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Short Communication

Replicate evaluation of drug exposure to study bioequivalence between two brands of phenytoin in patients

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ABSTRACT

Two multisource phenytoin products (Antepil® and Comitoína®) available for oral administration in Uruguay were authorized to continue in the market provided they could demonstrate similar pattern of efficacy and safety. To accomplish this, fifty-seven epileptic patients under chronic treatment with one of the two brands were enrolled in a bioequivalence parallel design study in which saliva concentration-time profiles of phenytoin were evaluated twice in each subject. Maximum and mean steady state concentrations (C_{max} and C_{ss}) and peak trough fluctuation (PTF) were obtained from the saliva concentration-time profiles. Two 90% confidence intervals (90%CI) for the ratio of the geometric brand means were calculated for each parameter using the total and the residual variance. The results show a narrower 90%CI when the residual instead of the total variance is used, making it possible to include the 90%CI obtained from the residual variance within the bioequivalence interval [0.80-1.25], in opposition to the wider 90%CI obtained from the total variance for the parameters C_{ss} and C_{max}. Regarding PTF, as the residual variability was similar to the total one, none of the 90%CI could be included within the bioequivalence interval. However, for parameters with high intrinsic coefficient of variation, a wider bioequivalence interval has been accepted by the WHO. The results obtained in this study allow us to conclude that Antepil® and Comitoina® are bioequivalent. Moreover, the procedure of parallel assay, with replicate evaluation of drug exposure, should be considered as a valuable solution to demonstrate bioequivalence of multisource drug products.

KEYWORDS: phenytoin, multisource products, bioequivalence in patients, parallel design study.

ABBREVIATIONS

WHO, World Health Organization; PHT, phenytoin; C_{max}, maximum concentration; C_{min}, minimum concentration; C_{ss}, mean steady state concentration; t_{max}, time to reach maximum concentration; AUC, area under the concentration-time curve; PTF, peak trough fluctuation; 90%CI, 90% confidence interval; CV, coefficient of variation.

INTRODUCTION

The concept of bioequivalence denotes equivalent bioavailability, not meaning identical but similar, with an acceptable degree of dissimilitude. The bioavailability is the fraction of the administered dose that is absorbed, and it refers not only to the extent of drug that reaches systemic circulation but also to the rate of the process. The conventional procedure to evaluate *in vivo* bioequivalence between two products is conducting a two-period, two-sequence, and a balanced crossover design study with healthy volunteers, with each volunteer receiving the two products in a randomized order. These trials are usually called average bioequivalence studies. When single-dose studies are conducted,

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the pharmacokinetic parameters that must be assessed are area under the concentration time curve from time zero to infinite (AUC_0^∞) for the extent of the absorbed drug, and maximum concentration (C_{max}) and time to reach maximum concentration (t_{max}) for the rate of the absorbed drug. In multiple-dose studies, the parameters are area under the curve for the dosing interval (AUC_0^τ) for the extent and C_{max} and t_{max} or peak trough fluctuation (PTF) for the rate [1]. These parameters must be estimated from experimental data, which is obtained by quantifying the drug in a biological fluid, mainly plasma. However, our group has vast experience in the use of saliva as a useful drug monitoring fluid, [2-8] even for bioavailability and bioequivalence studies [9-12].

According to most of the regulatory agencies, generic products need to undergo average bioequivalence studies in order to prove they are bioequivalent to the reference product before they obtain the marketing authorization. Conducting average bioequivalence studies is a way of guaranteeing the quality of the products. However, bioequivalence does not mean therapeutic equivalence, since the clinical response is not evaluated in this type of studies. In addition, similar bioavailability between two products concluded from a study in healthy volunteers might not be retrieved when the same products are evaluated in patients, as a consequence of the different physiopathological conditions of the subjects under study. Consequently, there is a need to evaluate the products in their natural context of use. One method could be to conduct bioequivalence studies in patients with a crossover design, as in healthy volunteers. Nevertheless, patients will be subject to a switch brand, entailing some risk of toxicity or therapeutic failure when the disease has already been under control. Therefore, an alternative way of evaluating the products in the clinical setting is conducting a parallel design study, in which two groups of patients are formed, with each group receiving one of the branded products. Characteristics such as physiopathological condition, age, sex and ethnicity, among others, should be similar between groups in order to avoid bias, since the subjects could not act as control of themselves as they would in a crossover study. Although the parallel design has not been established as a usual design for bioequivalence, it is the type of design recommended when products with long elimination half-lives are evaluated in healthy volunteers [1].

In Uruguay, in 2007, a regulation with technical recommendations to conduct bioequivalence studies was approved. Some of the products marketed before that year should have been evaluated following Regulatory Authority's priorities. However, mainly because of the costs associated with average bioequivalence studies, only a few products have been studied so far. This means that in this country there are multisource drug products marketed before 2007 that have not been evaluated as bioequivalent yet.

Phenytoin (PHT), an anticonvulsant used to treat epilepsy [13], was claimed as a priority for bioequivalence evaluation. Nowadays, two commercial brands of PHT are available in Uruguay for oral administration, both multisource drug products. Other brands quit the market once the bioequivalence requirement started. In order to maintain the offer of at least two products containing PHT, they were authorized to continue in the market provided they could demonstrate similar pattern of efficacy and safety. To accomplish this, a bioequivalence study between these two brands was carried out in patients following a parallel design.

MATERIALS AND METHODS

Subjects and study design

Fifty-seven epileptic patients (thirty-one women and twenty-seven men) between 18 and 76 years old under chronic treatment with the same dose for at least six months with one of the two brands of PHT available in Uruguay were enrolled in a bioavailability study. The patients were receiving doses of PHT every 12 hours that allowed them to control their pathology. Patients were grouped into two according to the commercial brands they were receiving: thirty-three patients were under treatment with Antepil® (Fármaco Uruguayo Laboratory) and twenty-four with Comitoina® (Roemmers Laboratory). The demographic characteristics of the groups are shown in Table 1. The study was conducted under a parallel design and saliva concentration-time profile of PHT was evaluated twice in each subject.

Table 1. Demographic characteristics of the patients included in the study.

	Antepil®	**Comitoina®**
Number of patients	33	24
Men	15	12
Women	18	12
Age (years)	18-75	18-76
Mean weight (kg) [range]	75.8 [45-140]	76.5 [45-108]

The study protocol was designed according to the clinical research guidelines and was approved by the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay). Written informed consent was obtained from all subjects before their entry into the study.

Sampling and chemical analysis

Saliva samples were obtained by citric acid stimulation. The samples were scheduled before dose intake and at 1, 2, 3, 4, 5, 6, 8, and 12 hours after dosing. The samples were stored at -25 ºC until analysis. Quantification of PHT in saliva was carried out by Chemiluminescent Microparticle Immunoassay (CMIA), using Architect (Abbot™) equipment, according to the instructions given in the package insert. The lower limit of quantification was determined to be 0.3 mg/L, intra-and-inter-day precision was below 20%, coefficient of variation and accuracy were below 15% and between 85% and 115%, respectively.

Pharmacokinetic and statistical analysis

Concentration values along the dosing interval were obtained twice during the study period for the fifty-seven patients included. The maximum and minimum PHT saliva concentrations (C_{max} and C_{min}) for each patient were computed from the experimental data. The area under the saliva concentration–time curve for the dosing interval, from zero to twelve (AUC_0^{12}), was calculated using the trapezoidal rule. The mean steady state concentration (C_{ss}) and PTF were calculated according to equations 1 and 2, respectively.

$$C_{ss} = \frac{AUC_0^{12}}{12} \quad (1)$$

$$PTF = \frac{C_{max} - C_{min}}{C_{ss}} \times 100 \quad (2)$$

Mean concentration-time profiles in saliva for each product were constructed and mean $C_{max,}$ C_{ss} and PTF (± standard deviation) were calculated for each product.

Bioequivalence between two products is claimed if the 90% confidence intervals (90%CI) for the Antepil/Comitonia (A/C) ratio of the geometric means for each parameter are within the range 0.80-1.25. 90%CI were calculated according to equation 3 [14].

$$90\%\text{CI} = e^{\left[Ln\left(X_1 / X_2\right) \pm t_{0.10}^{n_1+n_2-2} \times \sqrt{\frac{s^2}{n_1}+\frac{s^2}{n_2}} \right]} \quad (3)$$

where X_1/X_2 is the A/C ratio of geometric means for the pharmacokinetic parameter under study, n_1 and n_2 the number of patients under treatment with Antepil® and Comitoina®, respectively, $t_{0.10}^{n_1+n_2-2}$ the critic value for the t-distribution with a Type I error of 0.10 and n-2 degrees of freedom, and s^2 the combined variance, which was calculated according to equation 4 [14].

$$s^2 = \frac{sc_1 + sc_2}{n_1 + n_2 - 2} \quad (4)$$

where sc_1 and sc_2 are the sum of squares for Antepil® and Comitoina®, respectively, which were calculated as follows: $SC_i = (n_i - 1)s_i^2$, where n_i is the number of subjects and s_i^2 the variance [14]. The sum of squares for each product was calculated by two methods: I) using the total

variance; and II) using the residual variance obtained after processing the logarithmic of the parameters (C_{max}, C_{ss} and PTF) obtained in the two occasions by the analysis of variance (ANOVA, Microsoft Office Excel 2010 software), considering subjects and periods as the variation sources. Coefficient of variation (CV) was calculated according to equation 5 [14]. Therefore, two 90%CI were obtained, the first one using total variance and the second one using residual variance.

$$CV = 100 \times \sqrt{e^{s^2} - 1} \quad (5)$$

RESULTS

Both groups received similar daily body weight dose (mean ± 95%CI) of PHT (4.58 ± 0.43 and 4.34 ± 0.37 mg/kg.day for Antepil® and Comitoina®, respectively). The pharmacokinetic parameters for both formulations, and bioequivalence metrics are shown in Table 2.

Saliva concentration-time profiles of PHT for each formulation are shown in Figures 1 and 2.

DISCUSSION

Although the saliva concentration-time profiles of both brands are similar, a delay at the beginning of the absorption can be seen in Comitoina profile. A dissolution assay with both brands conducted by our research group (unpublished data), according to USP32 NF27, showed a more rapid dissolution pattern for Antepil®. This brand

Table 2. Geometric means of the pharmacokinetic parameters for Antepil® and Comitoina®, Antepil/ Comitoina (A/C) ratio of means, 90% confidence intervals (90%CI) and coefficient of variation (CV) calculated using total variance and residual variance.

Parameter	Antepil®	Comitoina®	A/C	Using total variance			Using residual variance		
				90%CI		CV (%)	90%CI		CV (%)
C_{ss} (mg/L)	0.700	0.698	1.00	0.754	1.33	64.2	0.844	1.19	36.7
C_{max} (mg/L)	0.953	0.964	0.989	0.768	1.27	55.8	0.859	1.14	29.7
PTF (%)	54.7	52.7	1.04	0.814	1.32	53.5	0.815	1.32	53.2

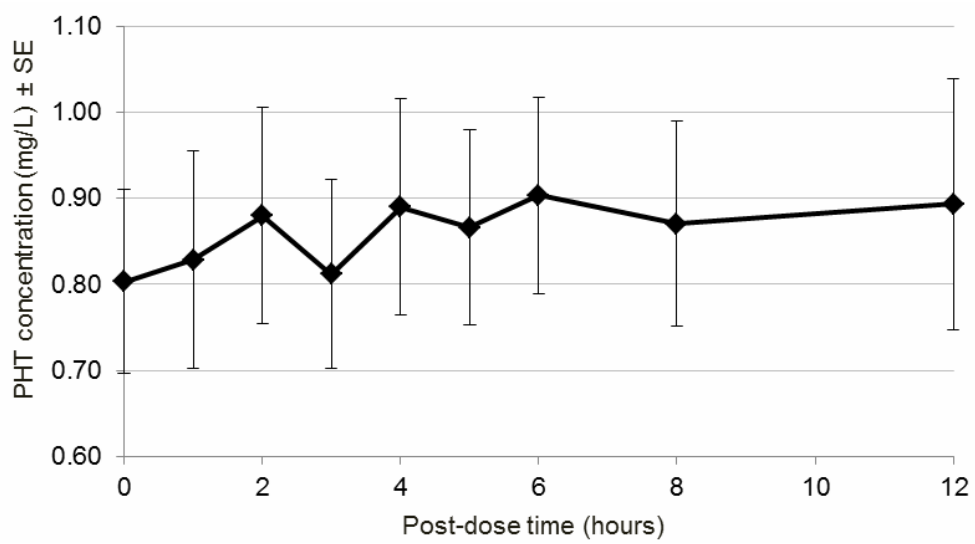

Figure 1. Mean (± standard error) saliva concentration-time profile of PHT for Antepil®.

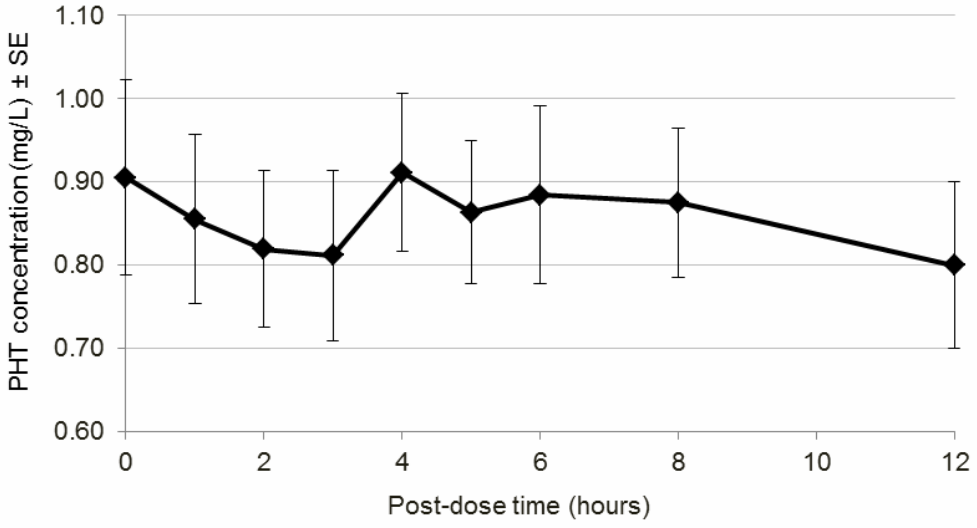

Figure 2. Mean (± standard error) saliva concentration-time profile of PHT for Comitoina®.

reached 72% of dissolved drug at 20 minutes, while Comitoína® was barely starting the dissolution process (only 4% of dissolved drug) at the same period of time. Comitoina® was able to reach 90% of drug dissolved at 60 minutes, while Antepil® reached the same percentage at 30 minutes.

Table 2 shows that the 90%CI for C_{ss} is narrower when the residual variance is used instead of the total variance. This was the expected result since the former is a fraction of the total variance. Consequently, it was possible to include the 90%CI obtained from the residual variance within the bioequivalence interval [0.80-1.25], in opposition to the wider 90%CI obtained from the total variance. The residual variance obtained in our trial is in fact the intraindividual variability, which was possible to estimate thanks to the replicated design implemented here for each formulation. This is not difficult to carry out in patients under chronic therapies, provided that the dose and the interval of dosing are kept constant. A non-replicated parallel design could demand a higher number of subjects in order to conclude equivalent amount of drug absorbed.

Despite using the intraindividual variance, the number of subjects included in a parallel study should not be low considering that the variability in patients is higher in comparison with healthy volunteers. The most important cause for this variability is the physiological condition of the patients, which is more subject to changes in comparison with healthy volunteers. Another point that should be considered is that patients do not necessarily receive the same daily dose in order to control their clinical responses. This is a problem when the drug follows a nonlinear pharmacokinetics, as PHT does. Therefore, drug exposure could have been conditioned not only by different bioavailabilities, but also by different clearances. Correcting the exposure by the administered dose received would not have solved the problem. Fortunately, the patients in each group received similar doses.

The analysis of C_{max} does not differ from C_{ss}, but the former refers to both the amount of drug absorbed and the rate of absorption. Analytical error should have contributed to a higher extent to the variance of a single point parameter as C_{max}, in opposition to C_{ss}, where the analytical random error becomes insignificant throughout the several concentration data that support AUC_0^{12}. Although a higher variability for C_{max} is always expected when the concentration-time profile is flat, as shown in Figures 1 and 2, the analytical error also reduces its contribution to C_{max} variance, increasing the variability over the t_{max} instead. This was the reason why CV for C_{max} was not higher than CV for C_{ss} in our study.

Regarding PTF, the intraindividual variability was similar to the total one, as it is shown in Table 2.

Table 3. Acceptance limits to determine bioequivalence according to the residual CV.

Residual CV (%)	Lower limit	Upper limit
30	0.80	1.25
35	0.77	1.30
40	0.75	1.34
45	0.72	1.39
≥ 50	0.70	1.43

Therefore, the 90%CI could not be included within the bioequivalence interval not only when it was calculated with the total variance but also with the residual variance. In fact, from the three pharmacokinetic parameters under evaluation, the PTF is the one that has the highest intrinsic variability. In this case, the analytical random error over C_{min} should have been the main cause.

Taking into account the proposal made by the World Health Organization [1] for those parameters with high intrinsic CV, it is possible to adopt a wider bioequivalence interval based on the residual CV observed, as it is shown in Table 3. Considering that the residual CV for the PTF was 53.2, the bioequivalence interval could be expanded to 0.70-1.43. Under this scenario, the 90%CI for PTF A/C ratio could be included in the bioequivalence interval.

According to the results obtained in this study, for the three parameters under evaluation, it can be concluded that Antepil® and Comitoina® are bioequivalent.

CONCLUSIONS

Considering the difficulty associated with conducting bioequivalence studies in healthy volunteers, and the huge amount of multisource drug product marketed in Uruguay before 2007, there is a critical need to perform such studies in patients. In order to address patient safety, this procedure of parallel assay, with replicate evaluation of drug exposure, becomes a valuable solution to demonstrate bioequivalence of such products.

In addition, this parallel design can also be used to follow up bioequivalent products in the clinical setting, by doing active pharmacovigilance of their switchability in different populations, even in those countries where the process for asking market authorization is already a common practice.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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