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Pharmacokinetics-Based Pediatric Dose Evaluation and Optimization Using Saliva – A Case Study

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Abstract

Understanding pharmacokinetics (PK) in children is a prerequisite to determine optimal pediatric dosing. As plasma sampling in children is challenging, alternative PK sampling strategies are needed. In this case study we evaluated the suitability of saliva as alternative PK matrix to simplify studies in infants, investigating metamizole, an analgesic used off-label in infants. Six plasma and 6 saliva PK sample collections were scheduled after a single intravenous dose of 10 mg/kg metamizole. Plasma/saliva pharmacometric (PMX) modeling of the active metabolites 4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA) was performed. Various reduced plasma sampling scenarios were evaluated by PMX simulations. Saliva and plasma samples from 25 children were included (age range, 5–70 months; weight range, 8.7–24.8 kg). Distribution of metamizole metabolites between plasma and saliva was without delay. Estimated mean (individual range) saliva/plasma fractions of 4-MAA and 4-AA were 0.32 (0.05–0.57) and 0.57 (0.25–0.70), respectively. Residual variability of 4-MAA (4-AA) in saliva was 47% (28%) versus 17% (11%) in plasma. A simplified sampling scenario with up to 6 saliva samples combined with 1 plasma sample was associated with similar PK parameter estimates as the full plasma sampling scenario. This case study with metamizole shows increased PK variability in saliva compared to plasma, compromising its suitability as single matrix for PK studies in infants. Nonetheless, rich saliva sampling can reduce the number of plasma samples required for PK characterization, thereby facilitating the conduct of PK studies to optimize dosing in pediatric patients.

Keywords

children, infants, metamizole, pharmacokinetics, pharmacometrics, saliva

Introduction

Despite numerous drugs being approved every year, data in infants and children are rarely collected and pediatric investigation plans are mainly implemented for novel therapies. In more than 50% of marketed drugs there is no information in their respective la-

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bels about how to use these drugs in this vulnerable population.[1,2] For only 38% of drugs approved by the European Medicines Agency from 1996 to 2019, the population studied also included children.[3] Up to 50%–80% of drug prescriptions for hospitalized children remain off-label, depending on age and indication, despite regulatory efforts for improvement.[4,5] Weight-proportional dosing is frequently used, although the relationship between weight and a drug's exposure is not necessarily linear and differences in pharmacokinetics (PK) between children of different ages and adults are not considered.[6,7] The development of safe and efficacious drug use in infants and children is commonly supported by PK-based pediatric dose evaluation and optimization.[5]

Most commonly, age-appropriate dose-finding studies are conducted with exposure data in plasma. However, plasma sampling in infants and children is ethically and technically challenging. Repeated invasive plasma sampling can often only be conducted in in-patient settings. Alternative matrices for pharmacological and PK studies in infants and children are required.[1,8,9] Several studies report that concentrations of drugs and metabolites can also be measured in saliva of adults and children.[10,11] Saliva collection is noninvasive and offers advantages for multiple collections in in-patient, out-patient, or home settings. For several antiepileptic drugs, favorable stability in saliva even outside controlled laboratory conditions has been shown, facilitating sample processing and transport.[12] Variability can impede its application as therapeutic drug monitoring matrix since accurate exposures are necessary for safe dose adjustments. However, in a controlled setting such as a clinical study, saliva can provide a valuable alternative to derive PK in a vulnerable population such as infants and children.[13]

Here we aimed at evaluating the suitability of saliva as an alternative PK matrix to facilitate the development of rational dosing recommendations in children, demonstrated by a case study with metamizole (dipyrone). Despite rare cases of agranulocytosis and market withdrawal in several countries,[14] metamizole remains widely used for treatment of severe pain and refractory fever due to its favorable tolerability profile, nonaddictive nature, and potential for analgesic combination therapy.[15,16] Administered orally or intravenously (i.v.), the prodrug is rapidly hydrolyzed to the active metabolite 4-methylaminoantipyrine (4-MAA), which is converted into another active metabolite, 4-aminoantipyrine (4-AA). Therefrom, the main inactive metabolites 4-formyl-aminoantipyrine (4-FAA) and 4-acetyl-aminoantipyrine (4-AAA) are formed.[7,17] Recently, plasma PK of metamizole were described for the first time in infants and children (3–72 months of age) after a single i.v. dose of 10 mg/kg for postoperative analgesia.[7] Elimination of 4-MAA was significantly reduced in 4 children <1 year, for whom i.v. administration is off-label, leading to an increased exposure compared to older children.[7] According to early studies in healthy adults, saliva concentrations of metamizole metabolites were highly correlated with plasma concentrations, and the saliva concentrations of the active metabolites with its analgesic effect.[18,19]

We report the quantification of metamizole metabolites in saliva in children of 7–70 months of age after receiving a single i.v. dose of metamizole as postoperative analgesic in a prospective clinical study. Further, saliva noncompartmental analyses (NCA) and pharmacometric (PMX) PK modeling of the main active metamizole metabolites in saliva and plasma is described. Different sparse plasma sampling scenarios

Table 1. Demographic Characteristics of Children Providing At Least 1 Plasma and/or Saliva Sample.

Characteristics	Statistics	Saliva	Plasma	Overall
		N = 16	N = 25	N = 26
Number of samples				
Number of samples per child	Median (IQR)	4 (2.3–5)	5 (4–5)	5 (3–5)[a]
Children with ≥5 samples	n (%)	7 (44)	19 (76)	6 (23)[a]
Sex				
Female	n (%)	5 (31)	7 (28)	8 (31)
Male	n (%)	11 (69)	18 (72)	18 (69)
Age [months]	Median (IQR)	57 (30.8–62.5)	43 (22.0–59.0)	46 (22.0–58.8)
	Range	7–70	5–70	5–70
Weight [kg]	Median (IQR)	17 (11.9–21.0)	15 (11.2–18.0)	15.5 (11.3–18.7)
	Range	8.7–24.8	7.8–24.8	7.8–24.8
Albumin concentration [mg/mL]	Median (IQR)	35.5 (33.8–36.5)	34 (32.0–36.0)	34.5 (32.0–36.0)
	Range	30.0–41.0	27.0–41.0	27.0–41.0

%, percentage of subjects; IQR, interquartile range; N, number of subjects in the population; n, number of subjects in the respective category.
[a] Number of samples per matrix and children with ≥5 plasma and saliva samples.

complementing rich saliva sampling were evaluated to further facilitate PK studies in infants and children.

Methods

Study Design and Population

The presented data originate from a single-center, open-label, prospective clinical study approved by the local ethics committee (clinicaltrials.gov number NCT02660177), as reported previously.[7] Briefly, infants and children from 3 months to 6 years, scheduled for surgery with standard postoperative metamizole pain management, were enrolled after informed consent was obtained from the parents and meeting the eligibility criteria as defined previously.[7] Clinical laboratory parameters (e.g., liver and kidney function parameters, albumin) were collected directly after anesthesia. Metamizole was administered as a single i.v. bolus dose of 10 mg/kg (Novalgin, metamizole injection, 500 mg/mL, Sanofi-Aventis SA, Vernier, Switzerland) prior to awakening from anesthesia or immediately after arriving at the postanesthesia care unit. Blood and saliva samples for PK analyses were taken 1, 2, 4, 6, 10 (±1), and 24 h post dose.[7] Saliva was collected with SalivaBio children and infant oral swabs consisting of a durable polymer, which are validated for sample recovery (Salimetrics, State College, PA) in infants and children <6 years. The end of the collection device was placed under the child's tongue for 1.0–1.5 min, collecting approximately 200 μL saliva. Immediately after saliva collection, tubes were centrifuged and the supernatant stored at −80°C.

Bioanalysis

The plasma concentrations of metamizole metabolites were determined using a fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as described previously.[7,20] The method was adapted for the analysis of saliva samples. In brief, an aliquot of 20 μL saliva was mixed with 150 μL methanol containing 0.5% formic acid and the internal standards (20 ng/mL 4-MAA-d_3, 50 ng/mL 4-AA-d_3, and 100 ng/mL 4-AAA-d_3). After centrifugation, an aliquot of 2.5 μL supernatant was injected into the LC-MS/MS system. Chromatography was performed using an Atlantis T3 C18 analytical column (3 mm × 50 mm, 3 μm, Waters, MA). Mobile phases A and B were water and methanol, respectively, both supplemented with 0.1 % formic acid. The sample was delivered at 0.1 mL/min and 2% mobile phase B onto the column. In the first 0.5 min, the solvent flow was diluted in-line with mobile phase A through a T-union installed in front of the analytical column (flow ratio: 0.1:0.4 mL/min). Afterward, the mobile phase B concentration was increased linearly to 95% within 2.5 min at 0.5 mL/min. The column was flushed at 95% for 1 min and reconditioned at 2% mobile phase B for 0.5 min. The resulting retention times of 4-MAA, 4-AA, 4-FAA, and 4-AAA were 2.07, 2.23, 2.56, and 2.60 min, respectively. Calibrations (n = 8) and quality control samples (n = 5 QC levels) were prepared in a pool of saliva (n = 5). The lower limit of quantification (LLOQ) was 100 ng/mL for 4-MAA and 10 ng/mL for the other metabolites. The method used was linear, resulting in an R value of ≥0.996 across all analysis batches. Inter-batch precision and accuracy were ≤12.6% and 96.3%–116.4% considering all metabolites. The results of the incurred sample reanalysis experiments indicate that the majority of the samples analyzed had a deviation of less than 20%. Specifically, 86% of the 4-MAA samples (7 out of 8), 89% of the 4-AA samples (8 out of 9), 100% of the 4-FAA samples (12 out of 12), and 100% of the 4-AAA samples (12 out of 12) met this criterion.

Descriptive Statistics

Plasma and saliva concentrations as well as saliva/plasma concentration ratios per metabolite were summarized using the geometric mean and 95% confidence interval (CI) and presented graphically over time. Linear regression between time-matched plasma and saliva concentrations was calculated per metabolite in R version 3.6.1.[21]

NCA of saliva concentrations of all 4 metabolites was performed with Phoenix Winnonlin version 8.3 (Pharsight Inc., Princeton, NJ), including all children providing ≥5 saliva samples. Due to the limited number of children <2 years providing saliva samples (N = 2, i.e., 7 and 10 months), PK parameters were not stratified by age.[7] Plasma PK parameters were recalculated for the same population.

Pharmacometric Pharmacokinetic Modeling

To investigate mechanistic aspects of the plasma/saliva distribution of the active metabolites (4-MAA and 4-AA) and to evaluate the comparable precision (intra-/interindividual variability) of saliva and plasma pharmacokinetics, a population PK model of 4-MAA and 4-AA in plasma and saliva concentrations of all study participants was developed using Monolix 2021R1 (Lixoft SAS, a Simulations Plus company, Antony, France).

The 1-compartment model developed previously for plasma concentrations was extended to describe saliva and plasma concentrations of 4-MAA and 4-AA simultaneously.[7] This model incorporated weight-based allometric scaling of total volume of distribution (V) as well as clearance (CL) for all metabolic pathways ($CL_{MAAtoAA}$, $CL_{AAother}$, and $CL_{MAAother}$). An age-effect <2 years on all 4-MAA metabolic CL parameters was included. $CL_{MAAtoFAA}$, $CL_{MAAtoAAA}$, and $CL_{MAAother}$ were combined in a single parameter in this analysis

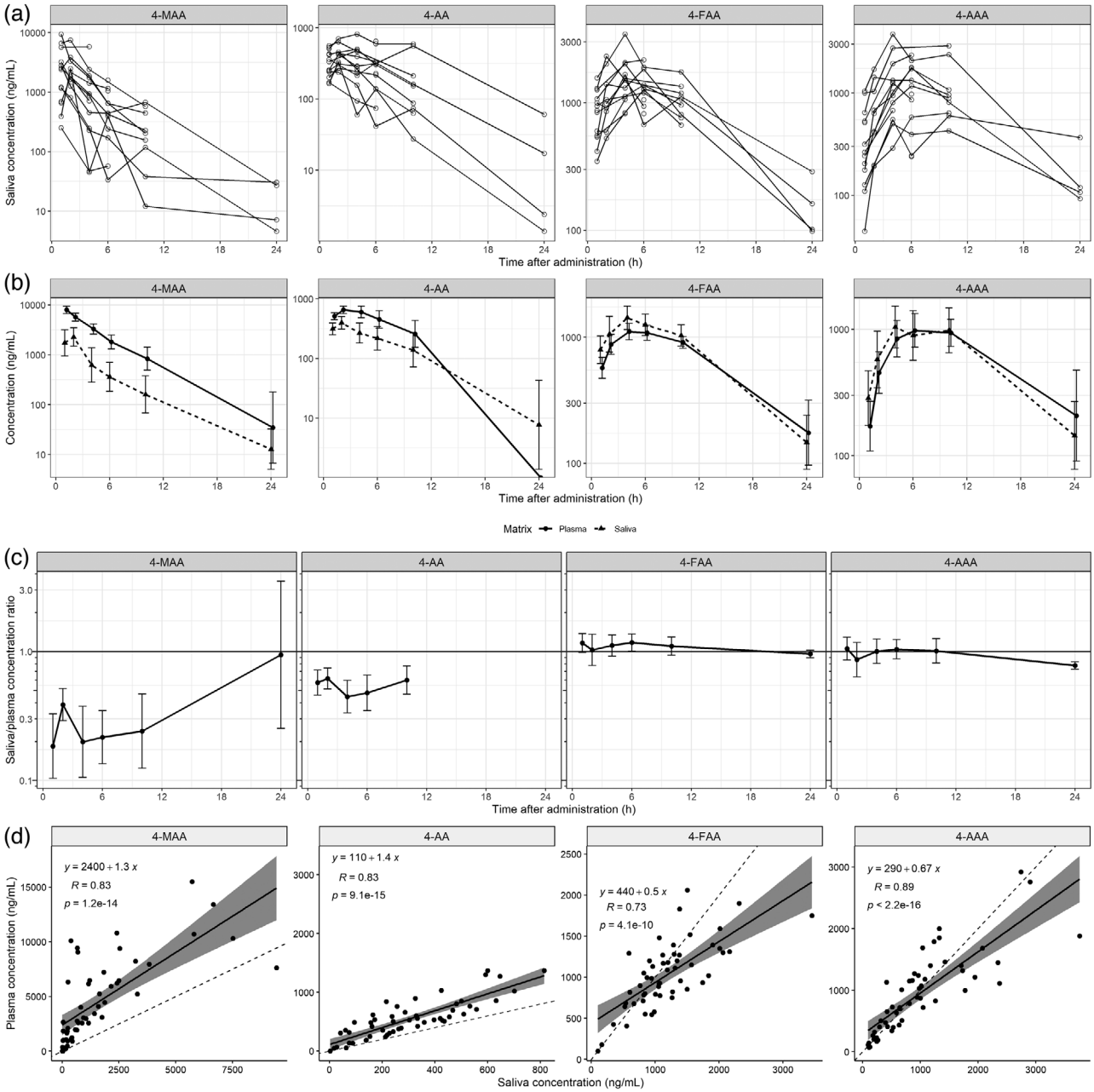

Figure 1. Metamizole metabolites in infants and children after a single intravenous dose of 10 mg/kg (N = 16 for saliva, 25 for plasma, and 15 for concentration ratios and correlations). (a) Individual saliva concentration–time profiles, (b) mean saliva (dashed line) and plasma concentration (solid line)–time profiles (geometric mean ± 95% CI), (c) mean saliva/plasma concentration ratio (geometric mean ± 95% CI); 8 h sample as well as 4-AA saliva/plasma data pair at 24 h available from only 1 child, therefore not included, (d) correlation of plasma versus saliva concentration (solid line = linear regression, grey-shaded area = 95% confidence interval, dashed line = line of identity, spearman correlation coefficients, and corresponding *P*-values).

as nonactive metabolites were not included. The metamizole hydrolysis rate constant to 4-MAA was fixed to 20/h.[7] Distribution of metabolites from plasma into saliva was assessed as a hypothetical effect compartment,[22] that is, assuming no or negligible elimination occurring from saliva. Alternatively, based on the observed rapid distribution of 4-MAA into saliva, saliva concentrations were predicted as a fraction of the plasma concentrations ($F_{S/P}$). Once 4-MAA was adequately described in plasma and saliva, 4-AA data were included similarly.

Between-subject variability was incorporated on V, CL of 4-MAA via metabolism to 4-AA, CL of 4-MAA via other routes, CL of 4-AA (log-normal distribution), and $F_{S/P}$ for both metabolites (logit-normal distribution).

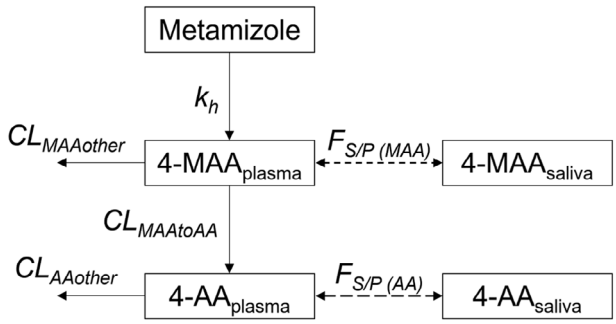

Figure 2. Structural model of metamizole and its active metabolites in plasma and saliva.

Residual intraindividual variability was assessed with constant, proportional, and mixed error models for each observation type. Different strategies to handle saliva concentrations below LLOQ were assessed such as using reported values above the detection limit, LLOQ, LLOQ/2, treating them as missing or censored (corresponding to "M3" or "M4" methods).[23]

Apart from the allometric scaling components for age and body weight, albumin concentrations (collected during clinical laboratory assessment prior to dosing) were evaluated as covariate on log-transformed V and logit-transformed $F_{S/P}$ graphically and in a linear relationship in the model.[7]

The final plasma–saliva model was selected based on the corrected Bayesian information criterion (BICc), precision of parameter estimates, visual predictive checks (VPC), and other standard goodness-of-fit (GOF) plots.

Pharmacometric Analysis to Evaluate Reduced Plasma Sampling Designs

Feasibility of reduced plasma sampling was explored by refitting the final saliva/plasma population-PK model to reduced datasets, in which plasma samples of specific time points were removed stepwise. From the 6 originally scheduled plasma samples (1, 2, 4, 6, 10, 24 h post dose), data from 1–6, 1–4, and 1–2 h as well as single plasma samples at 1, 4, and 6 h post dose were kept in respective reduced datasets. Relative accuracy and precision of estimated 4-MAA CL and $F_{s/p}$ parameters were compared to the values estimated from the full dataset to determine the residual precision of saliva samples and increasingly sparse plasma sampling.

Results

Patient and Data Characteristics

Out of 25 children with plasma PK data previously reported,[7] 15 provided at least 1 evaluable saliva sample, with 6 providing ≥5 saliva samples. In addition, 1 child provided ≥5 saliva samples at all scheduled time points, but no evaluable plasma data. All 7 children with ≥5 saliva samples were included in the NCA of both matrices, whereas all children were included in the combined population-PK modeling (Figure S1). Patient characteristics are given in Table 1. Median age of children providing saliva samples tended to be higher compared to those providing plasma (57 vs 43 months), but differences were not statistically significant (P = .273, determined by Mann–Whitney test).

For 3 saliva samplings in 2 patients, the actual sample collection time was missing and was imputed with the scheduled time. Metamizole metabolites could be quantified in saliva in all collected samples, while 10 4-MAA and 2 4-AA concentrations were below LLOQ (17 and 3%, respectively). For 4-FAA and 4-AAA, no saliva concentrations were below LLOQ.

Descriptive Analysis

The individual and geometric mean saliva concentration–time profiles are provided in Figures 1a,b (together with respective plasma profiles). Geometric mean ratios of saliva/plasma concentrations per time point were 0.18–0.39 (except for 0.94 at 24 h based on N = 2) and 0.39–0.62 for active metabolites 4-MAA and 4-AA, respectively, showing no evident concentration dependency in saliva/plasma ratios (Figure 1c). Time-dependent correlations of plasma versus saliva concentrations ranged from 0.73–0.89 (P-values <.001) for all metabolites (Figure 1d).

Results of the NCA are given in Table S1. Briefly, 4-MAA exposure parameters were approximately 3-fold higher in plasma than in saliva, whereas time to maximum concentrations (t_{max}) and terminal half-lives ($t_{1/2}$) were comparable between matrices. For 4-AA, saliva exposure was approximately 50% lower than plasma, although 95% CIs were overlapping. 4-AAA and 4-FAA showed similar exposures and profiles in both matrices.

Pharmacometric Pharmacokinetic Modeling

With an estimated plasma-to-saliva transfer rate constant of 6.6/h (RSE: 198%), a negligible distribution delay between 4-MAA could be quantified. Therefore, the combined plasma and saliva data were best described by including saliva concentrations as a fraction of plasma concentrations, with separate $F_{S/P}$ parameters for 4-MAA and 4-AA (Figure 2). Ranges of individual $F_{S/P}$ values were 0.05–0.57 for 4-MAA and 0.25–0.70 for 4-AA, respectively.

A correlation between the random effects of both individual $F_{S/P}$ parameters was included in the model (Table 2).[24] No correlation of any $F_{S/P}$ with age or albumin concentrations was observed or improved the model (Figure S2). Proportional error terms were applied for plasma and saliva concentrations of both metabolites, with concentrations below LLOQ treated

Table 2. Population Pharmacokinetic Parameter Estimates of Combined Plasma and Saliva Data of Active Metamizole Metabolites 4-MAA and 4-AA.

Parameter [unit]	Description	Population Parameter			Interindividual Variability	
		Estimate	SE	RSE (%)	Estimate (SD)	RSE (%)
k_h [1/h]	Metamizole hydrolysis rate constant	20 (fixed)				
V [L]	Total volume of distribution	9.54	0.72	7.59	0.34	15.0
$CL_{MAAother}$ [L/h]	Clearance of 4-MAA via other routes	1.72	0.31	18.2	0.79	19.7
$CL_{MAAtoAA}$ [L/h]	Clearance of 4-MAA via metabolism to 4-AA	0.82	0.11	13.3	0.55	15.8
$CL_{AAother}$ [L/h]	Clearance of 4-AA	3.84	0.63	16.5	0.71	18.3
SLP	Age-dependent power value for $CL_{MAAother}$ in children <24 months of age	0.68	0.065	9.46	-	-
SLP2	Age-dependent power value for $CL_{MAAtoAA}$ in children <24 months of age	0.28	0.088	31.2	-	-
$F_{S/P\ (MAA)}$	Distribution fraction parameter 4-MAA saliva to plasma	0.32	0.052	16.3	0.81	24.9
$F_{S/P\ (AA)}$	Distribution fraction parameter 4-AA saliva to plasma	0.57	0.043	7.55	0.55	0.13
Random effect correlations						
Corr V and $CL_{MAAtoAA}$	Random effect correlation between total volume of distribution and metabolic $CL_{MAAtoAA}$	0.79	0.092	11.6	-	-
Corr $F_{S/P\ (MAA)}$ and $F_{S/P\ (AA)}$	Random effect correlation between distribution fraction parameters of 4-MAA and 4-AA	0.89	0.13	14.8	-	-
Error model parameters						
b1	Proportional error (4-MAA plasma)	0.17	0.015	9.20	-	-
b2	Proportional error (4-AA plasma)	0.11	0.0099	8.94	-	-
b3	Proportional error (4-MAA saliva)[a]	0.47	0.061	12.8	-	-
b4	Proportional error (4-AA saliva)[a]	0.28	0.032	11.4	-	-

[a] Concentration values < lower limit of quantification: 10 for 4-MAA and 2 for 4-AA in saliva. RSE: relative standard error. SE: standard error. SD: standard-deviation of log-transformed (CL, V) and logit-tranformed ($F_{S/P}$) parameters, respectively.

as censored ("M4 method").[23] Besides allometric scaling and the effect of age on CL parameters, no covariate improved the model fit as defined in the BICc. All relative standard errors were below 32%.

Overall, the parameter estimates of the final model are presented in Table 2, with graphical evaluation diagnostics of the final model provided in Figure S3 (residual plots), Figure S4 (VPC), and Figure S5 (correlations between residual errors of 4-MAA and 4-AA in plasma and saliva).

Pharmacometric Analysis to Evaluate Reduced Plasma Sampling Designs

Estimated $CL_{MAAtoAA}$, CL_{other}, and their sum, CL_{total}, from a decreasing number of plasma samples included in the dataset (number of evaluable samples and included data per sampling time point provided in Table S2) in comparison with the original estimates are shown in Figure 3. Overall, no statistically significant differences were observed for any parameter considering overlapping 95% CI. Relative standard errors ranged between 7.6% and 68.0%, whereas point estimates varied by −33.5% to 40.1% from original estimates. Estimated CL_{total} tended to be higher compared to the original estimate of 2.53 L/h, the corresponding nonsignificant bias was highest when including a single plasma sample 1 h post dose (estimate: 3.19 L/h), and lowest when including a single plasma sample 4 h post dose (estimate: 2.94 L/h). The estimated $F_{S/P}$ parameters for both metabolites were comparable across the different tested scenarios, with estimates ranging between 0.32–0.35 and 0.54–0.63 for 4-MAA and 4-AA, respectively. For $F_{S/P\ 4\text{-}MAA}$, uncertainty (95% confidence interval) was largest when including a single plasma sample at 6 h post dose, while for $F_{S/P\ 4\text{-}AA}$, the dataset including plasma sampling time points from 1 to 6 h resulted in the widest confidence interval.

Discussion

In this case study, we evaluated the suitability of saliva as an alternative PK matrix to simplify studies in infants, investigating metamizole, an analgesic used off-label in infants. PMX analysis describes the combined population PK of metamizole metabolites in saliva

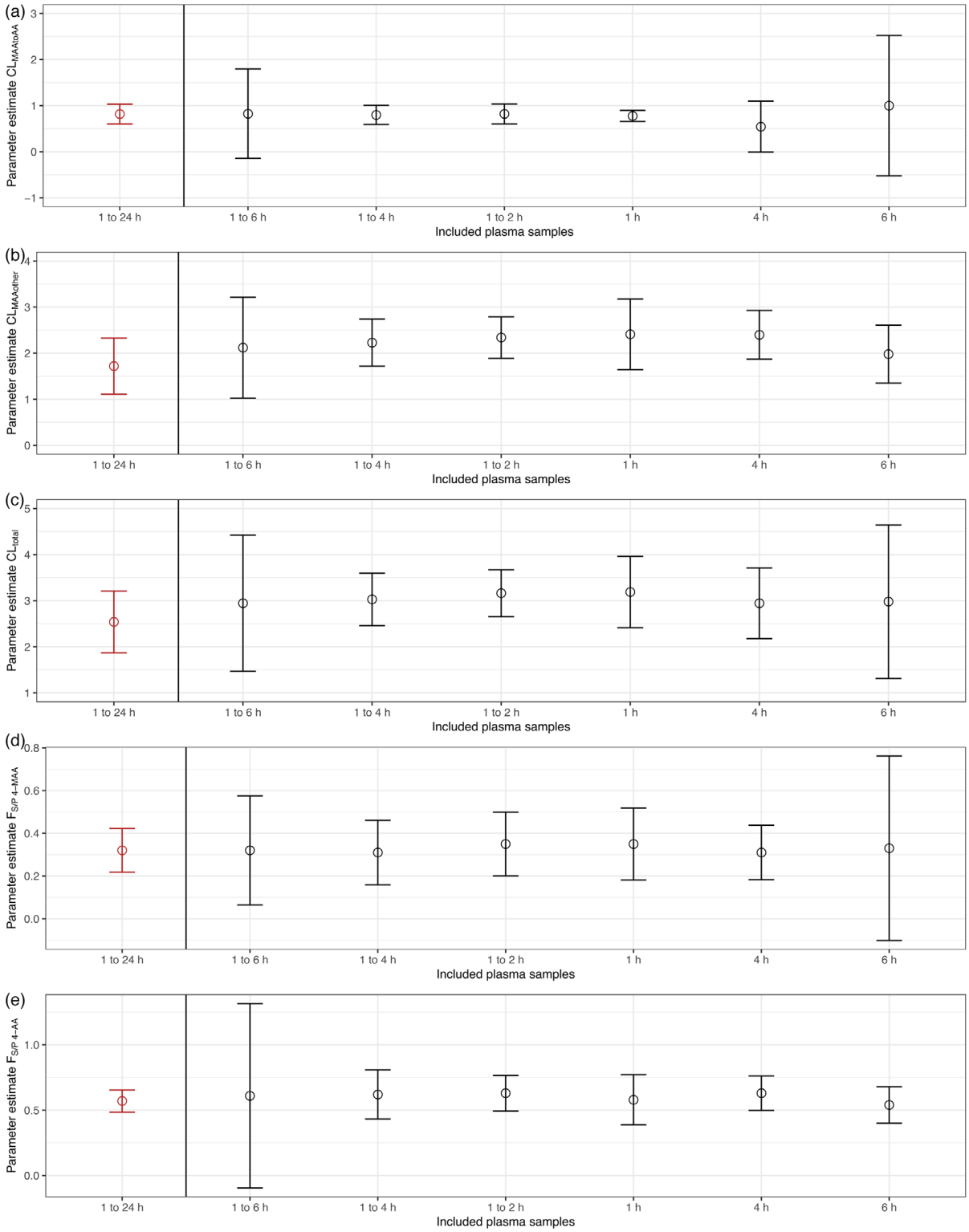

Figure 3. Main model parameter estimates (±95% CI) of original sampling scenario (left: complete 1 to 24 h plasma sampling included) and obtained from sampling scenarios with decreasing number of plasma samples included (1 to 6 h, 1 to 4 h, 1 to 2 h, and single sample at 1, 4, and 6 h, respectively). (a) 4-MAA clearance to 4-AA, (b) 4-MAA clearance via other routes, (c) total 4-MAA clearance, (d) distribution fraction parameter saliva to plasma (FS/P) for 4-MAA, and (e) FS/P for 4-AA.

and plasma of children for the first time, a strategy that may be further evaluated to facilitate noninvasive PK sampling strategies. Our data indicates rapid drug distribution from plasma into saliva, as supported by a nonquantifiable distribution delay in PMX analysis. Drug concentrations of the active metabolites 4-MAA and 4-AA were approximately 3- and 2-fold lower in saliva than in plasma, with $F_{S/P}$ estimates close to reported mean unbound fractions in plasma of 4-MAA and 4-AA.[25] Interindividual variability of $F_{S/P}$ was high. Together with high residual intraindividual variability, especially for the main active metabolite 4-MAA, this compromises the suitability of saliva as a single matrix for metamizole PK studies in children. Nonetheless, all 4 metabolites could be quantified in saliva, which can support reduced plasma PK sampling strategies, as illustrated by our feasibility analysis of such an approach.

Model-based $F_{S/P}$ estimates and saliva/plasma exposure ratios from NCA were consistent with data reported in adults after a single oral dose.[18,19,26] Since drugs are reported to mainly distribute into saliva via passive diffusion, the extent is mainly restricted to the unbound fraction.[27,28] For metamizole metabolites, the unbound fraction was highly variable in adults: the mean values were determined as 0.42 (range: 0.21–0.73), 0.52 (0.23–0.79), 0.82 (0.70–0.92), and 0.85 (0.78–0.95) for 4-MAA, 4-AA, 4-FAA, and 4-AAA, respectively.[25] Plasma protein binding of metamizole metabolites was never quantified in children or infants to the best of our knowledge. No correlation between $F_{S/P}$ parameters and albumin or age was found, indicating that potential age-related differences in albumin levels are not expected. This was also observed for the weak base clonazepam.[29,30] As such, we may hypothesize that $F_{S/P}$ estimated in our model might describe the actual unbound fractions in children, although we could not verify this hypothesis with the determination of the fraction unbound due to low remaining sample volume. Since the unbound fraction is pharmacologically active, $F_{S/P}$ may represent a suitable exposure correlate with efficacy in the absence of laboratory-derived data of fractions unbound. In adults, saliva concentrations could indeed be correlated to the analgesic effect of metamizole.[19]

Various other factors besides protein binding may contribute to the variability of drug distribution into saliva, for example, the salivary flow rate and composition.[1,9] In this study, saliva was collected with validated oral swabs not interfering with sample pH to avoid any impact on salivary composition as well as matrix effects.[31] Since mainly nonionizable compounds or the nonionized fraction at physiological pH can freely distribute across membranes, the acid dissociation constant (pKa) of a compound plays a major role.[10] The saliva pH range shows greater variation than in plasma, that is, 6.2–7.4.[32] Drugs with pKa values within the salivary pH range experience highly fluctuating amounts of nonionized molecules. Thereby, a variable correlation between plasma and saliva concentrations is expected,[31] potentially relevant for 4-MAA and 4-AA with pKa values of 5.0 and 4.9, respectively.[10,18,25]

In our population PK model, no drug elimination from saliva was assumed (high oral bioavailability of 85% reported).[33] No reports on CYP2C19 or N-acetyltransferase 2, enzymes involved in metamizole metabolism, were found in saliva.[34,35] Supported by a similar $t_{1/2}$ of 4-MAA and comparable terminal elimination phases of the other metabolites in plasma and saliva, further metabolism of the studied compounds within saliva was considered unlikely.

Saliva concentrations were best described as an instantaneously distributing fraction of the plasma concentrations, close to the expected unbound fraction in plasma, based on passive permeation of oral epithelium by molecules in nonionized state at salivary pH,[36,37] similar to models developed for other compounds before.[28,29,38]

There is still a need to generate PK data in infants and children to further inform safe and efficacious use of drugs. However, PK studies should apply the least invasive sampling techniques in such a vulnerable population. The impact of reduced plasma sampling scenarios in combination with rich saliva sampling on main population-PK model parameter estimates (CL_{MAA}, $F_{S/P}$ for MAA) was evaluated. All reduced plasma PK scenarios provided point estimates within the 95% CI of the original model estimated from full plasma PK sampling. The point estimate of the total 4-MAA CL deviated the least from the estimate obtained with the full dataset when including a plasma sample in the 4-MAA elimination phase (≥4 h post dose). The precision of parameter estimates appeared to vary specifically with the inclusion of the 6 h sampling time point. This could be due to higher variability in plasma concentrations at 6 h based on fluctuating concentrations in 1 child.[7] This highlights the potential of saliva as primary matrix complemented by sparse plasma sampling for PK studies in infants and children. Special care may need to be applied to minimize factors potentially increasing intraindividual variability in saliva distribution.[13]

Limitations of this PMX study are inherent to many PK studies in infants and children, that is, the sample size and the number of collected saliva samples were small, limiting suitability to stratify NCA by age. Longitudinal analysis by population-PK modeling increases the power to detect differences by including multiple samples per child. In general, saliva is not

easily accessible. The sample volume and analyte recovery are restricted, requiring more sensitive analytical methods. Imposing fasting conditions for sample collection in clinical trials in children is considered unethical, thus food and fluid intake might alter saliva collection. Saliva stimulation with citric acid could dilute samples and interfere with natural saliva pH and drug concentrations. Mechanical stimulation bears the risk of drug adsorption to the chewing item.[27,31,39] The collection device we applied thus did not employ stimulation methods. In our study, saliva sampling by the caregivers was well tolerated by the majority of children and their parents. Nonetheless, cooperation for saliva collection may still be a challenge for infants.[31] Still, high 4-MAA exposures were measured in saliva, suggesting that saliva is technically suitable to further evaluate age-dependent elimination kinetics.

Conclusions

Inter- and intraindividual variability associated with saliva distribution may limit suitability of saliva as a single matrix for PK studies in children. However, saliva concentrations appear to correlate with pharmacologically active unbound plasma concentrations, as the estimated fraction distributing from plasma into saliva was very close to previously reported fraction unbound in plasma.[25] Simulated sparse plasma sampling scenarios (only 1 plasma sample during the elimination phase of 4-MAA, approximately 4–6 h post dose) combined with rich saliva sampling (up to 6 saliva samples), resulted in PK parameter estimates comparable to the full plasma sampling scenario. Accordingly, serial saliva samples in combination with a single plasma sample during the elimination phase have the potential to reduce the invasiveness of PK studies and facilitate PK-based pediatric dose evaluation and optimization of new and existing medicines that share physiochemical properties with metamizole (intermediate plasma protein binding, pKa at lower limit of the salivary pH, and analytical feasibility for quantification in saliva).[31,32,40]

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Author Contributions

FR, MP, AA, TOE, MH, NG, and JvdA designed the clinical study. FR, VZ, and JAB conducted the clinical study. MA and VG analyzed the data, built the model, and wrote the manuscript. DR, UD, and MH developed the bioanalytical method and analyzed the samples. All authors reviewed and approved the final version of the manuscript before submission.

Conflicts of Interest

MA was a full-time employee of Idorsia Pharmaceuticals Ltd. at the time of this work and is a full-time employee of F. Hoffmann-La Roche Ltd. MP is a part-time consultant for Certara, L.P. JAB's husband is a senior corporate counsel at Novartis International AG, Basel, Switzerland, and holds Novartis stock and stock options. FR, VZ, AA, TOE, NG, SHC, UD, DR, MH, JvdA, and VG declared no competing interests in this work. University Children's Hospital Basel (MP) has received an unrestricted educational grant from Sanofi-Aventis Suisse SA. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the article.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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