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Effect of truncating AUC at 12, 24 and 48 hours when evaluating the bioequivalence of drugs with a long half-life

Running Title: Truncating AUC at 12, 24 and 48 hours in long half-life drugs

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Abstract

Bioequivalence studies of drugs with a long half-life require long periods of time for pharmacokinetic sampling. The latest update of the European guideline allows the area under the curve (AUC) truncated at 72 h to be used as an alternative to AUC_{0-t} as the primary parameter. The objective of this study was to evaluate the effect of truncating the AUC at 48, 24 and 12 h on the acceptance of the bioequivalence criterion as compared with truncation at 72 h in bioequivalence trials. The effect of truncated AUC on the within-subject coefficient of variation (CV_w) and on the ratio of the formulations was also analysed.

Twenty-eight drugs were selected from bioequivalence trials. Pharmacokinetic data were analysed using WinNonLin 2.0 based on the trapezoidal method. Analysis of variance (ANOVA) was performed to obtain the ratios and 90% confidence intervals for AUC at different time points.

The degree of agreement of AUC_{0-72} in relation to AUC at 48 and 24 according to the Landis and Koch classification was “almost perfect”. Statistically significant differences were observed when the CV_w of AUCs truncated at 72, 48 and 24 h was compared with the CV_w of AUC_{0-12}. There were no statistically significant differences in the AUC ratio at any time point. Compared to AUC_{0-72}, Pearson’s correlation coefficient for mean AUC, AUC ratio and AUC CV_w was worse for AUC_{0-12} than AUC_{0-24} or AUC_{0-48}.

These preliminary results could suggest that AUC truncation at 24 or 48 h is adequate to determine whether two formulations are bioequivalent.

Introduction

The European guidelines for conducting bioequivalence studies of immediate-release formulations state that two formulations are bioequivalent if the 90% confidence interval (CI) of the ratio of the means for the pharmacokinetic parameters (area under the plasma concentration curve from administration to the last observed concentration at time t [AUC_{0-t}] and maximum plasma concentration [C_{max}]) of the test and reference formulations is within the acceptance interval of 80.00-125.00% (1, 2). In the case of drugs with a long half-life, the latest update of these guidelines proposes truncating the AUC at 72 h (AUC_{0-72}) as an alternative to calculating the AUC_{0-t} for comparison of the extent of exposure, as the absorption phase is completed during the first 72 h (1). Furthermore, the absorption phase of a drug in its plasma concentration versus time is more sensitive and decisive and enables better detection of the differences between formulations before the drug is completely eliminated (3). Therefore, a sampling period longer than 72 h is not necessary for immediate-release formulations, irrespective of half-life (1).

Complete absorption of the drug formulation occurs mostly within the first 24 h; therefore, truncation of the AUC at 24 h or 48 h as the primary parameter should be enough. Moreover, since absorption is the most relevant parameter in shorter AUCs, these intervals could be more sensitive for calculating differences between formulations.

An AUC truncated at under 72 h may be interesting in drugs with a long half-life, not only in terms of volunteer participation, but also in terms of compliance with trial visits and in terms of the study analysis and costs (3).

The purpose of this study was to analyse the effect of truncating the AUC at 48, 24 and 12 h on the acceptance of the bioequivalence criterion as compared with truncation at 72 h in bioequivalence trials. The effect of truncated AUC on the within-subject coefficient of variation (CV_w) and on the ratio of the formulations is also evaluated.

Materials and methods

Selection criteria for clinical trials

Bioequivalence trials conducted from 2001 until August 2014 at Hospital Universitario de La Princesa were analysed on the basis of drug half-life.

The drugs were oral immediate-release drugs whose median half-life of the test and/or reference formulation in the clinical trials was ≥ 15 h. This value was chosen as a reference value because 5 half-lives is sufficient to ensure complete washout (1); consequently, samples could be taken up to 72 hours for these drugs. In this case, a total of 15 active substances (28 formulations) were analysed, as follows: amlodipine, aripiprazole, citalopram, desloratadine, digoxin, donepezil, efavirenz, flunarizine, mirtazapine, nevirapine, olanzapine, rosuvastatin, sertraline, tadalafil and telmisartan. The median half-life for the test and reference formulations of the trials included are shown in Table 1.

All drugs were presented as tablets, except for aripiprazole, desloratadine and olanzapine, which were also presented as orodispersible tablets. Metabolites were not taken into account, and sampling was performed at 12, 24, 48 and 72 hours for all selected trials.

Study design and subjects

All selected trials shared the same design. They were open-label (blinding was for the analyst only), single-dose, randomized, two-period, crossover studies held under fasting conditions.

The sample size was 12 to 48 healthy subjects of both sexes aged between 18 and 55 years. The selection criteria were similar for all studies.

The clinical trials were evaluated and approved by the Ethics Committee for Clinical Research at Hospital Universitario de La Princesa and the Spanish Agency for Medicines and Medical Devices. The studies were conducted in accordance with the Declaration of Helsinki (4) and the Guideline for Good Clinical Practice (5). Informed consent was obtained from all participants before any procedure was carried out.

Statistical analysis

Pharmacokinetic parameters were calculated using the non-compartmental method, as recommended by the European Medicines Agency (EMA) (1), using the pharmacokinetic software package WinNonlin Professional Edition, version 2.0 (Scientific Consulting, Inc., Cary, USA). The AUC truncated at the various time points was calculated using the linear trapezoidal rule.

The pharmacokinetic data of the selected formulations were analysed using the statistical package integrated in WinNonLin based on an analysis of variance (ANOVA) of the log-transformed pharmacokinetic parameters AUC and C_{max} and the application of the 90% confidence intervals. According to the recommendations of the EMA(1), four factors are considered in this ANOVA: sequence, subject within sequence, period and formulation. The

bioequivalence acceptance limits were 80-125% for all drugs, except for digoxin, whose acceptance limit for the AUC was 90-111% (1, 2).

The degree of agreement of the AUC truncated at various time points was determined by calculating the Kappa index according to the Landis and Koch classification (6). Moreover, the linear correlation of the mean AUC (test and reference formulations), AUC ratio and AUC CV_w were calculated using the Pearson correlation coefficient (PCC) with SPSS 19.0.

The CV_w was estimated using the formula recommended by the EMA (1) from the mean square error obtained from the ANOVA for each pharmacokinetic parameter.

Finally, repeated measures ANOVA was performed using SPSS, version 19.0 to investigate significant differences in the results for CV_w and in the ratios of the means of the AUC (test and reference formulations) at different truncation time points. Adjustment to the normal distribution of CV_w was necessary and was performed by transforming the data to the Naperian logarithm.

Results

Acceptance of bioequivalence criteria under the truncated AUC

Figure 1 shows that 4% of studies did not prove to be bioequivalent in the selection of drugs with a median half-life ≥15 h. This trial maintained the criterion of not being bioequivalent with the AUC truncated at any time point. Bioequivalent drugs maintained the acceptance criterion for all four time points, except for one case of aripiprazole tablets and efavirenz tablets, which ceased being bioequivalent for AUC_{0-12}.

When agreement between AUC_{0-72} and the other time points (AUC_{0-48}, AUC_{0-24} and AUC_{0-12}) were compared, bioequivalence was "almost perfect" between AUC_{0-72} and both AUC_{0-48} and AUC_{0-24}, according to the classification of Landis and Koch (6) (Kappa=1) (Table 2). Meanwhile, in the case of AUC_{0-12}, the degree of agreement in relation to AUC_{0-72} was "moderate" (6) (Kappa=0.472). Therefore, agreement was better for AUC_{0-48} and AUC_{0-24} than for AUC_{0-12}.

The PCC for the mean AUC of the test and reference formulations was worse for AUC_{0-12} than for the remaining truncations in relation to the mean AUC_{0-72}. In the case of the mean AUC of the test formulation, the PCC was 0.930 for AUC_{0-12} compared with 0.976 and 0.996 for AUC_{0-24} and AUC_{0-48}, respectively. Similar results were found for the AUC mean of the reference formulation, namely, a PCC of 0.944 for AUC_{0-12}, 0.976 for AUC_{0-24} and 0.997 for AUC_{0-48}.

Variation in CV_w in truncated AUCs

According to the results of the AUCs truncated at the different time points (Table 3), statistically significant differences were observed when the CV_w of the AUCs truncated at 72, 48 and 24 h was compared with the CV_w of the AUC_{0-12}.

The PCC in the CV_w is worse as we decrease the time of the truncated AUC in relation to AUC_{0-72}, namely, 0.969 for AUC_{0-48}, 0.929 for AUC_{0-24} and 0.862 for AUC_{0-12}.

Variation in ratios in truncated AUCs

No statistically significant differences were observed in the mean of the ratios of AUC for any of the truncation time points (Table 4). The PCC for the ratio of AUC_{0-72} versus AUC_{0-48}, AUC_{0-24} and AUC_{0-12} is worse as the truncation time points decrease: 0.980 for AUC_{0-48}, 0.951 for AUC_{0-24} and 0.909 for AUC_{0-12}.

Discussion

Evaluation of AUC truncated at 12, 24 and 48 h compared with the results for AUC_{0-72}

According to the latest update of the EMA guideline, the absorption phase is completed within the first 72 hours for immediate-release drugs, regardless of half-life (1). Accordingly, the criterion for evaluation of bioequivalence may be sufficient with sampling times of up to 72 h. But, most absorption process take place in the first 24 h and the sampling period could be shortened.

Our results showed better agreement in the bioequivalence assessment for AUC truncated at 24 h and 48 h than for AUC truncated at 12 h in relation to AUC_{0-72} (see Table 2). As shown in Figure 1, only two formulations (aripiprazole and efavirenz tablets) ceased being bioequivalent with AUC truncated at 12 h. These two factors determine that truncation of the AUC at 12 h could be hasty and would not ensure complete absorption of the drug.

While truncation of the AUC at 24 h or 48 h may seem less restrictive than at 72 h, it should be noted that, according to the results of Table 3, statistically significant differences were found when the CV_w of AUC_{0-12} was compared with the CV_w of AUC truncated at 72, 48 and 24 h. In addition, the PCC of both the AUC CV_w and the AUC ratio of AUC_{0-72} in relation to the other time points shows the worst correlation coefficient for AUC_{0-12} (see Tables 3 and 4). No statistically significant differences were observed in the mean of the ratios for any of the time points (Table 4). Therefore, AUC truncated at 24 h and 48 h could also be useful as a criterion to establish whether two formulations are bioequivalent.

Some authors have demonstrated bioequivalence with truncation at 24 h and/or 48 h using studies similar to those used here (7-13). Marzo et al. (13) were able to demonstrate bioequivalence for alprazolam ($t_{1/2}$ = 12.8 h) and naproxen ($t_{1/2}$ = 16.5 h) with the AUC truncated at 12 h. However, truncation of the AUC at less than 72 h was not the main objective of any of the studies cited above. Moreover, these studies did not analyse the effect of CV_w.

Oishi et al argue that in the case of immediate release formulations, the elimination phase after the time peak concentration depends on the pharmacokinetic property of the active substance rather than the dissolution profile of a formulation (14) and we would be focusing on the absorption phase of the drug, in which it is easier to detect differences between formulations.

In addition, when comparing very different formulations, Oishi et al show that the use of AUC from zero to the last measurable point (method of calculating AUC in Europe) is more sensitive than calculating by extrapolation to the last sampling point (method of calculating AUC in Japan) (13). Therefore, truncation of the AUC at 48 h or, even better, at 24 h, might be more sensitive for evaluating differences between formulations, since the absorption process has more impact on the parameter analysed. In theory, it should be easier to demonstrate bioequivalence with $AUC_{0-\infty}$ than with AUC_{0-72}, AUC_{0-48} or AUC_{0-24} because of the greater influence of elimination, which is not related to the formulation.

A major limitation of the present analysis is the small number of studies that did not prove bioequivalence. Using a simulation of two non-bioequivalent drugs, Mahmood (15) concluded that truncating the AUC at 72 h in drugs with a long half-life could be useful, although it could lead to drugs that are not bioequivalent being considered as such. Therefore, it would be interesting to validate whether our results are consistent in the case of non-bioequivalent drugs. Although this study has only analysed the effect of truncated AUC in long half-life drugs in crossover designs, it should also be evaluated whether these time points of truncation are valid in parallel designs or, on the contrary, whether the time points should be increased, as evidenced by El-Tahtawy et al (16).

Estimation of the effect on CVw of the AUC truncated at various time points

Najib (17) observed that out of six drugs that proved to be bioequivalent for $AUC_{0-\infty}$, AUC_{0-t} and C_{max}, four ceased being bioequivalent with the AUC truncated at 72 h. They also realized that truncation at 72 h made the CV_w increase in relation to the CV_w of the AUC at time t and up to infinity (regardless of whether they were bioequivalent). This led the authors to argue that the parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max} were more sensitive for establishing bioequivalence between two formulations, thus making it necessary to consider the method of truncation at 72 h recommended by the EMA (1) with caution. The reason they give is that while low CV_w with the parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max} is valid for determining an adequate sample size, when the AUC is truncated, the CV_w increases and the sample size may no longer be adequate, with the result that the drug becomes non-bioequivalent. This observation should be taken into account when deciding to apply this method of truncation as a main variable in a study (17). In the study by Najib (17), it is important to remember that not all the drugs used had a long half-life, as argued by Khandave et al. (3). In addition, they do not specify the formula or the method for estimating the CV_w.

The results in Table 3 show statistically significant differences when the CV_w of AUC_{0-12} was compared with the CV_w of AUC truncated at 72, 48 y 24 h. In addition, the CV_w mean was higher for AUC_{0-12} than for the other time points of AUC truncation. This increase in the variability could explain why some formulations cease to be bioequivalent with AUC truncation at 12 h (see Figure 1); therefore, more subjects could be needed to demonstrate that the formulations are bioequivalent (18). The other possibility is that this variation in CV_w occurs because the whole absorption phase of the drug is not being taken into account. The results are also consistent with those obtained in the calculation of PCC for the CV_w of AUC_{0-72} at the different time points, in which the correlation was worse for AUC_{0-12}. Consequently, the CV_w is not affected by truncation at 24, 48 and 72 h. The fact that CV_w does not affect truncation at 72 h was also demonstrated by Khandave et al. (3).

In conclusion, our preliminary results could suggest that truncation of the AUC at 24 h or 48 h in drugs with a long half-life is sensitive and adequate when determining whether two formulations are bioequivalent. Applying these time points in drugs with a long half-life for the assessment of bioequivalence reduces not only the number of extractions and site visits, but also study costs. However, these studies should be confirmed by analysing a higher and more diverse amount of drugs, as well as more non-bioequivalent studies.

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Table 1. Half-lives for each active substance analysed according to the median resulting from the pharmacokinetic analysis and number of volunteers.

Active substance	Median $t_{1/2}$ test (h)	Median $t_{1/2}$ ref. (h)	Number of volunteers and sex
Amlodipine tablet 1	34	32	36 (18 M/18 F)
Amlodipine tablet 2	37	37	36 (18 M/18 F)
Amlodipine tablet 3	33	33	23 (12 M/11 F)
Aripiprazole tablet 1	44	49	24 (15M/9F)
Aripiprazole tablet 2	47	53	26 (18M/12F)
Aripiprazole tablet 3	50	44	30 (17M/13F)
Aripiprazole (orodispersible) 1	38	41	34 (15M/19F)
Aripiprazole (orodispersible) 2	49	52	36 (18M/18F)
Aripiprazole (orodispersible) 3	50	47	36 (18M/18F)
Citalopram tablet	33	31	24 (12M/12F)
Desloratadine tablet	17	16	37 (19M/18F)
Desloratadine (orodispersible)	17	17	37 (19M/18F)
Digoxin tablet	88	93	40 (20M/20F)
Donepezil tablet	62	61	36 (18M/18F)
Efavirenz tablet 1	55	56	12 (6M/6F)
Efavirenz tablet 2	62	56	12 (6M/6F)
Efavirenz tablet 3	60	61	12 (6M/6F)
Flunarizine tablet	397	288	30 (15M/15F)
Mirtazapine tablet	24	23	36 (18M/18F)
Nevirapine tablet	28	31	24 (12M/12F)
Olanzapine tablet	31	29	30 (15M/15F)
Olanzapine (orodispersible)	33	33	30 (15M/15F)
Rosuvastatin tablet 1	15	13	36 (19M/17F)
Rosuvastatin tablet 2	14	16	36 (17M/19F)
Sertraline tablet 1	26	26	24 (12M/12F)
Sertraline tablet 2	23	24	24 (12M/12F)
Tadalafil tablet	26	25	36 (19M/17F)
Telmisartan tablet	22	20	48 (22M/26F)

Median $t_{1/2}$ test: Median of the half-life of the test formulation; Median $t_{1/2}$ ref: Median of the half-life of the reference formulation; M: male; F: female.

Table 2. Degree of agreement of AUC_{0-72} in relation to AUC at 48, 24 and 12 h according to the Landis and Koch classification [5]

	Measure of agreement Kappa	Landis and Koch Classification
AUC_{0-72} vs AUC_{0-48}	1	Almost perfect agreement
AUC_{0-72} vs AUC_{0-24}	1	Almost perfect agreement
AUC_{0-72} vs AUC_{0-12}	0.472	Moderate

Table 3. Means of CV_w for AUC_{0-72}, AUC_{0-48}, AUC_{0-24} and AUC_{0-12}.

	Mean and SD of CV_w %		p value in relation to AUC_{0-12}	p value in relation to AUC_{0-72}	PCC in relation to AUC_{0-72}
AUC_{0-72}	10.859	1.454	0.000		
AUC_{0-48}	10.794	1.490	0.000	0.732	0.969
AUC_{0-24}	11.201	1.499	0.000	0.264	0.929
AUC_{0-12}	12.692	1.449		0.000	0.868

SD: standard deviation; PCC: Pearson correlation coefficient; N=28 drugs

Table 4. Means of the ratios for AUC_{0-72}, AUC_{0-48}, AUC_{0-24} and AUC_{0-12}.

	Mean and SD of ratio		p value in relation to AUC_{0-12}	p value in relation to AUC_{0-72}	PCC in relation to AUC_{0-72}
AUC_{0-72}	102.494	6.003	0.769		
AUC_{0-48}	102.291	6.864	0.914	0.491	0.980
AUC_{0-24}	102.067	8.172	0.762	0.471	0.951
AUC_{0-12}	102.205	9.955		0.769	0.909

SD: standard deviation; PCC: Pearson correlation coefficient; N=28 drugs

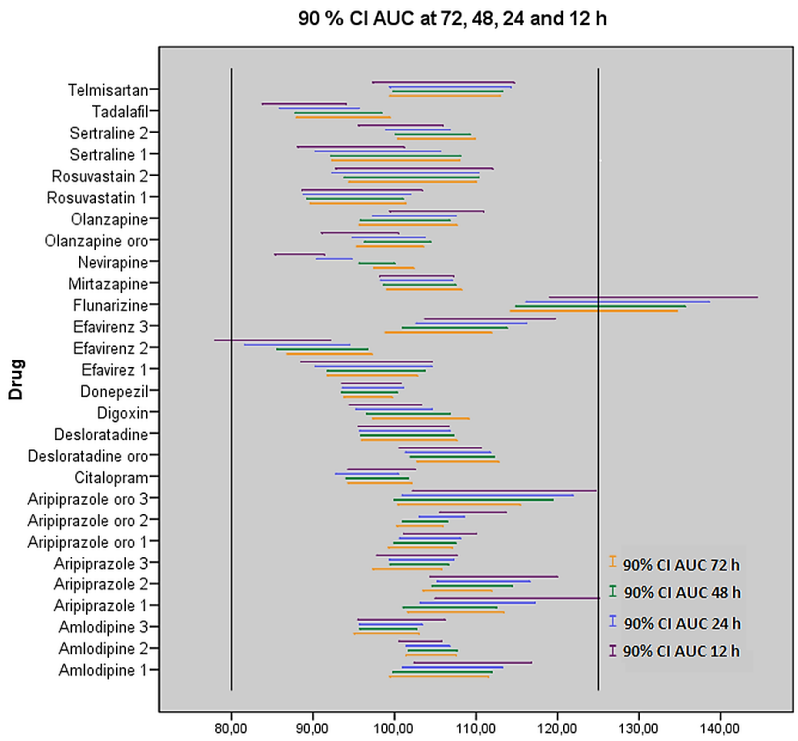

Figure 1. Acceptance of the bioequivalence criterion on the basis of an AUC truncated at various time points. The bioequivalence acceptance limits are 80-125% for 90% of confidence interval (CI), except for digoxin, whose acceptance limits for AUC are 90-111%. Flunarizine was the unique formulation that was never bioequivalent for any of the time points and Aripiprazol 1 and Efavirenz 2 tablets ceased being bioequivalent for $AUC_{0\text{-}12}$.