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Population Pharmacokinetic Modeling and Pediatric Exposure of Dexamethasone Sodium Phosphate Encapsulated in Erythrocytes (eDSP) Administered Monthly for Treatment of Neurological Symptoms of Patients With Ataxia Telangiectasia

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ABSTRACT

The EryDex System (EDS) is a drug/device combination, which has been tested in clinical trials for ataxia telangiectasia (AT). EDS encapsulates dexamethasone sodium phosphate (DSP) solution in autologous erythrocytes at the point of care, and encapsulated DSP (eDSP) is infused back into the patient. Low doses of dexamethasone are released from erythrocytes over a 30-day period. This study aimed to (1) characterize the pharmacokinetics (PK) of dexamethasone released from intravenously infused eDSP based on data collected in clinical trials of healthy adults and pediatric AT patients, and to (2) simulate and extrapolate exposure measures of dexamethasone following intravenous infusion of eDSP administered once per month over 6 months in a pediatric population. The population PK model was developed using dense PK data from a phase 1 study in healthy adults and sparse PK data from a phase 3 study in pediatric AT patients. Three dose levels were studied, and the overall PK population included 24 healthy adults and 109 AT patients. The PK of dexamethasone released from eDSP was described using a simplified two-compartment model, adequate for estimating systemic exposure despite not fully capturing RBC release kinetics indicative of a triphasic pattern. The model showed a good fit, and future refinement will include mechanistic release modeling as more in vitro and in vivo data become available. Monte Carlo simulations of eDSP showed a rapid peak at 0.67 h, followed by sustained dexamethasone release; faster in the first 24 h, then slower over 20–30 days. No accumulation occurred with once-monthly dosing.

1 | Introduction

Ataxia telangiectasia (AT) is a neurodegenerative disease caused by a biallelic pathogenic variant in the *Ataxia Telangiectasia Mutated* (*ATM*) gene, located on chromosome 11q22.3 [1]. The pathophysiological process that underlies the disease is not completely understood, but emerging evidence suggests that oxidative stress from sustained microglia activation, increased inflammation, mitochondrial exhaustion, and altered neuronal membrane polarization and neurotransmitter levels play a

Study Highlights

- What is the current knowledge on the topic?
  - PK characteristics of eDSP have been described in a healthy adult population from the phase I trial; however, pediatric PK data were not available prior to this study.
- What question did this study address?
  - This study addresses the development of a pediatric PK model based on data from the adult study and pediatric patients with AT administered monthly with eDSP and predicts the exposure data in the patient AT population administered monthly with eDSP for 6 months.
- What does this study add to our knowledge?
  - This study describes a pediatric PK model for eDSP infusion and demonstrated that multiple infusions of eDSP resulted in low, sustained plasma concentrations of dexamethasone for 20–30 days without accumulation.
- How might this change drug discovery, development, and/or therapeutics?
  - The eDSP's ability to maintain low, sustained plasma concentrations of dexamethasone for 20–30 days without accumulation may become a better option for the treatment of patients requiring long-term steroid administration. This approach may also enhance compliance with treatment, which may improve outcomes, as it reduces the frequency of dosing and minimizes steroid toxicity.

crucial role in the pathophysiology of AT [2–5]. Currently, there is no approved treatment that slows down or stops the progression of AT.

The role of steroids in the treatment of neurological symptoms in people with AT has been studied since 2006 [6]. Treatment with bethamethasone and other steroids attenuated neurological symptoms of the disease. However, this neurological improvement was transitory and disappeared when oral steroid administration was discontinued. Due to the development of adrenal insufficiency, the benefit-to-risk ratio of long-term administration of oral steroids in AT patients was unfavorable [7–9].

The EryDex System (EDS) encapsulates ex vivo inactive prodrug dexamethasone sodium phosphate (DSP) solution (25 mg/mL) into the patient's erythrocytes, resulting in a point-of-care prepared encapsulated DSP (eDSP). Blood collected from the patient is automatically processed ex vivo by EDS using hypotonic saline solutions to permit osmotic opening of pores and diffusion of DSP into the erythrocytes. Subsequent incubation in a hypertonic solution reseals the cells and encapsulates DSP within red blood cells (RBCs). The eDSP is infused into the patient after extensive washing to remove extracellular content, process solutions, and any non-encapsulated DSP. After infusion, eDSP is dephosphorylated by phosphatase enzymes within the RBC, and dexamethasone (active drug) passively diffuses into the plasma. The release of the active drug is an enzymatically limited step and occurs continuously over a period of 20–30 days [10]. eDSP enables administration of dexamethasone at stable, low, and systemic levels over an extended period of tim; the EDS is designed to avoid the need for daily administration of higher oral doses of systemic steroids. The low plasma exposure provided by the EDS suggests that this method of administration may reduce the well-known risks of steroid side effects [11, 12].

A phase 3 study (ATTeST; NCT02770807) evaluated the efficacy and safety of eDSP (formerly known as EryDex) in pediatric AT patients [13]. In this study, as well as prolonged use of eDSP in the Open Label Extension trial (NCT03563053) in AT patients, a favorable toxicity profile was observed. Side effects typical of steroid use, such as adrenal suppression or cushingoid features, were rarely reported even after several years of use [13, 14].

This study aimed to characterize the PK of dexamethasone and develop a population model to estimate systemic exposure and evaluate extended-release properties in pediatric patients aged 2–< 17 years. Given limited pediatric sampling, sparse PK data from phase 3 children ($n = 115$) were combined with dense adult data from phase 1. The resulting population PK model enabled simulation of detailed pediatric PK profiles with frequent sampling across age groups. This investigation aimed to assess whether eDSP maintains sustained release of dexamethasone (in children) over 20–30 days, following a monthly infusion.

2 | Methods

2.1 | Study Designs, Patients, and PK Sampling

Population PK analysis was run based on PK data from a phase 1 study in healthy adults (ClinicalTrials.gov, NCT01925859) and a phase 3 study in pediatric patients with AT (NCT02770807). Three validated bioanalytical methods were used for the quantification of dexamethasone in human plasma (EDTA K2), each covering ranges from 0.5 to 250 ng/mL. All methods employed ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS or HPLC-MS/MS). Full assay validation details are available upon request.

The phase 1 study was a single-center, open-label study in healthy volunteers (age ≥ 18 years) where eDSP was given as a single IV infusion with a duration of approximately 10 min at a rate of approximately 8 mL per minute. This study was conducted at two dose ranges in two sequential groups of patients. In group 1 ($n = 9$), subjects received a mean eDSP dose in the range of ~2.5–5 mg of DSP encapsulated in RBC in the final infusion bag (Half-Low dose- H-LD). In group 2 ($n = 9$), subjects received a mean eDSP dose in the range of ~14–22 mg (High dose-HD). PK samples were collected just prior to the start of the infusion (0 min) and at 15 and 30 min, and at 1, 2, 4, 8, 12, 24, and 48 h after the start of the infusion. Additional blood samples were collected at 3, 7 ± 1, 14 ± 1, 21 ± 1, 28 ± 2, 35 ± 2, and 42 ± 2 days post-infusion for measurement of plasma dexamethasone and determination of PK parameters.

The ATTeST study was a phase 3, international, multi-center, one-year, randomized, double-blind, placebo-controlled study in pediatric patients with AT (age ≥ 6 years) where eDSP was given as IV infusion with a duration of approximately 40 min

at monthly intervals. The active treatment groups received the treatment at two doses of eDSP. In group 1 ($n = 58$), subjects received a mean dose of 8.3 mg of DSP encapsulated in RBC in the final infusion bag (Low dose-LD). In group 2 ($n = 57$), the mean encapsulated DSP dose was 17.4 mg (High dose-HD). After the first dose of eDSP, PK samples were collected before infusion (0 min); 1 h and 4 h after the end of the infusion (Day 1); at 24 h after the end of the infusion (Day 2); and on Day 15, in the morning. After the 2nd, 3rd, 4th, and 5th dose of eDSP, PK levels were obtained prior to (trough) and 1 h after (peak) the infusion. For the 6th infusion, only a trough level was obtained prior to the infusion. Plasma concentrations of dexamethasone were determined using validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods.

2.2 | Ethics Statement

All studies were conducted in accordance with the ethical principles derived from the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council for Harmonization Good Clinical Practice guidelines, and applicable laws and regulations. Ethics approval was obtained from institutional review boards and ethics committees. Written informed consent was provided prior to study enrollment by either the participant or their parent/guardian.

2.3 | Dataset Construction

The population PK dataset combined dosing, concentration-time data, and covariates. It was initially based on phase 1 adult data and later updated with sparse pediatric data from the ATTeST phase 3 trial. Records with missed dosing or sampling times, below the limit of quantification (BLQ) concentrations following detectable values, and subjects with only BLQ concentrations were excluded. Pre-dose concentrations were set to zero, and one subject was removed due to missing dosing information.

2.4 | Population PK Model Development

Plasma concentration-time data of dexamethasone was analyzed using a nonlinear mixed effects modeling approach using NONMEM 7.4 (Icon Development Solutions, Ellicott City, MD, USA). The minimization algorithm of the First-Order Conditional Estimation with Interaction (FOCE-I) was used for model parameter estimation.

Various one-, two-, and three-compartmental models were evaluated, including linear and nonlinear elimination, dual absorption processes, and semi-physiological models. The models were initially fitted to phase 1 PK data and subsequently tested with pooled phase 1 and 3 data.

Body weight was incorporated as a fixed allometric scaling factor (0.75 power for clearance (CL) and inter-compartmental clearance (Q); 1 for central (V1) and peripheral (V2) volumes of distribution, centered to 70 kg) during structural base model development in the pediatric population [15, 16]. Inter-individual variability was modeled exponentially on most PK parameters, and residual error was described using combined proportional and additive components.

After selecting the final structural model, covariates such as age, weight, BMI, dose level, health status, ethnicity, race, age groups, and gender were evaluated on CL and V1 during covariate analysis using a standard stepwise approach. Correlation between covariates was assessed using correlation matrices and exploratory plots. The evaluation of the impact of covariates on the dexamethasone population PK model focused on clinically and biologically relevant covariates. Covariates of interest were tested using a stepwise forward additive approach with a p-value of 0.01 (MOF = Minimum Objective Function, $\Delta MOF = 6.635$, for one degree of freedom [df]) and a backward elimination with a p-value of 0.001 ($\Delta MOF = 10.828$, for one df). Highly correlated covariates were not included together on the same parameter. Covariates were incorporated multiplicatively based on their mathematical structures: continuous covariates using a power model and categorical covariates using a proportional structure.

2.5 | Model Evaluation and Validation

The final model's performance was assessed using goodness-of-fit (GOF) plots and visual predictive checks (VPC) of model-simulated serum concentration-time profiles. For VPC, a total of 1000 replicates of the original dataset (i.e., dose history, PK sample times) were simulated with the final PK model. Bootstrap 1000 resampling was conducted to assess model stability and estimate parameter confidence intervals.

2.6 | Monte Carlo Simulations and Extrapolating the Exposure Measures

The final population PK model was implemented in R to conduct Monte Carlo simulations generating PK profiles of dexamethasone following monthly IV infusions of eDSP over 6 months in pediatric patients. A virtual population was built based on the distribution of body weights within age groups provided by the Centers for Disease Control and Prevention (CDC) growth charts [17]. Body weights were randomly created with a generalized additive model for location scale and shape (GAMLS) applied on the CDC chart for a population between 2 and 17 years old. A sampling approach was then performed on this virtual population by selecting 1000 subjects within each age group (i.e., 2 to < 6 years, 6 to < 10 years, and 10–17 years). The final population PK model was used to perform Monte Carlo simulations for pediatric patients across three dose ranges: Half-Low Dose (H-LD) 4.2 mg eDSP/Inf, Low Dose (LD) 8.3 mg eDSP/Inf, and High Dose (HD) 17.4 mg eDSP/Inf. The model included typical values of PK parameters, inter-individual variability, and incorporated covariates. Dexamethasone concentration-time profiles were simulated over 6 months (4320 h), with PK samples taken every 0.2 h during the first hour after each dose and every hour thereafter within each dosing interval. Simulated concentrations were used to calculate PK exposures after the first dose (AUC_{0-720} and C_{max}) and multiple-dose PK exposures (C_{max}, C_{trough}, and AUC_{tau}) following the sixth dose for each dose level and age group.

3 | Results

3.1 | Study Population and PK Data

The population PK analysis dataset included a total of 133 subjects, comprising 18 subjects from the phase 1 study and 115 subjects from the phase 3 study. Among the phase 3 participants, there were 6 adults and 109 pediatric patients, as summarized in Table 1. There were sufficient numbers of subjects in the adult ($n = 24$), 6–9 years ($n = 64$), and 10–17 years ($n = 44$) age groups; however, only 1 subject was available in the 2–5 years age group. The overall population was fairly balanced in terms of gender (53% male and 47% female) and race (57% white and 43% African American and/or black). In terms of dose distribution, 9 subjects received the half-low dose, 58 subjects received the low dose, and 66 subjects received the high dose eDSP treatment.

Descriptive statistics of the observed PK concentrations are summarized in Table S1. Out of a total of 1719 available PK concentrations, 68% were above the limit of quantification threshold, while 17% were BLQ within the first month after dosing. Since this drug is intended for monthly administration, the BLQ samples observed after 1 month were expected and served to confirm the absence of drug accumulation over time. The mean PK concentrations were approximately 10.4, 80.7, and 140 ng/mL for the half-low, low, and high dose groups, respectively (Table S2).

3.2 | Population PK Model Development

On a semi-log scale, the dexamethasone plasma concentration–time profile for phase 1 exhibited a biphasic or triphasic decline, suggesting a multi-compartmental (two or more) model (Figure 1). The PK profile for phase 3 observations is presented in Figure S1. Several 2- and 3-compartmental models were evaluated, starting with a reference one-compartment model with linear clearance. While Figure 1 suggests that a 3-compartment model may better describe the PK profiles, the selection of the 2-compartment model was based on a combination of statistical criteria (e.g., Akaike Information Criterion/Bayesian Information Criterion) and goodness-of-fit diagnostics. The value of the objective function was 2652.543 for the 2-compartment model, while it was 2724.38 for the 3-compartment model. Additionally, the 3-compartment model did not provide a significant improvement in fit while increasing model complexity. The best-fitting model, a two-compartment model with linear elimination (Figure S2), was selected to describe the concentration–time profiles of dexamethasone.

Figure S3 illustrates schematic representations of the other models considered during model development. Three-compartmental models (Figure S3a), two-compartmental models with Michaelis–Menten elimination (Figure S3b) and with mixed (linear + Michaelis–Menten) elimination (Figure S3c) were also developed to fit the data; however, they were inferior to the two-compartmental model with linear clearance.

The encapsulation (loading) of DSP into autologous RBCs, subsequent dephosphorylation of DSP within the RBCs to dexamethasone (the active drug), and sustained release of dexamethasone with passive diffusion into the plasma render eDSP to act as a depot formulation. Hence, different models were developed to simulate the release of dexamethasone into the plasma. To this purpose, a depot compartment was introduced into the model with first-order rate process (Figure S3d), Michaelis–Menten rate process (Figure S3e), and dual absorption process (Figure S3f). The two-compartmental model with dual absorption process (Figure S3f) consisted of the 1st order absorption rate constant standing for the faster release process of dexamethasone from eDSP in the first 24 h, and Michaelis–Menten rate constant describing the slow long-term release process. None of the models with depot compartment and absorption process provided a better GOF compared to the two-compartmental model with linear elimination.

To describe the dephosphorylation of DSP within the RBCs to dexamethasone and sustained release of dexamethasone into the plasma of the subjects, a two-compartmental semi-physiological model with linear elimination was also developed. The semi-physiological model is depicted in Figure S4. The semi-physiological model adequately described dexamethasone PK released from eDSP using phase 1 data, with relevant population estimates validated by bootstrap and VPC. However, adding phase 3 data led to an unrealistically low Q estimate and poor estimation of its inter-individual variability, likely due to model overparameterization.

3.3 | Final PK Model

A two-compartmental model with linear elimination was selected as the final PK model, as it provided the best improvement of the minimum objective function (MOF) and more reliable PK parameter estimates compared to all examined models, including the semi-physiological model. The final PK model was parameterized in terms of CL, Q, V1, and V2, with allometric scaling of weight fixed to 0.75 on CL and Q, and to 1 on V1 and V2. Inter-individual variability was introduced only on CL and V1, as its addition on PK parameters of the second compartment did not improve model fit and would only over-parameterize the model. Two error models, one for each phase of the study, comprising additive and proportional components, were incorporated to describe the residual variability of the observed concentration-time profiles of dexamethasone.

Based on the ETA versus continuous covariate plots, no statistically significant relationships were observed between PK parameters and continuous covariates such as BMI, age, and dose. Similarly, the ETA versus categorical covariate plots did not indicate any statistically significant relationships between PK parameters and categorical covariates, with the exception of disease status (healthy volunteers vs. patient population) and CL. In the stepwise covariate analysis, disease status was identified as a statistically significant covariate on CL. This effect was estimated as 0.899 in the θ^{PTNT} relationship, where PTNT = 0 represents healthy subjects and PTNT = 1 represents the AT patient population, indicating approximately 10% lower clearance in patients compared to healthy subjects. The population PK parameter estimates of the final model are presented in Table 2. The precision of the final model parameter estimates and covariate effects on PK parameters were assessed with 90% confidence intervals obtained from bootstrapping, which was generated by

TABLE 1 | Summary of baseline intrinsic and extrinsic covariates in the population PK dataset in Phase 1—Healthy volunteers and in Phase 3 patients with ataxia telangiectasia.

	Phase 1	Phase 3				
	Adults (*N* = 18)	**2–<6 years (*N* = 1)**	**6–<10 years (*N* = 64)**	**10–<17 years (*N* = 44)**	**Adults (*N* = 6)**	**Overall (*N* = 133)**
Weight (kg)						
Mean (CV%)	74.7 (14.5%)	15.1 (NA%)	22.1 (23.0%)	29.8 (37.0%)	54.1 (25.8%)	33.2 (59.9%)
Median [Min, Max]	74.8 [58.1, 95.7]	15.1 [15.1, 15.1]	21.0 [15.2, 40.6]	26.7 [16.1, 63.9]	51.6 [40.0, 78.1]	25.0 [15.1, 95.7]
Height (cm)						
Mean (CV%)	167 (15.7%)	106 (NA%)	121 (6.02%)	136 (9.13%)	162 (8.90%)	134 (15.7%)
Median [Min, Max]	174 [66.8, 184]	106 [106, 106]	121 [106, 140]	134 [115, 167]	162 [145, 180]	129 [66.8, 184]
BMI (kg/m^2)						
Mean (CV%)	24.9 (12.1%)	13.4 (NA%)	14.9 (15.5%)	15.5 (19.2%)	20.3 (13.3%)	16.7 (26.0%)
Median [Min, Max]	25.3 [19.4, 29.5]	13.4 [13.4, 13.4]	14.3 [11.3, 22.6]	14.9 [9.60, 22.9]	20.2 [17.1, 24.1]	15.2 [9.60, 29.5]
Age (Year)						
Mean (CV%)	35.9 (31.3%)	5.00 (NA%)	7.64 (12.6%)	11.3 (15.6%)	22.3 (35.6%)	13.3 (79.0%)
Median [Min, Max]	31.0 [21.0, 55.0]	5.00 [5.00, 5.00]	7.00 [6.00, 9.00]	11.0 [10.0, 16.0]	18.5 [17.0, 37.0]	10.0 [5.00, 55.0]
Sex						
Male	12 (67%)	0 (0%)	35 (55%)	22 (50%)	2 (33%)	71 (53%)
Female	6 (33%)	1 (100%)	29 (45%)	22 (50%)	4 (67%)	62 (47%)
Race						
White	6 (33%)	0 (0%)	43 (67%)	23 (52%)	4 (67%)	76 (57%)
African American and/or Black	12 (67%)	1 (100%)	21 (33%)	21 (48%)	2 (33%)	57 (43%)
Ethnicity						
Non-Hispanic or Latino	17 (94%)	1 (100%)	59 (92%)	44 (100%)	6 (100%)	127 (95%)
Hispanic or Latino	1 (6%)	0 (0%)	5 (8%)	0 (0%)	0 (0%)	6 (5%)
Mean Dose[a] (mg)						
Mean (CV%)	10.6 (64.6%)	11.3 (NA%)	12.5 (52.1%)	10.9 (59.8%)	10.8 (67.2%)	11.6 (56.3%)
Treatment						
Half-low dose (H-LD)	9 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (7%)
Low dose (LD)	0 (0%)	0 (0%)	34 (53%)	21 (48%)	3 (50%)	58 (44%)
High dose (HD)	9 (50%)	1 (100%)	30 (47%)	23 (52%)	3 (50%)	66 (50%)

Note: 9 subjects were included in the half-low dose group. Interpretations for younger patients (aged 2 to <6 years) should be made with caution, as only one patient from this age group was included in the population PK analysis.
Abbreviations: BMI, Body mass index; CV, Coefficient of variation expressed as a percentage; Max, Maximum; Min, Minimum; *N*, Number of subjects; NA, Not applicable.
[a]DSP Dose Encapsulated in RBC (mg).

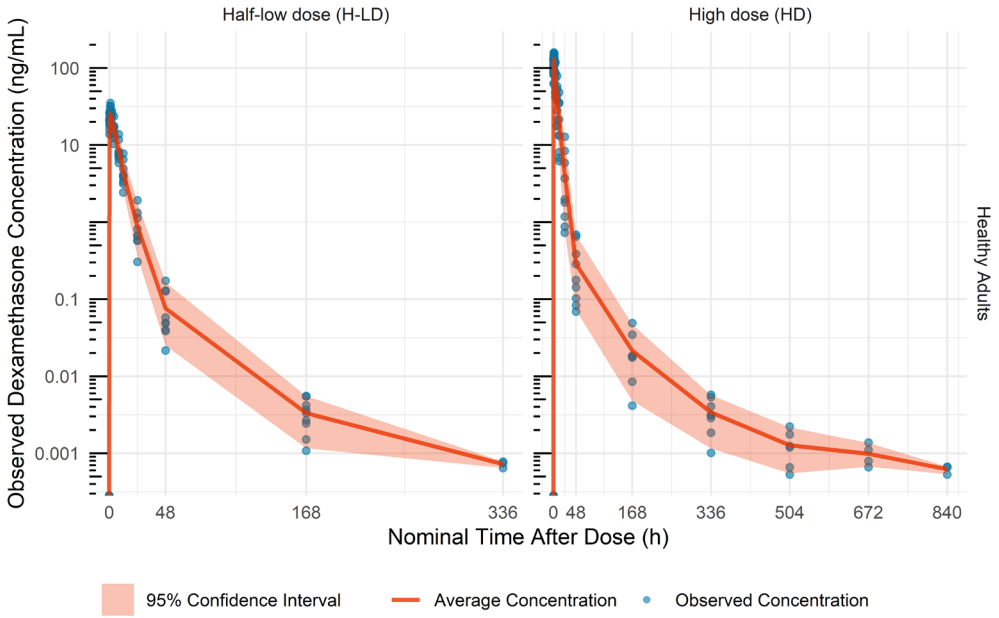

FIGURE 1 | Mean and Observed PK Concentrations Following a Single Dose of Dexamethasone Administered via the EryDex System (Semi-Logarithmic Scale)—Half-Low Dose and High Dose. 9 subjects were included in the half-low dose group. Interpretations for younger patients (aged 2 to < 6 years) should be made with caution, as only one patient from this age group was included in the population PK analysis.

TABLE 2 | Estimated PK parameters in the final population PK model for dexamethasone administered via the EryDex system.

Parameter Typical values	Population estimates	RSE%	Median [90% CI]
Clearance CL (L/h)	20.8	8%	20.9 [18.7–23.2]
Central volume V1 (L)	122	9%	123.6 [113.6–135.2]
Inter-compartmental clearance Q (L/h)	0.358	13%	0.362 [0.270–0.430]
Peripheral volume V2 (L)	16.8	9%	16.6 [14.0–19.1]
Weight effect on CL → * $(Weight/70)^{\theta}$	0.75 FIXED		—
Weight effect on V1 → * $(Weight/70)^{\theta}$	1 FIXED		—
Weight effect on Q → * $(Weight/70)^{\theta}$	0.75 FIXED		—
Weight effect on V2 → * $(Weight/70)^{\theta}$	1 FIXED		—
Patient effect on CL → * θ^{PTNT}	0.899	10%	0.887
Between Subject Variability (CV%)			
ETA_CL	41.4%	51% [26%][a]	37.9% [27.7%–51%]
ETA_V1	70.7%	21% [7%][a]	67.1% [46.4%–86.8%]
Residual error			
Proportional Error (%)—Phase 1	18.1%	10%	17.6% [14.9%–20.8%]
Additive error (ng/mL)—Phase 1	0.0013	18%	0.0012 [0.0008–0.0017]
Proportional Error (%)—Phase 3	29.5%	23%	28% [21%–33.5%]
Additive error (ng/mL)—Phase 3	24.3	58%	27.3 [21.9–41]

Note: Objective function value: 8584.636. PTNT = 0 for healthy subjects and PTNT = 1 for patient population. 9 subjects were included in the half-low dose group. Interpretations for younger patients (aged 2 to < 6 years) should be made with caution, as only one patient from this age group was included in the population PK analysis.
Abbreviations: CI, confidence interval; RSE, relative standard error.
[a][Shrinkage].

taking the range between the 5th and 95th percentiles of the parameter distribution.

3.4 | Model Validation

Overall GOF plots are shown in Figure 2, with pediatric-specific GOF plots in Figure S5. To improve visualization on the semi-log scale, 202 observations with IPRED values below LLOQ (0.0005 ng/mL) were excluded from Figure 2, leaving 969 non-BLQ concentrations. The complete GOF plot, including all data points, is available in Figure S6 (linear scale).

VPCs presented in Figure 3 demonstrate that the model adequately captures the concentration-time profiles of eDSP, with the observed 5th, 50th, and 95th percentiles mostly falling within the 95% confidence intervals of the simulated percentiles. Additional standard and prediction_corrected VPCs with an 80% prediction interval (10th to 90th percentiles) are provided in Figures S7 and S8, respectively.

GOF plots and VPCs indicate the model adequately captures dexamethasone concentrations, with no major bias or trends. Observed and simulated medians align well, particularly at lower and middle ranges. Limited high-range data reduced precision in matching the 95th percentile between observed and simulated values across treatment arms and age groups.

3.5 | Simulations and Extrapolating the Exposure Measures

Secondary PK parameters (AUC_{tau} and C_{max}) were derived from simulated PK profiles for both single-dose and multiple-dose regimens (after the 6th month). C_{trough} was also derived from the simulated PK profile at month 6. Descriptive statistics for these PK parameters are summarized in Table 3 (single dose) and Table S3 (multiple dose).

To better characterize C_{max}, which is expected to occur at the end of eDSP infusion (approximately 0.67 h), simulated PK data were generated with a sampling frequency of every 0.2 h during the first hour after dosing.

As shown in Figure 4, the comparison of C_{max} and AUC_{tau} between the single-dose and multiple-dose (6-dose) regimens indicated no drug accumulation with repeated eDSP administration. The slow release of dexamethasone was observed for

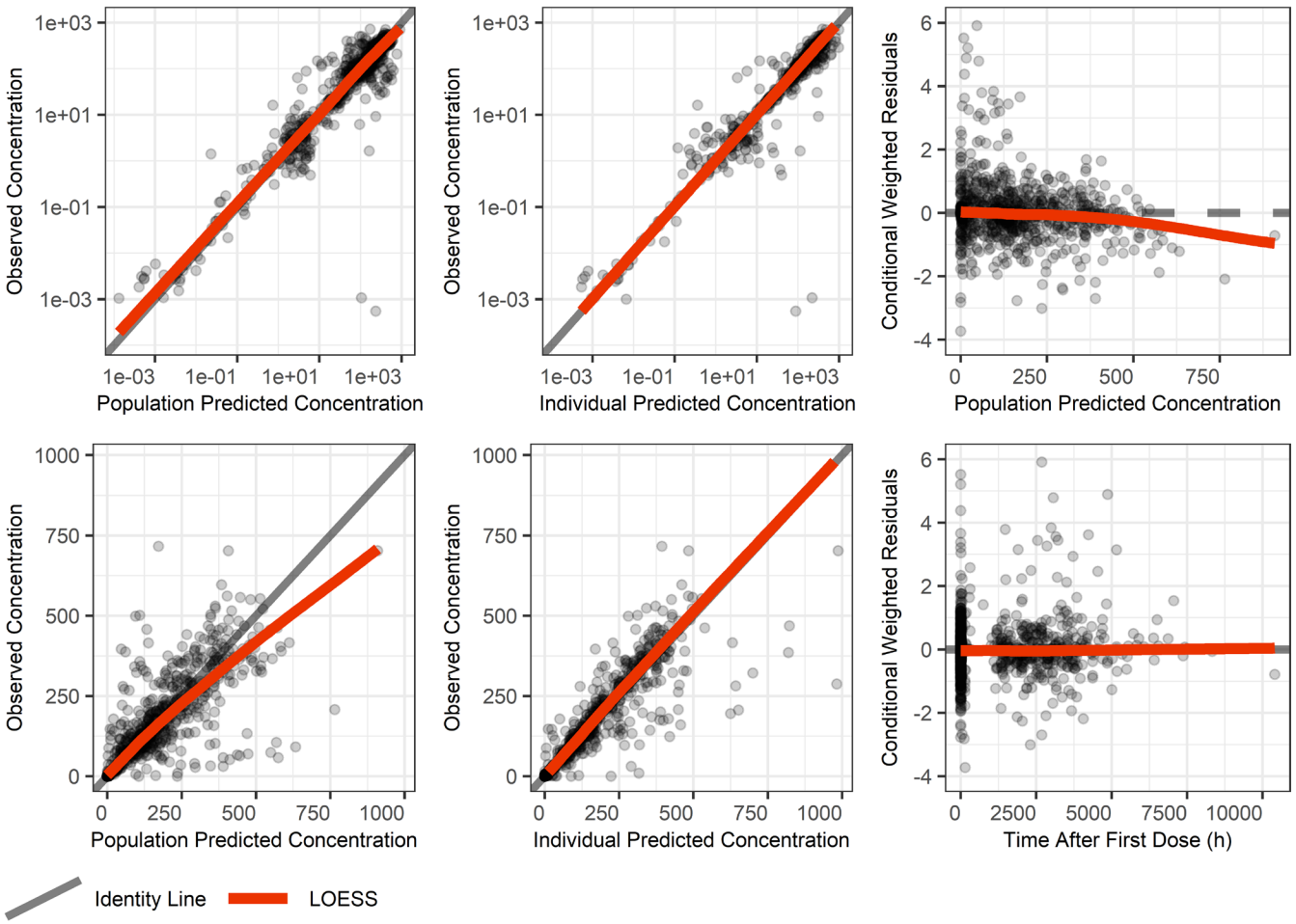

FIGURE 2 | Overall Goodness-of-Fit (GOF) Plots for the Final PK Model. LLOQ, Lower Limit of Quantification; LOESS, Locally Weighted scatter plot smoothing. A total of 202 observations with IPRED values below the LLOQ (0.0005 ng/mL) were excluded from the plot. These very low values were close to zero, making it difficult to visualize the remaining 969 non-BLQ observed concentrations in the semi-logarithmic plot. The Goodness-of-Fit plot including all data points (both above and below the LLOQ) is provided in Figure S6.

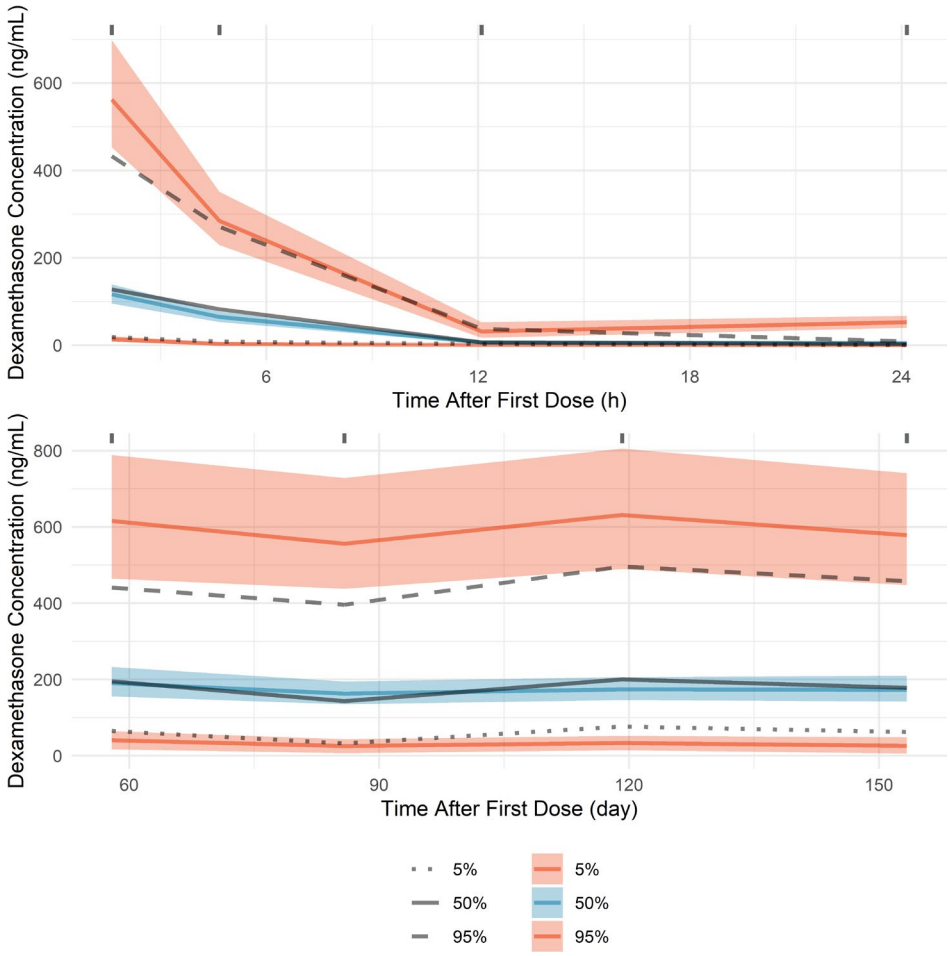

FIGURE 3 | Overall Visual Predictive Checks (VPC) Plot for the Final PK Model. The concentration-time profiles of DSP, with the observed 5th, 50th, and 95th percentiles mostly falling within the 95% confidence intervals of the simulated percentiles; additional VPCs stratified by age group and treatment group are provided in Figures S7 and S8, respectively. Due to the limited number of observations between Day 2 and Day 60, this time interval was excluded from the VPCs to enhance clarity.

TABLE 3 | Descriptive statistics of simulated pediatric PK exposures following a single dose of dexamethasone administered via the EryDex system.

Mean (CV%) Median [Min, Max]	DSP dose encapsulated in RBC	2–<6 years (*N* = 1000)	6–<10 years (*N* = 1000)	10–<17 years (*N* = 1000)
Weight (kg)		16.8 (20.8%) 16.3 [10.4, 31.9]	29.5 (26.4%) 28.1 [16.3, 65.6]	50.3 (21.3%) 50.7 [24.6, 69.9]
C_{max} (ng/mL)	Half-low dose (H-LD) (4.2 mg)	158 (63.9%) 132 [11.8, 898]	94.7 (65.9%) 80.0 [7.65, 583]	54.9 (71.0%) 44.6 [5.55, 468]
	Low dose (LD) (8.3 mg)	312 (63.9%) 260 [23.4, 1775]	187 (65.9%) 158 [15.1, 1153]	108 (71.0%) 88.1 [11.0, 925]
	High dose (HD) (17.4 mg)	654 (63.9%) 546 [49.1, 3720]	393 (65.9%) 331 [31.7, 2416]	227 (71.0%) 185 [23.0, 1938]
AUC (ng.h/mL)	Half-low dose (H-LD) (4.2 mg)	742 (44.0%) 685 [178, 2387]	477 (45.9%) 434 [107, 1842]	323 (46.9%) 292 [62.5, 1190]
	Low dose (LD) (8.3 mg)	1466 (44.0%) 1354 [352, 4717]	942 (45.9%) 857 [212, 3640]	638 (46.9%) 578 [124, 2351]
	High dose (HD) (17.4 mg)	3072 (44.0%) 2838 [739, 9888]	1975 (45.9%) 1796 [445, 7632]	1337 (46.9%) 1211 [259, 4930]

Note: 9 subjects were included in the half-low dose group. Interpretations for younger patients (aged 2 to <6 years) should be made with caution, as only one patient from this age group was included in the population PK analysis.
Abbreviations: AUC, Area under the curve of concentration-time over 1 month; C_{max}, Maximum Dexamethasone concentrations over 1 month; CV, Coefficient of variation expressed as a percentage; Max, Maximum; Min, Minimum; *N*, Number of simulated pediatric patients.

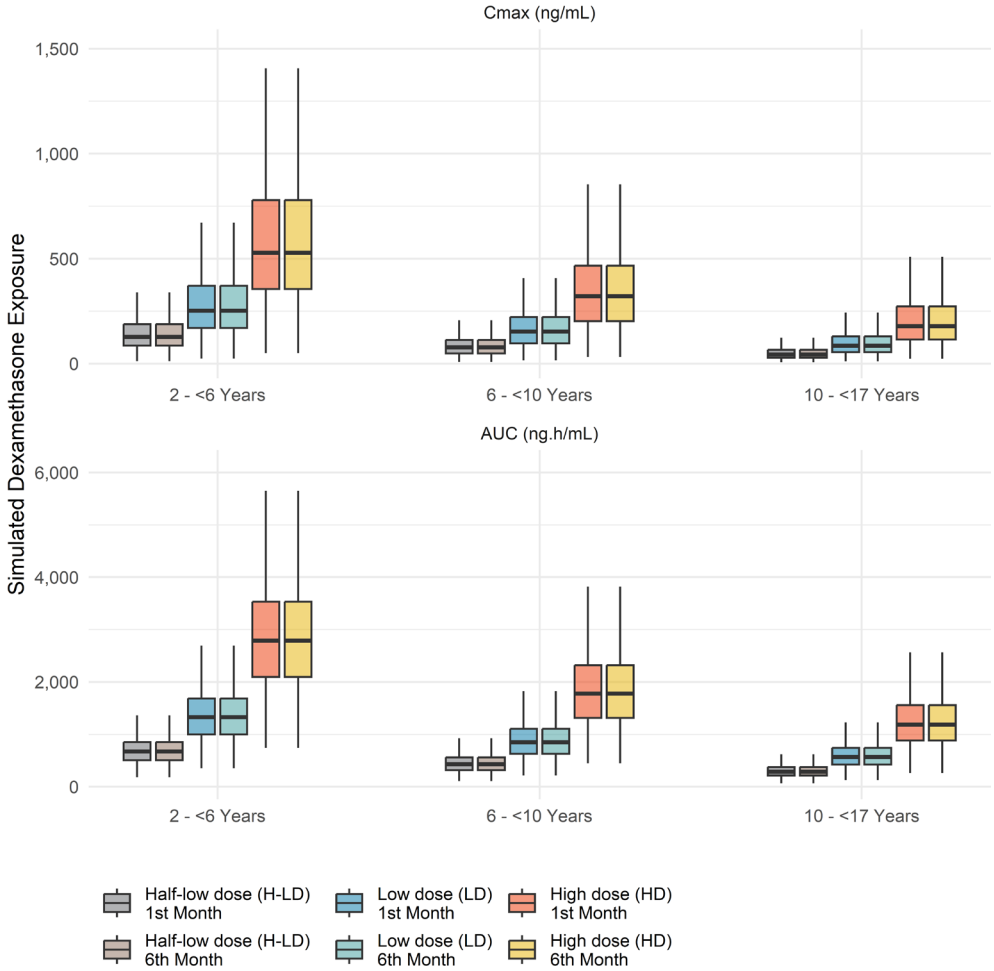

FIGURE 4 | Boxplot of Simulated Exposures Across Pediatric Age Groups and Different Treatment Dosing Schemes for Single and Multiple (6th) Doses. AUC, Area under the curve of concentration-time; Cmax, Maximum Dexamethasone concentrations. Half-low dose (H-LD) (4.2 mg); Low dose (LD) (8.12 mg); High dose (HD) (17.3 mg). Outliers were excluded from this plot for better visualization. 9 subjects were included in the half-low dose group. Interpretations for younger patients (aged 2 to < 6 years) should be made with caution, as only one patient from this age group was included in the population PK analysis.

up to 20–30 days following dosing, even at lower dose levels. Simulated PK profiles for different age groups at each dose level are presented in Figures S9 (single dose) and S10 (6th month multiple dose).

As shown in Table S4, the observed C_{max} values for subjects receiving the low dose indicate that while there is no significant difference between the observed and simulated C_{max} in the 6- to < 10 years age group, notable discrepancies were observed for the 10- to < 17 years age group. Specifically, the average simulated C_{max} values were 108 ng/mL and 227 ng/mL for the low and high doses, respectively, compared to the observed C_{max} values of 150 ng/mL and 314 ng/mL. These differences are primarily driven by differences in weight distribution between the observed and simulated populations. The average weight of the observed subjects in the 10- to < 17 years age group was approximately 30 kg, whereas the larger simulated population included heavier subjects, resulting in an average weight of approximately 50 kg. Given the effect of weight on CL, where heavier subjects exhibit higher clearance, the resulting C_{max} values are lower in the simulated population. The slightly lower clearance in patients compared to healthy subjects could be due to known fluctuation of CYP3A4 activity with age and body composition.

Additionally, it is important to interpret results for the younger age group (2–< 6 years) with caution, as the population PK model was developed based on only a single subject in this age range; no maturation functions were incorporated into the model.

4 | Discussion

Due to the sparse sampling in the phase 3 trial, pooled data from both the phase 1 and phase 3 trials were first used to characterize the PK by implementing population PK modeling. The model-derived PK parameters were then used to simulate rich sampling PK data in patient populations of three different age groups (2–< 6 years, 6–< 10 years, and 10–< 17 years).

When the rich sampling profile was simulated in different age groups using population PK estimates from the final model, the release of dexamethasone from encapsulated erythrocytes,

augmentation of dexamethasone concentration in plasma to C_{max} at 0.67 h from dosing, and its subsequent decline were adequately characterized (Figure S9). This supports the selection of the final model in characterizing the release of dexamethasone from eDSP, despite its simpler structure compared to a semi-physiological model.

In the simulated PK profile, a rapid, short-lived peak at 0.67 h was observed with a fast decline within the first 24 h after dosing and followed by a slow decline in plasma concentrations for the subsequent 20–30 days. The release of dexamethasone from erythrocytes is rapid initially but slows in later phases, contributing to sustained plasma levels up to 30 days. Dose-proportional AUC indicates no erythrocyte sequestration, suggesting most drug is released systemically over time, supporting the assumption of full bioavailability.

The rate of dephosphorylation of DSP to dexamethasone by resident phosphatase enzymes within erythrocytes is dependent on both the quantity of drug and the enzyme availability [15]. With a larger quantity of DSP in drug-loaded RBCs upon infusion, the rate of dephosphorylation is higher; therefore, a larger quantity of dexamethasone is released into the bloodstream by passive diffusion through the RBC membrane. As the quantity of DSP in drug-loaded RBCs decreases, the rate of dephosphorylation declines; therefore, a smaller quantity of dexamethasone is released at later timepoints, enabling dexamethasone administration at stable low systemic concentrations up to 20–30 days. This PK profile is similar to the one that Meduri et al. described as leading to improved outcomes in patients who received glucocorticoids for adult respiratory distress syndrome (ARDS) [18]. Optimal results in ARDS were achievable with an initial bolus glucocorticoid dose to achieve near maximal glucocorticoid receptorαsaturation, followed by a continuous infusion that maintained glucocorticoid receptor occupancy throughout the treatment period. For a more comprehensive understanding of the underlying mechanism of action, the development of a quantitative systems pharmacology (QSP) model or a semi-mechanistic model is recommended once additional data become available.

Simulated population PK profiles demonstrated no accumulation with once per month administration of eDSP in a multiple dose study scheme, consistent with data observed in the phase 1 and phase 3 clinical trials. The C_{max} values calculated from simulated PK profiles after the 1st dose (Table 3) were identical to those after the 6th dose within the same age group (Table S4). On the other hand, both LD and HD dose levels of eDSP show dexamethasone plasma concentrations of approximately 10^{-3} ng/mL after 20–30 days (corresponding to 500–720 h) from dosing time, while the H-LD dose level reaches this concentration in a shorter period of time. This is consistent with data from previous literature indicating that after conversion of DSP to dexamethasone by erythrocytes' phosphatases, dexamethasone is released into the bloodstream for about 20–30 days [19].

The final PK model adequately describes the observed data, predicts pediatric exposure, and supports the absence of accumulation after repeated infusions, with sustained low plasma concentrations for 20–30 days. While the simplified two-compartment model does not explicitly capture the controlled, likely triphasic release from RBCs, it provides stable exposure estimates without overparameterization. Rapid early plasma peaks suggest fast systemic appearance, with slower ongoing release possibly involving flip-flop kinetics. Future model refinement will incorporate additional in vitro and in vivo data to better characterize RBC-mediated release, terminal half-life, and formulation design.

Author Contributions

D.O., L.K., G.M., and P.O.T. wrote the manuscript and designed and performed the research. D.O. and L.K. analyzed the data.

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Conflicts of Interest

Deniz Ozdin, Leila Kheibarshekan, and Pierre-Olivier Tremblay were all employees of Syneos Health at the time this work was completed. Syneos Health received funding from Quince Therapeutics to perform the studies and analyses. Giovanni Mambrini is the Chief Technology Officer and a Board Member of Quince Therapeutics.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** psp470103-sup-0001-AppendixS1.docx.