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Short communication

How to combine non-compartmental analysis with the population pharmacokinetics? A study of tobacco smoke's influence on the bioavailability of racemic citalopram in rats

Wojciech Jawień[1], Jagoda Majcherczyk[2], Maksymilian Kulza[3], Ewa Florek[3], Wojciech Piekoszewski[4,5]

[1]Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

[2]Department of Refrigeration and Food Concentrates, University of Agriculture, Balicka 122, PL 30-149 Kraków, Poland

[3]Laboratory of Environmental Research, Department of Toxicology, University of Medical Science, Dojazd 30, PL 60-631 Poznan, Poland

[4]Department of Analytical Chemistry, Jagiellonian University, Ingardena 3, PL 30-060 Kraków, Poland

[5]Laboratory of High Resolution Mass Spectrometry, Regional Laboratory of Physicochemical Analysis and Structural Research, Faculty of Chemistry, Jagiellonian University, Ingardena 3, PL 30-060 Kraków, Poland

Correspondence: Wojciech Piekoszewski, e-mail: wojciech.piekoszewski@uj.edu.pl

Abstract:
Background: Citalopram (CIT) is an antidepressant drug from the group of selective serotonin reuptake inhibitors in which it is the most potent selective inhibitor of serotonin uptake currently available. Patients treated with CIT are often heavy cigarette smokers. Individual pharmacokinetic parameters cannot be directly estimated if full pharmacokinetic profiles are not available for each subject. Sparse sampling is common to experiments using small animals, such as the case that our study is concerned with.
Methods: The aim of the study was to demonstrate how the two (non-compartmental analysis (NCA) and nonlinear mixed-effect (NLME)) approaches, used simultaneously, can help overcome specific limitations of these separate methods whilst at the same time preserve their respective benefits.
Results: Despite the ultra-sparse design, the NLME approach enabled us to develop a pharmacostatistic model with the required covariate – exposition to the tobacco smoke.
Conclusions: A tobacco smoke slows down the absorption of the CIT and at the same time makes it more effective. The consistency of results obtained both with NCA and NLME decreased the risk of model misspecification and increased confidence in the final conclusions. Combining NLME with NCA may therefore be recommended for investigating pharmacokinetic properties of the drug in the sparse designs.

Key words:
citalopram, tobacco smoke, population pharmacokinetics, model independent pharmacokinetics

Introduction

Depression is a common mental disorder nowadays. By the year 2020, depression is expected to reach the 2nd place of the DALYs (Disability Adjusted Life Years) ranking, which is calculated for all ages and both sexes. The pharmacological treatment of this illness is based mainly on second generation antidepressant drugs.

Citalopram (CIT) is an antidepressant drug of the group of selective serotonin reuptake inhibitors (SSRI) where it is the most potent selective inhibitor of serotonin uptake currently available [4, 10, 16]. CIT is marketed as a racemate, but it is also the only S-enantiomer that possesses SSRI activity.

CIT is biotransformed by the cytochrome P 450 enzyme to desmethylcitalopram (DCIT), which is the main metabolite, and didesmethylcitalopram (DDCIT). Baumann and Larsen reported that metabolites of CIT inhibit the reuptake of serotonin at a level of four (DCIT) and thirteen (DDCIT) times less than the parent compound [2].

The bioavailability of the drug after oral administration is 80%. The plasma levels of CIT and DCIT range from 9 to 200 ng/ml and 10 to 105 ng/ml, respectively.

CIT is widely distributed among peripheral tissues and the volume of distribution is estimated between 12 and 16 l/kg [4]. Intrinsic clearance of CIT is low and its kinetic was found linear in the dose range of 10–60 mg [4, 11].

Patients treated with CIT are often heavy cigarette smokers. It is therefore interesting to investigate whether tobacco smoke has the potential to modify pharmacokinetic properties; especially the bioavailability of this drug. However, in the present paper, the above question is not the central one (as opposed to our previous work on CIT [15]). The main goal of this study is rather more of a methodological nature. We were trying to choose an approach that is best suited to the pharmacokinetic analysis of the available data.

Individual pharmacokinetic parameters cannot be directly estimated if full pharmacokinetic profiles are not available for each subject. Sparse sampling, as used in our study, is typical of experiments with small animals.

Fortunately, a number of possible solutions to the problems concerning sparse sampling exist. The naive averaged data (NAD) approach [7] is probably the simplest one. In this method, at each time point, several concentrations, each one coming from a different subject, are determined. These measurements are then averaged, giving a single pharmacokinetic profile. In the original NAD method, a pharmacokinetic model is fitted to this averaged profile and the obtained pharmacokinetic parameters are then considered as a population mean.

In the method of Bailer [1], further developed by Jawień [12], no specific pharmacokinetic model is assumed and the pharmacokinetic parameters are estimated in the framework of the model-independent pharmacokinetics; known more often (though less correctly) as NCA [17]. These model-independent parameters, with the most frequently used area-under-the curve (AUC) among them, can be estimated along with their standard deviations. This enables a statistical comparison between groups that underwent different treatment.

While the simplicity and model-independence are benefits of this approach, the numerous drawbacks of NAD have already been indicated in earlier literature [7].

At another extreme, one can find the NLME approach. It was introduced into the field of pharmacokinetics by Sheiner and Beal and was initially implemented in their famous NONMEM system [3]. The name – population pharmacokinetics (PPK) is often used for NLME modelling of pharmacokinetic parameters. This approach requires both a strict model specification and highly sophisticated software, but offers greater flexibility regarding the choice of statistical model components and creates an opportunity to extract almost all the information contained in the experimental data. This is especially important in the case of sparse data.

With the NLME, it is possible to include covariates (such as exposure to tobacco smoke) in the pharmacostatistical model. By doing this, one can formulate and verify statistical hypotheses on the factors influencing the pharmacokinetic parameters. A recent work on the pharmacokinetic profile of propofol [19] may serve as an example of NLME being applied for the pharmacokinetic-pharmacodynamic modelling.

In the present paper, we wanted to demonstrate how these two approaches, when used simultaneously, can help overcome specific limitations of the individual methods while at the same time preserve the benefits of each approach.

Materials and Methods

Animals and protocol of the study

Male Wistar rats with an average body weight of 225 g bred at the Department of Toxicology, University of Medical Sciences (Poznań, Poland), were housed in polycarbonate cages with hardwood chip bedding. A standard laboratory diet of Labofeed (Feeds and Concentrates Production Plant, Certificate of Quality System No 181/1/98, Kcynia, Poland) and water were available with no limitations. Throughout the entire study period, a 12/12 h light/dark cycle was maintained. After 14 days of initial acclimatization, the rats were randomized and divided into four groups containing 24 animals in each.

Rats from the groups I and III (experimental groups) were exposed to tobacco smoke in a dynamic toxicological chamber for 5 days (6 h per day) [8].

The tobacco smoke was generated by Polish cigarettes without a filter tip ("Poznańskie", 20 cigarettes per pack, Imperial Tobacco Poland S.A.). The CO concentration in the chamber reflected the smoke content in the inhaled air and was continuously monitored by a gas analyzer Infralyt 1110/1210 (infrared measurement), to maintain 1,500 mg CO/m^3 of air. The level of oxygen was established at 20 ± 0.5% of the air volume. The air in the chamber was exchanged 10 times per day.

After exposure, CIT (Lundbeck, Denmark) was administered intraperitoneally (*ip*) at a dose of 2 mg/kg body weight (b.w.) (group I) and by gavage at a dose of 10 mg/kg b.w. (group III).

In groups II and IV (controls) the CIT (Lundbeck, Denmark) was administered *ip* at a dose of 2 mg/kg b.w. (group II) and by gavage at a dose of 10 mg/kg b.w. (group IV).

After the administration of CIT, the rats were anesthetized (xylocaine 40 mg/kg and ketamine 5 mg/kg). Blood samples from the jugular vein were collected into tubes without an anticoagulant at eight timepoints after *ip* administration – 0.25; 0.5; 1; 1.5; 2; 4; 8; and 24 h, and after intragastric administration - 0.33, 0.66, 1, 1.5, 2, 4, 8 and 24 h with three rats per point. Plasma was subsequently separated for further analysis.

The protocol for this animal experiment was approved by the Local Ethics Commission for Animal Studies in Poznań (No. 32/2008, June 20th 2008).

Analytics

CIT and DCIT were determined by high performance liquid chromatography with a diode array detector (HPLC-DAD) that had been developed previously [16].

Pharmacokinetic analysis

Due to the destructive nature of the sampling, the concentration-time data are extremely sparse, and as such they are information-poor. In order to yield as much information as possible, two different approaches were applied: non-compartmental analysis of naive averaged data [7, 12, 17], and compartmental modelling in the population pharmacokinetics [3, 7, 9, 14, 19] framework. The NCA is insensitive to model misspecification and is more robust to fluctuations in experimental data. On the other hand, PPK enables researchers to model all of the available data at once and to investigate and exploit relations between them. Also, if the model is correct, the parameter estimates will be more accurate. This dual approach to the field of bioequivalence testing was recently investigated by Dubois et al. [6].

Non-compartmental analysis

The non-compartmental approach determines the model-independent parameters of the pharmacokinetic profile including AUC. Where sacrifice sampling is concerned, however, several methods exist to handle such data [1]. We have used an approach described elsewhere by one of us [12] in order to compare the AUC of CIT as well as DCIT in animals exposed to the tobacco smoke with the controls.

Population compartmental modelling

The pharmacokinetic model

The one compartment open model with first order absorption of the parent drug and first order elimination of both CIT and DCIT was chosen (Fig. 1). The parameters of the model were as follows: V – volume of distribution, F – ratio of the dose D that is absorbed, k_a – first order absorption rate constant, k_{10} – first order elimination rate constant of CIT (excluding metabolism to DCIT), k_{20} – elimination rate constant of DCIT, k_{12} – CIT into DCIT metabolism rate constant.

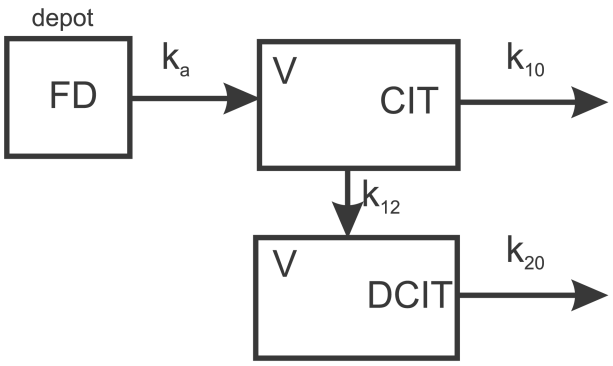

Fig. 1. The pharmacokinetic model for a drug-metabolite (CIT-DCIT) system. The two boxes to the right represent the same physical space, the central compartment. The two separate boxes were drawn to show the course of the drug and its metabolite

Because of the wide distribution and high volume of distribution, the two compartment model could seem more appropriate from a mechanistic point of view. However, from an empirical perspective, the one compartment model for CIT has been known to perform quite well in humans [5, 9]. The one compartment model is simpler, and has fewer parameters from which the estimates are more reliable; particularly for sparse data.

The biotransformation of CIT into DCIT is assumed to be a first order, irreversible process. The same model was used for both routes of administration. The F and k_a vary with the route, while the other model parameters are expected to be the same in both cases.

The system of differential equations describing the model is as follows:

$$\frac{dA_{CT}}{dt} = -\left(k_{10} + k_{12}\right)A_{CT} + k_a A_d$$

$$\frac{dA_{DCT}}{dt} = k_{12} A_{CT} - k_{20} A_{DCT}$$

$$\frac{dA_d}{dt} = -k_a A_d$$

where A_{CT} is an amount of CIT in a central compartment, and A_{DCT} is an amount of DCIT in that compartment. A_d is an amount of CIT in a dosing compartment (depot), and its initial value is FD, D being the CIT dose. All amounts should be expressed in molar units, otherwise an additional coefficient would be necessary. This is a linear system and a closed-form solution to it exists.

Mixed-effect population statistical model

Along with the pharmacokinetic model described above, the following mixed-effect model was assumed:

$$\log\left(V/F\right) = \log\left(V/F\right)_0 + \theta_{1s} smoke + \theta_{1r} route + \eta_{V/F}$$

$$\log k_a = \log\left(k_a\right)_0 + \theta_{2s} smoke + \theta_{2r} route + \eta_{ka}$$

$$\log k_{10} = \log\left(k_{10}\right)_0 + \theta_{3s} smoke + \eta_{\kappa 10}$$

$$\log k_{20} = \log\left(k_{20}\right)_0 + \theta_{4s} smoke + \eta_{\kappa 20}$$

$$\log k_{12} = \log\left(k_{12}\right)_0 + \theta_{5s} smoke + \eta_{\kappa 12}$$

where V/F is an individual value of the volume of distribution divided by F, the percentage of the dose that is absorbed. $(V/F)_0$ might be understood as a population-characteristic value of that parameter. In a similar manner, k_a is an individual value of a first order absorption rate constant, $(k_a)_0$ is a population-characteristic value of k_a, and so on. Indicator *smoke* equals 1 for animals exposed to the tobacco smoke and 0 otherwise. For intragastric administration, *route* = 0, and during the *ip* case, *route* = 1. θ_s and θ_r are the fixed-effect parameters. Quantities η are random inter-individual errors. Their statistical distribution is assumed to be multivariate normal with a zero mean and certain covariance matrix, Ω. Elements of this matrix are random-effect parameters of the model. Estimation of the fixed-effect and random-effect parameters is the main goal of PPK modelling. It is expected that rate constants k_{10}, k_{20} and k_{12} may depend only on the tobacco smoke, while the route of the administration may also influence the two remaining pharmacokinetic parameters: k_a and V/F.

Model validation

In order to reveal the potential model misspecification, simulated data were generated. The model parameter estimates were computed for both real and simulated data. The η-shrinkage for these parameters was computed to assess and compare the amount of information contained in these datasets [18]. The predictions *vs.* observations for both datasets were also plotted for diagnostic purposes [13].

Software

The NCA and statistical comparison was done with MathCad 11 software (MathSoft, Cambridge CA,

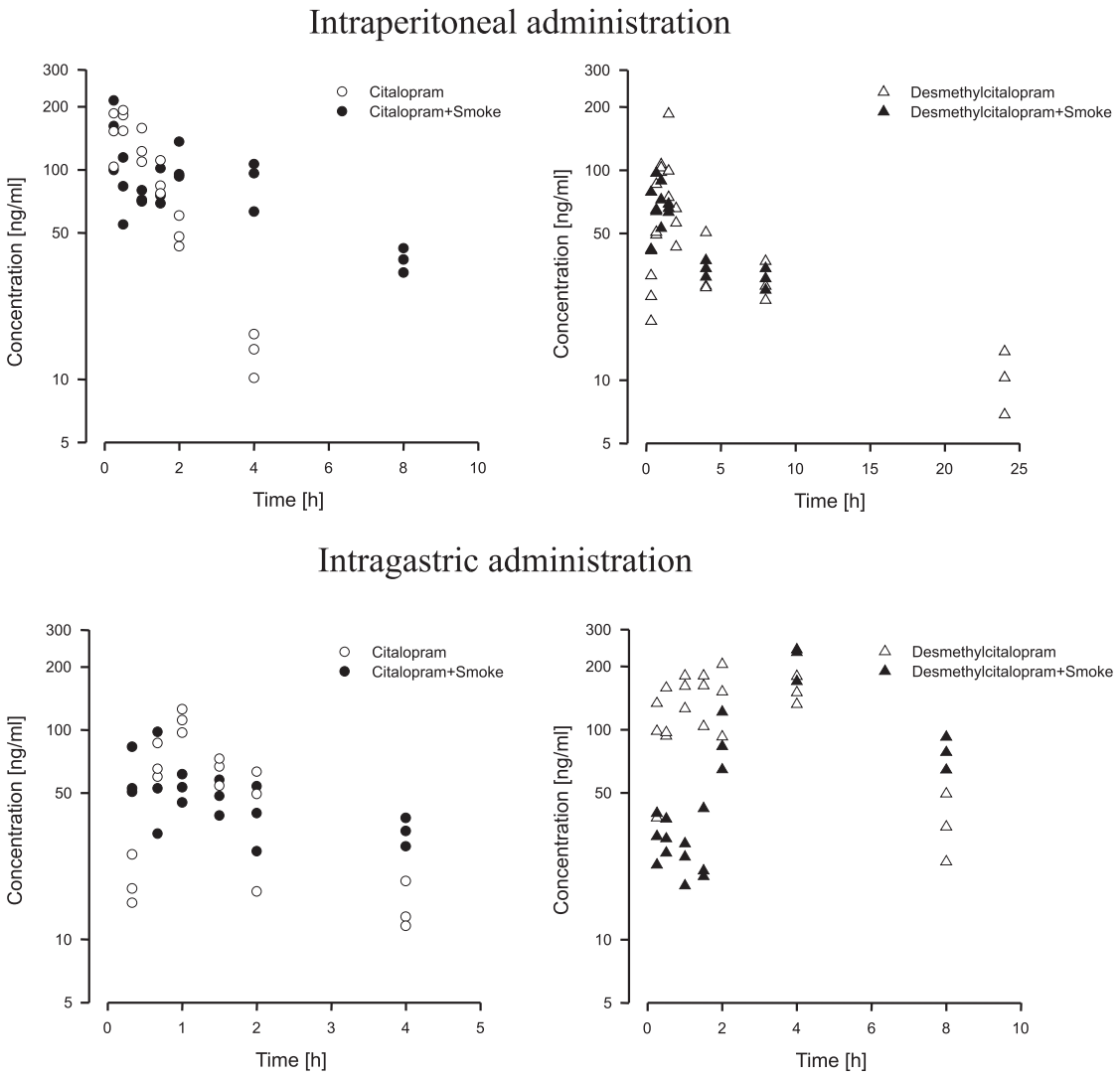

Fig. 2. Concentrations of CIT and DCIT in studied groups

USA). The MathCad spreadsheet developed by one of us [12] was applied for this purpose. The Monolix 3.2 program (developed by Inria, France) [14] was used for population pharmacokinetic modelling along with the related statistical analysis. Monolix runs were executed in a MATLAB 7.11 (R2010b) environment (MathWorks, Natick, MA, USA).

Results and Discussion

All measured concentrations of CIT and DCIT in the studied groups are presented in Figure 2. On each subplot data for animals exposed to tobacco smoke as well as for controls are depicted.

The results of NCA are compiled in Table 1. They include AUC values for each compound, route of administration and treatment. The p-values for statistical comparison between exposed and control groups are also given.

Table 2 contains estimates of PPK model parameters along with p-values indicating statistical significance of fixed effect parameters. In addition, the model diagnostic indicator, η-shrinkage of PK parameters for both real and simulated data is included.

The diagnostic plots of the predictions *vs.* observations are shown in Figure 3 to enable visual comparison of model performance for real and simulated data.

Tab. 1. Results of the non-compartmental analysis

Compound	Route[1]	$AUC_{0-\infty}$ (± SD) [μg h/l]		p
		Exposed to smoke	Control	
CIT	*ig*	506 (± 378)	203 (± 15)	0.24 NS
CIT	*ip*	795 (± 41)	309 (± 14)	< 0.001
DCIT	*ig*	1435 (± 901)	840 (± 81)	0.28 NS
DCIT	*ip*	1267 (± 75)	1037 (± 90)	0.042 < 0.05

[1] *ig* – intragastric, *ip*– intraperitoneal. $AUC_{0-\infty}$ – area-under-the curve estimated in the time range from 0 to infinity

Tab. 2. Estimates of the population pharmacokinetic model parameters

Parameter	Population mean (± SD)	$\theta_{.s}$		$\theta_{.r}$		η-shrinkage [%]	
		Value (± SD)	p	Value (± SD)	p	Real data	Simulated data
V/F [l/kg]	17.7 (± 3.7)	–0.883 (± 0.300)	0.003	–2.15 (± 0.14)	< 0.001	39	33
k_a [h^{-1}]	0.513 (± 0.092)	–1.12 (± 0.20)	< 0.001	0.235 (± 0.19)	0.22 NS	62	49
k_{10} [h^{-1}]	0.689 (± 0.240)	0.429 (± 0.500)	0.39 NS	Not applicable		57	38
k_{20} [h^{-1}]	0.690 (± 0.180)	0.187 (± 0.400)	0.64 NS			34	32
k_{12} [h^{-1}]	2.07 (± 0.38)	–0.107 (± 0.26)	0.68 NS			30	29

V – volume of distribution, F – ratio of the dose D that is absorbed, k_a – first order absorption rate constant, k_{10} – first order elimination rate constant of CIT (excluding metabolism to DCIT), k_{20} – elimination rate constant of DCIT, k_{12} – CIT into DCIT metabolism rate constant, $\theta_{.s}$ – fixed effect parameters for *smoke*, $\theta_{.r}$ – fixed effect parameters for *route*

The estimated mean AUC values for CIT as well as for DCIT were consistently higher in the group exposed to the tobacco smoke, although in the case of intragastric administration a high variability in the treatment group precluded the statistical significance of the results.

In turn, statistically significant results obtained in the framework of the population pharmacokinetic analysis apply only to the parameters of the absorption process, *V/F* and k_a. *V/F* is expected to be significantly lower in the subjects exposed to the tobacco smoke. Since the volume of distribution is assumed as not being dependent on the smoke exposure, this indicates that *F* is greater in the exposed group than with the control. *F* is also significantly lower where intragastric administration is concerned. The absorption rate constant was significantly lower in the treatment group.

It appears that tobacco smoke slows down the absorption of the CIT and at the same time makes it more effective.

High shrinkage values for the model parameters confirm that there is not very much information on these parameters contained in the experimental data. On the other hand, shrinkage for the simulated data was slightly lower. This might indicate a moderate discrepancy between the model and experimental data.

In the case of high shrinkage, Karlsson and Savic recommend the comparison of patterns in predictions *vs.* observations for real and simulated data [13], instead of using real data plots only as diagnostics. There were no apparent differences in these patterns for CIT as well as for DCIT (Fig. 3). Also, other diagnostics, such as the normalized prediction distribution error (NPDE [13], is not shown due to the space limitation) did not yield any clear evidence of model misspecification.

Despite the ultra-sparse design, the NLME approach enabled us to develop a pharmacostatistic model with the required covariates. All of the available data, CIT and DCIT concentrations from the

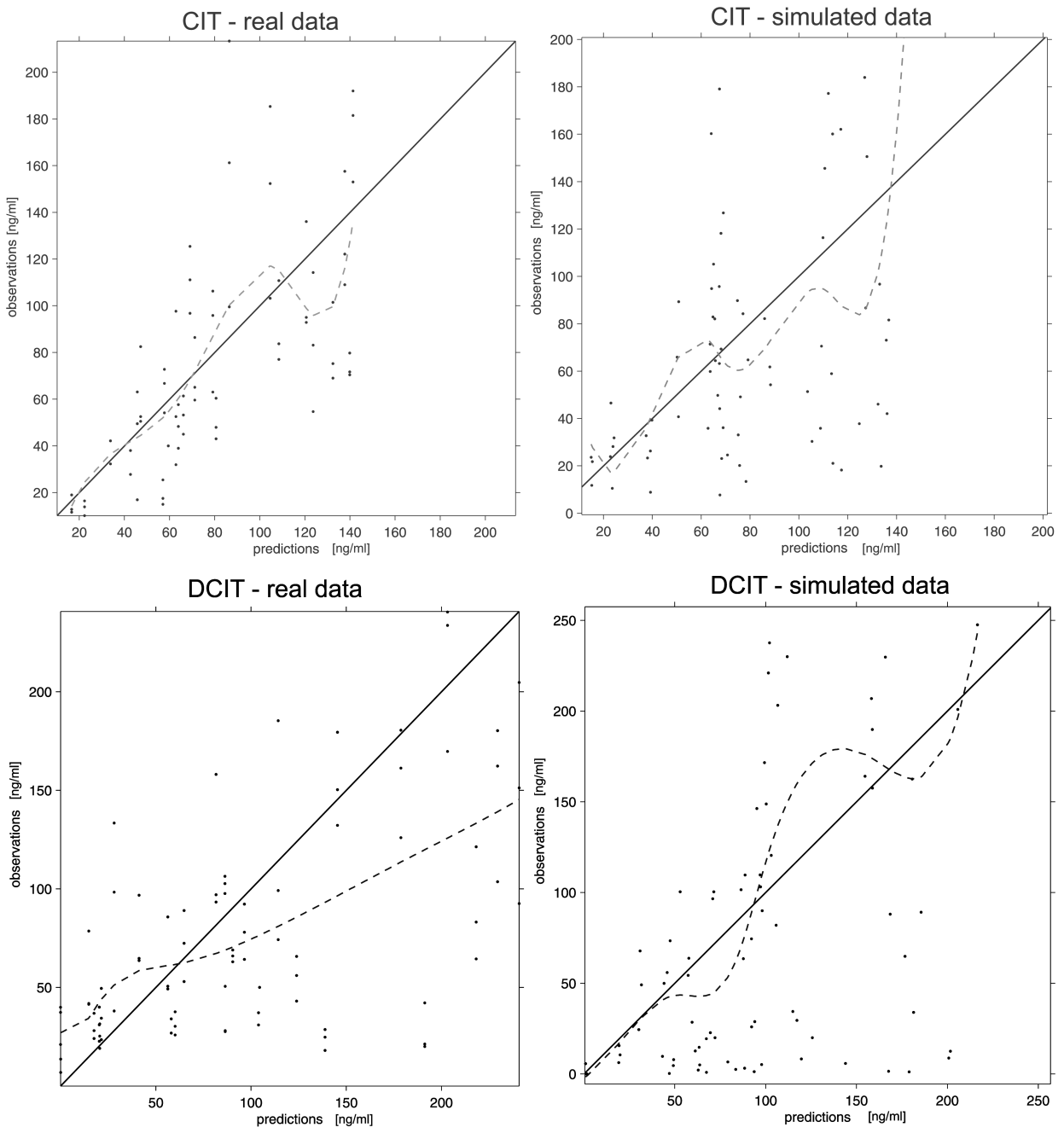

Fig. 3. Observed *vs.* predicted concentrations of CIT and DCIT for real and simulated data

treatment and control groups with both routes of administration, has been analyzed in the framework of one common model. While by no means perfect, this model appeared to be a useful approximation of reality. Consistent results obtained with the model-independent analysis decreased the risk of model misspecification and increased confidence in the final conclusions. Combining NLME with NCA may therefore be recommended for investigating pharmacokinetic properties of the drug in the sparse designs.

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References:

1. Bailer AJ: Testing for the equality of area under the curves when using destructive measurement techniques. J Pharmacokin Biopharm, 1988, 16, 303–309.
2. Baumann P, Larsen F: The pharmacokinetics of citalopram. Rev Contempor Pharmacother, 1995, 6, 287–295.
3. Beal SL, Sheiner LB: NONMEM User's Guide, University of California, San Francisco, 1989.
4. Bezchlibnyk-Butler K, Aleksic I, Kennedy SH: Citalopram – a review of pharmacological and clinical effects. J Psychiatry Neurosci, 2000, 25, 241–254.
5. Bies RR, Feng Y, Lotrich FE, Kirshner MA, Roose S, Kupfer DJ, Pollock BG: Utility of sparse concentration sampling for citalopram in elderly clinical trial subjects. J Clin Pharmacol, 2004, 44, 1352–1359.
6. Dubois A, Gsteiger S, Pigeolet E, Mentré F: Bioequivalence tests based on individual estimates using non-compartmental or model-based analyses: Evaluation of estimates of sample means and type I error for different designs. Pharm Res, 2010, 27, 92–104.
7. Ette EI, Williams PJ, Ahmad A: Population pharmacokinetic estimation methods. In: Pharmacometrics: The Science of Quantitative Pharmacology. Eds. Ette EI and Williams PJ, John Wiley & Sons, Hoboken, NJ, USA, 2006.
8. Florek E, Ignatowicz E, Piekoszewski W: Effect of pregnancy and tobacco smoke on the antioxidant activity of rutin in an animal model. Pharmacol Rep, 2009, 61, 935–940.
9. Friberg LE, Isbister GK, Hackett LP, Duffull SB: The population pharmacokinetics of citalopram after deliberate self-poisoning: a Bayesian approach. J Pharmacokin Pharmacodyn, 2005, 32, 571–605.
10. Garside D: Selective serotonin reuptake inhibitors are diverse group. Clin Toxicol, 2005, 5, 1–8.
11. Gutierrez MM, Abramowitz W: Pharmacokinetic comparison of oral solution and tablet formulations of citalopram: a single-dose, randomized, crossover study. Clin Ther, 2000, 22, 1525–1532.
12. Jawien W: Variability of the model independent AUC: The one sample per individual case. J Pharmacokin Biopharm, 1999, 27, 437–464.
13. Karlsson MO, Savic RM: Diagnosing model diagnostics. Clin Pharmacol Ther, 2007, 82, 17–20.
14. Lavielle M, Mentré F: Estimation of population pharmacokinetic parameters of saquinavir in HIV patients with the MONOLIX software. J Pharmacokin Pharmacodyn, 2007, 34, 229–249.
15. Majcherczyk J, Kulza M, Senczuk-Przybylowska M, Jawien W, Piekoszewski W, Florek E: The influence of tobacco smoke on pharmacokinetics of the enantiomers of citalopram (Polish). Przegl Lek, 2011, 68, 714–718
16. Majcherczyk J, Kulza M: Determination of citalopram and its enantiomers by means of chromatographic techniques. Problems Forensic Sci, 2002, 83, 68–77.
17. Muir KT, Gomeni RO: Non-compartmental analysis. In: Pharmacokinetics in Drug Development. Clinical Study Design and Analysis. Eds. Bonate PL, Howard DR, AAPS, Arlington, VA, USA, 2004, 235–265.
18. Savic RM, Karlsson MO: Importance of shrinkage in empirical Bayes estimates for diagnostics: problems and solutions. AAPS J, 2009, 11, 558–569.
19. Wiczling P, Bienert A, Sobczyński P, Hartmann-Sobczyńska R, Bieda K, Marcinkowska A, Malatyńska M et al.: Pharmacokinetics and pharmacodynamics of propofol in patients undergoing abdominal aortic surgery. Pharmacol Rep, 2012, 64, 113–122.

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