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The Use of Nonlinear Mixed Effects Models in Bioequivalence Studies: A Real Data Application

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Abstract

The aim of this study was to investigate the use of nonlinear mixed effects models in biequivalence studies and compare it to non-compartmental analysis which is proposed by regulatory agencies. Non-compartmental analysis requires few hypotheses but a large number of samples per subject. On the other hand, nonlinear mixed effects models approach is more complex than non-compartmental analysis but it has some advantages such as it requires few samples per subject. A real data application was provided for the study, which was get from Ege University Drug Development and Pharmacokinetics Research Center. According to real data analysis, nonlinear mixed effects models approach has smaller within subject error, narrower confidence interval and smaller p-value than non-compartmental analysis. In the light of results at this study, nonlinear mixed effects models approach was more effective than non-compartmental analysis. Thus, nonlinear mixed effects models approach was suggested as an efficient and alternative analysis tool for bioequivalence studies.

Keywords: Nonlinear mixed effects models; Bioequivalence; Non-compartmental analysis

Introduction

In many applications, particularly in the biological sciences, the time course of a response for an individual may be characterized by a function that is nonlinear in one or more parameters, where an "individual" may be a human subject, an animal, a plant, an agricultural plot, a laboratory sample or other observational unit [1]. Mixed effects models, which are widely used as a flexible and powerful tool, deal with repeated measures data. Non-linear mixed effects (NLME) models consist of both population and subject specific characteristics which are represent fixed parameters for population and random parameters for subjects. Mixed effects models for repeated measures data have become popular in part because their flexible covariance structure allows for nonconstant correlation among the observations and/or unbalanced data (designs that vary among individuals) [2]. To use drugs that containing same active substance interchangeably, these drugs must demonstrate similar chemical and therapeutic properties. Therefore, bioequivalence studies are utilized to determine effective and safe therapeutic properties of the first drug produced (original drug) and its copies (generic drugs) [3]. Bioavailability is defined by Food and Drug Administration (FDA) (2003) as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. Bioequivalence is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study [4]. Regulatory agencies are proposed to use non-compartmental analysis (NCA) for statistical calculations in bioequivalence studies [4,5]. Since the data that are used for bioequivalence studies consist repeated observations, these data also can be analyzed by using NLME models. Blood samples are collected to identify dosage of drug in body and how much of this drug has reached circulatory system. As the drug is absorbed and distributed, the plasma concentration rises and reaches a maximum (called the C_{max} or maximum concentration). Plasma levels then decline until the body completely eliminates the drug from the body. The overall exposure to drug is measured by computing the area under the plasma concentration curve (AUC) [6]. Some decision rules were proposed by the FDA between 1977 and 2003 [4,7] for testing the bioequivalence in terms of average bioavailability. FDA is proposed 80/125 rule for average bioavilability to asses bioequivalence. 80/125 rule is defined as

$$80\% < \frac{\mu_T}{\mu_R} < 125\% \quad (1)$$

where μ_T and μ_R represent average biovavilability of test formulation and reference formulation respectively. According to that rule, bioequivalence is concluded if the average bioavailability of the test formulation is within (80%, 125%) that of the reference formulation, with a certain assurance [8]. NCA method produce biased estimation when number of observations per subject is insufficient. Estimation with this method can also lead to missing data. In practice, a few missing values or unexpected observations may occur at some sampling time points owing to laboratory error, data transcription error, or other causes unrelated to bioequivalence [8]. Generally, missing values or unexpected observations between two end sampling time points have little effect on the comparison of bioavailability [9]. However, if many missing values or unexpected observations occur in the plasma concentration–time curve, especially at two end sampling time points, the bias of the estimated AUC could be substantial and, consequently, may affect the comparison of bioavailability. Thus, how to justify the bias in the calculation of AUC is an important statistical issue [8]. On the other hand, repeated measures data can also be analyzed using NLME approach. This approach can characterize the bioequivalence data with few observations per subject. Also, there are no missing data in this approach. The aim of this study is to examine the use of NLME

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approach in bioequivalence studies and compare results with NCA which is standard statistical procedure for bioequivalence studies.

Material and Methods

NCA method

In NCA method, the overall exposure to drug is measured by computing the area under the plasma concentration curve (AUC) using trapezoidal rule [6].

$$AUC_{0-t_n} = \sum_{i=2}^{n}\left[\frac{C_{i-1}+C_i}{2}\right]\left(t_i - t_{i-1}\right) \quad (2)$$

$$AUC_{0-\infty} = AUC_{0-t_n} + \frac{C_n}{\lambda} \quad (3)$$

where C_i, C_{i-1} and C_n are drug concentrations at times t_i, t_{i-1} and t_n respectively, n is the total number of control points and λ is terminal slope. The terminal slope is computed using the logarithm of the last concentrations to perform a log-linear regression. To avoid biased estimation of the terminal slope, the first point used for its computation should be on the descending side of the concentration curve and not too close to C_{max} [10]. If these assumptions are not satisfied, then there is no estimation of the total AUC for the subject and treatment concerned. C_{max} is estimated directly from the observed concentrations [8]. That is,

$$C_{max}=max\{C_0,C_1,\ldots,C_n\} \quad (4)$$

NLME approach

A general form of NLME models can be written as

$$y_{ij} = f\left(\phi_{ij}, x_{ij}\right) + \epsilon_{ij} \quad (5)$$

$i=1,2,\ldots,N$ and $j=1,2,\ldots,n_i$

where N is the number of subjects, n_i is the number of observations on the i_{th} subject, f is a general, real valued, differentiable function of a subject-specific parameter vector ϕ_{ij} and a covariate vector x_{ij}, and ϵ_{ij} is a normally distributed within subject error term. Individual parameter vector (ϕ_{ij}) can vary among subjects and can be written as

$$\phi_{ij}=A_{ij}\beta+B_{ij}b_i \quad (6)$$

$$b_i \sim N(0,\Sigma)$$

where β is a p-dimensional vector of fixed effects and b_i is a q-dimensional random effects vector associated with the i_{th} group with variance-covariance matrix Σ. The matrices A_{ij} and B_{ij} are of appropriate dimensions of some covariates at the j_{th} observation [11]. A one-compartment model with first-order absorption and first-order elimination adequately describes the data and can be written as

$$f\left(t,\theta\right) = \frac{FDk_a}{CL - Vk_a}\left(e^{-k_a t} - e^{-\frac{CL}{V}t}\right) \quad (7)$$

where D is the dose, F the bioavailability, k_a the absorption rate constant, CL the clearance of the drug and V the volume of distribution. AUC and C_{max} parameters can be obtained using NLME models

$$AUC_{0-\infty} = \frac{FD}{CL} \quad (8)$$

$$C_{max} = \frac{FD}{V}e^{-\frac{CL}{V}t_{max}} \quad (9)$$

where $t_{max} = \frac{log\left(k_a\right) - log\left(\frac{CL}{V}\right)}{k_a - \frac{CL}{V}}$ and other parameters as same as in equation (7). Iterative algorithms have been developed to obtain parameter estimations in NLME models. In this study, we used SAEM (stochastic approach to expectation-maximization) algorithm.

Real data application

The data were obtained from Ege University Drug Development and Pharmacokinetics Research Center for bioequivalence study after required permissions. The data have following characteristics:

1. 24 subjects were included to the study.

2. For each patient, fourteen blood samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 120 and 168 h after administration.

3. Each patient administered 10 mg oral dose.

4. A and B symbols were used for formulations.

5. In the first sequence (AB), subjects receive the A treatment and the B treatment in period one and two, respectively. In the second sequence, subjects receive treatments in the reverse order (BA).

R-2.9.2 and Monolix-2.4 statistical package programs were used for data analysis in this study.

AUC and C_{max} estimations

In this study, first of all, the data were analyzed using NCA method which is proposed by regulatory agencies. Then, same data set were analyzed using NLME method and finally, results were compared. Linear trapezoidal rule was used to analyze NCA method. For estimation of total AUC, the last six concentrations were used to compute terminal slope (λ) with log-linear regression. Then, estimation of $AUC_{0-\infty}$ were obtained using equation (3). Cmax is estimated directly from the observed concentrations as in equation (4). Equation (7) were used to analyze data set and SAEM algorithm were used to estimate parameter estimation in NLME approach. Equation (8) and (9) were used to estimate $AUC_{0-\infty}$ and C_{max}.

Bioequivalence estimations

The test procedures for the average bioavailability based on the interval hypothesis were proposed by Anderson and Hauck (1983) and Schuirmann (1987). The distribution of the observed test statistic proposed by Anderson and Hauck (1983) can be approximated by a central t-distribution [12]. Schuirmann's procedure uses two one-sided tests for assessment of equivalence in average bioavailability [8].

$$H_{01}: \mu_T-\mu_R \leq \theta_1 \quad H_{02}: \mu_T-\mu_R \geq \theta_2 \quad (10)$$

$$H_{11}: \mu_T-\mu_R > \theta_1 \quad H_{12}: \mu_T-\mu_R < \theta_2 \quad (11)$$

where θ_1 and θ_2 are bioequivalence limits, μ_T and μ_R are average bioavailability of test and reference formulations respectively. In this approach, two p-values are obtained to evaluate whether the bioavailability of the test formulation is not too low for one side (H_{01}-H_{11}) and not too high for the other side (H_{02}-H_{12}) [8]. Clearly, both of these null hypotheses would be rejected at the 5% level on a one-sided test [6]. Schuirman's two-one sided test is as follows [13]:

$$t_{01} = \frac{\left(\hat{\mu}_T - \hat{\mu}_R\right) - \theta_1}{s_{\hat{\mu}_T - \hat{\mu}_R}} \quad (12)$$

$$t_{02} = \frac{\left(\hat{\mu}_T - \hat{\mu}_R\right) - \theta_2}{s_{\hat{\mu}_T - \hat{\mu}_R}} \quad (13)$$

where $\hat{\mu}_T$, $\hat{\mu}_R$ and $s_{\hat{\mu}_T-\hat{\mu}_R}$ represent average bioavailability estimation of test formulation, average bioavailability estimation of reference formulation and standard deviation of difference between bioavailability estimations, respectively. In this study, we used average bioavailability and performed 80/125 rule. We used natural logarithm of $\overline{AUC}$ and $\overline{C}_{max}$ as proposed by EMEA (2001) and FDA (2003) and constructed linear mixed effects models as follows:

$$log(AUC) = \mu + F_i + S_j + P_{k(i,j)} + Sub_l \mid S_j + \varepsilon_{i,j,k,l} \quad (14)$$

$$log(C_{max}) = \mu + F_i + S_j + P_{k(i,j)} + Sub_l \mid S_j + \varepsilon_{i,j,k,l} \quad (15)$$

where μ, F_i, S_j, $P_{k(i,j)}$, $Sub_l|S_j$ and $\varepsilon_{i,j,k,l}$ represent population mean, effects of i_{th} formulation, effects of j_{th} sequence, effects of k_{th} period, random effects of l_{th} subject in j_{th} sequence and error term, respectively.

Results

Both analysis with NLME and NCA show that the 90% confidence intervals for the log-transformed parameters $AUC_{0-\infty}$ and C_{max} lie within the range 80-125%. Table 1 summarizes bioequivalence results.

According to real data analysis, NLME approach has smaller within subject error, narrower confidence interval and smaller *p*-value than NCA. In the light of results at this study, we concluded that NLME approach was more effective than NCA method.

Discussion

NCA method is easy to use and simple to apply. However, if there are missing values and few observations per subjects this method leads to bias estimations (Table 2). In our study, NLME approach has smaller within subject error than NCA. Thus, NLME method has narrower confidence intervals than NCA both for $log(AUC)$ and $log(C_{max})$. Statistically, a narrower confidence interval is always better. Hence, NLME approach was suggested as an efficient and alternative analysis tool for bioequivalence studies. NLME models, especially in recent years, is widely used to analyze repeated measures data. This method is more complex than NCA but has several advantages: it takes benefit of the knowledge accumulated on the drug and can characterize the PK with few samples per subject. This allows for analysis in patients, the target population, and in whom pharmacokinetics can be different from healthy subjects [10]. However, the use of NLME method is still rare in early phases of drug development or to analyze crossover studies. There are nine published studies which use NLME to analyze bioequivalence trials [10,14-21], and except in Zhou et al. (2004), Panhard and Mentre (2005) and Dubois et al. (2010), all analyze a dataset with many samples per subject. Seven papers [10,14-17,20,21] compare tests based on individual NCA estimates to tests based on NLME, and five of them conclude that the results are similar. Panhard and Mentre (2005) and Dubois et al. (2010) found that NLME is more efficient than NCA. Yet, they use different statistical approaches to test bioequivalence with NLME. Pentikis et al. (1996) propose the estimation of AUC and C_{max} by standard nonlinear regression as an alternative to the NCA, and Zhou et al. (2004) perform bioequivalence tests on the individual empirical Bayes estimates (EBE) of the volume of distribution and the steady-state through concentration. Otherwise, bioequivalence tests are performed on treatment effect parameters [14-18,20]. All authors agree that simulation studies are needed to evaluate bioequivalence tests based on NLME and to compare them to tests based on individual NCA estimates [10]. In this study, we used average bioequivalence method for bioequivalence analysis. Two more methods are available for bioequivalence studies: population and individual bioeaquivalence.

log(AUC)	NLME	NCA
within subject error	0.016	0.093
lower limit	98.7%	98.4%
upper limit	100.3%	107.9%
***p*-value**	2.11E-24	1.51E-07
result	bioequivalent	bioequivalent

Table 1: Bioequivalence estimations with NLME and NCA for *log*(*AUC*).

$log(C_{max})$	NLME	NCA
within subject error	0.035	0.143
lower limit	99.2%	91.2%
upper limit	102.7 %	105.1%
p-value	1.81E-16	3.29E-05
result	bioequivalent	bioequivalent

Table 2: Bioequivalence estimations with NLME and NCA for $log(C_{max})$.

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