Dose evaluation of intravenous metamizole (dipyrone) in infants and children: a prospective population pharmacokinetic study

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ABSTRACT

Purpose: The prodrug metamizole is prescribed intravenously for postoperative pain in children, including off-label use in infants <1 year. We aimed to assess the pharmacokinetics of the main metabolites of metamizole in children aged 3-72 months.

Methods: A single dose of 10 mg/kg metamizole was administered intravenously for postoperative analgesia. Pharmacokinetic samples were drawn at predefined time points. Pharmacokinetics of the main active metabolite 4-methylaminoantipyrine and three other metabolites was characterized by both non-compartmental and population pharmacokinetic analysis. AUC0-inf of 4-methylaminoantipyrine was calculated by non-compartmental analysis for two age cohorts (3-23 months, 2-6 years) and compared to the 80-125% range of adult dose-adjusted reference exposure (AUCref). Population pharmacokinetic analysis investigated age and weight dependency of the pharmacokinetics, and optimal dosing strategies to achieve equivalent adult exposure.

Results: A total of 25 children aged 5 months - 5.8 years (7.8-24.8 kg) with at least one concentration sample were included, 19 children had ≥5 predefined samples up to 10h after metamizole dose administration. AUC0-inf of 4-methylaminoantipyrine in children 2-6 years was 29.8 mg/L*h (95%CI 23.3-38.1), significantly lower than AUCref (80%-125% range: 39.2-61.2 mg/L*h). AUC0-inf of 4-methylaminoantipyrine in infants <2 years was 42.5 mg/L*h (95%CI 15.7-115.4), comparable to AUCref, while infants <12 months showed increased exposure. Observed variability could be partially explained by covariates weight and age.

Conclusions: Age-related changes in pharmacokinetics of 4-methylaminoantipyrine requires reduced weight-based IV dosing in infants <1 year compared to infants and children up to 6 years (5 versus 10-20 mg/kg) to achieve equivalent adult exposure.

Key words: Metamizole, dipyrone, pharmacokinetics, children, infants

(ClinicalTrials.gov Identifier: NCT02660177)
**Abbreviations:**

- **AA**: 4-aminoantipyrine
- **AAA**: 4-acetylaminoantipyrine
- **ADR**: adverse drug reaction
- **AE**: Adverse events
- **AIC**: Akaike information criterion
- **AUC**: area under the curve
- **BSV**: between-subject variability
- **CL**: clearance
- **Cmax**: maximal plasma concentration
- **COX**: cyclooxygenase
- **CYP**: cytochrome P450
- **FAA**: 4-formylaminoantipyrine
- **IV**: intravenously, intravenous
- **k_h**: hydrolysis rate of metamizole, MAA formation rate
- **LLOQ**: lower limit of quantification
- **MAA**: 4-methylaminoantipyrine
- **NAT2**: N-acetyltransferase 2
- **OFV**: objective function value
- **PACU**: post-anesthesia care unit
- **PK**: pharmacokinetics(s)
- **PPK**: Population PK
- **t_{1/2}**: elimination half-life
- **Tmax**: time of C_{max}
- **TV**: typical value
VPC  visual predictive check

WHO  World Health Organization
1 INTRODUCTION

Metamizole, or dipyrone, is a pyrazolone derivative used for treatment of severe pain and/or fever [1]. It has spasmylytic properties and a favorable safety profile regarding gastrointestinal, hepatic and renal adverse effects compared to other non-opioid analgesics [2, 3]. Its use is, however, questioned due to a rare risk of potentially life-threatening agranulocytosis, the reason why it has been banned in multiple countries [4]. The exact mechanism of analgesic action is not fully understood. Inhibition of cyclooxygenase isoforms 1 and 2 and of prostaglandin E1 and E2 synthesis has been demonstrated. Additionally, action on opioid and cannabinoid systems as well as activation of ATP-sensitive K+ channels are well documented [5-7].

Metamizole is a prodrug that is rapidly non-enzymatically hydrolyzed to an active metabolite, 4-methylaminoantipyrine [8]. MAA is further metabolized to another active metabolite, 4-aminoantipyrine, and an inactive end-metabolite, 4-formyl-aminoantipyrine (Figure 1). The influence of cytochrome P450 enzymes on the oxidative biotransformation of MAA to AA is not yet fully explained [9, 10]. In vitro and in vivo evidence has suggested a role for CYP2C19, and more recently, also of other CYPs isoforms and human myeloperoxidase in granulocytes [10, 11]. AA is acetylated to inactive 4-acetyl-aminoantipyrine by N-acetyltransferase 2 [12]. Also, AA is assumed to be metabolized to the inactive end-metabolite FAA. In total, more than 20 metabolites are currently known [8].

The analgesic effect of metamizole seems to correlate mainly with MAA exposure [13]. The drug has been shown to be an effective analgesic in children at doses of 15 mg/kg [14, 15]. Metamizole is one of the few non-opioid analgesics, along with paracetamol and ketorolac, that can be administered intravenously, which is a significant advantage in children postoperatively. But according to the current label, IV use is off-label in infants <12 months or with a body weight <9 kg and intramuscular administration is recommended in these patients [16]. In practice however, IV is favored over IM administration also in infants <12 months, since IV application allows for complete and rapid absorption, associated with a quick onset of action, whereas IM applications leads to erratic and delayed absorption, pain and risks of infection/inflammation at the injection site. The licensed parenteral pediatric dosing scheme is summarized in Table 1. However, dosing in mg/kg is more common with inconsistent dosing practices. Among Swiss pediatric hospitals for example, doses ranging from 5-20 mg/kg for repetitive dosing, or even up to 40 mg/kg for a single IV dose are used, including off-label IV use in infants of age 3-12 months [16].

Pharmacokinetics of metamizole metabolites is well described in adults (licensed dose: 500-1000 mg, max. 4 times daily), while such information is lacking for infants and children, despite its use for almost 100 years. A pharmacokinetic study in children aged 1-11 years reports increased...
urinary metabolite excretion in younger children compared to adults following a single oral dose of 8 mg/kg suggesting different pharmacokinetic properties [17]. No data in infants <1 year have been available.

The objective of this study was (I) to characterize the pharmacokinetics of the main metabolites of metamizole following a single IV dose for post-operative analgesia in infants and children 3 to 72 months of age (two age cohorts; infants 3-23 months and children 2-6 years), (II) to propose a rationale for an optimal mg/kg-dosing strategy in infants and children.

2 METHODS

2.1 Trial design

A single center, open-label, prospective study was conducted at the University of Basel Children's Hospital after approval by the local ethics committee (ClinicalTrials.gov Identifier: NCT02660177) between 01/2016 and 12/2017. Infants and children aged between 3 months and 6 years (72 months) of age with a body weight >5 kg, who were scheduled for elective in- or outpatient surgery with intended administration of IV metamizole as part of the local standard postoperative pain management, were eligible for the study.

Main exclusion criteria were premature birth, kidney or liver disease, hematological abnormalities, asthma, immunosuppression, treatment with strong CYP2C19 inhibitors or inducers or drugs known to induce agranulocytosis within 3 months prior to study, documented previous adverse drug reaction to metamizole, or treatment with metamizole within 30 days prior to study.

2.2 Intervention

After having obtained informed consent from parents of eligible patients, anthropometric parameters and medical history including concomitant treatments were recorded, and a physical examination was performed.

Following inhaled anesthesia, a first peripheral IV line was placed for the purpose of planned surgery and 0.7 mL of blood was drawn for biochemical and hematologic evaluation of exclusion criteria (differential blood count, urea, creatinine, ASAT, ALAT, bilirubin and albumin). A second peripheral IV line for repeated painless blood sampling was inserted at an extremity on the opposite side.

Before awakening from anesthesia, or immediately after arrival in the post-anesthesia care unit, patients received a single metamizole dose of 10 mg/kg (based on current body weight) through...
the first peripheral IV line (Novalgin® 50%, metamizole injection, 500mg/mL, Sanofi-Aventis SA, Vernier, Switzerland) as intravenous injection, followed by a saline flush. Further standard postoperative pain management consisted of regular administration of paracetamol (acetaminophen) and a non-steroidal anti-inflammatory agent (ibuprofen, mefenaminic acid or ketolorac), and opioids (nalbuphine, morphine) when required.

Blood samples, 0.5 ml each, were collected for pharmacokinetic analysis into EDTA tubes (Microvette 500 K3E, Sarstedt, Nümbrecht, Germany) at 5 predefined time points after dosing (1h, 2h, 4h, 6h, 10±1h). An additional sample at 24h was collected from inpatients; patients who underwent day-surgery were discharged home after the 10±1h sample.

At 6 hours, i.e. at the end of a regular dosing interval, an additional 0.7 mL blood sample was drawn for biochemical and hematologic safety assessment.

2.3 Pharmacokinetic analyses and dose evaluation

Concentrations of MAA, AA, FAA and AAA were analyzed using an LC-MS/MS method according to Bachmann et al., for details see supplement S2 [9]. The calibration range was 0.025-25 mg/L for MAA, AA and AAA, and 0.025-10 mg/L for FAA, i.e. a lower limit of quantification of 0.025 mg/L for all metabolites. Imprecision was max. 12.5%, inaccuracy ±15% (±20% at LLOQ).

Data were analysed both by non-compartmental analysis and population pharmacokinetic modelling. NCA included all patients having completed at least the predefined 5 blood samples (per protocol analysis), PPK all patients with at least one concentration sample (intention-to-treat analysis). NCA investigated exposure in two age cohorts: infants 3-23 months and children 2-6 years. Detailed information on performed analyses is provided in sections 2.3.3 and 2.3.4.

2.3.1 Reference exposure

Reference area under the plasma concentration-time curve from zero to infinity (AUC\(_{0-\infty}\)) was derived from 3 healthy volunteer studies in adults after a dose of 1000 mg metamizole IV (AUC\(_{1000}\)) [8, 18, 19]. The mixed effect estimate of adult MAA AUC\(_{1000}\) was re-scaled to a dose of 10 mg/kg, assuming a mean patient weight of 70 kg (reference AUC\(_{ref}\) = AUC\(_{1000}\) \cdot (10 mg/kg) / (1000 mg / 70 kg) = AUC\(_{1000}\) \cdot 0.7). Median exposure range in adults after an IV dose of 500-1000 mg (AUC\(_{500-1000}\)) was calculated (AUC\(_{500} = AUC_{1000} \cdot 0.5\).

2.3.2 Sample Size

The sample size was determined according to calculations proposed by Wang et al., i.e. the study was prospectively powered to target a 95% confidence interval (95% CI) of AUC\(_{0-\infty}\) as derived by NCA, within 80% and 125% of AUC\(_{ref}\) with at least 80% power. Accordingly, the choice of study
population consisted of 13 patients per age cohort (initially 3 age cohorts were defined: cohort 1: age 3-11 months, cohort 2: age 12-23 months, cohort 3: 24-72 months, but cohorts 1 and 2 needed to be combined as explained below) [20].

2.3.3 Non-compartmental analysis

NCA was conducted using the PKNCA package in R (Version 3.2.4, R Core Team, Vienna, Austria) [21, 22]. MAA AUC$_{0\text{-}\infty}$ was calculated as primary outcome according to the linear trapezoidal rule using log-transformed measured concentrations. The 95% confidence interval (95% CI) of the geometric mean AUC$_{0\text{-}\infty}$ of MAA was compared to the 80-125% interval of adult AUC$_{\text{ref}}$ (see above). Further parameters derived for MAA and the other metabolites were the AUC within a dosing interval of 8h (AUC$_{0\text{-}8\text{h}}$), maximal plasma concentration, time of C$_{\text{max}}$ and the elimination half-life. All parameters were estimated using the PKNCA package in R and then cross-checked visually using the plots. The half-life was estimated from the best fit line for all available points, again calculated using this package.

2.3.4 Population pharmacokinetic analysis

Population pharmacokinetic modelling was performed with the software package NONMEM (version 7.4.1, Icon Development Solutions, Ellicott City, MD).

All four metabolites were modelled simultaneously, starting from the structural model illustrated in Figure 3. MAA formation rate ($k_h$, hydrolysis of metamizole) was modelled as a first-order rate, which was fixed to 20/h (assuming a half-life of 2 min, i.e. complete hydrolysis within 10 min ≈ reported t$_{\text{max}}$ after IV administration) [18]. Both one and two-compartment models were considered to describe the distribution of metabolites. The apparent volume of distribution was set to equal values for all metabolites in the absence of IV metabolite administration data and information on fractions metabolized by different pathways.

Between-subject variability was assigned to all structural model parameters and was assumed to be log-normally distributed. A proportional error model was used for the residual variability.

Covariates considered were weight and age. Standard allometric scaling was used to model the relationship between weight and clearance and volume of distribution (fixed exponents of 0.75 and 1, respectively). The remaining correlation of individual model parameter estimates and patient demographics was attributed to age, considering (piece-wise) linear, power and sigmoidal (E$_{\text{max}}$) functions based on visual inspection. For sensitivity analyses, see supplement S4.

Nested models were compared by the likelihood ratio test (alpha=0.05), based on the NONMEM objective function value (corresponding to -2 x log-likelihood). Non-nested models were
compared by their Akaike information criterion. Further model diagnostics for model
development and selection included the decrease in inter-individual and residual variability,
correction in bias of individual random effects over covariates (for shrinkage <20-30%), standard
error of parameter estimates (target <30%), and goodness of fit plots (observations versus
predictions, residual diagnostics). The final model was internally evaluated using simulation-
based diagnostics (visual predictive check): empirical percentiles (median, 2.5\textsuperscript{th} and 97.5\textsuperscript{th}
percentiles) of observed concentrations over time were compared with the 95\% CI of simulated
percentiles.

2.3.5 Dose evaluation

PPK model simulations were performed to (I) evaluate the studied fixed weight-based dosing
strategy of 10 mg/kg IV, (II) the labelled dose range for 4 weight bands: 50-100 mg for 5-9 kg
(only IM administration licensed), 100-250 mg for 9-16 kg, 150-400 mg for 16-24 kg, 200-500 mg
for 24-30 kg (both IM and IV administration licenced), and (III) a new weight-based dosing
strategy accounting for lower MAA clearance in infants compared to children observed.

Step I. Deterministic model simulations (including parameter uncertainty) were performed to
illustrate the model-predicted influence of age and weight on the typical value of MAA total
clearance (TVCL\textsubscript{MAA,tot}= sum of all MAA clearances, eq. 1) and MAA exposure (area under the curve,
TVAUC\textsubscript{0-\infty} eq. 2) after a dose of 10 mg/kg. 95\% confidence intervals were calculated as 2.5\textsuperscript{th} and
97.5\textsuperscript{th} percentiles from 1000 multivariate simulations of the covariance matrix.

\begin{equation}
TVCL\textsubscript{MAA,tot} = TVC\textsubscript{CL\textsubscript{MAA,tot}AA} + TVC\textsubscript{CL\textsubscript{MAA,ofAA}} + TVC\textsubscript{CL\textsubscript{rest}} \quad (eq. 1)
\end{equation}

\begin{equation}
TVAUC\textsubscript{0-\infty} = \frac{D_{\text{metamizole}} \cdot MW\text{MAA}}{TVCL\textsubscript{MAA,tot} \cdot MW_{\text{metamizole}}} \quad (eq. 2)
\end{equation}

Where \(D_{\text{metamizole}}\) is the dose of metamizole in mg (=10 mg/kg \cdot weight in kg), and \(MW_{\text{MAA}}\) and
\(MW_{\text{metamizole}}\) are the molecular weights of MAA (217.27 g/mol) and metamizole (333.34 g/mol),
respectively.

\(TVAUC\textsubscript{0-\infty}\) was illustrated over weight, considering the age-specific weight distribution (3\textsuperscript{rd} to 97\textsuperscript{th}
percentiles) according to World Health Organization (WHO) percentile curves for children aged
3, 6, 12, 18, 24, 48 and 72 months, and was compared to median exposure in healthy adults
reported after a 500-1000 mg IV dose (AUC\textsubscript{500-1000}).

Step II and III. Stochastic model simulations (including inter-patient variability) of individual MAA
total clearance (CL\textsubscript{MAA,tot,i}) and corresponding individual AUC\textsubscript{0-\infty,i} were performed to illustrate the
expected exposure distribution (95\% prediction intervals) following administration of the
labelled dose range (II) or a weight-based dosing that accounts for age-dependent MAA clearance
observed (III). A dataset of 140'000 children aged 3 to 72 months old (1000 patients for each month and gender) was created according to WHO Box-Cox distribution parameters provided for weight for age. CL\textsubscript{MAA\textsubscript{tot},i} was then simulated, and corresponding AUC\textsubscript{0-inf,i} derived as described in step I. Pediatric exposures were compared to median exposure in adults with a 500-1000 mg IV dose.

2.4 Assessment of Adverse Events

Incidence, nature, and severity of clinical adverse events and laboratory parameter changes between time of drug administration and 6h post-dose were recorded systematically.

3 RESULTS

3.1 Demographics

Due to the lower than expected number of eligible patients for the two younger cohorts 1 and 2, the study was amended and these two cohorts were combined according to ICH-E11 age groups, with the aim of including 13 patients in the combined cohort [23]. At the end of the two-year study period, 25 patients with at least 1 concentration sample were included, and 19 patients completed the predefined sampling for NCA analysis, 6 infants <24 months (flow-chart: Supplemental Figure S1, demographics: Table 2).

3.2 Pharmacokinetics

Plasma concentration-time profiles of all metabolites are shown in Figure 2.

3.2.1 Reference exposure

MAA AUC\textsubscript{ref} in adults was 48.9 mg/L*h (95% CI 44.3, 53.4), resulting in a 80%-125% AUC\textsubscript{ref} range of 39.2-61.2 mg/L*h [8, 12, 18, 19]. AUC\textsubscript{1000} and AUC\textsubscript{500} were 69.9 and 34.9 mg/L*h.

3.2.2 Non-compartmental analysis

AUC\textsubscript{0-inf} and other estimates from NCA are summarized for each cohort in Table 3. AUC\textsubscript{0-inf} of MAA in the cohort of children aged 2-6 years was with 29.9 mg/L*h (95% CI 23.4, 38.2) significantly lower than the 80% limit of AUC\textsubscript{ref}. AUC\textsubscript{0-inf} of MAA in infants 3-23 months was with 43.6 (95% CI 15.8, 119.0) mg/L*h comparable to AUC\textsubscript{ref}, but the latter showed considerable variability.

3.2.3 Population pharmacokinetic analysis

Two samples with an MAA concentration increase >50% were observed, resulting in the exclusion of one patient (>24 months) for the primary PPK analysis. A one-compartment model was chosen
to describe the distribution of all metabolites. All metabolic rates were described by first-order constants (=CL/V), there was no evidence of saturable processes. The final structural model is illustrated in Figure 3.

More than half of inter-individual variability in MAA clearance could be explained by the covariates weight and age (CL\textsubscript{MAAtoAA}: decreased from 86% to 52% and 31%; CL\textsubscript{MAAtoFAA}: from 112% to 73% and 40%; CL\textsubscript{MAArest}: from 184% to 151% and 54%, Supplemental Figure S4.1). Both a piece-wise linear and power model with age could describe the observed lower weight-corrected clearance in patients <24 months (corresponding to the time when most enzyme maturation processes are considered complete, and time where no age-dependency could be observed in the present dataset) [24]. As final model a “piece-wise” power relationship with age was chosen (lowest OFV, exclusion of negative values in simulations):

\[
CL_{TV} = \theta_1 \cdot \left(\frac{\text{weight}}{15}\right)^{0.75} \cdot \left(\frac{\text{age}}{24}\right)^{\theta_{\text{age}}} \text{ for age <24 months, and}
\]

\[
CL_{TV} = \theta_1 \cdot \left(\frac{\text{weight}}{15}\right)^{0.75} \text{ for age \geq 24 months}
\]

where CL\textsubscript{TV} is the typical clearance parameter for the given covariates weight and age, \( \theta_1 \) is the typical clearance for a patient with weight = 15 kg (median in the analysed dataset) and age \( \geq 24 \) months, weight is given in kg, age in months.

A similar age relationship was also observed with CL\textsubscript{FAAother} (exponent: 0.84, RSE: 26%; BSV \rightarrow 0) and V (exponent: 0.51, RSE: 21%; BSV decrease by 35%) in infants <24 months (Supplemental Figure S4.2), but was not included in the final model (no influence on MAA total clearance estimate; unclear physiologic meaning of lower weight-adjusted volume in younger children - rather the opposite would be expected from a hydrophilic drug). Large inter-patient variability in metabolic clearance of AA to AAA (mediated by polymorphic NAT2) could be explained by a latent variable, corresponding to a slow or fast metabolizer phenotype (\( \approx 7 \) times faster clearance estimated in patients assigned to the rapid metabolizer, frequency of slow metabolizers estimated to 26%), which was not measured in the present study [12].

### 3.2.4  Model evaluation

Residual diagnostics and VPCs are illustrated in the Supplement (Figures S4.3-S4.4). VPC suggests good agreement between observed and simulated percentiles. Residual diagnostics indicate unbiased predictions of MAA, while some bias for other metabolites remained, which was considered acceptable, given the main purpose of the study, and satisfying VPC diagnostics.

Parameter estimates of the final selected model are summarized in Table 4.
### 3.3 Dose evaluation

Figure 4 illustrates model-predicted TVAUC\(_{0-\text{inf}}\) with 95%CI over weight for different ages; corresponding TVCL\(_{\text{MAA,tot}}\) and individual NCA and PPK AUC\(_{0-\text{inf}}\) estimates are shown in the Supplement (Figures S5.1-S5.2). Supplemental Figure 5.2 illustrates the expected exposure distribution for the labelled dose range (while for <1 year only IM administration is licensed), and for a weight-based dosing scheme accounting for lower clearance in infants.

### 3.4 Safety

AE were fever (n=4), nausea (n=1), vomiting (n=1), abdominal pain (n=1) and pain at the surgical site (n=1), all of which were classified mild to moderate and unlikely related to the study drug. There were no clinically significant changes in hematology and biochemistry parameters before, and 6h after, the administration of metamizole (see Supplement S3). No clinically significant drop in blood pressure requiring treatment was recorded. No serious adverse event occurred during the study. No patient developed agranulocytosis within the study period.

### 4 DISCUSSION

This is the first study that describes the pharmacokinetics of the main metabolites of metamizole after IV administration in infants and children younger than 6 years of age. After a single IV dose of 10 mg/kg, children aged 2-6 years had a significantly (39%) lower exposure (AUC\(_{0-\text{inf}}\)) than the 80% limit of adult AUC\(_{\text{Ref}}\) for the active metamizole metabolite MAA, suggesting that children receiving the recommended 10 mg/kg dose may be slightly under-dosed compared to a 70 kg adult receiving the same weight-based dose (700 mg for a 70 kg adult). On the other hand, infants <2 years had comparable average exposure to adults, with a large (~10-fold) variability in MAA AUC\(_{0-\text{inf}}\). Increased MAA concentrations were measured in infants <1 year, suggesting that they may be overdosed when receiving same weight-based IV doses. PPK modeling and simulation demonstrated that a dose of 5 mg/kg in infants <1 year and 10-20 mg/kg in children 1-6 years would achieve a more consistent exposure in infants and young children compared to that observed in adults at the approved dose of 500-1000 mg (corresponding to 7-14 mg/kg for a 70 kg adult). Considering a weight range of 50-100 kg in adults, such dose recommendations would lie within the corresponding adult weight-adjusted dose range of 5-20 mg/kg.

It has been suggested before that MAA metabolism occurs faster in children >1 year than in adults by Balogh et al., who studied 38 children aged 1-11 years after a single oral dose of metamizole (8 mg/kg) compared to healthy adults. Urinary excretion of the metabolites AA, FAA and AAA within 6h was significantly higher in younger children than in adults, but plasma concentrations were...
unfortunately not measured in their study [17]. In line with those findings, plasma C\textsubscript{max} of those metabolites tended to be lower and t\textsubscript{max} tended to be earlier in our study (Table 3), compared to mean values reported in adults after an IV dose of 1 g (AA: 1.5-1.6 mg/L and 3.1-4.8 h, AAA: 1.4-1.6 mg/L and 13-17.3 h, FAA: 1.4 mg/L and 7.2-8.2 h) [8]. No pharmacokinetic data in infants <1 year is available to compare our findings of slower metabolism in this age group. However, our results are in line with lower CYP activity seen in young children during the first 1-2 years of life. CYP specific isoforms, including CYP2C19, show developmental expression patterns that can affect drug metabolism [24-27].

Model-predicted MAA clearance for a 70 kg adult (167 mL/min) is in excellent agreement with reported values, suggesting usefulness of the model for extrapolation to older children [8]. Model-derived average half-lives for a 70 kg adult are as follows: MAA: 3.2 h, AA 10.5 h (slow metabolizers) and 1.4 h (fast metabolizers), AAA: 3.7 h and FAA: 5.6 h. Those extrapolated half-lives of active metabolites MAA and AA are in line with data reported in adults [12]. Predicted AAA and FAA (non-active metabolites) half-lives are shorter than reported from NCA, likely because of limited data available for the elimination phase of those metabolites [8]. The discrepancy may potentially also indicate age-dependent elimination in children that the model did not account for, and limited usefulness of the model for extrapolation of the pharmacokinetics of inactive metabolites. Data suggest potential for considerable accumulation of MAA in infants <1 year and of other metabolites (AA in slow metabolizers, AAA and FAA; exposure ≈10% of MAA, Figure 2) after multiple dosing. The relevance of AA, AAA and FAA for drug safety and efficacy is not well described. Additional clinical studies are needed to characterize multiple-dose pharmacokinetics and safety of metamizole in infants. Because of these uncertainties, use of metamizole should be limited to short-term use, or may be completely avoided in infants <1 year.

NAT2 genotypes were not determined in this study, but presence of two phenotypes (26% slow and 74% fast metabolizers) was suggested. Since age appeared unrelated to the metabolic activity, we may assume that maturation of this enzyme already is high in infants >3 months (no age-relationship shown in this study). Literature suggests that NAT2 genotypes may even be grouped into three phenotypes, but many pharmacokinetic studies have reported two phenotypes only (e.g. for sulfamethoxazole, isoniazide or caffeine)[28].

Therapeutic efficacy and concentration-dependency could not be evaluated in our study due to concomitant use of standard analgesic combination therapy. Effectiveness of our recommended dose of 10-20 mg/kg for children >1 year is however supported by studies having demonstrated effective pain relief in children after a dose of 15 mg/kg. [14, 15]. Our single dose study in a small number of children does also not allow characterization of the safety profile of metamizole, or evaluation of dose-dependency of AE in infants and children. Recorded AEs were deemed not
related to the study drug, due to the latency time between drug administration and AE occurrence, and alternative explanations for the AEs by the surgical procedures or administered co-medications. The use of metamizole is controversial due to its risk of agranulocytosis [29-31]. With an incidence rate of 0.46-1.63 cases per million person-days, and approximately 4% of reported cases in patients <19 years, the probability for observing such a severe AE in our study was very low [32-34]. Also, the probability to observe serious hemodynamic, anaphylactic or respiratory adverse AE was low (estimated incidence <0.3% after a single IV dose of metamizole) [35]. A recent adult study further reported a dose-dependent risk of acute kidney injury in an intensive care unit, which has not yet been studied in children [36]. As aforementioned, there are uncertainties regarding accumulation and pharmacological safety properties, especially in infants <1 year. For these reasons we recommend to limit administration to 1 or 2 days. If administered over several days regular monitoring for clinical and laboratory abnormalities is warranted [37].

Since only 4 infants below the age of 1 year could be included in this study, there remains uncertainty about the exact optimal dose for this age group (as illustrated by 95%CI in Figure 4). The requirement for dose reduction was still perceived highly appropriate for this age group, due to highest MAA exposure (≈2-fold higher than AUC_{1000}) observed in these patients and plausible maturation of metabolic enzymes. For older children aged 2-6 years, there is some uncertainty concerning the appropriate reference weight for scaling of AUC_{inf} (weight of healthy volunteers not reported in all studies). For a lower adult reference weight (reported range: 54-68kg), the relative difference to adults exposure would be slightly lower than the calculated 39% [8, 12, 18, 19]. It also has to be noted that AUC_{0-inf} estimates from NCA tended to be lower than from PPK, which is to be expected, since higher peak concentrations are assumed to occur within 10 min after IV administration in PPK analysis compared to those measured with the first sample at 1h post-dose (with the sampling scheme being designed to describe the elimination phase). The proposed doses for both age groups are hence also based on practical considerations, targeting a simple dosing scheme, which may reduce dosing errors.

We consider that polymorphisms of genes encoding for the enzymes involved in drug metabolism might have contributed to the above-mentioned variability. Genotyping of these enzymes, however, was not a goal of this study, and sample size of this pharmacokinetic study would be too small to draw valid conclusions.

In conclusion, this prospective single dose study reports for the first time plasma pharmacokinetics data of IV metamizole in infants and children up to 6 years old. Body weight-adjusted dosing in children, assuming a linear relationship between weight and dose, is arbitrary and does not account for any specific differences in drug pharmacokinetics between children of different ages and adults. Significant age-dependency of the elimination kinetics of the main active
metabolite MAA was found, resulting in higher exposure in infants <1 year compared to older children and adults. This suggests the need for a reduced weight-based (off-label) IV dose in infants <1 year compared to older children up to 6 years (5 mg/kg versus 10-20 mg/kg) to achieve equivalent adult exposure, and mitigate the risk for overdosing in young infants. Additional clinical studies are warranted to further evaluate efficacy and safety of proposed dosing in infants.
COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

V.C.Z.: none
F.R.: none
A.A.: none
V.G.: none
C.B.: none
J.A.B.: Husband is senior corporate counsel at Novartis International AG, Basel, Switzerland, and holds Novartis stock and stock options.
M.H.: none
T.O.E.: none
U.D.: none
F.B.: none
N.G.: none
S.H.-C.: none
J.N.v.d.A.: none
M.P. is part-time consultant for Certara, L.P..

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FUNDING

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ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.
AUTHOR CONTRIBUTIONS

F.R., M.P., A.A., T.O.E., M.H., N.G. and J.N.v.d.A. designed the research; V.C.Z., F.R., and J.A.B. performed the research; A.A., V.G., C.B., U.D., F.B. and V.Z. analyzed the data; M.H., U.D. and F.B. performed the bioanalysis; V.C.Z., F.R., V.G. and M.P. wrote the manuscript; J.N.v.d.A., T.O.E., M.H., N.G. and S.H.-C. critically revised the manuscript. All authors reviewed and approved the final version of the manuscript before submission.
REFERENCES


Table 1: Licensed parenteral dosing of metamizole (Novalgin®, 500 mg/mL, solution for injection) for children <6 years and adults. In children <1 year only IM administration is recommended. Injection may be repeated after 6-8 h.

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Route of administration</th>
<th>Single dose</th>
<th>Corresponding calculated weight-based dose range a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-8 kg</td>
<td>Only IM</td>
<td>0.1-0.2 mL = 50-100 mg</td>
<td>6.2-20.0 mg/kg</td>
</tr>
<tr>
<td>9-15 kg</td>
<td>IM or IV</td>
<td>0.2-0.5 mL = 100-250 mg</td>
<td>6.7-27.8 mg/kg</td>
</tr>
<tr>
<td>16-23 kg</td>
<td>IM or IV</td>
<td>0.3-0.8 mL = 150-400 mg</td>
<td>6.5-25.0 mg/kg</td>
</tr>
<tr>
<td>24-30 kg</td>
<td>IM or IV</td>
<td>0.4-1.0 mL = 200-500 mg</td>
<td>6.7-20.8 mg/kg</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td>1-2 mL = 500-1000 mg</td>
<td>10-20 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(max. single dose 5 mL = 2500 mg, max. daily dose: 5000 mg)</td>
<td>(max. single dose 25-50 mg/kg, max. daily dose 50-100 mg/kg)</td>
</tr>
</tbody>
</table>

IM: intramuscular; IV: intravenous.

a calculated as: minimal recommended single dose / upper limit of body weight range = minimal weight based dose and maximal recommended single dose / lower limit of body weight range = maximal weight based dose.
Table 2: Patient demographics. Continuous variables are given as median and interquartile range (IQR) for all patients with at least 1 concentration sample.

<table>
<thead>
<tr>
<th>Number of individuals (n)</th>
<th>Infants 3-11 months (cohort 1)</th>
<th>Infants 12-23 months (cohort 2)</th>
<th>Children 2-6 years (cohort 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>with at least 1 concentration sample a</td>
<td>3</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>with at least 5 predefined samples b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>3 m, 1 f</td>
<td>4 m</td>
<td>11 m, 6 f</td>
</tr>
<tr>
<td>Age (months)</td>
<td>8 (6.5; 9.3)</td>
<td>20.5 (17.8; 22.0)</td>
<td>56 (43; 64)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.9 (8.5; 9.7)</td>
<td>11.5 (10.8; 12.0)</td>
<td>17 (15; 19)</td>
</tr>
<tr>
<td>z-score weight (for age)</td>
<td>0.58 (0.41;1.10)</td>
<td>0.14 (-0.08; 0.31)</td>
<td>-0.09 (-0.83; 0.45)</td>
</tr>
<tr>
<td>Type of surgery (n)</td>
<td>Urologic (3), other (1)</td>
<td></td>
<td>ENT (12), urologic (3), other (2)</td>
</tr>
</tbody>
</table>

a all individuals included in population pharmacokinetic analysis.

b included in non-compartmental analysis.
**Table 3.** Non-compartmental analysis. Pharmacokinetic parameters of the metamizole metabolites after a single intravenous dose of 10 mg/kg metamizole.

<table>
<thead>
<tr>
<th></th>
<th>Infants 3-23 months (n=6)</th>
<th>Children 2-6 years (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAA (main active metabolite)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-inf} (mg/L*h)</td>
<td>43.6 (15.8, 119.0)</td>
<td>29.9 (23.4, 38.2)</td>
</tr>
<tr>
<td>AUC_{0-λ} (mg/L*h)</td>
<td>31.7 (14.8, 67.9)</td>
<td>22.7 (19.5, 26.5)</td>
</tr>
<tr>
<td>C_{1h} (mg/L)</td>
<td>10.6 [8.3, 15.0]</td>
<td>7.8 [6.5, 9.4]</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>2.4 [1.7, 3.9]</td>
<td>2.0 [1.9, 3.1]</td>
</tr>
<tr>
<td>λz (h⁻¹)</td>
<td>0.3 [0.2, 0.4]</td>
<td>0.3 [0.2, 0.4]</td>
</tr>
<tr>
<td><strong>Metabolite AA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-λ} (mg/L*h)</td>
<td>3.6 (2.0, 6.4)</td>
<td>3.1 (2.5, 3.9)</td>
</tr>
<tr>
<td>C_{max} (mg/L)</td>
<td>0.8 [0.6, 0.9]</td>
<td>0.6 [0.6, 1.0]</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>2.0 [2.0, 3.3]</td>
<td>2.0 [2.0, 4.0]</td>
</tr>
<tr>
<td><strong>Metabolite AAA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-λ} (mg/L*h)</td>
<td>4.6 (2.0, 10.9)</td>
<td>3.3 (2.0, 5.4)</td>
</tr>
<tr>
<td>C_{max} (mg/L)</td>
<td>1.6 [0.8, 1.8]</td>
<td>1.2 [0.7, 1.5]</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>6.0 [5.9, 6.0]</td>
<td>6.0 [5.8, 6.0]</td>
</tr>
<tr>
<td><strong>Metabolite FAA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-λ} (mg/L*h)</td>
<td>5.7 (4.4, 7.4)</td>
<td>5.1 [4.0, 6.6]</td>
</tr>
<tr>
<td>C_{max} (mg/L)</td>
<td>1.4 [1.3, 1.5]</td>
<td>1.3 [0.9, 1.4]</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>4.0 [4.0, 4.0]</td>
<td>5.8 [4.0, 6.0]</td>
</tr>
</tbody>
</table>

AUC_{0-inf} area under the plasma-concentration time curve from 0 to infinity; C_{1h} plasma concentration 1h after dosing; C_{max} maximal plasma concentration; T_{max} time of C_{max}; t₁/₂ elimination half-life; λz terminal elimination rate constant

a presented as geometric mean (95% confidence interval).

b presented as median [interquartile range].
Table 4: Estimates of population pharmacokinetic model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE)</th>
<th>Inter-individual variability (RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural kinetic model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_h$ (1/h)</td>
<td>20 (fixed)</td>
<td></td>
</tr>
<tr>
<td>$V$ (L) for 15 kg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.98 (5%)</td>
<td>21.6% (18%)</td>
</tr>
<tr>
<td>$CL_{MAAtoAA}$ (L/h) for 15 kg&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.07 (11%)</td>
<td>38%* (18%)</td>
</tr>
<tr>
<td>$CL_{MAAtoFAA}$ (L/h) for 15 kg&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.844 (13%)</td>
<td>51%* (17%)</td>
</tr>
<tr>
<td>$CL_{MAAother}$ (L/h) for 15 kg&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.26 (14%)</td>
<td>45% (21%)</td>
</tr>
<tr>
<td>$CL_{AAtoAAA}$&lt;sup&gt;fast&lt;/sup&gt; (L/h) for 15 kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.46 (14%)</td>
<td>51% (18%)</td>
</tr>
<tr>
<td>$CL_{AAtoAAA}$&lt;sup&gt;slow&lt;/sup&gt; (L/h) for 15 kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.972 (27%)</td>
<td>(same)</td>
</tr>
<tr>
<td>Proportion of slow metabolizers</td>
<td>0.259 (39%)</td>
<td>-</td>
</tr>
<tr>
<td>$CL_{AA}$ (L/h) for 15 kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.72 (11%)</td>
<td>39% (23%)</td>
</tr>
<tr>
<td>$CL_{FAA}$ (L/h) for 15 kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83 (8%)</td>
<td>25% (24%)</td>
</tr>
<tr>
<td><strong>Covariate model for age &lt;24 months</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta_{age,MAAtoAA}$ [-]</td>
<td>0.663 (29%)</td>
<td></td>
</tr>
<tr>
<td>$\theta_{age,MAAtoFAA}$ [-]</td>
<td>0.969 (25%)</td>
<td></td>
</tr>
<tr>
<td>$\theta_{age,MAAother}$ [-]</td>
<td>2.39 (24%)</td>
<td></td>
</tr>
<tr>
<td><strong>Error model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\varepsilon_{MAA}$ proportional (%)</td>
<td>23% (10%)</td>
<td></td>
</tr>
<tr>
<td>$\varepsilon_{AA}$ proportional (%)</td>
<td>13% (9%)</td>
<td></td>
</tr>
<tr>
<td>$\varepsilon_{AAA}$ proportional (%)</td>
<td>19% (11%)</td>
<td></td>
</tr>
<tr>
<td>$\varepsilon_{FAA}$ proportional (%)</td>
<td>10% (9%)</td>
<td></td>
</tr>
</tbody>
</table>

RSE: relative standard error. *estimated correlation: 96% (RSE: 36%). a: allometrically scaled and centered to 15 kg: $V_{TV} = V \cdot (weight/15)^1$. b: allometrically scaled and centered to 15 kg: $CL_{TV} = CL \cdot (weight/15)^{0.75}$. c: age as covariate included as follows for age <24 months: $CL_{TV} = CL \cdot (weight/15)^{0.75} \cdot (age/24)^\theta_{age}$. CV: coefficient of variation calculated as $\sqrt{\omega^2-1}$, where $\omega^2$ is the variance of log-normally distributed interindividual variability.
7 FIGURES

Figure 1: The metabolism of metamizole and its major metabolites

Figure 2: Measured individual concentration-time profiles of all metamizole metabolites. Three age groups are differentiated by color: <1 year (4 patients aged 5-10 months, among 3 with ≥5 samples), 1 year old (4 patients aged 14-22 months, among 3 with ≥5 samples), and 2-6 years (17 patients aged 28-70 months, among 13 with ≥5 samples). X: MAA concentrations increasing >50% from its previous value (physiologically not plausible and excluded in PPK analysis, but included in NCA). Dashed horizontal lines: lower limit of quantification (LLOQ). Concentrations measured below LLOQ are plotted at LLOQ/2.

Figure 3: Illustration of structural model of metamizole and its metabolites considered. Initially, all metabolic pathways (arrows) reported by Levy et al. [8] were considered. Grey dashed arrows indicate pathways that were not identifiable in this modelling work. $k_{f1}$: first-order hydrolysis rate. $CL_{MAAtoAA}$, $CL_{MAAtoAAA}$, $CL_{AAtoFAA}$: metabolic clearances. $CL_{MAAother}$, $CL_{Aother}$, $CL_{AAAother}$, $CL_{FAAother}$: sum of other clearance routes. Modelling work focussed on unbiased description of MAA, the main active metabolite of the prodrug metamizole. Volumes of distribution for all metabolites were assumed to be equal in the absence of data on single IV metabolite administration.

Figure 4: Illustration of model-predicted typical AUC for patients of different age and weight with 95% confidence intervals (shaded areas), receiving an intravenous (IV) dose of metamizole of 10 mg/kg. Weight for age bands were simulated according to WHO percentiles curves (extending from 3rd to 97th percentiles). Black horizontal lines: reference AUC in healthy volunteers receiving a dose of 500 mg or 1000 mg metamizole ($AUC_{500}$, $AUC_{1000}$). Dashed horizontal line: 2-fold increase in $AUC_{1000}$.

Figure 5: Illustration of model-predicted distribution of individual AUC$_{0\text{-}inf}$ for patients of different age (1000 individuals per month of age and gender simulated). Left: exposure following labelled dosing (Table 1, for 5-9kg only IM administration is licensed). Right: exposure following a new proposed weight-based IV dosing strategy for children 3-11 months and 1-6 years. Dashed lines: median. shaded area: 90% prediction interval.
MAA AUC (10 mg/kg i.v.)

- Age and weight distribution with AUC values
- Color coding for different age groups:
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**Supplementary Material**

Metamizole PopPK SUPPLEMENT V9_rev1-clean.docx