POPULATION PHARMACOKINETICS WITH A VERY SMALL SAMPLE SIZE

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SUMMARY

The objective of this study was to evaluate whether pharmacokinetic parameters (clearance and volume of distribution of the central compartment) from a sparse sampling population pharmacokinetic study can be obtained with a very small sample size. For this study, three drugs were selected from the literature. The pharmacokinetics of all three drugs were studied in healthy adult subjects and plasma concentrations versus time data for individual subjects from extensive blood sampling were available. For population PK analysis, only five subjects were chosen and each subject gave either one or two blood samples. The estimated PK parameters from population PK analysis were compared with the PK parameters obtained from extensive sampling. The results of the study indicated that a reasonable estimate of PK parameters can be obtained with two blood samples from each subject with a sample size of five. The population PK study with

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sparse sampling scheme may be useful for PK studies in neonates and very young children and in subjects with rare diseases where sample size is small.

KEY WORDS

population pharmacokinetics, sample size, clearance, volume of distribution, sparse sampling

INTRODUCTION

One of the most critical aspects of a pharmacokinetic (PK) study is blood sampling. Under normal circumstances it is possible to draw frequent blood samples from subjects and, depending on the route of administration, the sampling scheme includes absorption, distribution and elimination phases. There are, however, situations under which frequent blood sampling is not practical (critically ill patients, neonates and very young children, rare diseases, and elderly subjects). Under these circumstances, a limited number of blood samples (also known as sparse sampling) can be taken from subjects for the PK assessment.

Sparse-sampling population pharmacokinetics (POPPK) is one of the most suitable approaches that can be applied to estimate PK parameters. The POPPK approach can accommodate sparse and unbalanced PK data. POPPK quantifies population mean, inter-individual variability, residual variability and inter-occasion variability /1/. POPPK also seeks to identify the impact of certain covariates (such as weight, age, gender, concomitant medications, and disease states) on the estimated PK parameters /1/. For POPPK analysis, since the number of blood samples is sparse, it is widely believed that a large number of subjects is required for accurate estimation of pharmacokinetic parameters. As mentioned earlier, there are situations where frequent blood sampling may not be possible and yet estimation of PK parameters, especially clearance, is required for appropriate dosing. There are several situations where not only frequent blood sampling is not possible but sample size is also very small. These situations include neonates, infants, and rare diseases.

A rare (or orphan) disease is generally considered to have a prevalence of fewer than 200,000 affected individuals in the United States. Certain diseases with 200,000 or more affected individuals may be included in this list if certain subpopulations of people who have the disease are equal to the prevalence standard for rare diseases /2/. Rare disease can vary in prevalence between populations, so a disease that is rare in some populations may be common in others. This is especially true of genetic diseases and infectious diseases. An example is cystic fibrosis, a genetic disease. It is rare in most parts of Asia but relatively common in Europe and in populations of European descent. Many infectious diseases are prevalent in a given geographic area but rare everywhere else. Other diseases, such as many rare forms of cancer, have no apparent pattern of distribution but are simply rare.

Considering that under certain conditions there will be only few subjects (or children) available for POPPK study and frequent blood samples will not be available, the objective of this study was to evaluate whether one or two blood samples each from five subjects can be used to accurately estimate the clearance and volume of distribution of the central compartment for a given drug. The selection of a 5-subject sample size with one or two blood samples from each of the subjects is to emphasize the minimum requirements for a POPPK study. Nonetheless, it should be noted that it is highly likely that the data from more than five subjects with more than one sample may be available.

METHODS

Datasets

For this study, three drugs (cefodizime /4/, bisoprolol /5/, and nifedipine /6/) were selected from the literature. The pharmacokinetics of all three drugs were studied in healthy adult subjects and plasma concentrations versus time data for individual subjects from extensive blood sampling were available (Table 1). The total number of blood samples (extensive sampling) drawn from each subject for cefodizime, bisoprolol, and nifedipine was 9, 15, and 11, respectively. Cefodizime was given as an intravenous bolus injection whereas bisoprolol, and nifedipine were given orally. With extensive sampling, cefodizime followed a two-compartment model whereas bisoprolol and nifedipine

TABLE 1

Blood sampling scheme for three drugs for population pharmacokinetic analysis

Drug	Characteristics
Cefodizime	Extensive sampling = 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 hours
	1 sample at = 0.5, 1, 4, 8, 12 hours
	Intravenous administration
	Body weight range = 62 to 74.5 kg
Bisoprolol	Extensive sampling = 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, 24, 28, 32, 36 hours
	1 sample at = 1, 4, 12, 24, 36 hours
	Oral administration
	Body weight range = 66 to 73 kg
Nifedipine	Extensive sampling = 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 24 hours
	1 sample at = 0.5, 1, 4, 8, 24 hours
	Oral administration
	Body weight range = 58 to 74.8 kg

Body weight range is only for those five subjects who were included in the study.

followed a one-compartment model. The pharmacokinetic parameters thus obtained served as the observed values.

Population pharmacokinetic analysis

For population PK analysis, only five subjects were chosen and each subject gave either one or two blood samples. The subjects were selected randomly and blood samples from each subject were taken in a way that they covered the entire range of concentration vs time data profile from extensive sampling (from the beginning till the end of the sampling scheme in the extensive sampling profile).

The population analysis was performed using Nonlinear Mixed Effect Modeling (NONMEM), version VI 2.0 (GloboMax, ICON Development Solutions, Ellicott City, MD). PK parameters (clearance and volume of distribution of the central compartment) were estimated in the subjects using sparse sampling population as well as Bayesian (POSTHOC) approach. Initially, the structural model was developed without any covariate. Random effects for pharmacokinetic parameters were hypothesized to follow log-normal distribution as shown in the following equation:

$$P_j = P_{typ} \exp(\eta_j)$$

where P_{typ} is the population estimate of the parameter and P_j is the parameter from the j^{th} subject. η_j is interindividual error distributed with a mean of zero and a variance of ω^2.

Residual variability was examined using a proportional error model as in the equation:

$$C_{ij} = C^*_{ij} (1 + \xi_{ij})$$

where C_{ij} and C^*_{ij} are the observed and model predicted i^{th} concentrations in the j^{th} individual, and ξ_{ij} is the error term showing the differences between model predicted and observed i^{th} plasma concentrations in the j^{th} subject. ξ_{ij} is distributed with a mean of zero and a variance of σ^2.

The influence of body weight as a covariate on the pharmacokinetics of all three drugs was evaluated. Visual inspection of predicted and observed concentrations, precision of parameter estimates and estimates of random residual variances for the parameters were used to guide model development. A drop in objective function value of 3.8 ($p = 0.05$ at 1 degree of freedom) was used to discriminate between a structural model and a model with weight as a covariate. The estimated pharmacokinetic parameters using sparse sampling (one or two blood samples) were compared with the pharmacokinetic parameters obtained by extensive blood sampling (observed value) from the same five subjects.

WinNonlin analysis

Besides NONMEM, WinNonlin was also used to determine PK parameters from five subjects with one or two blood samples taken at one or two different time points. Individual subjects' concentration vs time data over a given time period were provided to WinNonlin for fitting. The intention was to evaluate whether the program can provide a reasonable estimate of PK parameters assuming that 5 or 10 concentrations at different time points were obtained from the same subject.

RESULTS

In Table 1, the name of the drugs, blood sampling scheme and demographic information is provided. The mean population PK parameters, individual PK parameters from POSTHOC, PK parameters from WinNonlin, and predicted vs observed plasma concentrations from NONMEM and WinNonlin are presented in Tables 2-7. A one-compartment model adequately described the plasma concentration vs time data for bisoprolol, and nifedipine, while a two-compartment PK model provided the best fit for cefodizime plasma concentration vs time data. Body weight as a covariate was only noted for bisoprolol. Inclusion of body weight as a covariate reduced the objective function for bisoprolol from 4.86 to 0.83 (for one sample per subject). However, with two blood samples, the objective function was not different with or without body weight.

Mean population clearance and volume of distribution obtained from one or two blood samples each from five subjects were comparable with the observed values obtained from the extensive blood sampling (Tables 2, 4, and 6). However, the mean population PK parameters obtained from two blood samples from each subject provided a better estimate of PK parameters than one blood sample population PK analysis.

The mean population clearance values for all three drugs using WinNonlin, from either one or two blood samples, were more accurate than NONMEM (Tables 2, 4, and 6). There was no difference in accuracy of clearance estimation between one or two blood samples when WinNonlin was used. On the other hand, the volume of distribution of the central compartment was estimated with less

TABLE 2

Cefodizime population mean (5 samples)

Parameters	Observed	Without wt	With wt	WinNONLIN
1 blood sample				
Objective function	NA	-23.62	0.874	NA
Clearance (l/h)	3.21	3.38	3.27	3.20
V_C (l)	4.92	4.78	3.32	4.79
Half-life (h)	2.76	NA	NA	3.32
2 blood samples				
Objective function	NA	29.07	33.76	NA
Clearance (l/h)	3.21	3.32	3.07	3.31
V_C (l)	4.92	5.59	5.78	4.65
Half-life (h)	2.76	NA	NA	3.01

NA = not applicable.

accuracy by WinNonlin than NONMEM. The volume of distribution was estimated more accurately by two rather than one blood sample by both NONMEM and WinNonlin. The percent prediction error in mean clearance and volume of distribution by one or two blood samples using POPPK approach and WinNONLIN is shown in Table 8.

Overall, the results of the study suggest that two blood samples taken from five subjects can provide a reasonably accurate estimate of mean population clearance and volume of distribution. However, individual PK parameters obtained from POSTHOC may not be accurate.

TABLE 3

Cefodizime individual pharmacokinetic parameters and predicted concentrations from POSTHOC

Obs CL (l/h)	Pred CL (l/h)	Obs Vc (l)	Pred Vc (l)	Obs Conc	Pred Conc
1 blood sample					
2.84	3.38	4.81	4.78	115	114
3.32	3.38	5.61	4.78	70	69
3.56	3.38	5.02	4.78	19	18
3.38	3.38	4.84	4.78	8	6.9
2.94	3.38	4.35	4.78	3.6	2.7
Mean = 3.21	**Mean = 3.38**	**Mean = 4.92**	**Mean = 4.78**		
2 blood samples					
2.84	3.22	4.81	5.28	115, 82	114, 81
3.32	3.24	5.61	6.56	98.5, 70	97.5, 69
3.56	3.19	5.02	3.92	31.8, 19	30.8, 18
3.38	3.29	4.84	6.53	36, 20	35, 19
2.94	3.21	4.35	6.62	9, 3.6	8, 2.6
Mean = 3.21	**Mean = 3.23**	**Mean = 4.92**	**Mean = 5.78**		

Obs = observed; Pred = predicted; Conc = concentration (μg/ml).

TABLE 4

Bisoprolol population mean

Parameters	Observed	Without wt	With wt	WinNONLIN
1 blood sample				
Objective function	NA	4.86	0.833	NA
Clearance (l/h)	17.6	18.7	21.8	17.3
V_C (l)	269	318	277	283
Half-life (h)	10.5	NA	NA	11.3
2 blood samples				
Objective function	NA	20.58	21.05	NA
Clearance (l/h)	17.6	19.1	21.2	17.1
V_C (l)	269	231	229	269
Half-life (h)	10.5	NA	NA	10.9

NA = not applicable.

TABLE 5

Bisoprolol individual pharmacokinetic parameters and predicted concentrations from POSTHOC

Obs CL (l/h)	**Pred CL** (l/h)	**Obs Vc** (l)	**Pred Vc** (l)	**Obs Conc**	**Pred Conc**
1 blood sample					
17.6	18.0	229	318	18.6	17.6
18.7	20.0	432	318	26.3	25.3
16.7	12.5	202	318	21.6	20.6
16.8	23.4	183	318	6.86	5.86
18.5	22.0	300	318	3.83	2.83
Mean = 17.6	**Mean = 19.2**	**Mean = 269**	**Mean = 318**		
2 blood samples					
17.6	18.8	229	195	18.6, 30.5	18.7, 28.5
18.7	19.7	432	287	26.3, 24.9	25.0, 24.7
16.7	18.1	202	222	28.0, 21.6	27.0, 20.1
16.8	20.4	183	213	10.9, 6.86	10.4, 5.87
18.5	18.4	300	245	7.54, 3.83	5.86, 3.21
Mean = 17.6	**Mean = 19.1**	**Mean = 269**	**Mean = 232**		

Obs = observed; Pred = predicted; Conc = concentration (μg/ml).

TABLE 6

Nifedipine population mean

Parameters	Observed	Without wt	With wt	WinNONLIN
1 blood sample				
Objective function	NA	5.950	5.646	NA
Clearance (l/h)	63.6	85.3	80.3	67.1
V_C (l)	790	993	1040	1207
Half-life (h)	9.1	NA	NA	12.5
2 blood samples				
Objective function	NA	13.0	12.4	NA
Clearance (l/h)	63.6	52.3	55.6	60.5
V_C (l)	790	869	869	1087
Half-life (h)	9.1	NA	NA	12.4

NA = not applicable.

TABLE 7

Nifedipine individual PK parameters and predicted concentrations from POSTHOC

Obs CL (l/h)	**Pred CL** (l/h)	**Obs Vc** (l)	**Pred Vc** (l)	**Obs Conc**	**Pred Conc**
1 blood sample					
54.6	91.4	551	993	5.2	4.62
42.1	74.8	806	992	9.3	8.03
58	66.0	976	992	16.0	14.93
98	161.8	984	993	8.4	7.46
65.3	64.0	632	993	5.9	4.87
Mean = 63.6	**Mean = 91.6**	**Mean = 790**	**Mean = 993**		
2 blood samples					
54.6	62.2	551	869	5.2, 7.3	3.7, 6.7
42.1	19.8	806	869	12.5, 16.2	11.5, 14.6
58	36.1	976	869	16.0, 18.5	15.9, 16.8
98	178	984	869	10.3, 8.4	9.43, 7.3
65.3	64.8	632	869	13.9, 5.9	12.9, 4.8
Mean = 63.6	**Mean = 72.2**	**Mean = 790**	**Mean = 869**		

Obs = observed; Pred = predicted; Conc = concentration (μg/ml).

TABLE 8

Percent prediction error in mean clearance and volume of distribution by one or two blood samples using POPPK approach and WinNONLIN

Drug	Population PK		WinNONLIN	
	One sample	Two samples	One sample	Two samples
Cifodezime				
Clearance	5	3	0	1
Volume	3	14	3	5
Bisoprolol				
Clearance	24	20	2	3
Volume	3	15	5	0
Nifedipine				
Clearance	26	13	6	5
Volume	32	10	53	38

DISCUSSION

To the best of our knowledge, a population PK analysis with such a small sample size has not been previously reported. The main objective of this study was to evaluate whether or not a limited number of blood samples obtained from a limited number of subjects (in this case only one or two blood samples from each subject with a total of five subjects) can be used to estimate clearance and volume of distribution using a population PK approach. Although population PK studies are conducted with sparse blood samples but with a large sample size, there may be situations (as mentioned earlier) where only a limited number of plasma concentration vs time data will be available for PK analysis. Under these circumstances, it becomes necessary to determine whether a relatively small sample size can be

used for accurate estimation of clearance and volume of distribution. The results of this study indicate that a population PK analysis from five subjects and two blood samples from each of the five subjects is possible. The mean population clearance and volume of distribution values can be reasonably accurately obtained from such a limited number of data. However, it is important to indicate that prior knowledge of the pharmacokinetics of the drug of interest is essential due to the following two reasons. One is that there is no way to determine the appropriateness of the structure model with such a small sample size. One has to be aware of the basic structure model before using this approach. The second is that the parameter estimates are significantly influenced by the initial estimates. If one has no prior PK knowledge of the drug of interest, inappropriately selected initial estimates may lead to considerably biased results.

Not only the population means, but also the individual parameter estimates can be obtained using the POSTHOC command in NONMEM. This is a major advantage of POPPK. However, under certain circumstances, this advantage may not be shown, such as in the case of cefodizime (one sample per subject). The estimated inter-subject variability was so small (at 0.001% level) that the individual parameter estimates could not show appreciable differences among the five subjects (all five subjects had the same clearance values). This means that in some cases the NONMEM program may not have enough information to estimate the inter-subject variability properly with such a small sample size. In this case, the population method is practically equivalent to the NPD (naïve pool data) approach /7/. It should be emphasized that in the case of such a small sample size the only advantage of NONMEM over WinNONLIN is that NONMEM can generate individual parameters whereas WinNONLIN only gives a mean value which will be comparable to the mean values obtained from NONMEM. One of the interesting observations of this study was that WinNONLIN-predicted clearance for all three drugs was superior to NONMEM-predicted clearance from one or two blood samples (Table 8). However, volume of distribution was predicted more accurately by NONMEM than WinNonlin.

Body weight did not show significance in two out of three cases of this study (except bisoprolol one sample per subject) and this can be explained by the small sample size and narrow body weight range. It

should be noted, however, that the 2-blood samples model did not show the influence of body weight on PK parameters for bisoprolol.

CONCLUSIONS

- This untraditional population PK study demonstrates that it is possible to estimate clearance and volume of distribution of the central compartment with reasonable accuracy from as few as five subjects and one blood sample from each subject.
- The accuracy of estimation for clearance and volume of distribution of the central compartment can be improved by taking two rather than one blood sample from each subject.
- Due to the small sample size the variability of the estimated PK parameter cannot be accurately estimated by NONMEM.
- It appears that individual subjects' clearance and volume of distribution cannot be reliably estimated by NONMEM (POST HOC approach) with such a small sample size.
- With a small sample size and one or two blood samples, mean population clearance and volume of distribution can be reasonably accurately estimated from WinNonlin. For clearance, one blood sample from five subjects provides a fairly accurate estimate but two blood samples will be required to estimate volume of distribution more accurately.
- WinNonlin provides a more accurate estimate of clearance from five subjects' data (irrespective of one or two blood samples) than NONMEM but volume of distribution is estimated more accurately by NONMEM than by WinNonlin.
- It should be noted that this study evaluated the performance of NONMEM and WinNonlin for the estimation of clearance and volume of distribution using a minimum number of five subjects and one or two blood samples, but in real life the data from more than five subjects and more than two blood samples may be available.
- The theme of this study is applicable and useful for the estimation of clearance and volume in children, especially neonates and infants, as well as critically ill patients where sample size and

number of blood samples can be issues. Further studies should be conducted in this direction.

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