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Population pharmacokinetic/pharmacodynamic modeling of histamine response measured by Histamine Iontophoresis Laser Doppler

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Abstract

The epicutaneous histamine (EH) test is the current gold standard method for the clinical evaluation of allergic conditions. However, the EH method is limited in providing an objective and qualitative assessment of histamine pharmacodynamic response. The histamine iontophoresis with laser Doppler (HILD) monitoring method, an alternative method, allows a fixed dose of histamine to be delivered and provides an objective, continuous, and dynamic measurement of histamine epicutaneous response in children and adults. However, due to the high sampling frequency (up to 40Hz), the output files are usually too cumbersome to be directly used for further analysis. In this study, we developed an averaging algorithm that efficiently reduces the HILD data in size. The reduced data was further analyzed and a population linked effect pharmacokinetic/pharmacodynamic (PK/PD) model was developed to describe the local histamine response. The model consisted of a one-compartment PK model and a direct-response fractional Emax model. The parameter estimates were obtained as follows: absorption rate constant (ka), 0.094 /min; absorption lag time ($Tlag$), 2.72 min; partitioning clearance from local depot to systemic circulation (CL_{par}), 0.0006 L/min; baseline effect ($E0$), 13.1 flux unit; maximum effect ($Emax$), 13.4; concentration at half maximum effect ($EC50$) 31.1 mg/L. Covariate analysis indicated that age and race had significant influence on $Tlag$ and $EC50$, respectively.

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Histamine iontophoresis with laser Doppler; histamine pharmacodynamics; PK/PD linked effect modeling; data averaging algorithm

Introduction

The epicutaneous histamine (EH) test is the current gold standard method for the clinical evaluation of allergic conditions (e.g. allergic rhinitis) [1]. EH testing assesses the microvasculature response to histamine through the observation and measurement of the ‘wheal and flare’ response which results from vasodilatation and vascular leakage after activation of histamine receptors. EH testing is also utilized in clinical trials to assess antihistamine efficacy and duration of effect [1, 2]. However, the EH method is limited in providing an objective and quantitative assessment of histamine pharmacodynamic response. Limitations include operator subjectivity and inter-operator variability; discontinuous measurements; and variable histamine dosing.

We have shown that the Histamine iontophoresis with laser Doppler (HILD) monitoring method allows a fixed dose of histamine to be delivered and provides an objective, continuous, and dynamic measurement of histamine epicutaneous response in children and adults [3, 4]. Characterization of histamine response in patients with allergic disease may be important in defining disease phenotypes whereby therapeutic treatment with antihistamines may be more beneficial. Among children with allergic rhinitis, we have identified distinct histamine response phenotypes via the HILD method [3]. These response phenotypes may be important in future pharmacological studies of antihistamines in children and adults with allergic disease.

As mentioned one of the advantages of HILD over EH method is that the former allows continuous histamine measurements over time. However, it has also been a challenge to expand the use of its output results. Due to the high sampling frequency (up to 40Hz), the output files are usually too cumbersome to be directly used for further analysis. This has limited the analysis of HILD results for broader clinical implications. In addition, no attempts have been made to assess the HILD measured endpoints from an advanced pharmacometric angle. In this study, we developed an averaging algorithm that efficiently reduces the HILD data in size. The reduced data was further analyzed and described using a population linked effect pharmacokinetic/pharmacodynamic (PK/PD) model.

Methods

Subjects

All study participants were enrolled in an IRB-approved protocol after obtaining parental permission and, when appropriate (i.e. age ≥ 7), child assent. Convenience sampling was utilized for this study. Children with a diagnosis of asthma were enrolled from allergy, asthma and immunology outpatient clinics at The Children’s Mercy Hospital in Kansas City, MO. Asthma was defined by $\geq 12\%$ post-bronchodilator reversibility in forced expiratory volume in 1 second (FEV1) or by an Allergy/Asthma specialist diagnosis based on clinical

symptoms in children unable to perform spirometry. Exclusion criteria included: (1) history of immunodeficiency, mastocytosis, chronic abnormal conditions of the skin, liver or kidney, neoplastic disease, or movement or neurologic disorders; (2) active eczema on the forearms at the time of study; (3) history of a previous anaphylactic episode; and (4) evidence of pregnancy (by urinary hCG) or lactation at the time of the study.

HILD method

HILD was performed in 169 participants in an identical fashion. A detailed description of the HILD technique used was previously published [3]. Baseline blood flow was assessed and blood flow measurement (provided in units of flux) was continuously conducted after histamine administration. Blood flow measurements were obtained until return to baseline or for up to a maximum duration of 2 hours.

Data processing

Data collected from HILD monitoring was re-sampled using an averaging algorithm. The raw individual data (up to 3600 data points per subject) was first evenly divided into 200 segments with each containing approximately 18 observations. Within each segment, every data point was examined for potential outliers based on interquartile range rule [5]. An averaged signal was calculated by taking the mean of signals within each segment. The corresponding time was assigned by taking the median time point within the segment. With this approach, each individual data set was reduced to 200 observations (averaged data). A final screening was conducted to select 10–15 data points per subject to further reduce the size (reduced data), while maintaining a realistic representation of the histamine response over time curve. Data from 13 patients were excluded due to data quality and integrity. The re-sampled data sets were then pooled together and subjected to pharmacometric modeling. An example of the individual data processing is shown in Figure 1.

Software

NONMEM v7.3 (ICON Dev. Soln., Ellicott City, MD) was used to build the population PK/PD model. First-order conditional estimation with eta-epsilon interaction method (FOCEI) was used to run the models. Pearl-speaks-NONMEM (PsN) 4.4.0 and Pirana 2.9.0 were used to facilitate the model developing and conduct the bootstrap and visual predictive check (VPC) analysis. Goodness-of-fit plots were generated by running user-defined codes in R studio (R version 3.2.1). Xpose4 package under R environment was used to plot the results of VPC.

PK/PD linked effect model

A PK/PD linked effect model was developed to describe the histamine response over time monitored by HILD. The model is composed of a one-compartment PK model and a direct-response fractional Emax PD model (Figure 2A). The PK model is characterized by one compartment, first-order elimination, and first-order absorption with a lag time. The PD model is a direct-response fractional Emax model with gamma fixed to 1. The model is expressed mathematically as below:

$$\frac{dA_0}{dt} = -ka \times A_0$$

$$\frac{dA_1}{dt} = ka \times A_0 \times F - \frac{A_1}{V_1} \times CL_{par}$$

$$\frac{dA_2}{dt} = \frac{A_1}{V_1} \times CL_{par} - \frac{A_2}{V_2} \times CL$$

$$E = E_0 \times \left(1 + \frac{E_{max} \times \frac{A_1}{V_1}}{EC50 + \frac{A_1}{V_1}} \right)$$

where A_0 is the amount of histamine remaining to be absorbed; A_1 is the amount of histamine in the local depot; A_2 is the amount of histamine in the central plasma compartment; V_1 is the local volume of distribution; V_2 is the central plasma volume of distribution; CL_{par} is the partitioning clearance from local depot to systemic circulation; CL is the central clearance; ka is the absorption rate constant; F is the fraction of histamine absorbed; E is the local response observed; E_0 is the baseline effect; E_{max} is the maximum effect; $EC50$ is the histamine concentration at half of the maximum effect. Since F was not determined in the current model, the clearance and volume of distribution parameters were estimated with F adjustments (CL_{par}/F , CL/F and V_1/F). A delay of absorption ($Tlag$) was also included in the software setting but not shown in the above equations. A_0 was initialized to dosing amount after accounting for $Tlag$, while A_1 and A_2 were initialized to 0.

Between-subject variability (BSV) was initially investigated for all PK and PD parameters assuming an exponential error model:

$$P_i = \theta \times \exp(\eta_i)$$

where P_i is the individual parameter value for i th subject; θ is the population mean of the parameter; η_i is the individual variability from the population mean in the i th subject.

Residual variability was separately assessed as additive error, proportional error and additive/proportional combined error model. The variability was best described with an additive error model:

$$Obs_{ij} = E_{ij} + \varepsilon_{ij}$$

where Obs_{ij} denotes the j th HILD measurement in the i th subject. E_{ij} is the model-predicted j th HILD read for the i th subject. ε_{ij} is the residual error of the j th measurement in the i th subject.

Both η and ε were assumed to be normally distributed with expectation of 0 and variance of ω^2 and σ^2 respectively.

Patient age, weight, height, BMI, sex, race, ethnicity and diagnosis of asthma were evaluated for their potential effects upon histamine pharmacokinetic and pharmacodynamic parameters. A stepwise covariate search was performed based on improvement of OFV with forward selection ($p=0.05$) and backward elimination ($p=0.01$). Power relationship was assumed for continuous covariates (age, weight, height, BMI), and linear relationship was assumed for categorical covariate (sex, race, ethnicity, diagnosis of asthma):

$$\theta^* = \theta \times (\text{Cov}/\text{Median})^{\beta_{co}} \times (1 + \beta_{ca})$$

where θ^* is covariate adjusted population mean of the parameter; θ is the population mean of the parameter; Cov is the continuous covariate value; Median is the median of the continuous covariate values in the dataset; β_{co} is the estimated power value for the corresponding continuous covariate; β_{ca} is the estimated value assigned to each level of the categorical covariate. The final covariate model was used to describe the local histamine response in studied patients.

Results

The demographic information of patients is listed (Table 1). The original data for each subject contained up to 3600 observations (measured at 40Hz, sampled at 0.5Hz) for up to 120 min. To reduce the data size to a suitable and manageable level for pharmacometric modeling, an averaging algorithm was applied to reduce the size to 15 – 20 observations per subject. The final data set contained a total of 2494 observations from 156 subjects.

The histamine response by HILD monitoring was described by a PK/PD linked effect model (Figure 2A). The model is composed of a one-compartment PK model with first-order absorption, and a direct-response fractional Emax PD model. Different error models (additive, proportional, combined, etc.) were tested and additive error model was found to best describe the data.

BSV was investigated for all parameters and was retained for CL_{par} , $E0$ and E_{max} . The base model was reached with an OFV of 17402.4. All parameters were estimated with high confidence (Table 2). The local volume of distribution (VI) was fixed to be 0.006 L to increase the model identifiability. No substantial eta- or epsilon- shrinkage was observed for any random-effect parameters: η_{CL} shrinkage=3.8%, η_{E0} shrinkage=19.4%, η_{EMAX} shrinkage=36.9% and ε shrinkage=6.4%. Goodness-of-fit plots demonstrated a good description of data by the model (supplemental figure 1). Simulations of the residual histamine amount in absorption depot ($A0$) and local histamine concentration ($A1/VI$) are plotted (Figure 2B).

Stepwise covariate search returned age and race as significant covariates on *Tlag* and *EC50*, respectively (Table 2). Goodness-of-fit plots demonstrated improved model fitting, especially for the population prediction, after including these covariate effects in the final model (supplemental figure 2). Individual fitting plots are also included (supplemental figure 6). A bootstrap analysis with 500 simulations was conducted to assess the model stability and finished with 99.2% success rate. The bootstrap mean and 95% confidence intervals of the parameter estimates were in close agreement with the final model estimates (Table 2). A VPC analysis was done and the result is plotted (Figure 3), which demonstrates that the variability of the data was sufficiently explained by the model.

Discussion

The use of laser Doppler flowimetry for assessment of histamine response test has previously been described [6]. However, we have utilized HILD to both deliver a fixed dose of histamine and to provide a more robust measurement of response than has previously been reported. We have taken advantage of the very rich data obtained from the HILD method to more fully characterize the histamine response. Furthermore, we have developed an algorithm to reduce the data size to a suitable level for population pharmacometric modeling while still maintaining important characteristics that allows classification of differing response phenotypes. A population PK/PD linked effect model was developed and described the histamine response over time data with high confidence.

While HILD method provides much more quantifiable measurements, the rich sampled data has been difficult to use for pharmacometric analysis due to several reasons. First, measurements may be redundant. The sampling frequency is very high (up to 40 Hz) and histamine response occurs over a prolonged period. Therefore, it is not necessary to take all the data points to build a sufficiently accurate model. Second, due to the high sampling frequency, individual data sets often reach over 3000 observations. Dealing with such a large data size is a major challenge for population modeling approaches using currently available software packages. Third, due to the intensive sampling and the measurement variability, sampled data are very noisy. Dramatic outliers caused by measurement disturbance (e.g. participant movement) are also recorded and will lead to biased parameter estimates if not detected and excluded. These have been the major challenges to utilizing HILD data for pharmacometric analysis purposes.

To overcome the challenges and, more importantly, to take advantage of the richness of data, we developed an averaging algorithm that reduces the data size while maintaining a realistic representation of the histamine response over time. The essence of the algorithm is to take the average of signals within relatively small time segments and at the same time scan for potential outlier data points. We divided each individual data set into 200 segments (about 20 seconds of measurement each). Considering the histamine response feature and sampling frequency, this number was chosen so that the signals within each segments were at comparable levels and therefore the averaging strategy was feasible. The averaged data set is characterized by a smooth response curve with much fewer data points. Following this step, an easy reduction step was conducted to select representative data points. With this

treatment, the data redundancy was reduced and the data sets were brought to a manageable size for population modeling.

During the segmentation step, a screening process for potential outliers was conducted simultaneously. The exclusion was based on the interquartile range rule [5]. Observations falling outside ($Q1 - 1.5 \times IQR$, $Q3 + 1.5 \times IQR$) range were identified as potential outliers and therefore excluded. Such a criterion was efficient in excluding outliers for two reasons. First, the histamine response is overall continuous in spite of measurement noise. Second, outliers usually deviate from true signals dramatically due to physical disturbance. The criterion was shown to successfully retain almost all true signals while excluding most potential outliers.

Although previous investigators have utilized the PD model to describe antihistamine effect [1, 2, 7, 8], this study is the first to explore the feasibility of modeling histamine response measured by HILD method. HILD method has been used to monitor the histamine-induced local blood flow modification after antihistamine treatment [9, 10]. However, interpretation of such measurements has traditionally been simple and descriptive. Here we applied more advanced pharmacometric approaches to further analyze the results.

To our knowledge, this study is the first attempt to access local histamine response over time through a population modeling approach. A PK/PD linked effect model was developed. The choice of the model structure reflected the fact that the measured local response was a function of loading dose and time after dose [11]. In recognition of the Occam's razor principle [12] and due to the lack of systemic or local (at measurement site) histamine exposure data, the simplest 1-compartmental model structure was assumed and applied. Nonetheless, the current model structure still has a mechanistic presentation of the local histamine PK [13]. For the PD, a direct-response fractional E_{max} model was used to describe the response that is driven by local histamine concentrations. When evaluating candidate model structures, the structurally simpler direct response PD model was chosen over the indirect model in order to minimize the number of parameters to be estimated. Initial attempts of modeling the data using the indirect model structure (featuring kin and $kout$ in addition to E_{max} and EC_{50}) were unsuccessful (data not shown). Error messages and convergence failure indicated over-parameterization. This was likely due to the fact that local histamine concentrations were not available in the current study design, and therefore the more complex indirect model structure could not be sufficiently supported by the data structure.

Due to the lack of plasma PK data in current study, we took a series of approaches in order to increase the model identifiability and to confirm the validity of the current model structure. First, the local volume of histamine distribution was assumed to have similar value as observed in the traditional 'wheal-and-flare' test. A typical 'flare' surface area lies around 22 cm^2 [2]. The three layers of dermal structures at forearm have typical thickness of 0.2 mm (epidermis), 1.2 mm (dermis) and 1.2 mm (hypodermis including subcutaneous fat) [14, 15]. A typical local volume of distribution can then be calculated as $22 \text{ cm}^2 \times (0.2+1.2+1.2) \text{ mm} = 0.0057\text{L}$. Therefore, a volume of 0.006L was fixed in current model to reflect this physiology. Second, the partitioning clearance estimate was compared against corresponding

parameter estimates from published study. Middleton et al. and Li et al. reported histamine plasma PK after subcutaneous injection [16, 17]. The published data were digitized, and fitted into a population model (supplemental figure 3A) to estimate histamine partitioning rate constant (ka_2), apparent central clearance (CL/F) and apparent central volume of distribution (V_2/F) (supplemental table 1 and supplemental figure 4). In this model, ka_2 describes the rate of histamine partitioning from local subcutaneous depot to systemic circulation, and corresponds to CL_{par}/V_1 in our local model (Figure 2A). Comparison of the values of ka_2 estimate (0.095/min) with CL_{par}/V_1 (0.1/min) revealed very close resemblance. This suggests that the partitioning process (from local histamine into circulation) described by our current model agrees well with published evidence. Third, a model linking local and plasma histamine concentrations was constructed and fitted for the digitized plasma PK data after local subcutaneous injections (supplemental figure 3B). The model was intended to demonstrate that assuming current local partitioning model structure, the corresponding plasma PK should mimic the published results. Therefore, in this model, V_1 and CL_{par} were fixed to 0.006 L and 0.0006 L/min as in our local base model, while other parameters were estimated. The central PK parameter estimates agreed well with those from modeling digitized data (Table 3 and supplemental table 1). VPC analysis also showed that the predicted plasma PK profile overlay very well with published data (Figure 4). All these evidence have brought confidence to the current model in correctly describing the partitioning process of local histamine to circulation.

Covariate analysis revealed that $Tlag$ was significantly associated with age, while EC_{50} was significantly associated with race. Example plots of key PD parameter values vs. covariates and selected individual PD profiles are presented (Figure 5 and supplemental figure 5). Early investigation of Laser Doppler flowimetry have revealed minimal differences related to age and race [18]. Presumed race related differences may instead be related to observed genetic variation in the histamine production, response, and degradation pathway [19]. The advanced model provides a much more precise way to describe the variability of histamine response in population and suggests broader application of HILD method in allergy pharmacodynamic research.

Conclusion

The richly sampled HILD data was reduced to a suitable size for pharmacometric analysis using an averaging algorithm. The reduced data set was best described by a population PK/PD linked effect model. The model consists of a one-compartment PK model with first-order absorption, first-order elimination and an absorption lag time, and a direct-response fractional Emax model. This study has demonstrated the feasibility of modeling HILD data for pharmacodynamic purpose.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Data reduction process example

HILD read (flux unit) vs. time after dosing (min) is plotted for a single participant. **A:** raw data. **B:** averaged data. **C:** reduced data.

Figure 2. PK/PD model structure

The PK/PD linked effect model structure is presented. **A:** The model consists of a one-compartment local PK model with absorption lag time, and a direct-response fractional Emax model. The histamine partitioning process is also depicted. *A0*, histamine amount in absorption depot; *A1*, histamine amount in local subcutaneous compartment; *A2*, histamine amount in plasma. Definitions of the other symbols can be referred to Table 2 and 3. **B:** Simulation of residual histamine amount in dosing depot (*A0*) and local histamine concentration (*A1/V1*) over time.

Figure 3. Visual predictive check plot of local histamine response over time

Circles: reduced observation data points. Solid line: 50th simulation quantile. Dashed lines: 5th and 95th simulation quantiles. Shaded area: 95% confidence intervals of corresponding quantiles.

Figure 4. Visual predictive check plot of digitized histamine plasma PK data after single subcutaneous injections

Averaged plasma histamine concentrations after single doses at subcutaneous site published by Middleton et al. (1mg, squares) and Li et al. (0.5mg, circles; 1mg, triangles) were analyzed. Solid line: 50th simulation quantile. Dashed lines: 5th and 95th simulation quantiles. Shaded area: 95% confidence intervals of corresponding quantiles.

Figure 5. Selected key PD parameters vs. covariates

Individual EC_{50} and E_{max} vs. Race (boxplots) and BMI (scattered plots). Boxplots: middle line, median; box limits, interquartile range; error bars, maximum and minimum interquartile range; circles, outliers.

Table 1
Demographic characteristics of all participants

Values are presented in number (N) and percentage (%), or mean with range. BMI, body mass index.

Characteristics	Mean (Range)
Age (years)	12.2 (7 – 18.9)
Weight (kg)	52.3 (18.6 – 130.9)
Height (cm)	151.1 (113.5 – 188.2)
BMI	22.1 (13.8 – 50.1)
	N (%)
Sex	
Male	88 (56.4%)
Female	68 (43.6%)
Race	
Caucasian	83 (53.2%)
African American	57 (36.5%)
Asian	3 (1.9%)
More than one race	13 (8.3%)
Ethnicity	
non-Hispanic	136 (87.2%)
Hispanic	20 (12.8%)

Table 2

Parameter estimates and bootstrap results

Base and final parameter estimates are presented with values and 95% confidence intervals. Bootstrapping results of final model are presented with mean and 95% CI. BSV, between-subject variability; RSE%, residual error percentage.

Parameter	Description	Base Model Estimates			Final Model Estimates			Bootstrap Results		
		Value	95% CI		Value	95% CI		Mean	95% CI	
ka (1/min)	Absorption rate constant	0.094	0.085 – 0.104		0.094	0.084 – 0.104		0.094	0.083 – 0.104	
$CL_{par/F}$ (L/min)	Apparent partitioning clearance	0.0006	0.0005 – 0.0007		0.0006	0.0005 – 0.0007		0.0006	0.0005 – 0.0007	
$V1/F$ (L)	Apparent local volume of distribution	0.006 fixed	-		0.006 fixed	-		0.006 fixed	-	
EO (flux unit)	Baseline effect	16.8	14.5 – 19.1		13.1	10.4 – 15.8		13.0	9.2 – 16.9	
E_{max}	Maximum effect	13.7	9.1 – 18.3		13.4	10.1 – 16.7		13.9	8.2 – 18.5	
$EC50$ (mg/L)	Concentration at half the maximum effect	39.9	32.8 – 47.0		31.3	25.2 – 37.4		31.1	25.5 – 38.9	
$Tlag$ (min)	Absorption lag time	2.66	2.62 – 2.70		2.72	2.67 – 2.77		2.72	2.60 – 2.83	
$\beta 1$ (Age- $Tlag$)	Age influence on $Tlag$	-	-		0.148	0.087 – 0.209		0.146	0.053 – 0.243	
$\beta 2$ (Race- $EC50$)	Race influence on $EC50$	-	-		0	-		0	-	
Caucasian or more than one race		-	-		-	-		-	-	
African American		-	-		0.79	0.13 – 1.44		0.81	0.09 – 1.48	
Asian		-	-		1.01	0.08 – 1.92		1.09	-0.49 – 2.50	
Parameter	Description	Value	RSE%		Value	RSE%		Mean	RSE%	
$\omega^2 - CL_{par/F}$	variance of apparent partitioning clearance BSV	0.41	14.9%		0.42	14.3%		0.42	13.6%	
$\omega^2 - E_{max}$	variance of maximum effect BSV	0.08	66.4%		0.07	33.6%		0.10	38.4%	
$\omega^2 - EO$	variance of baseline effect BSV	0.12	35.4%		0.09	26.1%		0.11	30.6%	
σ^2 (additive)	variance of additive residual variability	284	12.0%		277	12.0%		271	11.2%	

Table 3
Histamine plasma PK parameter estimates using digitized data, assuming local partitioning model structure

BSV, between-subject variability; 95% CI, 95% confidence intervals; RSE%, relative standard error percentage.

Parameter	Description	Estimate	95% CI
k_a (1/min)	Absorption rate constant	0.094 fixed	-
CL_{par}/F (L/min)	Partitioning clearance from local depot to circulation	0.0006 fixed	-
$V1/F$ (L)	Apparent local volume of distribution	0.006 fixed	-
CL/F (L/min)	Apparent plasma clearance	5.09	2.96 – 76.2
$V2/F$ (L)	Apparent plasma volume of distribution	50.5	24.8 – 7.23
Parameter	Description	Estimate	RSE%
$\omega^2 - CL/F$	Variance of apparent plasma clearance BSV	0.124	64.3%
$\omega^2 - V2/F$	Variance of apparent central volume of distribution BSV	0.306	34.3%
$\omega^2 - CL_{par}/F$	Variance of apparent partitioning clearance BSV	0.488	42.4%
σ^2 (additive, $\mu\text{g/L}$)	Variance of additive residual variability	0.98	41.2%