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Application of a population pharmacokinetic modeling to bioavailability/bioequivalence study of cefadroxil preparations

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Abstract

The bioavailability of cefadroxil after administration of two immediate-release preparations, Cedrox and Duricef, to 12 healthy volunteers was studied. Bioequivalence of the preparations was assessed using model-independent parameters and a non-parametric confidence interval method. No difference in the extent of bioavailability and in the absorption rate was detected. However, after applying pharmacokinetic models and a population approach to estimate their parameters, significant differences in absorption kinetics were evident. A one-compartment model with a time lag and two consecutive first-order absorption steps were found to be consistent with the data, and the first step absorption rate constant was greater in the case of Cedrox while in the second step Duricef demonstrated more rapid absorption. No significant differences in the absorption time lag were found. Despite the fact that the difference in the absorption kinetics of cefadroxil for these two formulations is clinically insignificant, the study clearly demonstrated the usefulness of a population approach in determining the intimate details of the drug absorption process.

Keywords: Cefadroxil preparation; Cefadrox; Duricef; Bioequivalence; Relative bioavailability; Population modeling approach

1. Introduction

Standard bioequivalence testing is usually designed as a full-scale pharmacokinetic study with frequent blood sampling in a relatively small number of participating subjects (12–18). Model-independent pharmacokinetic parameters are calculated on an individual basis and analysed for statistical significance of differences between formulations (Chow and Liu, 1992; Schulz and Steinijans, 1992). An alternative approach has recently been suggested (Graves and Chang, 1990; Kaniwa et al., 1990; Miller and Ludden, 1993; Li et al., 1994) in which pharmacokinetic modeling has been used and model parameters have been estimated by means of the so-called population method or nonlinear mixed effect modeling (NONMEM). In the studies cited, a computer program of the same name (Beal and Sheiner, 1980) which enables one to estimate mean values of model parameters, their variability across the subpopulation and to test the significance of dif-

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ferences between parameters influenced by formulations under study has been used. It has been demonstrated that statistically valid conclusions on bioequivalence can be made on the basis of a few samples per subject where the application of the standard approach implying the evaluation of individual parameter values is restricted (Kaniwa et al., 1990).

The unique ability of the population approach to separate inter- and intra-individual variability in pharmacokinetic parameters may be of great value when performing more elaborate data analysis with the aim of evaluating those parameters intimately related to the absorption process independently of the disposition. It is of importance in bioavailability studies which differ from bioequivalence testing in their goals. The former are directed to gathering any information on the absorption kinetics that may help the producer to optimise the composition of a formulation and/or the technology and require detailed pharmacokinetic analysis by contrast with bioequivalence testing in which the absence (or presence) of significant differences in the extent and/or the rate of absorption is a final aim.

The goals of this study were (i) to test the bioequivalence of two cefadroxil preparations (Cedrox and Duricef) produced by different companies, (ii) to quantitate any differences in cefadroxil absorption kinetics between these formulations and (iii) to demonstrate the usefulness of a population approach in bioavailability studies. Model-independent methods recommended by health authorities were used to achieve the first goal and pharmacokinetic modeling with the application of the population approach to estimate parameters was utilised in the bioavailability assessment. A newly developed population pharmacokinetic-dynamic program (P-Pharm, 1993) was used for this.

2. Material and methods

2.1. Preparations

Cedrox tablets (C, test formulation) produced by Hikma Pharmaceuticals, Amman, Jordan (batch no. 7711) contain 1000 mg of cefadroxil monohydrate per tablet. Duricef tablets (D) from Mead Johnson, Evansville, Indiana, USA (cefadroxil monohydrate 1000 mg per tablet, batch no. B2W23DK3) was used as a reference formulation. Both formulations were of immediate release.

2.2. Subjects

12 healthy volunteers (six males and six females) were enrolled in the study. Average age was 28 years (range 21–40 years), height 173 cm (160–183 cm), and body weight 73 kg (53–95 kg). The internal examination consisting of complete anamnesis, physical examination, ECG and laboratory screening (blood count, glycemia, ALT and AST activities, creatinine, serum cholesterol and urinalysis) was performed within 14 days prior to the study and its result was within the normal range. The study was approved by the local ethical committee and written informed consent was obtained from each participant.

2.3. Study protocol

The study was designed according to a randomised cross-over scheme. The volunteers were randomly distributed into two groups one of which received C first (nos 1, 3, 5, 7, 9 and 11) while the other took D first (nos 2, 4, 6, 8, 10 and 12). The interval between administrations was not shorter than 1 week. One tablet of each preparation was given in the morning after overnight fast. Smoking was prohibited, and no coffee, alcohol or sweet drinks were allowed during the study day. The volunteers were put on a standardised rational diet. They rested in bed during the first 2 h after the dose.

Blood samples were collected into heparinized tubes before dosing and 15, 30, 60, 80, 100, 120 min, 3, 4, 6, 8, 12 and 24 h thereafter using an indwelling canal. Plasma was separated and stored at −70°C until analysis performed within 28 days.

2.4. Cefadroxil assay

Plasma cefadroxil was assayed by high-performance liquid chromatography according to Lind-

gren (1987). In brief, 12.5 μl of 200 mg/l internal standard solution (cephalexine monohydrate, Slovakofarma, Hlohovec, Slovakia) were added to 500 μl of plasma. After deproteinization by adding 500 μl of 6% trichloroacetic acid and centrifugation for 3 min at 5000 × *g* 80 μl of supernatant were injected onto the column. A Nucleosil 5 SA (5 μm) 4 × 200 mm column (Phenomenex, USA) was used with 20 mmol/l $NH_4H_2PO_4$-methanol-acetonitrile 40:30:30 (v/v) as mobile phase. pH was adjusted to 3.0 using phosphoric acid. Flow rate was 0.6 ml/min. UV detection was carried out at 240 nm. Cefadroxil and cephalexine retention times were 13.4 and 21 min, respectively. The recovery was 99.5% (CV = 5.08%, $n = 8$). The within-day precision was 4.8% at 3.5 mg/l and 4.0% at 21.5 mg/l. Between-day precision was 6.23% at 10.2 mg/l. The limit of detection was 0.2 mg/l and the calibration graph was found to be linear up to 35 mg/l.

2.5. Model-independent data analysis

The following parameters recommended by health authorities (Schulz and Steinijans, 1992) were calculated from cefadroxil plasma concentration-time measurements in each subject after administration of C and D: AUC, total area under the concentration-time curve from zero to infinity (in mg l^{-1} h^{-1}); C_{max}, maximal concentration achieved (in mg/l); T_{max}, time to reach the concentration maximum (in h); MRT, mean residence time (in h).

C_{max} and T_{max} were obtained directly from the measured concentrations. AUCs were calculated by combined linear/logarithmic trapezoidal rule with extrapolation to infinity. The same method was applied when calculating areas under the first moment curve (AUMC) needed to estimate MRTs according to the common formula: MRT = AUMC/AUC.

To test the bioequivalence a distribution-free procedure to construct 90% confidence intervals for the ratios of (or difference between) the expected medians of test (C) and reference (D) formulations as described by Hauschke et al. (1992) was used. Before applying the statistical analysis AUC, C_{max} and MRT values were converted into logarithms and the ratio test was used. In the case of T_{max} no conversion was made and the differences were tested.

In addition, routine statistical tests (paired *t*-test and Wilcoxon rank test) were applied with $P < 0.05$ as the level of significance.

2.6. Pharmacokinetic modeling

Two models were applied:

(1) An open one-compartment model with first-order absorption and an absorption lag time (one-step absorption, OSA). The Bateman function was used to describe concentration-time curves $C(t)$:

$$C(t) = (F/V)D\frac{k_a}{k_a - k_e}\left\{\exp\left[-k_e(t - T_{lag})\right] - \exp\left[-k_a(t - T_{lag})\right]\right\}$$

where F/V (in l^{-1}) is the fraction ultimately absorbed divided by the apparent volume of distribution; this ratio was taken as a single parameter inasmuch as the separate estimation of F and V is not possible; D is the dose administered (in mg); k_a and k_e denote absorption and elimination rate constants, respectively (in h^{-1}); T_{lag} is the absorption time lag (in h).

(2) An open one-compartment model with two consecutive first-order inputs and an absorption lag time. This model (two-step absorption model, TSA) is a simplification of a more general model with a two-compartment disposition derived by Wagner (1975). The following equation was used to fit concentration-time data:

$$C(t) = (F/V)Dk_{a1}k_{a2}\left\{\frac{\exp\left[-k_{a2}(t - T_{lag})\right]}{(k_e - k_{a2})(k_{a1} - k_{a2})} + \frac{\exp\left[-k_{a1}(t - T_{lag})\right]}{(k_e - k_{a1})(k_{a2} - k_{a1})} + \frac{\exp\left[-k_e(t - T_{lag})\right]}{(k_{a2} - k_e)(k_{a1} - k_e)}\right\}$$

where F/V, D, T_{lag} and k_e have the same meaning as before and k_{a1} and k_{a2} are rate constants of two consecutive absorption processes.

Both models were fitted to the total concentration-time data set that contained cefadroxil concentration measurements in all subjects after administration of C and D. In each volunteer the elimination rate constant was made equal for both preparations. Other parameters may be equal or different (see below). The basic OSA model contained seven parameters: one k_e constant, two F/V, k_a and T_{lag}, one for each preparation. The basic TSA model had nine parameters: one elimination rate constant k_e and four pairs of F/V, k_{a1}, k_{a2} and T_{lag}.

The population pharmacokinetic-dynamic data modeling program P-Pharm version 1.0 was used throughout this work (P-Pharm, 1993). The program explores two step EM-type iterative algorithms to find estimates of (sub)population means of parameters, their standard deviations and the residual error ϵ reflecting the intra-individual variability due to analytical errors and other reasons. The program also calculates the Akaike information criterion (AIC) (Akaike, 1974; Yamaoka et al., 1978) that enables one to choose an optimal (or minimal in the sense of the principle of parsimony) model among a set of similar models.

The latter feasibility was employed in this study to detect possible differences in absorption kinetics of cefadroxil from the preparations under study. After fitting the basic models described above to the data, the models were simplified by making one or more twine parameters common for both preparations. Below, a list of the submodels used in the case of the OSA model is given:

Submodel no. 1, the basic model; submodel no. 2, F/V common; submodel no. 3, k_a common; submodel no. 4, T_{lag} common; submodel no. 5, both F/V and k_a common; submodel no. 6, F/V and T_{lag} common; submodel no. 7, k_a and T_{lag} common; submodel no. 8, all parameters are common for both preparations.

A basic model with T_{lag} fixed equal to zero was also tested.

In the case of the TSA model the following submodels were compared:

Table 1
Parameters of cefadroxil pharmacokinetics after oral administration of Cedrox (C) and Duricef (D) tablets (1000 mg)

Volunteer no.	T_{max} (h)		C_{max} (mg/l)		AUC (mg l^{-1} h^{-1})		MRT (h)	
	C	D	C	D	C	D	C	D
1	0.75	1.00	27.71	29.26	87	83	4.05	4.02
2	1.33	1.33	28.02	25.26	83	78	4.80	4.90
3	2.00	2.00	19.22	20.60	71	72	5.29	5.32
4	2.00	3.00	20.02	17.14	79	71	4.95	5.75
5	2.00	1.33	22.48	26.25	91	100	5.18	5.40
6	1.67	1.67	26.44	20.81	115	89	5.44	5.50
7	2.00	0.75	25.67	24.58	113	101	4.69	4.87
8	3.00	1.33	24.19	27.03	102	96	6.46	5.27
9	1.33	2.00	25.47	27.31	88	99	4.51	4.88
10	1.00	2.00	29.28	25.88	107	105	4.61	5.40
11	3.00	1.33	18.31	23.96	76	81	5.38	4.71
12	1.67	2.00	25.22	27.21	112	110	4.88	5.11
Mean	1.81	1.65	24.34	24.61	94	91	5.02	5.09
SD	0.69	0.60	3.60	3.48	16	13	0.61	0.46
Median	1.83	1.50	25.35	25.57	90	93	4.91	5.19
Minimum	0.75	0.75	18.31	17.14	71	71	4.05	4.02
Maximum	3.00	3.00	29.28	29.26	115	110	6.46	5.75
Paired t-test	$P = 0.612$		$P = 0.838$		$P = 0.578$		$P = 0.756$	
Paired Wilcoxon test	$P = 0.450$		$P = 0.530$		$P = 0.556$		$P = 0.480$	

Submodel no. 1, the basic model; submodel no. 2, the basic model without T_{lag}; submodel no. 3, F/V common; submodel no. 4, k_{a2} common; submodel no. 5, k_{a1} common; submodel no. 6, T_{lag} common; submodel no. 7, F/V and k_{a2} common; submodel no. 8, F/V and k_{a1} common; submodel no. 9, F/V and T_{lag} common; submodel no. 10, all parameters common.

Submodels with the lowest AIC were selected as optimal and the difference in cefadroxil absorption parameters not common in the optimal submodel was regarded as significant.

The Wagner-Nelson method (Wagner and Nelson, 1964) was used to construct the absorption plots.

2.7. Statistical analysis

Standard methods of descriptive statistics were used. The results are presented as means and standard deviations (SD) as well as medians and ranges (minimum–maximum).

3. Results

3.1. Model-independent analysis

The fraction of the extrapolated areas in estimated AUC and AUMC was not higher than 4 and 10%, respectively.

Table 1 shows individual estimates of model-independent parameters obtained after administration of C and D to the volunteers. P values given at the bottom of Table 1 indicate no significant differences in any parameters between the formulations under study.

Nonparametric 90% confidence intervals for the ratios of the expected medians of AUC, C_{max} and MRT and for the differences in T_{max} are presented in Table 2. In the case of AUC, C_{max} and MRT they are well within the bioequivalence range of 0.8–1.25 adopted in a recent CPMP guidance on bioequivalence studies (CPMP, 1991). However, the expected difference in median T_{max} for C and D is outside the stipulated bioequivalence range of $\pm 20\%$ (Chow and Liu, 1992). The 90% confidence interval is rather wide and practically overlaps the bioequivalence range, and because of this no definitive conclusion could be made. Nevertheless, since no statistically significant differences in other parameters were found, one can conclude that C and D are bioequivalent.

3.2. Pharmacokinetic modeling

The bioavailability of cefadroxil was assessed first by applying a common one-compartment model with the first-order absorption (OSA model according to the notation accepted in this work). Eight versions of the basic model were fitted to the data, and the AIC values obtained were compared (Fig. 1, upper graph). The submodel without T_{lag} was also tested, but the resulting AIC was much greAter than in other submodels (not shown). Submodel no. 2 gave the lowest AIC and was selected as optimal. In this model both k_e and F/V were common for C and D. Mean parameter estimates and their coefficients of variation as well as other characteristics of the model are listed in Table 3.

Inasmuch as in the optimal submodel F/V was common for both formulations, one can con-

Table 2
Expected ratios of median AUC and C_{max} and differences in median MRT and T_{max} and their 90% confidence intervals for Cefadroxil and Duricef

	AUC (multiplicative)	C_{max} (multiplicative)	MRT (multiplicative)	T_{max} (additive)
Ratio/difference	0.980	0.919	1.027	0.271
90% confidence interval	0.922 – 1.020	0.889 – 0.976	0.984 – 1.102	−0.167 – 0.830
Significance	NS	NS	NS	– [a]

[a] See section 3.

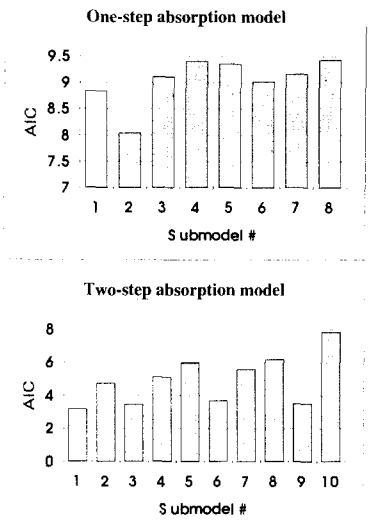

Fig. 1. Values of the Akaike information criterion estimated by the P-Pharm program for the one-compartment model with the first-order absorption (one-step absorption model) and the one-compartment model with two consecutive first-order absorption steps (two-step absorption model). Submodels differ in parameters made common for Cedrox and Duricef (see section 2 for explanations).

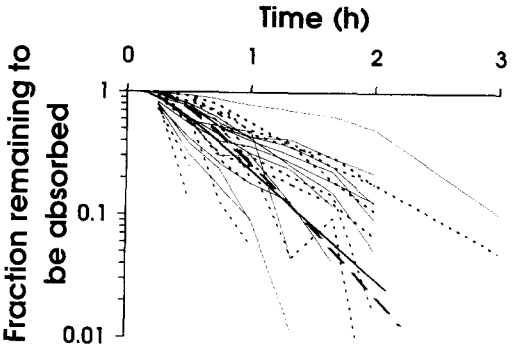

Fig. 2. Individual semilogarithmic absorption plots produced by means of the Wagner-Nelson method for Cedrox (continuous lines) and Duricef (dashed lines). Bold lines show absorption plots predicted by the two-stage absorption model.

clude that the OSA model did not detect any difference in the extent of bioavailability of cefadroxil from C and D. However, the difference in the absorption rate was significant, since submodels with common k_a and T_{lag} resulted in higher AIC values. The absorption from D was more rapid, but T_{lag} was about 2-fold longer than in the case of C (0.36 vs 0.19 h).

Semilogarithmic absorption plots produced by the Wagner-Nelson method are shown in Fig. 2. Most of them are convex, indicating that first-order kinetics may be poor approximation for the absorption in this case.

Table 3
Estimates of subpopulation means and coefficients of variation of parameters of the one-compartment model with first-order absorption (OSA) and with two consecutive first-order absorption steps (TSA)

Parameter (dimension)	OSA model		TSA model	
	Mean	CV (%)	Mean	CV (%)
k_e (h^{-1})	0.42	7.0	0.41	5.7
F/V_c (l^{-1})	0.039	15.9	0.039	16.7
F/V_d (l^{-1})	0.039	15.9	0.037	12.7
$k_{a,c}$ (h^{-1})	1.69	50.6	–	–
$k_{a,d}$ (h^{-1})	1.87	34.0	–	–
$k_{a2,c}$ (h^{-1})	–	–	5.79	57.9
$k_{a2,d}$ (h^{-1})	–	–	4.70	48.9
$k_{a1,c}$ (h^{-1})	–	–	2.07	48.5
$k_{a1,d}$ (h^{-1})	–	–	2.53	41.0
$T_{lag,c}$ (h^{-1})	−0.19	19.5	0.09	63.0
$T_{lag,d}$ (h^{-1})	0.36	24.4	0.21	49.3
σ	0.00136	–	0.00376	–
Maximum likelihood	−2159	–	−840	–
AIC	8.04	–	3.178	–

F/V, fraction ultimately absorbed to the apparent volume of distribution ratio; k_e, elimination rate constant; T_{lag}, absorption time lag; k_a, absorption rate constant in OSA model; k_{a1} and k_{a2}, absorption rate constants in TSA model; σ, residual error; AIC, Akaike information criterion. Subscripts c and d refer to Cedrox and Duricef, respectively.

The more complex TSA model was found to fit the cefadroxil concentration-time data much better. AIC values for all submodels tested were lower than those for the OSA submodels (Fig. 1). The basic TSA submodel had the lowest AIC among all 10 submodels tested and hence was selected as the optimal model, its parameters being presented in Table 3. In contrast to the OSA model, the TSA model detected some difference in the extent of bioavailability since submodel no. 3 in which F/V is common resulted in a slightly higher AIC than the basic model with different F/V. Submodel no. 2 (without T_{lag}) gave a substantially higher AIC, therefore, the inclusion of a second absorption step into the model did not eliminate the time lag. The submodel with $T_{lag,c} = 0$ and $T_{lag,d}$ open for iterations was also tested, but was found to be worse than the basic submodel (results not shown).

The difference in both absorption rate constants was significant: k_{a1} was found to be greater in the case of D while k_{a2} was higher for C (Table 3). This means that the absorption kinetics of cefadroxil for C and D were different. Differences are illustrated by the model-predicted absorption plots shown in Fig. 2. In the case of D the absorption was slower within approx. 0.5 h after administration, but then became more rapid as compared with C.

4. Discussion

Bioequivalence testing carried out by commonly accepted methods using model-independent parameters demonstrated no significant differences between C and D in the extent of bioavailability and the absorption rate of cefadroxil. However, an alternative approach consisting in the use of pharmacokinetic modeling, a population method for parameter estimation and AIC criterion for optimal model selection led to another conclusion: there was a small difference in the extent of bioavailability, but the absorption kinetics substantially differed. Despite the apparently conflicting conclusions, in essence, there are no contradictions because the aims of bioequivalence and bioavailability studies are far from being identical and the methods used differ substantially. Bioequivalence testing is aimed at discovering clinically important differences in bioavailability between drug products and relatively insensitive methods based on model-independent data processing are usually sufficient. In contrast, (relative) bioavailability studies are aimed at the investigation of any differences in absorption kinetics, and even small and clinically insignificant differences in absorption may be of importance, since they potentially can help a manufacturer to change the composition and/or the technology of a formulation to improve absorption characteristics.

Bioavailability studies usually require pharmacokinetic modeling including the assessment of mean values of model parameters and measures of their inter-individual variability in a subpopulation studied. There are two alternative ways to obtain such values which are statistically valid (Sheiner and Ludden, 1992). One consists in fitting a model to individual data with subsequent statistical analysis of individual parameter estimates (a standard two-stage approach). According to the second one a model is fitted to the total data set which includes concentration-time data pairs obtained from all subjects studied using a special program that enables one to separate fixed and random effects (nonlinear mixed effects modeling, NONMEM). The latter method, a population approach, provides direct estimates of (sub)population means of parameters and their variabilities. It may be of value not only in a routine clinical pharmacokinetic data analysis, as was first claimed by its authors (Sheiner et al., 1977; Sheiner and Beal, 1980), but also in other fields of pharmacokinetics including bioavailability studies (Graves and Chang, 1990; Kaniwa et al., 1990; Miller and Ludden, 1993; Li et al., 1994). Until recently, only one population program called NONMEM (Beal and Sheiner, 1980) has been available commercially, but it requires a mainframe computer. An alternative program, P-Pharm, for personal computers has been developed recently which employs different mathematical methods than NONMEM, but the basic principles of population pharmacokinetic analysis used are the same (P-Pharm, 1993).

In this study, we applied the P-Pharm program to estimate the parameters of two different models fitted to cefadroxil concentration-time data after C and D administration: the common one-compartment model with first-order absorption and the one-compartment model with two consecutive first-order absorption steps (OSA and TSA models, respectively). The third model, namely the one-compartment model with zero-order absorption followed by first-order absorption, was also tested, but resulted in much higher AIC values than two above-mentioned models (results not shown).

The pharmacokinetics of cefadroxil has been demonstrated to be dose-dependent (Sanchez-Pico et al., 1989; Garrigues et al., 1991), nevertheless, as the doses used were equal for both preparations, the application of linear models in this study was justified. However, the parameters evaluated are empirical and can hardly be used for predictions, especially to forecast the kinetics after the administration of various doses or multiple dosing.

In this study the elimination half-life of cefadroxil was found be 1.7 h which is longer than that reported by Welling et al. (1985) (1.1 h); this may be attributed to the saturable elimination of the drug (Garrigues et al., 1991), since a 2-fold greater dose was used in this study (1000 mg) as compared to that by Welling et al. (1985).

According to the principle of parsimony, the lower AIC value corresponds to the optimal model, and the TSA model was shown in this study to be preferable over the simple OSA (Fig. 1). The former provided estimates of rate constants of two consecutive absorption steps, the first of which might correspond to the accumulation of drug in the intestinal wall while the other reflects the rate of cefadroxil appearance in the systemic circulation. Or, alternatively, the first step may correspond to drug transport from the stomach to the intestine, and the second one to the systemic absorption from the intestine. However, these interpretations are speculative since the model is purely empirical.

To compare the absorption characteristics for two formulations the method typical to population pharmacokinetic data analysis was applied. Several submodels differing in parameter number were tested. In the basic submodel all parameters were assumed to be different in both formulations except that corresponding to the disposition (k_e). Other submodels were constructed by making two matched parameters identical in both formulations. If AIC decreased as compared to that of the basic submodel, one could assume no difference in the specific parameter. In the case of the TSA model the basic submodel had the lowest AIC, indicating significant differences in all parameters including the fraction absorbed. However, the difference in F/V was too small to be taken into consideration (5%). The differences in parameters reflecting the absorption rate were substantial (see Table 3) indicating a significant distinction in the rate of cefadroxil systemic absorption for C and D. T_{lag} was found to be 2-fold shorter in the case of C which favours this formulation as compared to D.

The reason(s) for these differences is(are) not clear at present, but, in all probability, they are relatively unimportant for the clinical use of C which is the test preparation. Nevertheless, these results may be of interest for the manufacturer of the preparation because they reflect intimate details of the absorption of cefadroxil from the gastro-intestinal tract.

In conclusion, this study has clearly demonstrated that a population approach in pharmacokinetic modeling is a powerful means for gathering valuable information on drug bioavailability from routine bioequivalence studies.

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