test and reference) were taken as a representative example to show the validity of this new approach. Experiments were performed on 24 healthy humans, after signing informed consents, in the International Pharmaceutical Research Center. Study design was a single dose, randomized, fasting, two-treatment, two-phase, two-sequence crossover with a two-week washout period between phases. In addition, all subjects were medication free, including over-the-counter agents, for 7 days prior to the study. Study protocols were approved by the Jordanian Drug Directorate and the Institutional Review Board of the study site. After an oral dose of 100 mg, venous blood samples were collected at $0_{pre\text{-}dose}$, 0.5, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.5, 4, 5, 6, 8, 10 and 12 hr post dosing. Serum samples were assayed for diclofenac sodium using a sensitive and specific high performance liquid chromatographic procedure (3). In brief, the assay involves protein precipitation of the serum samples with acetonitrile, followed by elution from a 5-micrometer C-8 reversed phase column with a mobile phase consisting of acetonitrile-water (50:5, v/v) adjusted to pH 3.3 with glacial acetic acid, at a flow rate of 2 ml/min, with ultra-violet detection at 280 nm. Quantitation was done by the measurement of the peak-height ratio of diclofenac sodium to the internal standard flufenamic acid. Limit of detection was 20 ng/ml with a concentration range of 0.02-7.0 mcg/ml.

Results

$AUC_{0-\infty}$ was calculated using the linear trapezoidal rule without adjustment. $AUC_{0-\infty}$ was then recalculated after accounting for the percent of AUC from zero time to first time of drug appearance in plasma (T_{lag}). In addition, the 90% confidence intervals of ($AUC_{Test}/AUC_{Reference}$) formulations were calculated after logarithmic transformation. Results are shown in Tables 1 and 2.

Discussion

Delayed release formulations, such as enteric coated formulations, can exhibit a fairly long T_{lag} before appearance in plasma. Hence, drug regulations require that the time to reach maximum plasma concentration (T_{max}) values for such formulations to be adjusted according to the T_{lag} values (1). However, no adjustment is mentioned about the calculation of AUC, which is a major parameter in bioequivalency studies.

As shown in Table 1, the direct linear trapezoidal rule method to calculate $AUC_{0-\infty}$ resulted in large percent of AUC from zero time to first time of drug appearance in plasma in our experiment. This bias results from the calculation of the first segment in AUC from time zero to the lag time which in turn over estimates the $AUC_{0-\infty}$ (Fig. 1).

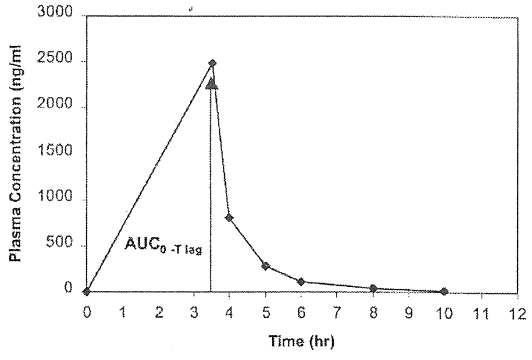

Fig. 1. A representative plot showing high $\%AUC_{0\text{-}Tlag}$ after administration of test formulation to Subject 7

Table 1. $AUC_{0-\infty}$ and the percent AUC from zero time to T_{lag} values for all human subjects

	Reference		Test	
Subject	$AUC_{0-\infty}$(ng.hr/ml)	$\%AUC_{0-Tlag}$	$AUC_{0-\infty}$ (ng.hr/ml)	$\%AUC_{0-Tlag}$
1	2517	2.0	2570	1.6
2	1134	6.8	800	4.0
3	1130	55.1	1288	21.6
4	1848	9.8	2591	61.1
5	1081	26.1	1986	58.7
6	1720	1.6	1536	3.6
7	1828	7.6	6191	70.2
8	1889	41.9	950	1.3
9	613	8.7	957	29.7
10	3899	74.3	1741	23.8
11	1920	1.4	4048	42.9
12	1442	50.1	721	10.8
13	1965	23.3	2110	1.9
14	1537	15.3	2351	42.5
15	1980	2.6	1980	21.8
16	1966	1.0	1321	29.2
17	1153	19.6	2057	15.2
18	1658	6.4	1849	6.7
19	1729	3.2	1891	8.8
20	1475	3.3	1188	65.9
21	1583	4.4	2826	35.5
22	2375	34.8	2069	21.8
23	1619	3.3	2026	3.6
24	2037	1.6	1728	51.2

Table 2. 90% Confidence Interval of Test/Reference AUC ratio after logarithmic transformation using different methods

	Using $AUC_{0-\infty}$	**Using $AUC_{0-\infty}$ for subjects of %AUC<10%**	**Using $AUC_{Tlag-\infty}$**
Point estimate	108.8	99.7	95.0
Lower limit	91.7	86.5	81.4
Upper limit	129.0	114.8	110.8
S.D.	1.6	1.2	1.6
Sample size	24	6	24
Decision	**Bio-inequivalent**	**Bio-inequivalent**	**Bio-inequivalent**

Hence, in order to obtain unbiased AUC values, we recalculated AUC after subtracting the first segment contribution, under the scientific assumption that drug concentrations will be negligible or essentially zero before the lag time. Another method was also tested in which all subjects having large AUC

contribution from the first segment (>10%) were omitted from confidence interval calculations. However, a major drawback to the second method is the small sample size used in calculations.

As shown in Table 2, a major result was obtained after applying this approach in which the final decision was changed from bio-inequivalence to bio-equivalence in terms of $AUC_{0-\infty}$.

Hence, if the computer program used does not correct the error in AUC calculation for delayed release formulations, this new approach is suggested to be used for further human and animal bioavailability and bio-equivalence studies in order to obtain more accurate and unbiased decisions.

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