Protocol

1 Title of the trial

Paracetamol and cefazolin disposition in normal-weight and overweight children

Acronym: TISSUE

2 Objective of the study

Primary outcome measures

To investigate if drug disposition is altered in overweight and obese children :

- in plasma/blood: paracetamol (blood), paracetamol metabolites (blood) and cefazolin (plasma)
- in subcutaneous interstitial fluid: cefazolin

Secondary outcome measures

To investigate achievement of target concentrations in plasma (paracetamol) and tissue (cefazolin)
To identify predictors for interpatient variability in drug disposition
To characterize plasma protein binding of cefazolin in normal weight and overweight children

3 General information

3.1 Investigator(s)

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3.2 Sponsor

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3.3 Departments/laboratories involved in the study

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Department of Anesthesiology, Ghent University Hospital, Belgium
Department of Pediatrics, Ghent University Hospital, Belgium
Department of Orthopedics, Ghent University Hospital, Belgium
Department of Urology, Ghent University Hospital, Belgium
Department of Otorhinolaryngology, Ghent University Hospital
Department of Pediatric Surgery, Ghent University Hospital
Department of Pharmacy, Erasmus Medical Centre, Rotterdam, the Netherlands
Laboratory of Toxicology, Ghent University

4 Introduction

Background

Over the past decades, obesity in children of all ages has been on the rise. In 2016 it was estimated that in just 40 years, the number of school-age children and adolescents with obesity worldwide has risen from 11 million to 124 million. In addition, 216 million children were estimated to be overweight but not obese. The condition also affects younger children, with over 38 million children aged under 5 living with overweight or obesity in 2017.[1] In Flanders, depending on the age, 8.6% to 21% of children has overweight.[2] This evolution worries health care professionals and policy makers worldwide as obesity and obesity related complications represent a large burden for the individual patient but also for the health care system in general.

Growth and development lead to major changes in drug disposition in children. Most maturational changes occur under the age of 2 years and are most pronounced during the first weeks of life.[3] Pathophysiological changes in children with obesity can also affect drug pharmacokinetics (PK) during the whole age span.[2] (Figure 1) The volume of distribution

(Vd) is probably the most impacted parameter in obesity because it relies on the drug physicochemical properties, body composition, blood flow and plasma protein binding.
All these patient factors are (potentially) affected by obesity.[4] Obese individuals have a decreased total body water per kg total body weight ratio compared to their lean counterparts due to the lower extracellular water volume in adipose tissue. Hydrophilic compounds mainly distribute into the extracellular volume and as a result of obesity the Vd of these compounds might be altered.[5] The distribution of more lipophilic compounds has also been shown to be more unpredictable, making the choice of an appropriate scaling method for dosing difficult. Next, obese adults have shown a markedly reduced tissue perfusion, as well as an impaired endothelium-dependent vasodilatation, which leads to an impaired peripheral blood flow.[4]

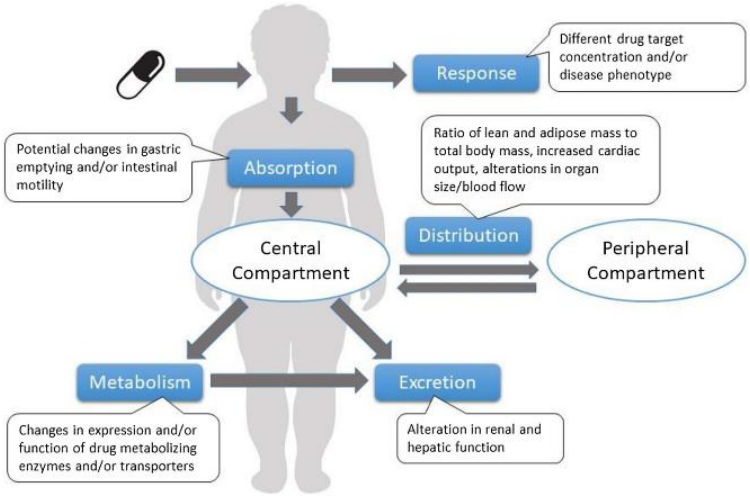

Figure 1: (Patho)physiological changes impacting drug PK in children with obesity [6]

Next to changes in Vd, obesity may affect drug clearance (CL) as well.[4-6] The metabolic capacity of the liver, the perfusion of the liver and kidney and plasma protein binding are the most important determinants of CL. Altered liver blood flow, reduced CYP3A4 enzyme and increased CYP2E1 enzyme activity (phase I) and increased phase II metabolization have been observed in obese adults.[4] In obese children, a similar increased CYP2E1 activity has been observed; however, the few data on the impact of obesity on CYP3A4 activity are mixed.[5] Besides, obesity is a risk factor for non-alcoholic fatty liver disease in children (NAFLD). NAFLD also seems to have an effect on hepatic metabolism in children, but data on this topic are scarce [7] Regarding renal CL, obese adult individuals often present with augmented renal clearance, with indications that it could be present in children as well.[4,5] Finally, obese children seem to have a higher incidence of albuminuria and b2-microglobinuria, prognostic of glomerular and tubular dysfunction, respectively.[5] Clinical data to guide dosing in this context of an increased GFR and altered glomerular and tubular function, are lacking in children.[5]

Consequently, pediatric dosing regimens for these children cannot be linearly derived from adult dosing regimens on a milligram per kilogram approach but require proper investigation. The lack of evidence-based drug dosing regimens for obese children still puts care providers for a dilemma: either depriving the child from the drug or prescribing the drug off-knowledge.

In this study, we will investigate whether the PK of two model compounds with a different PK profile, paracetamol and cefazolin ,is altered in children with overweight and obesity. Cefazolin is a hydrophilic prophylactic antibiotic that is eliminated unchanged through the kidneys . It is mostly used to prevent postoperative infections such as surgical site infections. Paracetamol, a commonly used analgetic and antipyretic drug in children, is a more lipophilic component that is metabolized by the liver.

As secondary objective, we will study whether target concentrations are reached in plasma (paracetamol and cefazolin) and in subcutaneous tissue (cefazolin). This knowledge is important to develop evidence-based dosing regimens.

For studying cefazolin penetration in subcutaneous interstitial fluid, we will use the microdialysis technique. In microdialysis a small probe is implanted into the tissue or organ of interest. The microdialysis probe is designed to mimic a blood capillary and consists of a shaft with a semipermeable hollow fiber membrane at its tip (Figure 2).

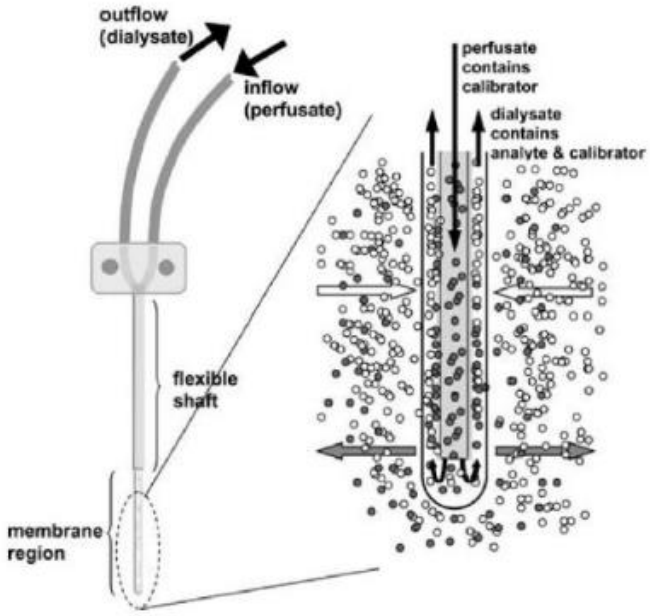

Figure 2: Schematic overview of the microdialysis technique [8]

The probe is continuously perfused with an aqueous solution (perfusate) that closely resembles the composition of the surrounding tissue fluid at a low-flow fixed rate. Small solutes cross the semipermeable membrane by passive diffusion along the concentration gradient. The dialysate, i.e. the solution leaving the probe, is collected at specified time intervals and the unbound drug concentration is quantified in these aliquots of dialysate. For most analytes the equilibrium between interstitium and the perfusion medium is incomplete, which is why microdialysis probes need to be calibrated [8].

Its minimally invasive character and the fact that a minimal volume is being removed from the patient during sampling makes microdialysis a state-of-the-art tool to measure the ISF drug concentrations in children.

Note: the microdialysis methodology is currently being used in a similar observational PK trial studying tissue disposition of beta-lactam antibiotics in critically ill children (TACTIC trial ; BC-09534).

References:

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[8] Marchand S., Chauzy A., Dahyot-Fizelier C., Couet W. Microdialysis as a way to measure antibiotics concentration in tissues. Pharmacological research. 2016 Sep;111:201-7.

5 The present study

5.1 Study design

Monocentric, prospective observational study

5.1.1 Number of subjects

Maximum 120 children

60 children per study compound*: 30 obese children and 30 children with normal weight (matched-controls for age, gender and type of surgery).

*a patient and his/her legal representatives can consent for study participation in the paracetamol plasma PK study compound and cefazolin plasma PK study compound.

5.1.2 Inclusion criteria

- Patient admitted to the operation room for surgery
- Patients undergoing general anesthesia for surgery*
- Patient age: children aged 2 year to 15 years
- Patient receiving cefazolin or paracetamol as per standard-of-care
- Intra-arterial (preferred) or intravenous access available for blood sampling
- Extra for the overweight/obese study cohort: patient body surface area above the body surface area-for-age threshold for overweight (85^{th} percentile or greater according to BMI-for age growth charts of the Center for Disease Control and Prevention)
- Extra for the matched-control cohort: BMI between 5^{th} and 85^{th} percentile according to BMI-for age growth charts of the Center for Disease Control and Prevention and matched for age (+/-3 years), gender and type of surgery to participants in the overweight/obese study cohort

*this is an inclusion criteria for microdialysis catheter insertion. If the patient is weaned from the anaesthesia after the catheter has been inserted, the microdialysis probe can remain *in situ* until the end of the study.

5.1.3 Exclusion criteria

- Personal or family history of excessive bleeding
- Pre-existing coagulopathy and/or thrombocytopenia
- No catheter available for blood sampling

- Absence of parental consent
- Known hypersensitivity to one of the study compounds
- Pregnancy

5.1.4 Replacement of subjects

If a drop-out occurs, replacement of subject will be done until the predefined number of subjects is reached.

6 Procedures

6.1 Procedures

6.1.1 Blood sampling

At predefined time points through an arterial (preferred) or venous catheter already in place, with a maximum total volume to be withdrawn of maximum 10 ml. This is far below the guideline of the European Medicines Agency of 2.4 ml/kg/patient.

Cefazolin:
In all patients a maximum of 6.5 mL (= 3 x 0.5mL + 2 x 2.5 mL) of blood will be sampled to measure total cefazolin plasma concentrations.

Maximum number of cefazolin samples: 5

The blood samples with a volume of 2.5 mL will be used to measure the unbound fraction of cefazolin and the following biochemical parameters: serum creatinine, CRP, albumin, total protein in plasma, liver enzymes, bilirubin.

Paracetamol and paracetamol metabolites:
For paracetamol and paracetamol metabolites (paracetamol-glucuronide, paracetamol-sulfate, paracetamol-mercaptopurate, paracetamol-cysteine), we will use a validated volumetric absorptive microsampling (VAMS) technique to sample venous or arterial blood. For each sample only 10 microliter of blood is absorbed by the tip of a specific sample device from a 100 microliter blood sample drawn from an arterial or venous catheter already in place.
The dried blood samples will be frozen at -80°C in the biobank of the Heymans institute until bio-analysis.

Maximum number of paracetamol samples: 5 x 100 microliter

If the patient is receiving cefazolin and paracetamol, time points for blood sampling for both molecules are the same, to minimize manipulations of the sampling catheter.

6.1.2 Microdialysis

6.1.2.1 Catheter placement

A 63 Microdialysis Catheter (M Dialysis AB, Solna, Sweden) with a molecular weight cut-off of 20kDa and a membrane length of 30 mm will be used. This is a flexible catheter, appropriate for use in resting and active tissue. Minimum 15 minutes before scheduled dosing of cefazolin, the catheter will be inserted in the patient's subcutaneous tissue in the upper arm or thigh.
The skin at the site of the microdialysis catheter insertion will be cleaned and disinfected. The subcutaneous tissue will be punctured by a steel guidance needle. The microdialysis probe will be placed into the tissue by using this guidance needle. The guidance needle will subsequently be removed, leaving the probe in the tissue layer. After insertion and throughout the sampling schedule, the microdialysis systems will be connected and perfused with 0.9% physiologic saline solution by means of microinfusion precision pumps. During sampling of cefazolin, an internal calibrator cefuroxime be added to the physiologic saline perfusion solution to determine the relative recovery (see 6.1.2.3.1).

6.1.2.2 Sampling

We will start to perfuse the microdialysis catheter with the internal standard solution. After an equilibration period of minimum 15 minutes, cefazolin will be administered as intravenous infusion (the dose and infusion time are prescribed by the treating physician). Subsequently, plasma and microdialysis sampling will be performed over one dosing interval as per sampling schedule (see separate document: Annexe – sampling protocol). After the sampling, the microdialysis probe will be removed. The microvials in which the dialysate is collected during the experiment will be stored at –80°C until the bio-analysis of the samples.

Maximum number of microdialysate samples: 8

6.1.2.3 In vivo probe calibration

To obtain the interstitial concentrations from dialysate concentrations, probe calibration will be assessed according to the internal standard method.

6.1.2.3.1. Internal standard

The internal standard method determines the in vivo relative recovery during the experiment. The internal standard is added to the perfusion solution during the course of the experiment. It is a molecule which matches the physicochemical characteristics of the studied drug as close as possible, so that the concentration loss of the internal standard will predict the concentration recovery of the drug.

The relative recovery value of the antibiotic of interest will thus be calculated as:

Relative recovery antibiotic (%) = loss internal standard (%) = 100 - (100 * internal standard concentration out / internal standard concentration in)

Interstitial cefazolin concentrations will be calculated according to the following equation:

ISF concentration = 100 * (sample concentration / relative recovery)

For this study we selected cefuroxime as internal standard for cefazolin.

A small amount of antibiotic in the perfusion fluid will pass the membrane to end up in the surrounding interstitial fluid. This is not a therapeutic intervention. The absolute amount of antibiotic crossing the membrane is very low, much lower than the therapeutic range.
We calculated that by adding the internal standard to the perfusion solution, the patient would be exposed to the following approximate amount of cefuroxime :
Cefuroxime: ± 20 microgram (therapeutic dose: 25 milligram/kg/dosis)

6.2 Data collection

Patient-specific characteristics : age, length, weight, body surface area, primary reason for admission, pre-existing co-morbidity, lab values at day of blood sampling (serum creatinine, C Reactive Protein, albumin, total protein in plasma, liver enzymes, bilirubin)

Treatment-specific characteristics : dosing regimen of study compounds, start and stop time of administration, duration of treatment, co-medication on days of blood sampling, pain scores during treatment with paracetamol or until microdialysis catheter removal (whichever lasts longer.)

Sampling-specific characteristics: catheter used for blood sampling, location of microdialysis catheter, time of insertion and removal of microdialysis catheter

6.3 Flowchart

D-1 Planning for surgery by treating surgeon requiring treatment with cefazolin or paracetamol during surgery

D-1: Screening for trial eligibility

D-1 or D1: Written parental informed consent – patient informed consent for patients older than 12

D1:

- Administration of cefazolin and paracetamol, according to current hospital dosing guidelines
- Microdialysis probe insertion
- Microdialysis, blood sampling: see Annexe for sampling scheme
- Removal of microdialysis probe after last sampling
- Data collection

End of trial

The expected total duration of this trial part is 3 years.

6.4 Ethical and legal aspects

The study will be performed in accordance with the Declaration of Helsinki (1964) and Good Clinical Practice (GCP) guidelines of the European Commission. Approval from the Ghent University (Hospital) ethics committee will be sought before initiating the study.

6.5 Expected risks/inconveniences

Patients under anesthesia should experience no pain or discomfort from the microdialysis catheter insertion. While *in situ* in resting and active muscle tissue, the flexible catheter does not cause discomfort for the patient. Pain scores (Comfort score<4 years of age; 4-6 years of age: Faces Pain Scale; 6-15 years Visual Analogue Scale) which are recorded as a part of the routine clinical care, will be monitored during the study.

7 Study analysis

7.1 Sample size calculation

The sample size calculation for the current study was well considered. For a comparison of the PK data between normal weight and obese children (paired t test, two-sided) the following parameters were considered: a power of 0.9, an alpha value of 0.05, a correlation factor of 0.5, and an effect of obesity on the AUC of 25% (bio-equivalence criterium).

- Cefazolin plasma PK: Assuming a standard deviation of 0.102 on the log10 scale (estimated based on Schmitz et al. [1]), a total sample size of 14 pairs is sufficient to obtain 90% power to detect a decrease of 25% of the AUC (corresponding to a difference in mean ratio of 0.097 on the log10 scale) when comparing normal to overweight/obese children.
- Cefazolin tissue PK: Assuming a standard deviation of 0.144 on the log10 scale (estimated based on Palma et al. [2]), a total sample size of 26 pairs is sufficient to obtain 90% power to detect a decrease of 25% of the AUC (corresponding to a difference in mean ratio of 0.097 on the log10 scale) when comparing normal to overweight/obese children.
- Paracetamol plasma PK: Assuming a standard deviation of 0.119 on the log10 scale (estimated based on Zuppa et al. [3]), a total sample size of 18 pairs is sufficient to obtain 90% power to detect an decrease of 25% of the AUC (corresponding to a difference in mean ratio of 0.097 on the log10 scale) when comparing normal to overweight/obese children.

Conclusion: the minimal sample size needed to perform the envisioned statistical comparisons of the various PK data is 26 pairs (cefazolin tissue PK).

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Surgery Based on Evidence from Subcutaneous Microdialysis and Populational Pharmacokinetic Modeling. Pharmaceutical research. 2018;35:116.
[3] Zuppa AF, Hammer GB, Barrett JS, Kenney BF, Kassir N, Mouksassi S, et al. Safety and population pharmacokinetic analysis of intravenous acetaminophen in neonates, infants, children, and adolescents with pain or Fever. The journal of pediatric pharmacology and therapeutics : JPPT : the official journal of PPAG. 2011;16:246-61.

7.2 Analysis of the samples

Separation the bound and unbound cefazolin fraction in cefazolin samples will be performed using a validated ultracentrifugation method at the Department of Pharmacy, Erasmus MC, Rotterdam, the Netherlands.
Bio-analysis of cefazolin blood samples for unbound and total will be performed using a validated HPLC method at the Department of Pharmacy, Erasmus MC, Rotterdam, the Netherlands.

Bio-analysis of paracetamol microsamples will be performed through a validated HPLC method at the Laboratory of Toxicology, Faculty of Pharmacy, Ghent University.

7.3 Statistical analysis

Pharmacokinetic data will be analyzed using a non-linear mixed effects modelling approach. For evaluation of target attainment, Monte Carlo simulations will be performed using the predefined PK/PD target for cefazolin and paracetamol.

Statistical analysis will be performed using a commercially available computer program (IBM SPSS® Statistics). Pharmacokinetic outcome variables AUC, AUC tissue/plasma ratio, C_{max}, T_{max}, $T_{1/2}$ will be tested for normal distribution by means of the Shapiro-Wilk test and/or P-P and Q-Q plots. Normally distributed data will be presented as mean ± standard deviations, non-normally istributed data will be described using median and interquartile range.

For correlation and comparison of parameters between statistical entities an adequate scientific significance test (e.g. dependent-samples t-test for comparison of means between first and steady state dose, as well as between the different compartments, given normally distributed data) will be used.

8 Duration of the trial

The expected total duration of this trial part is 3 years.

9 Indemnity insurance

'No fault' Insurance Ghent University Hospital

10 Publication policy

Results will be published in peer-reviewed A1 journals.

The Principal Investigator will be given the choice to be the first, or the last author on any publication. The order of the subsequent authors is to be based on significant scientific input and patient recruitment to the study. The Sponsor will retain the right to include in the authorship list, names other than Investigators.

Drafts of these publications will be discussed with all co-authors before submission allowing constructive comments. Researcher shall give its final approval in writing before submission and within a reasonable time of thirty (30) days.

Study Sponsor will have the right, in narrow collaboration with, to disclose the results orally or in writing (including but not limited to: submission of a manuscript, abstract, patent application, presentation at seminars, symposia, national or regional professional meetings, or to publish in journals, theses or dissertations etc.).

11 Signature page

Investigator:

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