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Research Article

EVALUATION OF COVARIATES INFLUENCE ON EUPRESSYL PHARMACOKINETICS USING LINEAR REGRESSION MODEL

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ABSTRACT
The identification and quantification of covariates, particularly using population pharmacokinetics is now seen as an integral part of drug development. This present study was aimed to assess the influence of subject specific parameters (covariates) on pharmacokinetics of Eupressyl (Urapidil) from typical pharmacokinetics studies. The influence of covariates (age, height, body weight, body mass index) on the pharmacokinetics of Urapidil was evaluated analyzing the data pooled from three different pharmacokinetic studies. The influence of covariates on Urapidil pharmacokinetics was evaluated using linear mixed effect model. Covariate analysis was carried out following a two-stage approach. Results from the first stage analyses showed that there is no significant effect ($P > 0.05$) on Urapidil pharmacokinetic parameters against evaluated covariates. However, at second stage following linear mixed effect models, subject specific parameters were correlated with obtained pharmacokinetic parameters. The results evidencing that the reasonable influence of covariates on Urapidil pharmacokinetics parameters were observed for different lot of innovator products. Thus, characterizing effect of few of the covariates on pharmacokinetics outcome will definitely reduce the number of pharmacokinetic studies using healthy human subjects and also development time and cost in generic drug developments. However, further pharmacokinetic models for Urapidil to be developed and validated using non-linear mixed effectmodels, as it is considered one of the standard method for evaluating drug variability.
Keywords: Covariates, Eupressyl, Linear regression model, Variability

INTRODUCTION

Variability in the design and analysis of bioequivalence studies has been the topic of discussion for many years[1]. Variability is an inherent property and it can be decreased or theoretically eliminated by implementing "ideal" experiments and data-processing techniques[2]. All drugs exhibit between-subject variability in exposure and response and many studies performed during drug development are aimed at identifying and quantifying this variability to improve the safety and efficacy of a drug agent. Variability is usually characterized in terms of fixed and random effects. The fixed effects are the population average values of pharmacokinetic parameters. The random effects quantify the amount of pharmacokinetic variability that is not explained by the fixed effects[3]. A number of factors can contribute to high variability in bioequivalence parameters[4]. The formulation factors that may impact on bioavailability and bioequivalence can be classified into two categories: (a) In the first group belong factors that can affect drug dissolution or release which is considered as a prerequisite to the drug absorption process. (b) The second category comprises factors related to excipients or inactive ingredients which can influence drug stability, absorption and metabolism[5]. The variability is an inherent property of the system of interest; it can be observed and recorded but not changed. Accordingly, uncertainty in the information available can be decreased and theoretically eliminated by implementing "ideal" experiments and data-processing techniques[6]. Hence, the identification and quantification of covariates, particularly using population pharmacokineticsis now seen as an integral part of drug development. However, many pharmaceutical companies go through unnecessary cycles of clinical studies involving formulation optimization without attention to the feasibility of reducing inter-individual variability and the source of such variation[7]. The *in-vivo* absorbability of drugs categorized as BCS Class II is very difficult to predict because of the large variability in the absorption or dissolution kinetics[8]. Urapidil comes under the category of BCS Class II. The pharmacokinetic parameters are well defined after oral or intra-venous administration. Pharmacokinetic parameters after oral and intra-venous administration are similar and are linearly proportional to dose. In summary, the plasma concentration decreases for 10 minutes and then remains at that level for about 1 hour. The mean serum half-life of elimination is 2.7 hours. The plasma protein binding is 80 %[9]. This present study was aimed to assess the influence of subject specific parameters (covariates) on pharmacokinetics of Urapidil from typical pharmacokinetics studies.

MATERIALS AND METHODS

All materials used in this study were complied with current European Pharmacopoeia compendial specifications.

Formulations

Formulation 1: Eupressyl 60 mg (Urapidil Retard Capsules 60 mg), Manufactured by Altana Pharma, France, Lot no.126662.
Formulation 2: Eupressyl 60 mg (Urapidil Retard Capsules 60 mg), Manufactured by Altana Pharma, France, Lot no.113494.

Pharmacokinetic studies

An open-label, randomized, fasting, single-dose, parallel/two way crossover studies were performed with 30 healthy, non-smoking, male subjects. The study protocols for Urapidil were approved by the Institutional Review Board at the clinical site (approval no. 12-09/2009/BE EXP/LUPIN-28/DC)[10]. Written informed consent was obtained from all subjects prior to enrolment in the study. A total of 30 healthy adult human male subjects (10 subjects each) were enrolled in 3 different studies (study 1-3). In each study period, after an overnight fast of at least 10 h, single oral dose of Urapidil Retard Capsules 60 mg was orally administered with 240 mL of drinking water in sitting posture at ambient temperature in the morning, as per the randomization schedule. In each period, 21 blood samples were collected. The pre-dose blood sample (1 x 5-mL) was collected within 1 hour prior to dosing. The post-dose blood samples (1 × 5-mL each) were collected at 1.00, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, 20.00, 24.00, 30.00 and 36.00 hours after dosing. Subjects were seated upright for the first two hours following drug administration and prohibited from any strenuous or athletic activity during housing period of the study. Plasma samples were stored at -80^{o}C before analysis. Plasma samples were separated and analyzed for Urapidil plasma concentrations.

Analytical methods

The in-house validated UPLC-MS/MS method was applied to determine the concentration of Urapidil in human plasma using Urapidil D4 as internal standard. The method was validated for selectivity, linearity, reproducibility, recovery, precision accuracy and stability. The study sample analysis was performed on UPLC-MS/MS system using Betasil C 18 (50 x 4.6 mm, 5 µ). Electrospray ionization was carried out in positive ion mode $[M + H]^+$ using triple quadruple mass spectrometer and multiple reaction monitoring (MRM) transition used for detection of Urapidil and Urapidil D4 were m/z 388.3/190.1 and 392.3/190.1, respectively. The data was acquired and calculated by using Masslynx version 4.1 Software. The slope, intercept and correlation of coefficient were determined by least squares linear regression with 1/ x 2 (1/conc2) weighting for the calibration curve standards. The measured concentrations for each subject for all the time points are calculated against the calibration curve prepared with known standards.

Pharmacokinetic data analysis

The Urapidil plasma concentration versus time data were evaluated using the WinNonlin software version 5.3. Pharmacokinetic parameters C_{max}, the maximum observed concentration, $AUC_{0-\infty}$, Area under the plasma concentration versus time curve from time 0 to infinity, AUC, the area under the concentration time curve, CL/F, total body clearance, Vd/F, volume of distribution and $t_{1/2}$, half-life of the drug were determined for each subject and formulation.

Analysis of covariates

The influence of covariates (age, height, body weight, body mass index) on the pharmacokinetics of Urapidil was evaluated analyzing the data from three different pharmacokinetic studies. The final data for covariate analysis includes 30 south Asians, adult male healthy subjects. Covariate analysis was carried out following a two-stage approach. At first stage, individual pharmacokinetic parameters (C_{max}, AUC_{inf}, CL/F, Vd/F and $t_{1/2}$) for Urapidil were calculated. The relationships between log transformed pharmacokinetic parameters versus effect of covariates were analyzed using linear mixed effects model. The linear mixed effects models were carried out using WinNonlin Software version 5.3. In the model definition, pharmacokinetic parameters used as categorical independent variables and covariates were used as continuous independent variables.

RESULTS AND DISCUSSION

Subjects' baseline characteristics are presented in Table 1. Samples from all 30 subjects (20 subjects from parallel studies and 10 subjects from cross over study) were analyzed to determine the plasma concentrations of Urapidil. Pharmacokinetic and statistical analyses were performed on data obtained from 30 subjects, who completed the studies as per the protocol. The pharmacokinetic parameters C_{max}, AUC, AUC_{inf}, CL/F, Vd/F, K_{el} and $t_{1/2}$ for Urapidil were calculated by non-compartmental method using Win Nonlin Professional Software (Version 5.3). Mean pharmacokinetic parameters are presented in Table 2.

Evaluation of covariates influence on Urapidil pharmacokinetics

The Linear Mixed Effects function is a statistical analysis system for analysis of variance for crossover and parallel studies, including unbalanced designs[11] and performs analyses using linear mixed effects models. A two-stage pharmacokinetic analysis approach was used in this study to evaluate the covariates influence on pharmacokinetics of Urapidil. Results from the first stage analyses showed that there is no significant effect ($P > 0.05$) on Urapidil pharmacokinetic parameters against evaluated covariates. However, obtained inter and intra-subject coefficient of variation for pharmacokinetic parameters C_{max} and AUC from parallel and two way cross-over study indicates that significant influence of covariates effect on Urapidil pharmacokinetics is expected, though obtained P value is not statistically significant in the regression model (Table 3). At second stage, using linear mixed effect models, subject specific parameters were correlated with obtained pharmacokinetic parameters. From the observed results R^2 values associated with linear regression model were found less (i.e., 0.0012 to 0.0137) for age effect and (i.e., 0.0013 to 0.0406) for body weight effect, respectively. The p-values associated with each covariate from log-linear regression analysis are presented in Table 4. The effect of age and body weight on the rate and systemic exposure of Urapidil following single dose administration is shown in Figure 1 and 2.

Table 1: Subjects' baseline characteristics

Parameters	Study 1 (N = 10)	Study 2 (N = 10)	Study 3 (N = 10)
	Mean ± SD (Range)		
Age (years)	30.50 ± 5.16 (25-38)	27.80 ± 4.13 (22-34)	31.70 ± 5.53 (20-39)
BMI (kg/m^2)	20.81 ± 2.24 (18-24)	21.58 ± 2.11 (18-24)	21.68 ± 1.72 (19-24)
Height (cm)	166.90 ± 7.90 (154-176)	166.25 ± 3.79 (161-172)	167.80 ± 4.96 (160-176)
Weight (kg)	58.53 ± 7.41 (50-71)	59.56 ± 6.43 (50-72)	61.06 ± 4.84 (55-70)

Table 2: Mean obtained pharmacokinetic parameters for Urapidil

Parameters	Mean	SD	Min	Median	Max	CV%	Geo Mean
K_{el} (h^{-1})	0.135	0.029	0.080	0.134	0.230	22.014	0.132
T_{half} (h)	5.338	1.171	3.008	5.151	8.574	21.945	5.219
T_{max} (h)	4.233	0.878	2.000	4.500	6.000	20.745	4.125
C_{max} (ng/mL)	715.305	217.595	420.961	624.158	1191.000	30.420	685.348
AUC_{0-t} (ng*h/mL)	5622.222	1892.117	2608.744	5484.278	10877.623	33.654	5330.616
AUC_{inf} (ng*h/mL)	5708.741	1943.585	2627.958	5621.776	11125.668	34.045	5406.680
Vd/F (L)	0.087	0.028	0.041	0.084	0.148	32.157	0.083
Cl/F (mL/min)	0.011	0.004	0.005	0.010	0.022	35.009	0.011

Geo Mean – Geometric Mean

Table 3: Obtained inter-subject and intra-subject coefficient of variation for pharmacokinetic parameters

PK Parameters	Inter-subject CV (%)				Intra-subject CV (%)	
	Study 1 Form-1 (N = 10)	Study 2 Form-2 (N = 10)	Study 3 - Form-1 (N = 10)	Study 3 - Form-2 (N = 10)	Study 1 and 2 Form 1 vs 2	Study 3 Form 1 vs 2
C_{max}	39	32	53	33	36	17
AUC_{inf}	33	44	41	30	36	15
Cl/F	21	19	26	19	35	15
Vz/F	24	28	23	17	31	23
$t_{1/2}$	15	41	15	10	19	12

Table 4: Results of regression analysis for age and body weight effects

Parameters	Age effects		Body weight effects	
	p-value	R^2	p-value	R^2
AUC_{0-t}	0.6359	0.0081	0.2855	0.0406
C_{max}	0.5372	0.0137	0.7437	0.0039
CL/F	0.8000	0.0023	0.7352	0.0041
T1/2	0.4690	0.0189	0.4243	0.0229
Vd/F	0.8571	0.0012	0.8491	0.0013

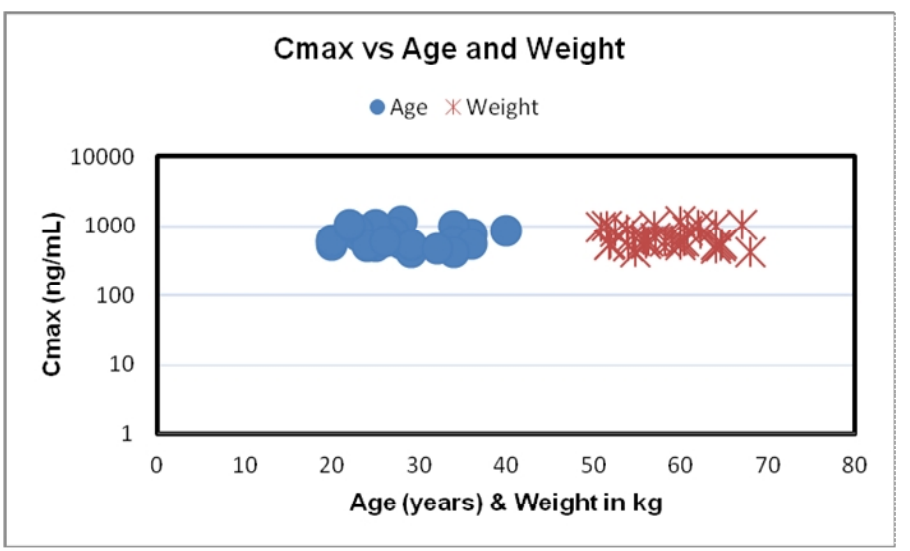

Figure 1: Effect of Age and Body weight on C_{max} (rate of absorption) of Urapidil

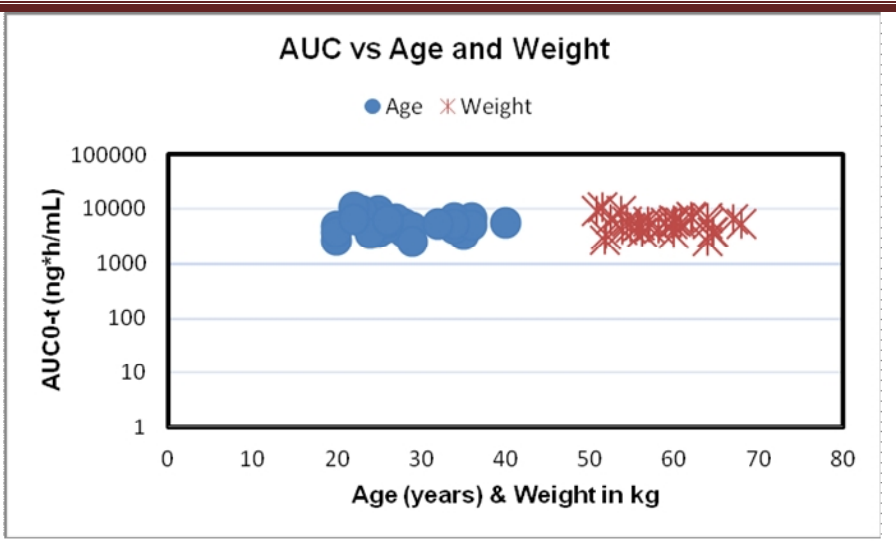

Figure 2: Effect of Age and Body weight on AUC (extent of absorption) of Urapidil

CONCLUSION

The influence of covariates on Urapidil pharmacokinetics was evaluated using linear mixed effect model. From the observed results R^2 values associated with linear regression model were found to be less for age effect and for body weight effect, respectively. However, results evidencing that the reasonable influence of covariates on Urapidil pharmacokinetics parameters may expected for different lot of innovator products. Thus, characterizing effect of few of the covariates on pharmacokinetics outcome will definitely reduce the number of pharmacokinetic studies using healthy human subjects and also development time and cost in generic drug developments. However, to confirm this results, for Urapidil further pharmacokinetic models to be developed and validated using non-linear mixed effect models, as its considered one of the standard method for evaluating drug related variability.

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