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RESEARCH PAPER

Mechanism - Based Translational Pharmacokinetic - Pharmacodynamic Model to Predict Intraocular Pressure Lowering Effect of Drugs in Patients with Glaucoma or Ocular Hypertension

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ABSTRACT

Purpose To develop a mechanism based translational pharmacokinetic-pharmacodynamic (PKPD) model in preclinical species and to predict the intraocular pressure (IOP) following drug treatment in patients with glaucoma or ocular hypertension (OHT).

Methods Baseline diurnal IOP of normotensive albino rabbits, beagle dogs and patients with glaucoma or OHT was collected from literature. In addition, diurnal IOP of patients treated with brimonidine or Xalatan® were also obtained from literature. Healthy normotensive New Zealand rabbits were topically treated with a single drop of 0.15% brimonidine tartrate and normotensive beagle dogs were treated with a single drop of Xalatan®. At pre-determined time intervals, IOP was measured and aqueous humor samples were obtained from a satellite group of animals. Population based PKPD modeling was performed to describe the IOP data and the chosen model was extended to predict the IOP in patients.

Results Baseline IOP clearly depicts a distinctive circadian rhythm in rabbits *versus* human. An aqueous humor dynamics based physiological model was developed to describe the baseline diurnal IOP across species. Model was extended to incorporate the effect of drug administration on baseline IOP in rabbits and dogs. The translational model with substituted human aqueous humor dynamic parameters predicted IOP in patients following drug treatment.

Conclusions A physiology based mechanistic PKPD model was developed to describe the baseline and post-treatment IOP in animals. The preclinical PKPD model was successfully translated to predict IOP in patients with glaucoma or OHT and can be applied in assisting dose and treatment selection and predicting outcome of glaucoma clinical trials.

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KEY WORDS aqueous humor dynamics · diurnal IOP · glaucoma patients · intraocular pressure · translational PKPD model

ABBREVIATIONS

IOP	Intraocular pressure
OFV	Objective function value
OHT	Ocular hypertension
PD	Pharmacodynamic
PK	Pharmacokinetic
RSE	Relative standard error
VPC	Visual predictive check

INTRODUCTION

Glaucoma, a leading cause of irreversible blindness in the world, is commonly characterized by progressive optic neuropathy with associated visual field defects and elevated intraocular pressure (IOP). Treatment with IOP lowering drugs delay disease progression and onset of glaucoma (1). Better prediction of drug efficacy and safety in humans based on *in vivo* animal studies would be very helpful to reduce the high attrition rate in drug discovery and development. Translational pharmacokinetic-pharmacodynamic (PKPD) modeling plays a vital role in early development of drugs where prior information from pre-clinical *in vitro* and *in vivo* studies are utilized in predicting the outcomes in human. Mechanism based PKPD modeling utilizes the drug-specific and biological system-specific parameters which are crucial for the accurate prediction of drug effects in human.

Aqueous humor is a clear liquid secreted by the ciliary body, circulates through the anterior chamber, and drains through the trabecular meshwork (2). Secretion and regulation of aqueous outflow is essential for maintaining the normal physiology of the eye, while impaired aqueous outflow results

in elevated IOP. As most of the currently available therapies for glaucoma lower IOP by inhibiting the aqueous inflow or by stimulating the aqueous outflow (3), understanding the dynamics of aqueous humor production and dissipation is imperative for elucidating the differences in treatment outcomes across species. Also, IOP is known to exhibit diurnal variation in human and animal species including rabbits, dogs and monkeys (4). In this study, a mechanistic baseline IOP model was developed for normotensive rabbits, normotensive dogs, and patients with glaucoma or ocular hypertension (OHT), taking into account diurnal variation and aqueous humor dynamics. The objective of this study was to develop a mechanism based PKPD model in preclinical species that incorporates the physiology of aqueous humor turnover, to predict the IOP in glaucoma or OHT patients by extrapolating the established PKPD model to account for differences in human aqueous turnover.

MATERIALS AND METHODS

Baseline IOP Data Collection

All baseline mean IOP data were collected from published studies where 24-h IOP profile was reported. Baseline mean IOP dataset for albino rabbits were obtained from references (5–8) and were assigned to rabbit IDs 1–6 (two IDs from Akaishi *et al.* (5), and Zhao *et al.* (7)). Except for data from Zhao *et al.* (7) (obtained directly from the table), IOP was extracted from the figures in articles and used for modeling. Diurnal baseline IOP from normotensive beagle dogs were extracted from references (9–11) and assigned to dog IDs 1–11 (two from Giannetto *et al.* (9) and eight from Piccione *et al.* (10)). Mean IOP data from right (OD) and left (OS) eyes were treated as individual eyes. Baseline mean IOP data of patients with glaucoma or OHT were obtained from references (12–19) and were assigned to patient IDs 1–26 (ten from Stewart *et al.* (17), three from Orzalesi *et al.* (18) and eight from Lee *et al.* (19)). Except for IOP data from Fogagnolo *et al.* (16) and Orzalesi *et al.* (18) (extracted from figures), all data were obtained from tables in the publications. IOP data from figures were extracted using Plot Digitizer, an open source Java program used to digitize scanned plots of functional data (plotdigitizer.sourceforge.net).

Data Source for IOP from Treated Patients

Brimonidine-treated IOP data were extracted from Orzalesi *et al.* (18) and Quaranta *et al.* (20) where patients with glaucoma or OHT were treated twice daily with 0.2% brimonidine tartrate drops for 6 weeks or 1 month. IOP data from latanoprost treated patients were obtained from references (14,15,21–23) and assigned to patient IDs 1–7 (two from Konstas *et al.* (14) and five from Orzalesi *et al.* (15)). All latanoprost treated patient IOP data were directly taken from the tables except Dubiner *et al.* (22) (extracted from figure).

Animals and Study Design

All animal studies were conducted in accordance with the Association of Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic research and approved by the Allergan's Animal Care and Use Committee. As aqueous humor sampling is known to interfere with IOP (24), study animals were stratified into two groups. One group was assigned to pharmacokinetic sampling and the other group was assigned to IOP measurement. Animals were acclimated to IOP measurement prior to the start of the study by going through the measurements at least twice weekly for 2 weeks. Animals used for IOP measurement were pre-screened to identify positive responders following a single topical administration of Alphagan® or Xalatan®.

Pharmacokinetic Study in Rabbits

Eighteen New Zealand White rabbits (Myrtle's Rabbitry Inc., Thompson Station, TN) received a single 35 μL drop of 0.15% Alphagan® (brimonidine tartrate) in the left eye. At pre-determined time intervals, rabbits were anesthetized and approximately 50 μL aqueous humor was collected at 0.5, 1, 2, 4, 6 and 24 h post-dose. Three animals were used for aqueous humor collection at each sampling point and one sample was obtained from each animal. Sample extraction for quantification of brimonidine involved addition of 25 μL of 1 M ammonium hydroxide solution and 1 mL of 95/5 methanol/water followed by vortexing and centrifuging at 3,000 rpm for 10 min. An aliquot 100 μL of supernatant was transferred and added with 20 μL of internal standard solution (10 ng/mL of brimonidine-d4). The mixture was evaporated to dryness under a gentle stream of nitrogen and reconstituted with 100 μL of reconstitution solvent (80/20 water/methanol). A 20 μL aliquot of the reconstituted sample was injected into LC-MS/MS for analysis. The analytical error was within ±20% and the lower limit of quantitation was 0.1 ng/mL.

Pharmacodynamic Study in Rabbits

Three New Zealand White rabbits (Myrtle's Rabbitry Inc., Thompson Station, TN) were topically administered with a single 35 μL drop of 0.15% Alphagan® in the left eye. IOP was measured in conscious animals at 0 (pre-dose), 0.5, 1, 2, 4, 6 and 24 h post-dose using a rebound tonometer (TonoVet®, Icare Finland Oy, Finland). Measured IOP (in mmHg) was

used for PKPD modeling without any further data transformation.

Pharmacokinetic Study in Dogs

Fourteen healthy normotensive Beagle dogs of both sex (Covance Research Products, Inc., Cumberland, VA) received a single 35 μL drop of 0.005% Xalatan® (latanoprost) in both eyes. At pre-determined time intervals, animals were anesthetized and approximately 50 μL aqueous humor was collected at 0.5, 1, 2, 4, 6, 10 and 24 h post-dose. Two animals (four eyes) were used for aqueous humor collection at each sampling point and only one sample was obtained from each animal. Following addition of internal standard (latanoprost acid-d_4) and ammonium hydroxide, aqueous humor samples were extracted using a solid phase extraction plate with methanol to elute latanoprost acid followed by evaporation under a gentle nitrogen stream. Residue obtained was reconstituted in 30% methanol solution and analyzed for latanoprost acid by high pressure liquid chromatography/tandem mass spectrometry. The analytical error was within ±20% and the lower limit of quantitation was 0.05 ng/mL.

Pharmacodynamic Study in Dogs

Eight healthy normotensive Beagle dogs of both sex (Covance Research Products, Inc., Cumberland, VA) were topically administered with a single 35 μL drop of 0.005% Xalatan® in both eyes. IOP was measured in both eyes of conscious animals at 0 (pre-dose), 2, 4, 6, 8, and 24 h post-dose using a rebound tonometer (TonoVet®, Icare Finland Oy, Finland). Measured IOP (in mmHg) was used for PKPD modeling without any further data transformation.

PKPD Modeling and Simulation

Software, Model Selection and Model Validation

All modeling and simulations were performed using NONMEM, version 7.2 (Icon Development Solutions, Ellicott City, MD). The first-order conditional estimation method (FOCE) with interaction was used for PK, PKPD and IOP baseline modeling. Perl-speaks-NONMEM (PsN) version 3.5.3 (25), Xpose program version 4.2.1 (26) implemented into R version 2.10.1 were used to guide the model building and evaluation process.

Model selection was based on the decrease in NONMEM objective function value (OFV), precision of parameter estimates (estimated from standard errors of NONMEM output) and visual inspection of goodness-of-fit plots. For nested models, a drop in OFV of 3.84 for 1degree of freedom was considered significant ($p<0.05$).

Final models were evaluated using a nonparametric bootstrap that involves re-sampling the original dataset 200 times (with replacement). The mean and standard errors obtained from the bootstrap analysis were compared with the estimates obtained from the covariance step in NONMEM. Visual predictive check (VPC) was performed to assess the fitness of final model to adequately describe the observed data. For VPC, 1,000 datasets were simulated from the final model parameters and wherever applicable, non-parametric 95% confidence intervals for the median, 5th and 95th percentiles were computed and presented for visual inspection along with the original dataset.

Mechanistic Modeling of Baseline IOP

Understanding the regulation of IOP and the dynamics of aqueous humor turnover is important for constructing a mechanistic model. Ciliary body is involved in the secretion of aqueous humor from where it passes through the ciliary epithelium into the posterior chamber (27). Aqueous humor drains from the eyes through trabecular meshwork (also called conventional or pressure sensitive pathway) and uveoscleral pathway, with the former accounting for 80–90% of aqueous outflow in most species (28). Equilibrium between the production and outflow of aqueous humor sustains the IOP which can be expressed using a modified Goldmann's equation (27) as mentioned below:

$$IOP = \frac{F_{in} - F_{us}}{C_{trab}} + P_{ev} \quad (1)$$

where F_{in} is aqueous humor production rate (μL/min), F_{us} is the uveoscleral outflow (μL/min), C_{trab} is the facility of outflow *via* the trabecular meshwork and Schlemm's canal (μL/min/mmHg), and P_{ev} is the episcleral venous pressure (mmHg).

IOP is not constant but known to exhibit diurnal variation in many species including human, dogs, rabbits and primates (4). Circadian measure in IOP is contributed by the diurnal variation of aqueous humor dynamics. For instance, aqueous humor production is decreased at night in humans including glaucoma patients (29) and increased in rabbits (7). This diurnal variation in aqueous humor dynamics can be modeled using a dual cosine functions as mentioned below (30):

$$IOP_{baseline} = \frac{F_{in(0)} + \alpha_1\left[\cos\left(2\pi\cdot\frac{t-\Phi_1}{24}\right)\right] + \alpha_2\left[\cos\left(2\pi\cdot\frac{t-\Phi_2}{12}\right)\right] - F_{us}}{C_{trab}} + P_{ev} \quad (2)$$

where α is the amplitude and Φ is the acrophase of the dual cosine functions, $F_{in(0)}$ is the aqueous humor production rate

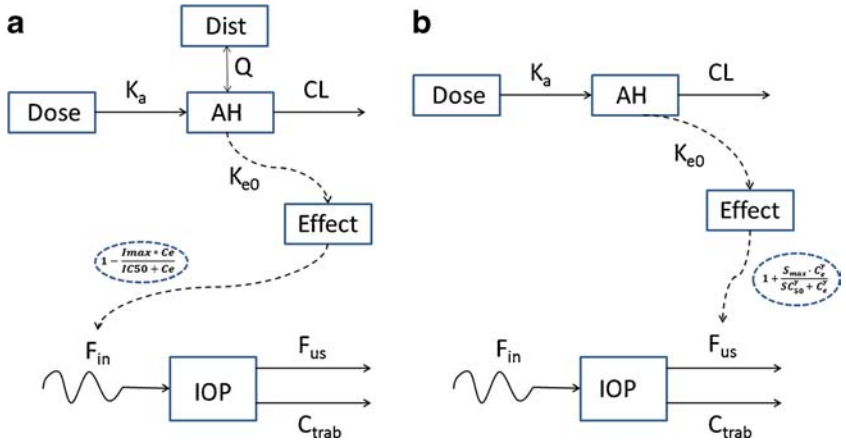

Fig. 1 Schematic representation of mechanistic PKPD models of drug effect on baseline IOP following topical administration: (**a**) PKPD model for brimonidine in rabbits: A two- compartment PK model with first-order absorption and a distribution compartment was fit to aqueous humor brimonidine concentration. Drug effect was mediated through an effect compartment and the drug action was included as an inhibitory action on the production of aqueous humor. (**b**) PKPD model for latanoprost in dogs: A one-compartment model with first-order absorption with time lag was fit to the aqueous humor latanoprost acid concentration. Drug effect was mediated through an effect compartment and the drug action was included as a stimulatory action on the uveoscleral outflow. See text for more details.

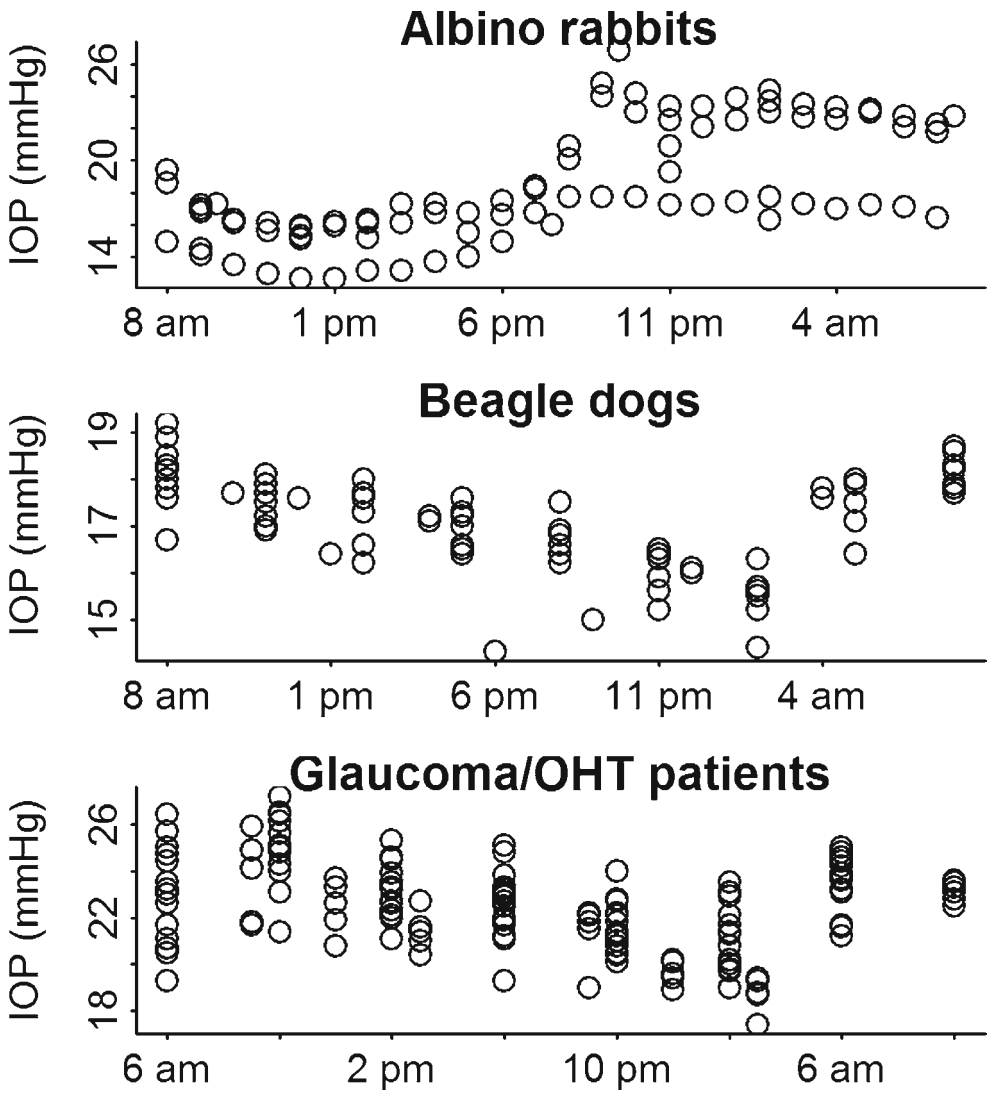

Fig. 2 Baseline diurnal IOP collected over 24-h period from albino rabbits, beagle dogs and patients with glaucoma or ocular hypertension. Each *circle* represents the average values obtained from literature.

Table I Summary of Model Development for Baseline IOP in Rabbits, Dogs, and Human

Model no.	Description	OFV	Status
Rabbit			
1	One cosine function (α_1, Φ_1)	164.8	• Minimization successful • Covariance step completed
2	Two cosine functions (α_1, Φ_1, α_2, Φ_2)	156.0	• Minimization successful • Covariance step completed • Final model selected
3	Three cosine functions (α_1, Φ_1, α_2, Φ_2, α_3, Φ_3)	156.0	• Minimization successful • No covariance step
Dog			
1	One cosine function (α_1, Φ_1)	83.9	• Minimization successful • Covariance step completed
2	Two cosine functions (α_1, Φ_1, α_2, Φ_2)	28.0	• Minimization successful • Covariance step completed • Final model selected
3	Three cosine functions (α_1, Φ_1, α_2, Φ_2, α_3, Φ_3)	24.0	• Minimization successful • Covariance step completed • Large RSE on α_3 and Φ_3
Human			
1	One cosine function (α_1, Φ_1)	269.1	• Minimization successful • Covariance step completed
2	Two cosine functions (α_1, Φ_1, α_2, Φ_2)	205.8	• Minimization successful • Covariance step completed • Final model selected
3	Three cosine functions (α_1, Φ_1, α_2, Φ_2, α_3, Φ_3)	240.6	• Minimization successful • Covariance step completed

at the first observed time (μL/min), F_{us} is the uveoscleral outflow (μL/min), C_{trab} is the facility of outflow *via* the trabecular meshwork and Schlemm's canal (μL/min/mmHg), and P_{ev} is the episcleral venous pressure (mmHg). Since the uveoscleral outflow accounts only for a portion of the total aqueous elimination, F_{us} was estimated as a fraction of the total aqueous humor production (F_{in}).

Mechanistic PKPD Modeling of Drug Effect on Baseline IOP

Since PK samples and IOP measurements were obtained from different group of animals in this study, a sequential PKPD modeling was performed. An appropriate PK model (one or two-compartments) was fit to the aqueous humor concentrations with inter-individual variability either fixed to zero or estimated. Effect of drug administration on baseline IOP was described by an indirect response model where drug effect was hypothesized to be mediated through an effect compartment.

Brimonidine, a selective alpha2-adrenergic receptor agonist, has dual mechanism of action where it lowers IOP by reducing aqueous humor production and stimulates aqueous humor outflow through the uveoscleral pathway (31). The predominant effect of short-term brimonidine treatment is inhibition of aqueous production while chronic treatment also stimulates uveoscleral outflow pathway. In this modeling exercise, brimonidine effect was described by an inhibitory model on aqueous humor secretion as mentioned in the following equation:

$$IOP = \frac{\left\{F_{in(0)} + \alpha_1\left[\cos\left(2\pi\cdot\frac{t-\Phi_1}{24}\right)\right] + \alpha_2\left[\cos\left(2\pi\cdot\frac{t-\Phi_2}{12}\right)\right]\right\}\cdot\left(1-\frac{I_{max}\cdot C_e}{IC_{50}+C_e}\right) - F_{us}}{C_{trab}} + P_{ev} \quad (3)$$

where I_{max} is the maximal capacity of drug inhibition on aqueous humor production, IC_{50} is the drug concentration corresponding to 50% inhibition of production, and C_e is the drug concentration in the effect compartment (Fig. 1a).

Latanoprost is a prostanoid selective FP receptor agonist that reduces IOP by increasing the outflow of aqueous humor (32). Based on the mechanism of action, effect of latanoprost administration on the baseline IOP is included as a stimulatory model on the uveoscleral pathway as described in the following equation:

$$IOP = \frac{F_{in(0)} + \alpha_1\left[\cos\left(2\pi\cdot\frac{t-\Phi_1}{24}\right)\right] + \alpha_2\left[\cos\left(2\pi\cdot\frac{t-\Phi_2}{12}\right)\right] - F_{us}\cdot\left(1+\frac{S_{max}\cdot C_e^{\gamma}}{SC_{50}^{\gamma}+C_e^{\gamma}}\right)}{C_{trab}} + P_{ev} \quad (4)$$

Table II Pharmacodynamic Parameters for the Baseline IOP Model

Parameter (Unit)	Estimate (RSE)	
	NONMEM	Bootstrap
Rabbit		
$F_{in(0)}$ (μL/min)	1.75 (7.49)	1.65 (9.70)
α_1	0.832 (11.3)	0.795 (11.9)
Φ_1 (h)	01:12 am (1.16)	01:12 am (1.28)
α_2	0.083 (40.2)	0.086 (54.6)
Φ_2 (h)	07:48 pm (1.88)	08:24 pm (7.88)
[a]F_{us} (% of F_{in})	18.4 (30.9)	14.0 (23.0)
[b]IIV-$F_{in(0)}$ (%)	24.7 (44.6)	21.8 (60.2)
[b]Residual variability (%)	7.5 (31.1)	7.42 (50.2)
C_{trab} (μL/min/mmHg)	0.2 (Fix)	–
P_{ev} (mmHg)	11 (Fix)	–
Dog		
$F_{in(0)}$ (μL/min)	1.72 (2.55)	1.66 (3.61)
α_1	0.354 (3.56)	0.341 (4.11)
Φ_1 (h)	10:24 am (0.47)	10:24 am (0.49)
α_2	0.157 (7.39)	0.151 (8.61)
Φ_2 (h)	07:54 am (0.76)	07:54 am (0.79)
[a]F_{us} (% of F_{in})	17.5 (1.81)	14.1 (16.03)
[b]IIV-$F_{in(0)}$ (%)	24.5 (Fix)	–
[b]Residual variability (%)	3.5 (10.9)	3.46 (34.1)
C_{trab} (μL/min/mmHg)	0.25 (Fix)	–
P_{ev} (mmHg)	11 (Fix)	–
Human		
$F_{in(0)}$ (μL/min)	2.48 (1.91)	2.41 (2.49)
α_1	0.272 (4.19)	0.266 (4.89)
Φ_1 (h)	11:18 am (1.04)	11:18 am (1.02)
α_2	0.156 (9.36)	0.153 (10.5)
Φ_2 (h)	08:06 pm (1.74)	08:06 pm (1.70)
[a]F_{us} (% of F_{in})	10.3 (2.17)	7.73 (21.9)
[b]IIV-$F_{in(0)}$ (%)	8.6 (31.7)	8.53 (55.9)
[b]Residual variability (%)	3.9 (13.4)	3.87 (37.5)
C_{trab} (μL/min/mmHg)	0.17 (Fix)	–
P_{ev} (mmHg)	9.5 (Fix)	–

$F_{in(0)}$ represents the initial aqueous humor production rate at time 0, α is the amplitude and Φ is the acrophase of the dual cosine functions, F_{us} is the uveoscleral outflow, C_{trab} is the facility of outflow *via* the trabecular meshwork and Schlemm's canal, P_{ev} is the episcleral venous pressure, IIV is the inter-individual variability and RSE is the relative standard error of parameter estimates

[a] F_{us} was estimated as a fraction of the total aqueous humor production (F_{in}) and converted to percentage

[b] Inter-individual and residual variabilities are expressed as percent coefficient of variation (%CV)

where S_{max} is the maximal capacity of drug stimulation on uveoscleral outflow, SC_{50} is the drug concentration corresponding to 50% stimulation of uveoscleral outflow, C_e is the drug concentration in the effect compartment and γ represents the curvature of response curve (Fig. 1b).

Prediction of IOP in Patients with Glaucoma or OHT

For simulation of IOP in patients, it was assumed that the variation in IOP response across species can be attributed to their physiological differences in aqueous humor dynamics. Thus by fixing the PKPD parameters to estimated values in the respective animal models, simulations were performed using the human aqueous humor dynamic parameters (F_{in}, F_{us}, C_{trab}, P_{ev}, α and Φ) for the various dosing regimen of brimonidine (18,20) and latanoprost (14,15,21–23) specified in the literature.

RESULTS

Baseline IOP Model

The baseline IOP profiles for rabbit, dog and patients with glaucoma or OHT are shown in Fig. 2. IOP peaks during the night in rabbits and is the lowest in dogs and patients. Models with varying cosine functions were evaluated to select the ones that best describe the diurnal variation in baseline IOP. In the case of rabbits, model with two cosine functions provided the best-fit to rabbit IOP with an OFV of 156 (Table I). Addition of one more cosine function (3 cosines) did not result in improved fit and the OFV remained unchanged. For dog IOP data, although the model with 3 cosine functions resulted in the lowest OFV (24), the OFV drop was not significant when compared to the model with 2 cosine functions (OFV= 28). Also, the RSE for third cosine function was higher (>90%) indicating over-parameterization of the model. The choice of model with 2 cosine functions was obvious for human IOP data, as increasing or decreasing the cosine function resulted in higher OFV. The best-fit parameters for the final baseline IOP model are summarized in Table II along with the bootstrap results. The initial aqueous humor production rate ($F_{in(0)}$), percent of aqueous humor cleared *via* the uveoscleral pathway (F_{us}) and the circadian parameters (amplitude and acrophase) are estimated from the data while trabecular outflow facility (C_{trab}) and episcleral venous pressure (P_{ev}) were fixed to values reported in literature (7,33,34). For the ease of interpretation, acrophase estimates are converted and reported in clock time. From the 200 bootstrap runs for the rabbit model, 7 runs had parameter estimates near the boundary and hence were excluded from the bootstrap analysis. Results from all 200 runs were included in the bootstrap analysis for dog and human. A comparison of the bootstrap and NONMEM estimates suggest that the parameters in the final models were reasonably well determined and the model was robust. As evident from Fig. 3, model

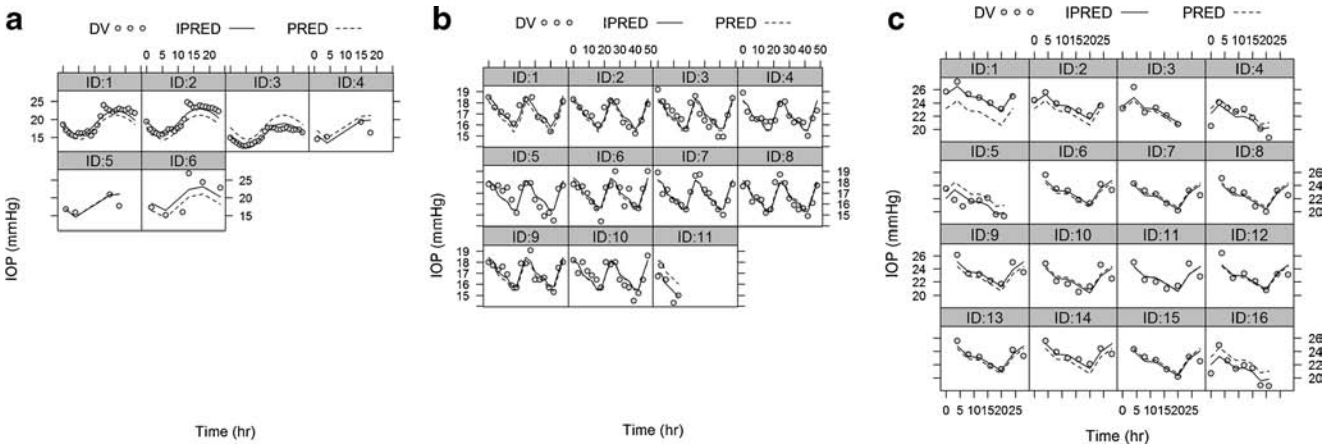

Fig. 3 Individual plots of IOP in (**a**) rabbits, (**b**) dogs and (**c**) patients along with model prediction. *Diamonds* are observed IOP (DV), *dashed lines* represent typical model prediction (PRED) and *solid lines* represent model individual predictions (IPRED). Time 0 on the x-axis corresponds to 6 am in human and 8 am in rabbits and dogs.

predictions adequately describe the observed baseline IOP data in all three species.

PKPD Model for Brimonidine

Brimonidine aqueous humor concentration was well-fit by a two compartment model with first-order absorption (Fig. 4a). Use of a one compartment model did not provide adequate fit for the elimination phase and the OFV was significantly higher than the two compartment model (346 *vs.* 339.3). Due to the limited sample size and non-serial nature of sample collection, it was difficult to estimate both the inter-individual and residual variabilities in the PK model. Initial attempts to estimate the residual variability indicated a good drop in OFV, when residual variability was around 10%. Since other values increased the OFV, residual variability was fixed at 10% (Table III). Inter-individual variability on clearance was estimated to be 63.6%. In the mechanistic PKPD model, PK parameters were fixed to the estimated values from rabbit PK study and the baseline IOP circadian parameters were fixed to the estimated values from rabbit baseline IOP model. Maximum inhibition of IOP (I_{max}) was fixed to 0.28 and the concentration of brimonidine to obtain 50% of inhibition (IC_{50}) of aqueous humor secretion was estimated to be 1.44 ng/mL. The bootstrap estimates summarized in Table III include results from 196 runs that were minimized successfully. In general, the bootstrap results were in close agreement with the final model estimate except for the IC_{50} value (4.60 ng/mL). VPC plot generated using the final PKPD model for rabbits is shown in Fig. 5, where the individual observed IOP (circles) is covered by the median (solid line) and the 5th and 95th quantiles (dashed lines) of simulated IOP.

Simulated IOP in patients administered with 0.2% brimonidine twice daily for 1 month or 6 weeks is shown in Fig. 6. The open circles represent the mean of IOP values

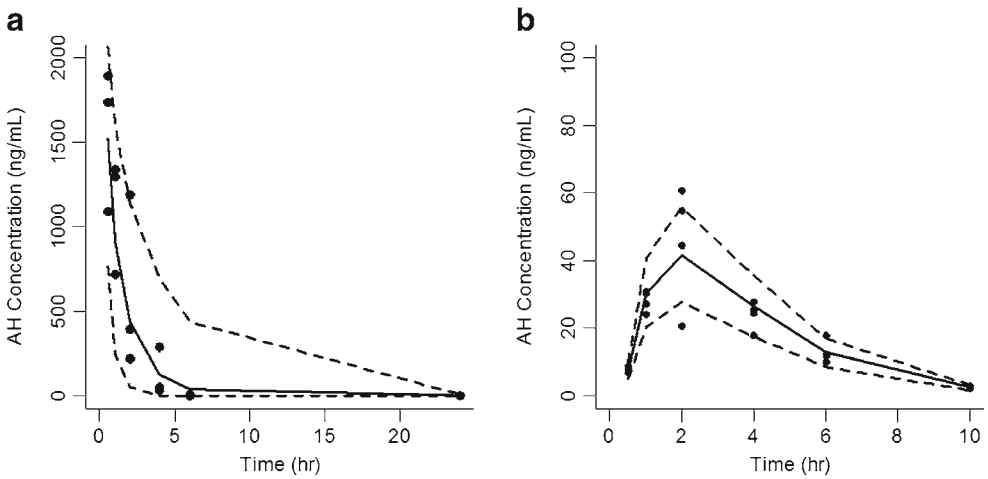

Fig. 4 Predicted and observed aqueous humor concentration of (**a**) brimonidine and (**b**) latanoprost acid. *Solid line* represents the median and the *dashed lines* represent the 95th percentile (upper) and 5th percentile (*lower*) of the 1,000 datasets simulated from the final PK models. The *closed circles* denote the observed individual data.

Table III Pharmacokinetic and Pharmacodynamic Parameters of Brimonidine Following a Single Topical Administration of 0.15% Brimonidine Tartrate to New Zealand White rabbits

Parameter (Unit)	Estimate (RSE)	
	NONMEM	Bootstrap
PK model		
CL/F (mL/h)	19.6 (10.2)	20.3 (14.5)
V2/F (mL)	16.8 (18.7)	16.2 (20.7)
Q/F (mL/h)	19.6 (39.4)	16.9 (51.1)
V3/F (mL)	10.3 (48)	10.1 (3.96)
K_a (1/h)	6.17 (14.2)	5.97 (2.39)
[a]IIV-CL (%)	63.6 (26.2)	63.3 (46.3)
[a]Residual variability (%)	10 (Fix)	–
PD model		
I_{max}	0.28 (Fix)	–
IC_{50} (ng/mL)	1.44 (68.1)	4.60 (28.2)
K_{e0} (1/h)	0.002 (8.7)	0.003 (27.7)
[a]Residual variability (%)	14 (16.9)	14 (11.8)

CL represents the clearance, V2 is the volume of distribution in the aqueous humor compartment, V3 is the volume of distribution in the distribution compartment, Q is the inter-compartmental clearance, F is the fraction bioavailable in aqueous humor after topical dosing, K_a represents the first-order absorption constant, IIV stands for the inter-individual variability, I_{max} represents the maximal inhibition of aqueous humor secretion, IC_{50} describes the drug concentration corresponding to 50% inhibition of secretion, K_{e0} is the elimination rate of drug from the effect compartment and RSE is the relative standard error of NONMEM parameter estimates

[a] Inter-individual and residual variabilities are expressed as percent coefficient of variation (%CV)

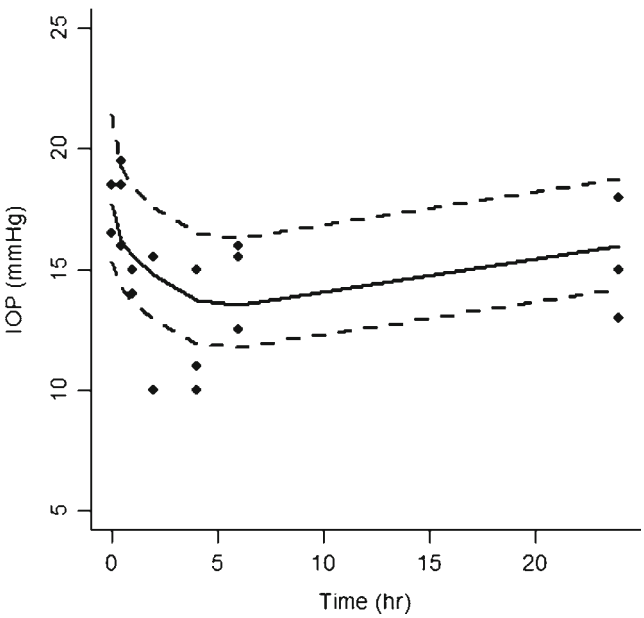

Fig. 5 VPC plot for the brimonidine PKPD model in rabbits. *Solid line* represents the median and the *dashed lines* represent the 95th percentile (*upper*) and 5th percentile (*lower*) of the 1,000 datasets simulated from the final model. The *closed circles* denote the observed individual IOP data.

obtained from 20 to 27 patients and the lines represent the simulated IOP (solid—median; dashed—5th and 95th quantiles). It can be inferred from Fig. 6, majority of the observed mean IOP data are well within the 5th and 95th percentiles of the model simulated data.

PKPD Model for Latanoprost

Latanoprost is an ester prodrug that gets rapidly converted to its free acid following topical administration (35). A one compartment model with first-order absorption and time lag provided the best-fit to latanoprost acid aqueous humor data (Fig. 4b). Inter-individual variability on all PK parameters was fixed to zero and residual variability was estimated to be 20.6%. The PKPD parameters obtained from the final model and bootstrap analysis are presented in Table IV. The maximal stimulation on uveoscleral outflow was 5.25 and the concentration of latanoprost acid to cause 50% of maximal stimulation was estimated to be 2.91 ng/mL in the effect compartment. Parameters estimated from the bootstrap procedure are closer to the final model estimate suggesting the model was stable. Figure 7 shows the 95% confidence intervals of the median (middle shaded area), 5th percentile (lower shaded area) and 95th percentile (upper shaded area) distribution of the simulated IOP along with the individual observed IOP data (circles). The lines represent the median (solid) and 5th and 95th percentile (dashed) distribution of observed data. As evident from the VPC plot in Fig. 7, simulated IOP from the final model adequately covers the observed IOP in dogs indicating the goodness-of-fit.

IOP in patients following latanoprost topical treatment were simulated using the final PKPD model from dog with substituted human aqueous humor dynamics parameters. Simulated IOP for patients treated with latanoprost for 2 weeks are shown as solid (median) and dashed (5th and 95th percentiles) lines in Fig. 8. Observed mean IOP (circles) values were within the 95th percentile distribution of simulated data and closer to the median values indicating the competency of the model to simulate clinical trials.

DISCUSSION

Translational PKPD model assimilates the knowledge from *in vivo* preclinical studies to comprehend the outcome in human. Incorporating the physiological distinction and mechanistic perception into translational PKPD models will greatly improve their predictive ability in human. In ophthalmology, the most commonly used preclinical species for IOP evaluation of drug candidates include rabbit, dog, and monkey. Often times, studies are performed in normotensive animals that have different dynamics with respect to aqueous humor turnover. Besides, there is a lack of translational model that

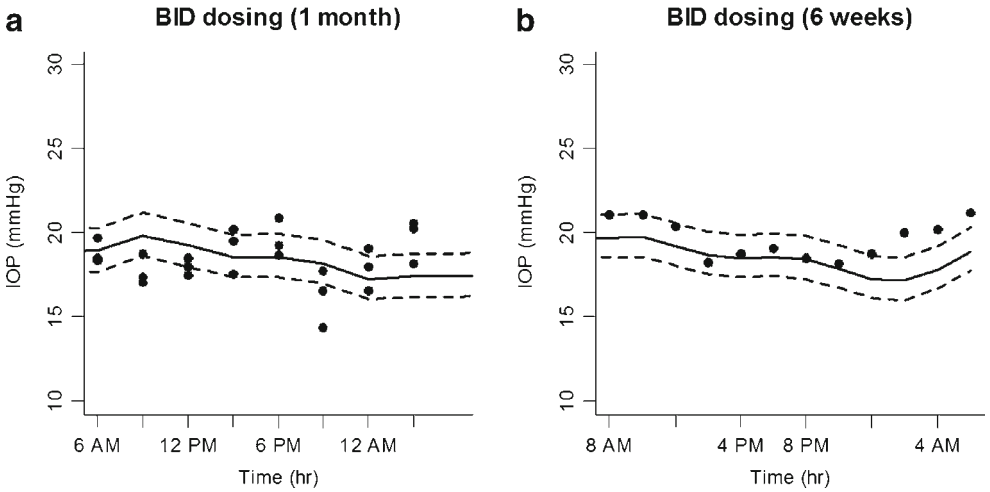

Fig. 6 Translational model predicted IOP in glaucoma/OHT patients treated with 0.2% brimonidine twice daily for (**a**) 1 month or (**b**) 6 weeks. *Solid line* represents the median and the *dashed lines* represent the 95th percentile (*upper*) and 5th percentile (*lower*) of the 1,000 datasets simulated from the final model. The *closed circles* denote the observed mean IOP data from 20 to 27 patients.

can link the preclinical findings to outcome in human. In this manuscript, for the first-time, we developed an aqueous humor dynamics based physiologically relevant model for baseline IOP in rabbit, dog and patients with glaucoma or OHT. Also, a mechanistic PKPD model was developed to comprise the effect of drug administration on baseline diurnal IOP that takes into account the drug's mechanism of action. Furthermore, the PKPD model was translated to predict the IOP in patients.

Table IV Pharmacokinetic and Pharmacodynamic Parameters of Latanoprost Acid Following a Single Topical Administration of Xalatan® to Beagle Dog Eyes

Parameter (Unit)	Estimate (RSE)	
	NONMEM	Bootstrap
PK model		
CL/F (mL/h)	9.22 (5.79)	9.21 (6.97)
V/F (mL)	22.2 (11.5)	21.8 (15.7)
K_a (1/h)	0.973 (19.7)	1.01 (45.1)
T_{lag} (h)	0.39 (4.25)	0.39 (11.2)
[a]Residual variability (%)	20.6 (34)	19.2 (37.8)
PD model		
S_{max}	5.25 (13.3)	5.42 (16.5)
SC_{50} (ng/mL)	2.91 (5.88)	2.98 (13.1)
Gamma	8.3 (52.7)	8.48 (58.9)
K_{e0} (1/h)	0.0597 (9.03)	0.0594 (11.0)
[a]IIV- S_{max}	38.3 (42.3)	37.9 (44.4)
[a]Residual variability (%)	8.90 (17.1)	8.57 (18.3)

CL represents the clearance, V is the volume of distribution in the aqueous humor compartment, F is the fraction bioavailable in aqueous humor after topical dosing, K_a represents the first-order absorption constant, T_{lag} is the lag time for the drug to reach aqueous humor after topical dosing, S_{max} represents the capacity for maximal drug stimulation of outflow, SC_{50} describes the drug concentration corresponding to 50% stimulation of outflow, gamma indicates the curvature of the IOP curve, K_{e0} is the elimination rate of drug from the effect compartment, IIV stands for the inter-individual variability and RSE is the relative standard error of NONMEM parameter estimates

[a] Inter-individual and residual variabilities are expressed as percent coefficient of variation (%CV)

The baseline IOP collected over 24 h period clearly displays different circadian rhythm in rabbits when compared to dog and human. In rabbits, IOP peaks in the night when they are usually most active (7). In contrast, beagle dogs and human have lower IOP in the night. The aqueous humor secretion rate estimated in the baseline model is within the expected range for albino rabbits (1.46–2.53 μL/min) (7) and dogs (1.5–6.8 μL/min) (34). Similarly, the estimated percent of aqueous humor cleared *via* the uveoscleral route in both the species is similar to the reported values in literature (13–25%) (28) indicating the competency of baseline IOP model. Also, the model estimated aqueous flow (2.48 μL/min) and uveoscleral outflow (10.3%) in patients is closer to those reported for OHT patients (2.46 μL/min and 12.6%) (36). The prominent difference in the aqueous humor dynamics between normotensive animals and patients with glaucoma or OHT is noticeable in the aqueous secretion rate/flow and the uveoscleral outflow. The increased aqueous flow (2.5 *vs.* ~1.7 μL/min) and lower uveoscleral outflow (10% *vs.* ~18%) in glaucoma/OHT patients might contribute to the elevated IOP in patients.

Since brimonidine causes a slight contraleteral decrease in IOP (31,37), only one eye per rabbit was treated with brimonidine while both eyes of dog were treated with latanoprost which does not have a contralateral effect (38). In the PKPD model, drug effect on baseline IOP was mediated through an effect compartment. Inclusion of the effect compartment improved the model fit and convergence. Drugs lowering IOP act by either reducing aqueous humor secretion (β-adrenergic antagonists, carbonic anhydrase inhibitors, α_2-adrenergic agonists) or by stimulating the aqueous humor outflow through uveoscleral route (prostaglandin FP receptor agonists, prostamides). The effect compartment in the PKPD model can be the target site for drug action which is primarily

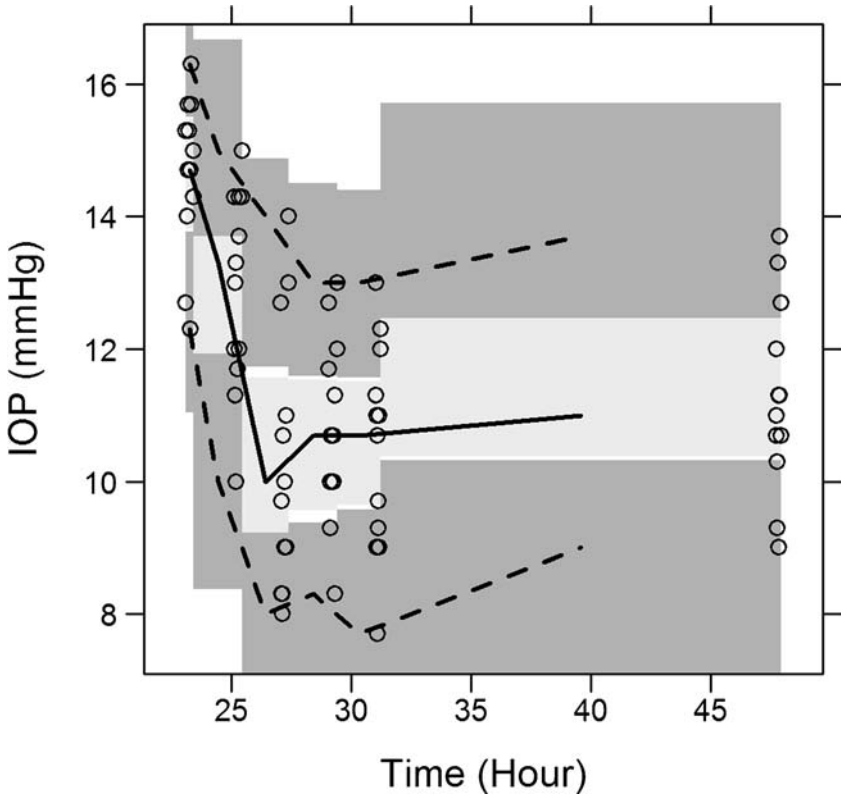

Fig. 7 VPC plot for the latanoprost PKPD model in dogs. The shaded areas represent the 95% confidence interval of median (*middle*), 95th percentile (*upper*) and 5th percentile (*lower*) of the 1,000 simulated datasets based on the final model. The median (*solid black line, middle*), 95th (*dashed black line, upper*) and 5th (*dashed black line, lower*) prediction intervals of the observed data are displayed along with the individual IOP data (*open blue circles*).

the ciliary body where aqueous humor is secreted or the vicinity of uveoscleral pathway to promote the outflow.

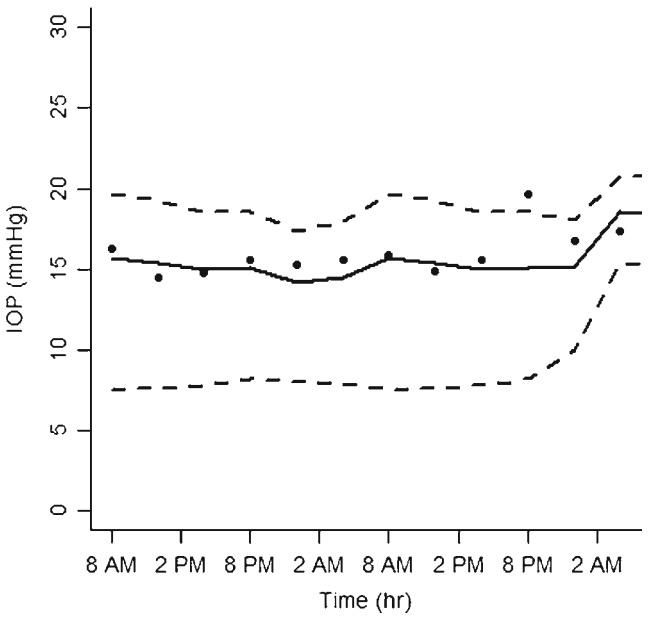

Fig. 8 Translational model predicted IOP in glaucoma/OHT patients treated with Xalatan® once daily for 2 weeks. *Solid line* represents the median and dashed lines represent the 95th percentile (*upper*) and 5th percentile (*lower*) of the 1,000 datasets simulated from the final model. The *closed circles* denote the observed mean IOP data from 18 patients.

Brimonidine IC_{50} estimated in the rabbit PKPD model (1.44 ng/mL; 5 nM) is comparable to the reported *in vitro* EC_{50} value (2 nM (39)) for functional activity at the α_2-adrenergic receptor. However, the brimonidine IC_{50} obtained from the bootstrap evaluation was higher (4.60 ng/mL) than the model prediction. This discrepancy could be attributed to the relatively smaller sample size used in the rabbit experiment which is known to provide biased estimates. *In vivo* EC_{50} estimated by the PKPD model (2.91 ng/mL; 7.4 nM) is closer to the *in vitro* EC_{50} of latanoprost acid for FP receptors (10 nM (32)). The differences in diurnal baseline IOP across species is the outcome of variation in aqueous humor dynamics (Table II). To extrapolate the animal PKPD model, we envisioned that IOP in patients can be predicted using the human aqueous humor dynamic variables assuming similar PKPD of drugs in both human and animals. Translational model predicted the diurnal IOP in patients treated with brimonidine or Xalatan® as indicated in the VPC plots (Figs. 6 and 8).

Given the nature of baseline IOP data collection, sparse PK sampling and use of satellite groups for PK and PD experiments, it is worth to consider several limitations of the translational model. The baseline IOP data collected from literature are average values that are prone to limitations of averaging individual data. For instance, inter-individual variability in the circadian oscillation cannot be determined that

could mask subtle changes in the diurnal rhythm of IOP. Since non-serial aqueous humor samples were collected from limited animals, inter-individual variability was not determined on all PK parameters. As PK information was collected from a satellite group of animals, individual contribution of PK to PD cannot be estimated in the respective animals. This results in lumping of PK variability into PD variability where the two cannot be differentiated. PK information was collected from few animals due to the destructive nature of sample collection which could influence the precision of parameter estimates. The number of animals used was reasonably and ethically limited which is common in preclinical ophthalmology studies. The differences in the body posture while IOP was measured in human and animals were not taken into account in the current model. Human predictions should be read with caution as body posture is shown to affect the IOP (40). Besides, the preclinical PKPD model was extracted following a single dose and translated to predict IOP in patients after repeat dose administration. This might undermine any PD desensitization or tolerance, if any, due to repeat administration. Despite the above stated limitations, the translational PKPD model predicted the IOP in patients with glaucoma or OHT with good accuracy. Moreover, the current model can be modified for other ocular dosing routes (eg., intra-ocular, periocular, *etc.*) and different dosage forms (eg., eye drops, suspensions, gels, implants).

In summary, we developed a physiology based population model for baseline IOP in rabbit, dog and human utilizing the aqueous humor dynamics in these species. Also, a mechanistic PKPD model was developed to describe the IOP after drug administration in rabbits and dogs. The preclinical PKPD model was successfully translated to predict the IOP in patients with glaucoma or OHT. Finally, the translational modeling approach adapted in this study may play a vital role in selecting dose and treatment regimen for designing clinical trials in patients using prior knowledge from preclinical studies.

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