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ASSESSMENT OF DIFFERENT BIOEQUIVALENCE METRICS IN RIFAMPIN BIOEQUIVALENCE STUDY

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ABSTRACT

The use of secondary metrics has become of special interest in bioequivalency studies. The applicability of partial area method, truncated AUC and C_{max}/AUC has been argued by many authors. This study aims to evaluate the possible superiority of these metrics to primary metrics (i.e. AUC_{inf}, C_{max} and T_{max}). The suitability of truncated AUC for assessment of absorption extent as well as C_{max}/AUC and partial AUC for the evaluation of absorption rate in bioequivalency determination was investigated following administration of same product as test and reference to 7 healthy volunteers. Among the pharmacokinetic parameters obtained, C_{max}/AUC_{inf} was a better indicator of absorption rate and the AUC_{inf} was more sensitive than truncated AUC in evaluation of absorption extent.

Keywords: Bioequivalence, Secondary metrics, Truncated AUC, Partial area method, C_{max}/AUC, Rifampin

INTRODUCTION

Bioequivalence of two formulations of a drug with systemic effect is demonstrated when the plasma (or other biological fluids) concentration-time profiles are sufficiently similar. Ratios of test to standard area under the curves (AUC) have been widely used to determine the equivalence of the extent of absorption. It has been claimed that for complicated absorption models, the use of AUC_{inf} (AUC from zero to infinity) is erroneous (1). The calculation of AUC_{inf} also involves prolonged sampling. In addition of costs, risks and insensitivity associated with bioequivalence studies for some drugs like hydroxychloroquine with $t_{1/2}$>50 days collection of samples for several months is required (2). Thus it has been suggested that in such conditions truncated AUCs are better indicators of bioequivalence of generic products than the total AUC (1-4). The peak drug concentration (C_{max}) and time to peak (T_{max}) obtained from plasma and/or serum concentration-time profiles have been utilized as a measure of the rate of absorption. In practice, these parameters are determined experimentally which heavily depend to sampling time schedule (3). T_{max} is a discrete parameter and there is a lack of statistical methods for T_{max} comparisons. Therefore, C_{max} becomes the only parameter used for estimation of absorption rate in most cases. The use of C_{max} for bioequivalence assessment seems inappropriate, since in addition of being insensitive and nonspecific in the assessment of absorption rate it reflects also the extent of absorption (5). Another parameter, C_{max}/AUC has been recommended as a metric of enhanced specificity, since it is independent of the extent of absorption (6-8) and also has smaller variation than C_{max} (8-9). However the superiority of C_{max}/AUC_{inf} or C_{max}/AUC_{lqc} (AUC from zero to last quantifiable concentration) is under question (5,10). So far, evaluation of bioequivalence metrics has been investigated by simulation methods (5,11,12) or by using two different formulations which, at first their bioequivalency should be cleared. Using one product as both test and reference products makes it possible to be sure of bioequivalence and using real experimental data, therefore the precise evaluation of different metrics would be possible. In this study Rifampicin-Hefa, 150 mg (Hefa Pharma-Germany) was used as test and reference product. This experiment was instructed to investigate the priority of the above parameters in bioequivalence evaluation.

MATERIALS AND METHODS

Materials

Pure rifampin and ascorbic acid were kindly donated by Alhavi Co. (Iran) and Daroupakhsh (Iran) respectively. HPLC grade acetonitrile and

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analytical grade KH_2PO_4 and double distilled water were also used throughout the study.

Instrumentation

HPLC system consisted of a Waters Model 600 E pump and a Rheodyne (7725i, California, USA) injection valve fitted with a 20 µl sample loop. The effluent was monitored at 334 nm with a Waters Model 486 variable wavelength UV detector. Separation was performed at ambient temperature on a MosHypersil C8 analytical column (5 µ, 150×3.9 mm) preceded by a guard pack module with a C8 insert (Waters). The mobile phase consisted of acetonitrile: KH_2PO_4 (100 mM, pH 4), 34:76 V/V with the flow rate of 0.7 ml/min. Peak areas were measured using a Waters Model 746 integrator.

Human study

Seven healthy adult volunteers, aged 23-40 years, and weighing between 60-80 kg were selected. A complete medical history, physical examinations, urine analyses and hematology were obtained from all volunteers before initiation of study. The volunteers were instructed to abstain from taking any medication for 1 week before and during the study period. They were administered the same product, Rifampicin Hefa 150 (Hefa Pharma-Germany), as both test and reference in two different periods. This would eliminate the subject by formulation effect. Volunteers given their written consent were received rifampin capsules (300 mg) after an overnight fasting. The washout period was considered 10 days. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hr intervals and serum rifampin concentration after protein precipitation by acetonitrile, were determined as described by Ishi and Ogata (13) with slight modification.

Data analysis

The linear trapezoidal rule that used for calculation of AUC_{0-t} and the $AUC_{t-\infty}$ was estimated by extrapolation. The AUC_{inf} was thus the sum of AUC_{0-t} and $AUC_{t-\infty}$. In order to evaluate the bioequivalency, parameters of C_{max}, T_{max}, AUC_{lqc}, AUC_{inf}, C_{max}/AUC_{lqc}, C_{max}/AUC_{inf}, AUC_{0-tmax} and AUC_{0-3hr} were calculated.

RESULTS AND DISCUSSION

The mean serum profiles of rifampin for two periods of study are shown in Fig.1. The mean pharmacokinetic parameters calculated in two periods of study and 90% confidence interval for their mean ratios and P values of paired t-test for mean of pharmacokinetic parameters are summarized in table I. The parameters AUC, C_{max} and T_{max}, have been widely used for assessing bioequivalence in generic products. According to report of Niazi et al (1) for most noncomplicated absorption models, the AUC correlates well with the extent of absorption.

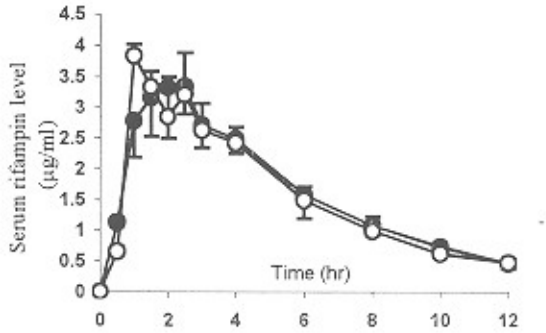

Fig 1. Mean serum concentrations of rifampin following a single oral dose of rifampin (300 mg) in period 1 (○) or period 2 (●). Values represent the mean±SE of 7 subjects.

However in nonlinear models of absorption, with mechanisms involving recycling of drugs and for drugs with long half life, the use of total AUC can give erroneous and clinically irrelevant results since the area is mostly influenced by elimination and drug recycling. Calculation of total AUC in addition of prolonged sampling, is associated with problems of bioequivalence studies. Thus it has been suggested that partial AUCs are better indicators of bioequivalence of generic products than the total AUC. By comparing AUC_{inf} with AUC_{lqc} in this study, it was found that the parameter of AUC_{inf} is a better indicator of the extent of rifampin absorption than AUC_{lqc} (confidence interval of 0.97-1.12 versus 0.96-1.22). Other parameters, C_{max} and T_{max} from plasma and/or serum concentration-time profiles have been utilized as a measure of rate of absorption that give minimal information about the absorption rate and absorption process of the drug and depends heavily upon the sampling time schedule. These parameters are not well defined in the presence of multiple peaks or when the plasma concentration curve around the peak is flat (3). T_{max} being a discrete parameter and lack of statistical methods for its comparison is not sensitive enough for estimation of the absorption

rate, and therefore C_{max} remains as only parameter that may be used for this purpose. Since it reflects only the extent of absorption, it is insensitive and nonspecific in the assessment of absorption rate. It has been suggested that the incremental area under the drug level curve (AUC) representing 10-30% of the total AUC would be more sensitive than either C_{max} or T_{max} in differentiating rate of absorption of drugs due to differences in formulation (8). Endrenyi et al have indicated that calculation of the partial AUC until the earlier of the two contrasted peaks (test or reference product) is the most effective (11), and Chen has suggested that the choice of cut off point for bioequivalence comparisons depends on both the peak time of the drug concentration curve and the therapeutic use of the drug under study (3).

Table 1. Summary statistics of pharmacokinetic parameters after a single oral dose of rifampin (300 mg)

Parameter	Mean±SD		90% confidence interval	P Value
	Period 1	Period 2		
C_{max} (μg/ml)	4.27±1.11	4.25±0.68	0.82-1.25	0.608
AUC_{lqe} (μg hr/ ml)	19.73±2.60	18.55±4.11	0.96-1.22	0.470
AUC_{∞} (μg hr/ ml)	22.19±2.96	21.38±3.62	0.97-1.12	0.456
C_{max}/AUC_{lqe} (1/hr)	0.22±0.04	0.23±0.04	0.77-1.14	0.511
C_{max}/AUC_{∞} (1/hr)	0.19±0.03	0.20±0.04	0.82-1.14	0.888
$AUC_{0\text{-}tmax}$ (μghr/ ml)	4.17±2.32	3.58±2.21	0.77-2.71	0.378
$AUC_{0\text{-}3\,hr}$ (μghr/ml)	7.23±2.43	6.93±2.07	0.78-1.50	0.750

According to table 1, 90% confidence interval for the mean C_{max} ratio lies within the range of 0.82-1.25 and for T_{max} of 0.90-1.72, which both of them are out of acceptable range. This result is in agreement with the above statement. $AUC_{0\text{-}tmax}$ and $AUC_{0\text{-}3hr}$ have been also calculated for evaluation of the rate of absorption. The results of $AUC_{0\text{-}tmax}$ (confidence interval of 0.77-2.71) represent that this metric is very sensitive to variations of early stages in absorption phase, and in the case of rifampin in which a high intra- and inter-individual variation in the early phase of absorption have been observed, the results would be misleading in evaluation of bioequivalence. Calculation of AUC to 1-1.5 hr after T_{max} ($AUC_{0\text{-}3hr}$) gave better confidence interval (0.78-1.50), which is not satisfying yet. C_{max}/AUC is recommended as a specific parameter for estimation of the absorption rate, because of its independence to the extent of absorption and its smaller variation than C_{max}. Bois (10) found that C_{max}/AUC_{inf} with various scenarios involving two compartmental models is sensitive to measurement errors. However Tothfalusi and Endrenyi (5) have demonstrated that C_{max}/AUC_{lqe} is generally superior to both C_{max} and C_{max}/AUC_{inf} for assessment of the equivalence of absorption rates.

Our study indicates that C_{max}/AUC in assessment of absorption rate is more suitable than C_{max}, when AUC_{inf} has been used (confidence interval of 0.82-1.14). This ratio metric has smaller variation than C_{max} (confidence interval of 0.82-1.25). The parameter C_{max}/AUC_{inf} is more reliable than C_{max}/AUC_{lqe} (confidence interval of 0.77-1.14) for comparison of rifampin absorption rates.

According to the results of this study it may be suggested that for rifampin and other class II drugs (14), AUC_{inf} is a better indicator of absorption extent and C_{max}/AUC_{inf} is more sensitive than C_{max} and T_{max} in evaluation of the absorption rate, and partial area method is not recommended for evaluation of absorption rate in rifampin bioequivalence study.

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