**SUPPLEMENTAL DATA CONTENT**

**Model Development**

Aripiprazole (ARI) and dehydro-aripiprazole (DHA) plasma concentration-time data were used for nonlinear mixed-effect modeling with NONMEM® program versions 7.2 and 7.3, with version 7.3 used for the final analysis. PDx-Pop version 5.1 was used as the NONMEM interface. The model was developed utilizing from a total of 21620 aripiprazole and dehydro aripiprazole plasma concentration measurements obtained from 616 patients with schizophrenia. The ARI plasma concentration-time curve after oral dosing was shown previously to be well-described by a two-compartment model with first-order absorption.1,2 This served as a starting point for model development.

Untransformed data were used to test four residual error models (additive error model, multiplicative error model, combination additive and multiplicative error model, and exponential error model). Because individual parameter estimates appeared unduly influenced by exclusion of samples with concentrations below the lower limit of quantification (LLOQ), proportional error models using M3 methodology3 were also evaluated to determine whether they provided more accurate individual parameter estimates. Plots of weighted residuals were evaluated for homoscedasticity relative to the predicted concentrations and time after dosing.

First Order Conditional Estimation with Interaction (FOCEI) failed to converge on reliable parameter estimates, thus Monte Carlo Importance Sampling (IMP) and IMP with maximum a posteriori (IMPMAP) Expectation Maximization was used for parameter estimation. Models were “mu referenced”4 to improve the efficiency of the IMP computations. Initial evaluations indicated that IMPMAP estimation provided the most stable objective function path, and consequently, it was used as the estimation method for base model evaluation. Iterative Two Stage and Stochastic Approximation Expectation Maximization estimation methods were also used during base model development.

Both inter‑individual variability (IIV) and inter‑occasion variability (IOV) were regarded as random quantities and were modeled in terms of eta () variables. The etas across individuals (*i\_P*) for each model parameter (*P*) was generally assumed to have a mean of zero; the variance () described in IIV and IOV for each model parameter identified the expected distribution of the individual parameter values (*P*i) around the typical population value (TVP). In the present modeling, IIV was incorporated exponentially, where . This approach had the convenient property that the square root of the variance approximated the coefficient of variation (CV) of TVP when the variance was small (ie, <0.15). When the variance exceeded 0.15, the CV was computed from .

The variance-covariance matrix () for all parameters with modeled IIV first took a diagonal form. After accounting for the influence of covariates, off-diagonal elements were added to  as appropriate to account for observed correlations. Decisions regarding the inclusion of off-diagonal elements were based on goodness-of-fit criteria. Preference was given to models with off-diagonal elements when goodness-of-fit criteria demonstrated no clear difference and when the addition of these elements did not introduce numerical instability to the estimation process. During the base model development it was found to be advantageous to include a full OMEGA block in the model to account for the correlation between etas. Two OMEGA blocks were included into the model, one for structural model parameters and another separate block for baseline ARI and DHA [ARI(0) and DHA(0), respectively].

The goodness-of-fit for a model was assessed by a variety of plots and computed metrics, including plots of population and individual predictions vs observations and vs time, plots of conditional/individual weighted residuals vs predictions and vs time, histograms and quartile-quartile plots of conditional/individual weighted residuals and of the etas, scatter plots of eta pairs and eta vs modeled covariates, and plots overlaying observed and predicted values vs time. The structure of the base model was expanded as necessary to best reflect the characteristic shape of the observations over time. Characterization of treatment specific drug input functions were explored as necessary to accommodate alternative dosing conditions represented in the data (eg, oral vs IM absorption, and lag time to represent delayed absorption for the long-acting formulation). When a base model had been identified, the influence of covariates was assessed.

**Covariate Evaluation**

Baseline covariates were obtained from observations on the first day of dosing, or if not available, from screening. They included continuous covariates [dose, age, body weight, bodymass index (BMI), total bilirubin, serum albumin, serum alkaline phosphatase (ALP), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum creatinine, creatinine clearance (CLCR) calculated using the Cockcroft-Gault formula5, and aripiprazole lauroxil injection volume] and categorical covariates [race, ethnicity, gender, injection site, injection site reaction, needle length, formulation, aripiprazole lauroxil production scale , CYP2D6 phenotype, CYP3A4 genotype, concomitant use of CYP2D6 or CYP3A4 inhibitors or inducers, and smoking status]. Only categorical covariates represented by at least 10% of the population were evaluated for inclusion in the full covariate model; those with low representation were explored to estimate trends rather than to provide precise parameter estimates. The available covariates were evaluated and selected for inclusion in the full covariate model based on one or more of the following criteria: plots of individual estimates vs covariates demonstrated a trend; a statistically significant covariate effect was found by univariate analysis of variance for categorical covariates or by regression analysis for continuous covariates; physiological or pharmacological rationale; or information from prior analyses or published sources. Continuous covariates were centered at their typical values, and categorical covariates were tested and incorporated in the model as a series of index variables taking on values of zero or one.

The full model with backward deletion was used for covariate modeling. All covariate-parameter relationships of interest were entered into the model simultaneously. A backward deletion was performed where the relative influence of each covariate on the model was re-evaluated by deleting it from the full model on an individual basis. The difference in the objective function value (OFV) between models was used to compare competing hierarchical models, in which the more complex model could be reduced to a simpler model by removing or fixing the value of its estimated parameters. A finite number of iterations that produced a stable OFV and minimal movement in proportional error terms for the base model was used for the covariate analysis. For any covariate to have a significant influence, the OFV must have decreased by >2 times the standard deviation (SD) of the OFV compared to the model without the covariate. The changes in OFV were considered in conjunction with other goodness-of-fit plots and metrics. Significant differences in OFV that were not associated with improvements in goodness-of-fit were critically reviewed for indications of model misspecification.

**Final Model Evaluation**

The predictive performance of the final model was assessed by applying a posterior visual predictive check (VPC), and by calculating the percentage of the observations outside the 90% prediction intervals (PI). The final model parameters generated based on the entire dataset were used to simulate 100 datasets based on the covariates, sampling times, and dosing histories contained in the original dataset. The median, 5th, and 95th percentiles of the original concentration data were then compared to the 95% confidence interval (CI) of the median, 5th and 95th percentiles of the simulated data for each time point. This comparison was used to evaluate whether the derived model and associated parameters were consistent with the observed data.

**Description of Base Model**

The base model for ARI and DHA including data from all five studies contained four IM depot compartments, an oral dose depot compartment, and central and peripheral compartments for ARI and DHA (Fig.1). Four IM depot compartments were included to represent each IM dose for up to a maximum of four injections that were administered in clinical studies. The inclusion of an IM depot compartment for each administration best addressed the prolonged release characteristics of aripiprazole lauroxil. Additionally, it was unlikely that administration occurred into exactly the same intramuscular space with each injection, thus having a new dosing compartment for each injection was a more accurate reflection of the actual administration. The parameters associated with each IM dosing compartment were assumed to be equivalent.

Conversion of aripiprazole lauroxil to aripiprazole was described by a zero-order process with the duration (D1) of appearance estimated, and a lag time (ALAG) from the IM depots to the appearance of aripiprazole in the central compartment. Aripiprazole absorption following oral dosing was described by a first-order process (Ka), as were the movement between the central and peripheral compartments for each analyte, conversion of ARI to DHA, and elimination of DHA. It was assumed that all ARI was converted to DHA; therefore, all DHA parameters were expressed as the fraction of ARI metabolized to DHA (/fm).

The base model estimated 14 structural parameters: D1, ALAG, Ka, central apparent volume of distribution for ARI (VC/F) and DHA (VCM/F/fm), peripheral apparent volume of distribution for ARI (VP/F) and DHA (VPM/F/fm), apparent clearance of ARI (CL/F) and DHA (CLM/F/fm), inter compartmental CL for ARI (Q/F) and DHA (QM/F/fm), baseline concentrations of ARI and DHA [Ari(0) and DHA(0), respectively], bioavailability of the IM injection relative to oral aripiprazole (FIM) and bioavailability of oral aripiprazole (FPO) which was fixed to 1.0 as the reference treatment. Because 26 patients had quantifiable predose concentrations, ARI(0) and/or DHA(0) were estimated in the model. Rather than excluding subjects or applying a baseline correction to quantifiable predose concentrations all data were used unchanged in the analysis using the baseline term.

**Description of Covariate Model**

Inspection of the eta vs covariate plots suggested that both injection site (gluteal vs deltoid) and aripiprazole lauroxil formulation (Formulation 1 vs Formulation 2) may differ in terms of IM absorption parameters, and that VC/F appeared to increase with increasing weight. As a result, the effects of injection site and formulation were evaluated on D1, ALAG, and FIM, and the effect of weight was explored on CL/F as well as VC/F due to its correlation with CL/F. Formulation 1 was administered in the first clinical study and Formulation 2 in the other four studies. As Formulation 2 is the commercial formulation, and the one for which the majority of data were from, this was used as the reference formulation and the change in parameters from Formulation 1 relative to Formulation 2 estimated. The effect of WT on both VC/F and CL/F was evaluated using the power model with values centered for a weight of 70 kg.

The covariates that were tested and those that were retained in the model following a backwards deletion approach after all covariate-parameter relationships of interested were entered in the model simultaneously are summarized in Table 1.

After exploring these covariate effects in the full model, five covariate effects were retained in the reduced full model and used in the backward elimination approach. The change in OFV on backward elimination exceeded 2-times SD in OFV for three covariate effects [weight on VC/F (35.6-points) and formulation on ALAG (30.4-points) and FIM (48.1-points)] but not for the two other covariate effects [injection site on ALAG (–20.1-points) and D1 (16.0-points)]. The resultant model containing the three significant covariate effects was then used to evaluate inclusion of IOV on CL/F. However, inclusion of this term in the model yielded an OFV that was 36.5-points higher than prior to its inclusion, and therefore IOV was not retained in the model.

Although an obvious difference was not apparent for CL/F among the different CYP2D6 phenotypes based on visual inspection, it was evaluated in the model because aripiprazole CL/F had been previously shown to be decreased in CYP2D6 poor metabolizers (PM).6 Inclusion of this effect resulted in an OFV that was 14.6-points lower than prior to its inclusion, which was within the range of Monte Carlo noise in the OFV indicating that the effect was not significant. However, the point estimate of CL/F in PMs compared with non-PMs was 0.760 (95% CI: 0.685–0.845), and therefore this effect was retained in the model.

**Final Model**

The final model parameter estimates are reported in Table 2. The final model retained the structure of the base model (Suppl. Fig. 1), and contained covariate effects describing higher FIM and slower ALAG of Formulation 1 compared to the intended commercial formulation (Formulation 2), higher VC/F with increasing weight, and lower CL/F in CYP2D6 PMs.

The final model estimated relative bioavailability of aripiprazole following aripiprazole lauroxil administration of Formulation 2 to be 58% compared with oral aripiprazole administration. The typical lag time of 129 hours plus the typical zero-order duration of conversion of 854 hours suggest that peak concentrations of aripiprazole will typically occur at least 41 days after administration of aripiprazole lauroxil. The final model described VC/F to increase with body weight with the power effect fixed at 1.0 according to the following relationship: . Therefore, for the minimum, median, and maximum weight of 43, 81.7, and 144 kg in the PopPK dataset, VC/F would be estimated at 165, 313, and 551 L, respectively. The model also estimated CL/F to be 23% lower in CYP2D6 PMs compared with non-PMs.

The model estimated IIV in all 14 estimated parameters with IIV for FPO fixed to 0 as used as the reference treatment. The IIV in parameters ranged from 49.7% for D1 and 209% for Ka (Table 2). As expected, with few patients having unexplained quantifiable predose concentrations, estimates of IIV for ARI(0) and DHA (0) were high. Compared with the base model, estimates of IIV were reduced for seven of the 14 parameters, including a 4% reduction for VC/F, 2% reduction for CL/F, and 6% reduction for FIM due to the included covariates. Four other estimates of IIV were almost identical between the base and final models. Shrinkage for IIV estimates was moderate-to-low for all parameters, ranging between 8.3% for CLM/F/fm and 41.5% for DHA(0), indicating the model was adequate to perform simulations. Three correlations between structural model parameters had coefficients >0.7, including those between CL/F and CLM/F/fm (0.892), CLM/F/fm and VCM/F/fm (0.806), and ARI(0) and DHA(0) (0.784). The model estimated two proportional residual error terms, one per analyte (26.1% and 22.1% for ARI and DHA, respectively).

**SUPPLEMENTAL TABLE 1.** Covariate Effects and Backward Elimination Analysis

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Model | Parameter | Covariates Tested/Removed | OFV | 95% CI Included Null Value | Retain Effect |
| Full Modela | FIM | Injection site | −234 | Yes | No |
|  | Formulation | No | Yes |
| D1 | Injection site | No | Yes |
|  | Formulation | No | Yes |
| ALAG | Injection site | No | Yes |
|  | Formulation | No | Yes |
| VC/F | Weight | No | Yes |
| CL/F | Weight | Yes | No |
| Full Model: Injection site on FIM and Weight on CL/F removeda | FIM | Formulation | 13.5 relative to the full model | No | Yes |
| D1 | Injection site | No | Yes |
|  | Formulation | Yes | No |
| ALAG | Injection site | No | Yes |
|  | Formulation | No | Yes |
| VC/F | Weight | No | Yes |
| Reduced Full Modela | FIM | Formulation | 59.0 relative to the full model | No | Yes |
| D1 | Injection site |  | No | Yes |
| ALAG | Injection site |  | No | Yes |
|  | Formulation |  | No | Yes |
| VC/F | Weight |  | No | Yes |
| Backward Eliminationb | VC/F | Weight removed | 35.6 | -- | Yes |
| ALAG | Formulation removed | 30.4 | -- | Yes |
| ALAG | Injection site removed | -20.1 | -- | No |
| FIM | Formulation removed | 48.1 | -- | Yes |
| D1 | Injection site removed | 16.0 | -- | No |

aFor any covariate(s) to have a significant influence on the base model their inclusion required a >25.3 (2 x SD) decrease in the base model objective value function (OFV) and for it not to include the null value. Where significant covariate effects were identified, assessment of effect magnitude over a relevant range, along with confidence intervals was considered. A covariate could be retained in the final model, without meeting the aforementioned criteria, if there was a strong pharmacological or physiological rationale for its inclusion.

bCovariate effects were retained if the OFV was >2 x SD relative to the reduced full model.

**SUPPLEMENTAL TABLE 2**. Parameter Estimates in the Final Population-Pharmacokinetic Model

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Parameter estimates | | | Inter-individual variability | | | |
| Parameter | Value | % RSE | 95% CI | Value | % RSE | 95% CI | CV% |
| Ka (h-1) | 0.574 | 24.3 | 0.440–0.748 | 4.36 | 9.08 | 3.58–5.14 | 209 |
| FPO\* | 1.00 | — | — | 0 | — | — | — |
| FIM FORM 2a | 0.581 | 6.30 | 0.543–0.621 | 0.394 | 9.72 | 0.319–0.469 | 62.8 |
| FIM FORM1b | 1.57 | 21.6 | 1.29–1.89 |  |  |  |  |
| D1 (h) | 854 | 0.387 | 812–898 | 0.247 | 8.38 | 0.206–0.288 | 49.7 |
| ALAG (h) FORM 2 | 129 | 1.17 | 116–144 | 0.866 | 7.69 | 0.735–0.997 | 93.1 |
| ALAG FORM 1c | 0.247 | 12.3 | 0.176–0.346 |  |  |  |  |
| CL/F (L/h) non-PM | 2.02 | 4.02 | 1.91–2.14 | 0.329 | 7.42 | 0.281–0.377 | 57.4 |
| CL/F PMsd | 0.767 | 22.7 | 0.682–0.863 |  |  |  |  |
| VC/F (L) | 268 | 0.537 | 252–284 | 0.396 | 9.77 | 0.320–0.472 | 62.9 |
| WT on VC/Fe | 1.00 | — | — |  |  |  |  |
| Q/F (L/h) | 0.423 | 7.37 | 0.374–0.479 | 1.16 | 8.88 | 0.958–1.36 | 108 |
| VP/F (L) | 2122 | 1.21 | 1772–2540 | 1.74 | 11.6 | 1.34–2.14 | 132 |
| CLM/F/fm (L/h) | 5.16 | 1.84 | 4.85–5.47 | 0.389 | 7.02 | 0.335–0.443 | 62.4 |
| VCM/F/fm (L) | 354 | 0.756 | 324–388 | 0.685 | 7.91 | 0.579–0.791 | 82.8 |
| QM/F/fm (L/h) | 0.868 | 74.6 | 0.705–1.07 | 3.06 | 11.2 | 2.39–3.73 | 175 |
| VPM/F/fm (L) | 351 | 1.86 | 284–433 | 0.938 | 18.1 | 0.605–1.27 | 96.9 |
| ARI(0) (ng/mL) | 0.915 | 134 | 0.725–1.15 | 2.55 | 11.3 | 1.99–3.11 | 160 |
| DHA(0) (ng/mL) | 0.188 | 7.07 | 0.150–0.237 | 2.16 | 12.5 | 1.63–2.69 | 147 |
|  |  |  |  | Residual Variability | | | |
| 2prop aripiprazole | |  |  | 0.261 | 1.01 | 0.256–0.266 | 26.1 |
| 2prop dehydro-aripiprazole | |  |  | 0.221 | 1.17 | 0.216–0.226 | 22.1 |

\*Fixed as 1.0

aIn reference to FPO; bIn reference to FIM FORM2; cIn reference to ALAG FORM2; dIn reference to CL/F non-PM; 5power effect = VC/F · (WT/70)1.0

ALAG, absorption lag time; ARI(0), baseline amount of ARI; CL/F, apparent clearance of ARI; CLM/F/fm, apparent clearance of DHA; DHA(0), baseline amount of DHA; D1, input duration; FIM, IM bioavailability; FORM, formulation; FPO, oral bioavailability; Ka, first-order rate of absorption; PM, CYP2D6 poor metabolizer; Q/F, inter-compartmental clearance of ARI; QM/F/fm, inter-compartmental clearance of DHA; VC/F, apparent volume of ARI central compartment; VCM/F/fm, apparent volume of DHA central compartment; VP/F, apparent volume of ARI peripheral compartment; VPM/F/Fm, apparent volume of DHA peripheral compartment; WT body weight

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