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POPULATION PHARMACOKINETICS OF DOXOPHYLLINE IN BRONCHIAL ASTHMA PATIENTS.

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ABSTRACT

The intention of our research work was to illustrate the population pharmacokinetics of oral doxophylline (DOXO) in bronchial asthma patients using a population pharmacokinetic (POPK) modeling approach. The study also aimed to identify and evaluate the role of covariates affecting inter-individual variability. Blood samples were collected from 109 patients and DOXO concentrations were analyzed from archived serum samples. Population PK modeling was performed using Phoenix NLME 6.2. The model used to describe the DOXO pharmacokinetics is a two-compartment pharmacokinetic model following first-order absorption and elimination. Boot strapping and visual predictive check methods were implied for model evaluation. The estimated value of clearance, CL and CL2 were 2.49 and 5.19 L/h with an inter-individual variability (IIV) of 1.3 and 4.8% respectively. The estimated value of volume of distribution, V and V2 were found to be 5.6 and 30.2 L with an inter-individual variability of 11.6 and 11% respectively. The absorption rate constant was calculated as 1.59/h with an inter-individual variability of 8.5%. The gender (ISM), smoking habit and co-morbid diabetes were identified as significant covariates affecting the clearance of DOXO. -2Log-likelihood profiling method indicated that the kinetic parameters could be estimated with good precision. Boot strap and visual predictive check model qualification methods proved that the developed model sufficiently described the data observed. This is the first

ever population pharmacokinetic study of DOXO in Indian patients. A population pharmacokinetic model from the observed data was developed for doxophylline successfully.

KEYWORDS: Doxophylline, population pharmacokinetics, bronchial asthma.

INTRODUCTION

In spite of the wide classes of drugs employed in treatment, 1 in 250 deaths occur worldwide due to asthma. Most of these deaths could be prevented as they are caused either due to suboptimal long-term medical care or delayed help during final attack. Report also state that over-reliance on acute care is a barrier in reducing the burden of asthma. The report suggest to pursue further research in the areas of asthma cause and the current primary and secondary intervention strategies used in asthma treatment.[1] In spite of the accepted inhaler therapy as the first line therapy in developed countries, the acceptance level is poor in developing countries like India.[2] Even though we know that most of the lung function could be reversed or reduced significantly as evident from the individual's degree of bronchoconstriction (expressed as FEV_1 and FEV_1/FVC ratio), complete asthma care is still an unanswered question. In spite of all the established treatment guidelines and therapy advancements there is still lag in the effective asthma control achievement. This has been out veiled by a large cross sectional study which assessed using the Asthma Control Test (ACT) scores and another one named Gaining Optimal Asthma Control (GOAL) study. In the latter study 30% of 3500 asthma patients failed to achieve optimal asthma control. Such a treatment failure occurred despite the treatment of the asthmatics with the standard drug combination of corticosteroids by inhalation and sustained acting beta2-agonists. One other study revealed a 59% of treatment failure even though they had received primary care.[3-6] Hence there is a need for research to help in providing successful asthma care. The use of traditional xanthines is restricted in asthma treatment highly due to its increased occurrence of adverse events and narrow therapeutic index. The evolution of newer xanthines like doxophylline thus resulted.

Doxophylline was discovered by Dr. Frank Lloyd Dini in 1980s in Italy and launched in to market by ABC pharmaceuticals in the year 1990. Doxophylline is a derivative of Theophylline with a dioxolanic ring at position 7. The chemical name of doxophylline is 7-(1,3dioxolar-2-ylmethyl)-theophylline.[7,8] It has both bronchodilator and anti-inflammatory activity. [9,10] This pharmacological action was proven in the studies carried out in both animals[11-13] and in patients with chronic asthma/chronic obstructive pulmonary disease.[14,15] This novel bronchodilator inhibits phosphodiesterase enzyme selectively and also possesses

less affinity towards the adenosine (A1 & A2) receptors when compared with theophylline. Doxophylline inhibits the PDE2A and A2A receptors only at supramaximal concentrations in an *invitro* study. This adenosine receptor antagonism claims the drug to be a bronchodilator with less adverse events and thereby more patient friendly. The drug acts by partly inhibiting the phosphodiesterase enzymes thereby increasing the intracellular concentrations of cyclic AMP and smooth muscle relaxation thereof.[16-19] The daily oral dose of Doxophylline for the treatment of asthma in adults is 400 mg twice or thrice and the pediatric dose is 200mg twice or thrice. The US FDA recommends doxophylline for class III adults and children in the treatment of asthma. Even though the drug is claimed to be selective with less adverse events, it has been reported to cause adverse events equivalent to theophylline in research.[20] Doxophylline has to be used with necessary precautions in patients with hypertension, hypoxemia, heart disease, chronic right ventricular failure, congestive heart failure, liver disease, renal disease and peptic ulcers.[2] The undesirable effects of doxophylline include nausea, vomiting, dyspepsia, anorexia, sweating, palpitation, precordial pain, epigastric pain, headache, anxiety, irritability, tachycardia, nervousness and insomnia.[2,21] Among the newer xanthines, the present literature depicts very scanty evidence in the research carried out worldwide with respect to the pharmacokinetics of drug Doxophylline. The previous authors have also commented that the potential of Doxophylline has not been fully explored. Moreover no population pharmacokinetic research has been carried out in India giving importance to the influence of covariate relations on Doxophylline pharmacokinetic parameters in bronchial asthma patients. This population study will help in better understanding of the pharmacokinetic factors affecting the efficacy of Doxophylline in bronchial asthma treatment. Further the pharmacokinetic model developed as a result of the study will aid in the individualization of doxophylline dose in bronchial asthma patients. This study is further a step towards effective utilization of doxophylline in asthma management.

MATERALS AND METHODS

Patients

Bronchial asthma patients who were on treatment with oral doxophylline tablets (400 mg, twice daily) and had attained steady state concentrations were included in the study. 436 samples were collected from 109 bronchial asthma patients after obtaining informed consent. Sparse sampling design was adopted for this study. All the patients were confirmed to be compliant in taking the medication as recommended by the physician. This research work was approved by the ethics committee of Sri Ramachandra University. Descriptive data of all

the patients were collected which includes name, age, gender, body weight, height, smoking habit, concomitant diseases and concurrent medications taken along with doxophylline. The clinical demographics of patients who took part in this study are represented in Table 1.

Bio-analytical method

The blood samples obtained from patients were let to coagulate for 30 minutes at room temperature and then the samples were subjected to centrifuge at 1500 rpm for 10 min at 25° C in refrigerated centrifuge equipment. Doxophylline was quantified from the serum samples by reverse-phase high performance liquid chromatography technique (RP-HPLC) at the Instrumental analysis lab of the pharmaceutical chemistry department at Faculty of pharmacy, Sri Ramachandra University, Chennai. Shimadzu liquid chromatography analytical system equipped with a LT 10AT VP pump, a SPD 10A VP.

Table 1: Patient characteristics.

Demography	Values
No. of patients	109
Male/Female	68/41
Age (yr)	55* (Range; 25-65)
Body weight (kg)	53.4* (Range; 29-74)
Total number of serum sampling time points	436
No of observation per patient	4* (Range; 2-5)
Doxo plasma concentration (µg/L)	0.7 to 37
Dose (mg/day)	800
Smokers	38
Co-morbid conditions	
Diabetes	21
Hypertension	24
Co-medication frequency (%) DOXO	
DOXO + Montelukast	31
DOXO + Ranitidine	44
DOXO + Pantoprazole	40

variable wavelength ultra violet ray visible spectrophotometric detector and an automatic injector system was employed (Shimadzu Kyoto, Japan) for analysis. An INERTSIL ODS-3V C-18, 150x5mm (Thermo Scientific) chromatography column was used in the instrument as stationary phase. The column temperature was adjusted at 25°C. The mobile phase consisted of acetonitrile and 12.5mM Dipotassium hydrogen ortho phosphate buffer (PH - 3) with ortho phosphoric acid in the ratio 18:82. 1 mL/min was the employed flow rate and the detection wavelength was 275 nm with a run time of 10 minutes. The mobile phase was filtered by a 0.45 m membrane filter and used for analysis. The flow rate of the mobile phase

was maintained at 1.0 mL/min and the detection wavelength was 275 nm with a runtime of 15 minutes. Metronidazole (1.5µg/ml) was used as an internal standard. Pure samples of doxofylline and metronidazole were gifts from Mars Therapeutics Ltd, Secunderabad, India and Torrent Pharmaceuticals, India respectively.[22]

Population pharmacokinetic modeling

PK analysis was performed using Phoenix NLME 6.2. An open two compartment model with first-order elimination was used. The first order conditional estimation with extended least squares - (FOCE-ELS) was used throughout the modeling process. Between subject variability (BSV) in CL and V1 was modeled assuming a log-normal distribution (Eq. 1).

$P_i = THETA_{Pc} .exp (ETA_i)$ *[Eq. 1]*

where P_i is the estimate for a PK parameter in the i^{th} individual as predicted by the model without covariate effects; $THETA_{Pc}$ is the typical (population) value of the (individual covariate adjusted) population PK parameter; and ETA_i represents a random variable (for BSV) with mean 0 and variance ω_i^2. Residual variability was modeled using a multiplicative error model (Eq. 2).

$C_{obs} = C_{pred} . EPSILON_{mul,ij}$ *[Eq. 2]*

Where $EPSILON_{add,ij}$ is a random variable (for residual error) with mean 0 and variance of σ_{add}^2.

Initially, a base model was developed without inclusion of covariates. Subsequently, the covariate model was developed using Covariate search option in NLME with a stepwise forward additive approach followed by a stepwise backward elimination approach. For each addition of a covariate to the base model, the improvement in fit was assessed. Covariates were included in the model if the change in objective function value (dOFV) was larger than 3.84, which equals to a statistical significance of $p<0.05$, based on a Chi-squared distribution (df =1). During subsequent backward elimination of covariates, a more stringent significance criterion was used (dOFV> 6.84, $p<0.01$). Covariates such as age, gender, weight, smoking, co-morbid conditions and concomitant medications were evaluated as possible explanatory variables in the PK model parameters. Additional criteria for evaluating the covariates included were: a reduction in inter-individual variability which is unexplained, diagnostic plots of the weighted residuals, and goodness of fit.

Covariate Model

In this step of model building, demographic covariates such as age, sex, body weight, smoking habit, co-morbid diabetes and hypertension, and concomitant medications were assessed as possible variables affecting the population pharmacokinetic parameters. The base model was used in the identification and quantification of the covariates influencing the PK parameters. Thus the effects of identified covariates were evaluated in NONMEM using Phoenix NLME 6.2 interface by -2 log likelihood stepwise forward additive and backward deletion approach. Continuous covariates were included in the structural model with linear and exponential function.

Statistical analysis

For the addition of every covariate to the base model, the improvement in the model fit was assessed and the validity of the covariate was evaluated. The change in the objective function value was considered as the most important tool. The change in OFV between two hierarchical models is chi-square distributed asymptomatically, with degrees of freedom equal to the difference in the number of parameters between the two models and this difference should be at least 3.84 (if degrees of freedom = 1) to achieve the desired level of significance ($\alpha = 0.05$). A change of 6.84 is considered significant for a p value of <0.01. (Vozeh, 1990) The other criteria for the evaluation of covariates were: reduction in unexplained inter-individual variability, diagnostic plots of randomly distributed weighted residuals and better relationship between the predicted Vs observed concentrations.

Model qualification

The model qualification was performed by bootstrap re-sampling and visual predictive check methods in order to validate the developed population pharmacokinetic model.

Bootstrap re-sampling

1000 bootstrap replicates were re-sampled from the original patient values and thereafter used to estimate the final parameters using the final model. This non-parametric bootstrap re-sampling mode of model evaluation was performed using the Phoenix NLME software interface. Median pharmacokinetic parameter and 95% confidence interval was calculated for the assessment of closeness and distribution of the PK parameters.

RESULTS

Base model

The data set subjected to population pharmacokinetic modeling using Phoenix NLME software led to the following significant results. The base model was derived by employing two-compartment first order conditional estimation with extended least squares (FOCE-ELS) method. The population pharmacokinetic parameter estimates have been listed in Table 2.

Covariate model

The effect of covariates such as age, gender, weight, smoking, co-morbid conditions and concomitant medications on pharmacokinetic parameters like clearance (CL) and volume of distribution (V) were examined in order to develop a suitable population pharmacokinetic model. The base model was modified by the step-wise -2log likelihood forward addition/backward deletion method. During the analysis a reduction in OFV by 3.84 or more was considered significant at $P<0.05$. The covariate gender, smoking habit and diabetes co-morbidity reduced.

Table 2: Results of forward addition and backward elimination of covariates in the model.

Model	Structural model	OFV	Change in OFV	Statistical significance
Forward Inclusion Steps			**↓ in OFV**	
Base Model	$Ka = \theta_1$ $CL = \theta_2 e^{\eta 1}$ $CL2 = \theta_3 e^{\eta 2}$ $V = \theta_4 e^{\eta 3}$ $V2 = \theta_5 e^{\eta 4}$ $Tlag = \theta_6$	946.261	–	–
Whether female gender affects clearance? (ISM = 0)	$CL = \theta_7 \times (ISM) \times [\theta_2 e^{\eta 1}]$ $CL2 = \theta_8 \times (ISM) \times [\theta_3 e^{\eta 2}]$	887.176 937.249	59.085 9.012	<0.01 <0.01
Whether smoking habit affects clearance?	$CL = \theta_9 \times (SMOK) \times [\theta_2 e^{\eta 1}]$ $CL2 = \theta_{10} \times (SMOK) \times [\theta_3 e^{\eta 2}]$	933.624 942.412	12.637 3.849	<0.01 <0.05
Whether diabetes co-morbidity affects clearance?	$CL = \theta_{11} \times (DIAB) \times [\theta_2 e^{\eta 1}]$ $CL2 = \theta_{12} \times (DIAB) \times [\theta_3 e^{\eta 2}]$	932.158 943.632	14.103 2.629	<0.01 NS
Whether montelukast comedication affects volume of distribution?	$V = \theta_{13} \times (MONT) \times [\theta_4 e^{\eta 3}]$ $V2 = \theta_{14} \times (MONT) \times [\theta_5 e^{\eta 4}]$	942.216 946.368	4.045 -0.107	<0.05 NS

Backward elimination steps		**868.367**	**↑ in OFV**	**_**
Deleting gender from the final clearance model	CL = θ_9 x (SMOK) x θ_{11} x (DIAB) x [$\theta_2 e^{\eta 1}$] CL2 = θ_{10} x (SMOK) x θ_{12} x (DIAB) x [$\theta_3 e^{\eta 2}$]	901.263	32.896	<0.01 NS
Deleting smoking habit from the final clearance model	CL = θ_7 x (ISM) x θ_{11} x (DIAB) x [$\theta_2 e^{\eta 1}$] CL2 = θ_8 x (ISM) x θ_{12} x (DIAB) x [$\theta_3 e^{\eta 2}$]	879.472	11.105	<0.01 NS
Deleting diabetes co-morbidity from the final clearance model	CL = θ_7 x (ISM) x θ_9 x (SMOK) x [$\theta_2 e^{\eta 1}$] CL2 = θ_8 x (ISM) x θ_{10} x (SMOK) x [$\theta_3 e^{\eta 2}$]	881.354	12.987	<0.01 NS
Deleting montelukast co-medication from the final volume model	V = $\theta_4 e^{\eta 3}$ V2 = $\theta_5 e^{\eta 4}$	871.257	2.89	NS NS
*As a result of significant correlation gender, smoking habit and diabetes were the covariates valid for inclusion in the final model. *Effect of montelukast on volume of distribution was insignificant and dropped from the final model Significant levels (3.84 ↑ or ↓ in OFV represents α=0.05, 6.84 ↑ or ↓ in OFV denotes α=0.01, NS denotes Not significant)				

The OFV value to a greater extent when introduced for its influence on clearance. Out of the covariates tested, concurrent montelukast therapy showed marked decrease in the OFV when included against volume of distribution in the base model. The decrease in the OFV value for gender (ISM on CL) was 59.085, smoking habit was 12.637 and diabetes co-morbidity was 14.103. During the backward deletion stage, the covariates that had significant influence were removed from the covariate model and tested for its significance with an OFV reduction of 6.84 or more. Montelukast co-medication lost its result on volume of distribution during the backward deletion stage. The structural model of the forward inclusion and backward elimination step are tabulated in Table 2. The parameter estimates of the final covariate model are tabulated in Table 3.

Table 3: Population pharmacokinetic parameter estimates of Base and Covariate model.

Population PK parameters	**Base Model**		**Covariate Model**	
	Typical value	**% RSE**	**Typical value**	**% RSE**
Ka(1/h)	1.58122	6.3	1.59441	8.51
V (L)	5.61967	9.63	5.64363	11.66
V2 (L)	30.3183	11.82	30.2147	10.96

CL (L/h)	2.39225	1.18	2.48965	1.29
CL2 (L/h)	5.072819	4.68	5.19176	4.76
Tlag (h)	0.681651	2.76	0.683713	2.57
Covariate model				
Effect of gender on CL	-	-	0.114353	18.9
Smoking on Clearance	-	-	0.065841	13.4
Diabetes on Clearance	-	-	0.036147	7.5
Between subject variability				
BSV on CL/F%	23.02	16.2	11.29	19.7
BSV on Vd/F%	13.9	39.3	14.2	41.6
Residual variability				
Proportional error %	0.086	12.7	0.087	12.9

The predictions of the covariate model were assessed based on the scatter plots as follows:

1) Observed drug concentrations (DV) versus population predicted (PRED) compared with individual predicted concentrations (IPRED). “Figure 1”
2) Weighted residual concentration (WRES) versus time after dose (TAD). “Figure 2”

The distribution of the points on the plot was symmetric around the central identity line. This indicates that the developed model satisfactorily describes the serum concentration of DOXO in the said patients. Hence the FOCE ELS two compartment with effects of ISM, smoking habit.

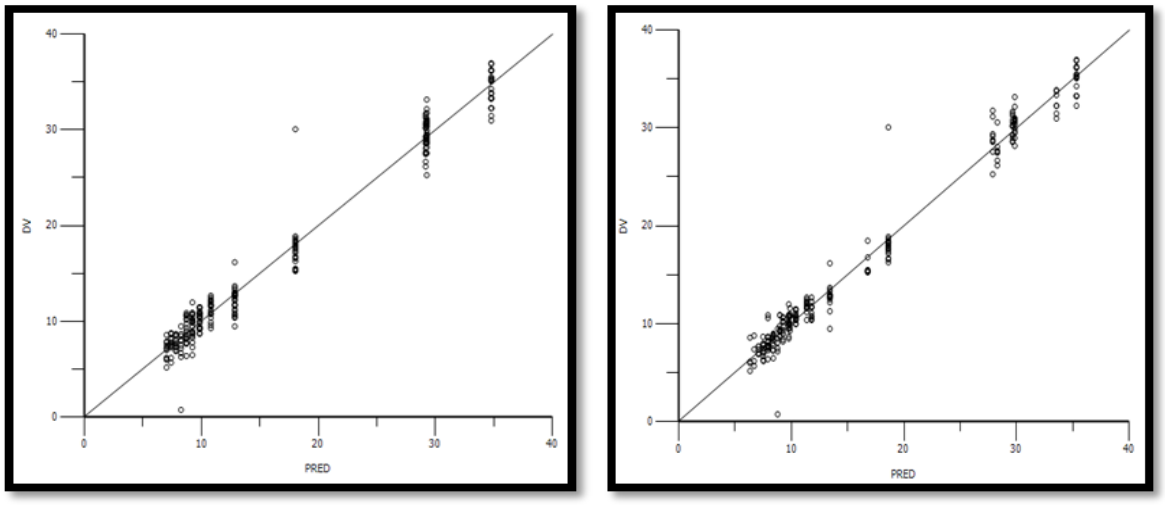

Figure 1: DV versus PRED (Base & Final model).

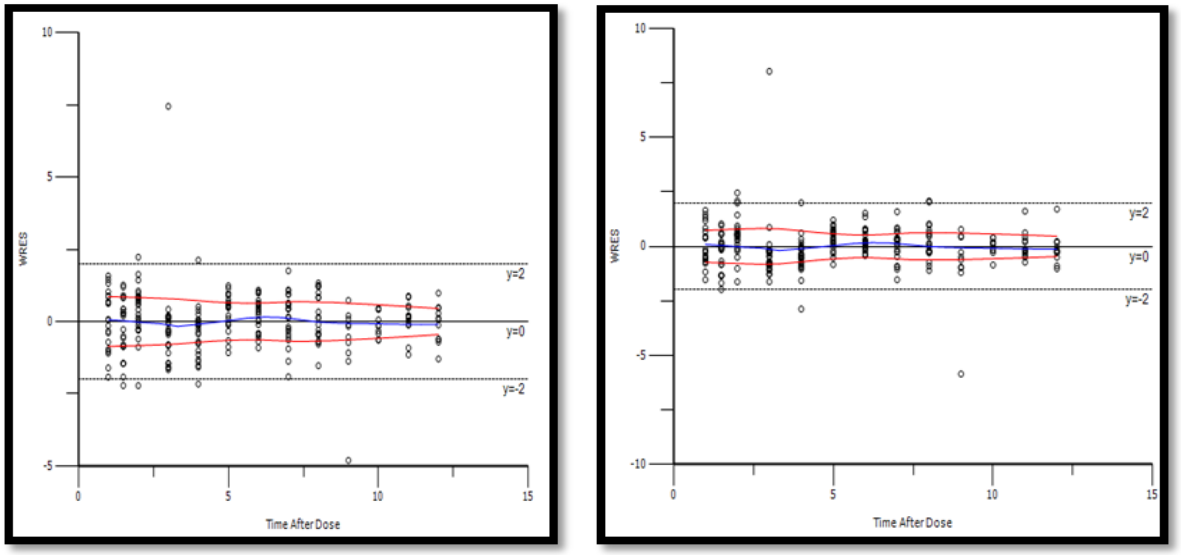

Figure 2: WRES versus TAD (Base & Final model).

and diabetes on CL model was decided to be the final population pharmacokinetic model. The population estimate of clearance, CL and CL2 were 2.49 and 5.19 L/h with an inter-individual variability (IIV) of 1.3 and 4.8% respectively. The population estimate of volume of distribution, V and V2 were found to be 5.6 and 30.2 L with an inter-individual variability of 11.6 and 11% respectively. The absorption rate constant was calculated as 1.59/h with an inter-individual variability of 8.5%.

Model qualification

Boot strapping

The final covariate model was evaluated by estimating the pharmacokinetic parameters and their 95% CI from 1000 re-sampled simulated using a non-parametric boot strap approach. In the qualification step of boot strapping, the generated 95% confidence interval (CI) values were similar to that of the final covariate model (Table 4). This signifies that the developed model was adequately precise.

Table 4: Final parameter estimates for bootstrap qualification method.

Parameter	Population model Naïve estimate	95% CI Naïve estimate	95% CI Boot strap estimate
Ka (1/h)	1.59	1.28-1.94	1.33-1.86
V (L)	5.64	4.22-7.18	4.35-6.94
V2 (L)	30.21	23.52-37.03	23.69-36.74
CL (L/h)	2.49	2.42-2.55	2.43-2.55
CL2 (L/h)	5.19	4.66-5.67	4.7-5.68

Tlag (h)	0.68	0.65-0.71	0.65-0.72
Covariate Model			
CL-ISM	0.11	0.07-0.15	0.07-0.16
CL-SMOK	0.07	0.06-0.13	0.062-0.14
CL-DIAB	0.04	3.92-5.39	3.96-5.42
Between subject variability			
BSV on CL/F%	11.00	8.56-12.12	8.63-12.24
BSV on Vd/F%	14.20	12.02-16.83	11.57-16.98
Residual variability			
Proportional error, %	0.09	0.07-0.18	0.072-0.19

DISCUSSION

The determination of serum doxophylline concentrations may provide vital information in monitoring patients with respiratory disorders. The principal aim of population pharmacokinetic analysis is to quantify the inherent kinetic variability in a patient population that occurs due to physiological, pathological and treatment-related factors (e.g. age, sex, weight, hepatic function, co-morbid conditions, concurrent medication, etc). Such intricate information can be utilized in the design of rational dosage guidelines in order to achieve therapeutic drug concentrations. In case of doxophylline, the achievement of effective drug concentration is complicated. Treatment with this theophylline derivative drug results in either lower therapeutic concentrations or adverse drug reactions in many cases. The limited knowledge about the influence of covariates on the disposition of the drug informs the need for the study.

The current study was designed to study the pharmacokinetic parameters in Indian bronchial asthma patients. In this study, CL/F, Vd/F and Ka were estimated from the observed drug concentrations. A two-compartment kinetic model with first order elimination was used to assess the pharmacokinetics of doxophylline. The total clearance estimate was 7.68 L/h with an inter-individual variability of 6.05% (CV). Clearance was found to be influenced by a number of covariates. The covariates that were subjected for screening are: age, gender, body weight, smoking status; diabetes and hypertension co-morbidity; concurrent use of ranitidine, pantoprazole and montelukast. The covariates gender, smoking status and diabetes co-morbidity were found to have significant effect on clearance. The effect of concurrent montelukast therapy on volume of distribution lost its significance in the backward -2log likelihood step of model building. The covariates age, body weight, hypertension co-morbidity, concurrent ranitidine and pantoprazole therapy did not possess any significant effect on clearance or volume of distribution. The volume of distribution estimated was

higher in our study group than reports published earlier in Chinese[23] and Caucasian[24] population. The clearance was higher than the Caucasians and lower than the values published in the above said Chinese study.

CONCLUSION

The population pharmacokinetic covariate model developed for doxophylline in this study estimated the fixed effect parameters such as volume of distribution, absorption rate constant and clearance in Indian bronchial asthma patients. The covariates identified in this study may play an imperative role in the optimization of doxophylline dose in bronchial asthma patients. Further studies in a larger population with additional covariate information such as genetic polymorphism, patient response, etc could lead to the finest population pharmacokinetic model for doxophylline in patients with chronic airway disorders.

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REFERENCES

1. Masoli M, Fabian D, Holt S, Beasley R. Global burden of asthma. Medical research institute of New Zealand (New Zealand) and University of Southampton (United Kingdom), 2004 May.
2. Mohan A, Prasanth MLL. A review on Doxophylline in pediatric asthma. Universal Journal of Pharmacy, 2014; 03(04): 17-26.
3. Rabe KF, Adachi M, Lai CK, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol, 2004; 114: 40-7.
4. Bateman ED, Boushey HA, Bousquet J, et al. Can guideline-defined asthma control be achieved? The gaining optimal asthma control study. Am J Respir Crit Care Med, 2004; 170: 836-44.
5. Chapman KR, Boulet LP, Rea RM, Franssen E. Suboptimal asthma control: prevalence, detection and consequences in general practice. Eur Respir J, 2008; 31(2): 320-5.
6. Partridge MR, van der Molen T, Myrseth SE, Busse WW. Attitudes and actions of asthma patients on regular maintenance therapy: the INSPIRE study. BMC Pulm Med, 2006; 6:13.

7. Dini FL, Cogo R. Doxofylline: a new generation xanthine bronchodilator derived and major cardiovascular side effects. Curr Med Res Opin, 2001; 16: 258-68.
8. Istituto Biologico Chemioterapico. Segnalazioni di reazioni avverse e dati di farmacovigilanza: 1990- 1993. ABC Data on File.
9. Franzone JS, Cirillo R, Biffignandi P. Doxofylline exerts a prophylactic effect against bronchoconstriction and pleurisy induced by PAF. Eur J Pharmacol, 1989; 165: 269-77.
10. Cogo R, Castronuovo A. Effects of oral doxofylline on inflammatory changes and altered cell proliferation in chronic obstructive bronchitis. Eur Rev Med Pharmacol Sci, 2000; 4: 15-20.
11. Franzone JS, Cirillo R, Barone D. Doxofylline and theophylline are xanthines with partly different mechanisms of action in animals. Drugs Exp Clin Res, 1988; 14: 479-89.
12. Franzone JS, Cirillo R, Biffignandi P. Doxofylline exerts a prophylactic effect against bronchoconstriction and pleurisy induced by PAF. Eur J Pharmacol, 1989; 165: 269-77.
13. Sugeta A, Imai T, Idaira K, Horikoshi S, Okamoto M, Adachi M. Effects of theophylline and doxofylline on airway responsiveness in beagles. Arerugi, 1997; 46: 7-15.
14. Bucca C, Rolla G, Fonzo D, Franzone JS, Carlo RD. Acute clinical-pharmacological findings in obstructive pneumopathy following 2(7,-theophyllinmethyl) 1,3-dioxolane (doxofylline). Int J Clin Pharm Res, 1982; 4: 101-3.
15. Poggi R, Brandolese R, Bernasconi M, Manzin E, Rossi A. Doxofylline and respiratory mechanics. Short-term effects in mechanically ventilated patients with airflow obstruction and respiratory failure. Chest, 1989; 96: 772-8.
16. Mastbergen JV, Jolas T, Allegra L, Page CP. The mechanism of action of doxofylline is unrelated to HDAC inhibition, PDE inhibition or adenosine receptor antagonism. Pulm Pharmacol Ther, 2012; 25: 55-61.
17. Cirillo R, Barone D, Franzone JS. Doxofylline, an anti-asthmatic drug lacking affinity for adenosine receptors. Arch Intde Pharmacodynamic et Therapie, 1988; 295: 221-37.
18. Cirillo R, Barone D, Franzone JS. Doxofylline, an antiasthmatic drug lacking affinity for adenosine receptors. Arch Int Pharmacodyn, 1988; 295: 221- 237.
19. Barnes PJ, Pauwels RA. Theophylline in the management of asthma: time for reappraisal? Eur Respir J, 1994; 7: 579-591.
20. DISEASEDEX^{TM} – General Medicine [Internet database]. Greenwood Village, Colo: Thomson Micromedex. Updated periodically.

21. Akram MF, Nasiruddin M, Ahmad Z, Khan RA. Comparative evaluation of Doxophylline and theophylline in patients of mild bronchial asthma. Int J Basic Clin Pharmacology, 2013; 2: 386-91.
22. Gannu R, Bandari S, Sudke SG, Rao YM, Shankar BP. Development and validation of a stability –indicating RP-HPLC method for analysis of doxophylline in human serum. Application of the method to a pharmacokinetic study. Acta Chromatographica, 2007; 19: 149-60.
23. Li D.-L, Kan Q.-C and Zhang K. Effect of levofloxacin on pharmacokinetics of doxofylline in chronic obstructive pulmonary disease patients. Chin Pharm J China, 2008; 43(23): 1807-10.
24. Bologna E, Lagana A, Terracino D, Bolignari P and Biffignandi P. Oral and intravenous pharmacokinetic profiles of doxophylline in patients with chronic bronchitis. Journal of International Medical Research, 1990; 18: 282-88.