The CYTONOX trial

Christina Gade[1], Gerd Mikus[2], Hanne Rolighed Christensen[1], Kim Peder Dalhoff[1], Jens-Christian Holm[3] & Helle Holst[1]

ABSTRACT

INTRODUCTION: In Denmark, it is estimated that 3-5% of children are obese. Obesity is associated with pathophysiological alterations that may lead to alterations in the pharmacokinetics of drugs. In adults, obesity was found to influence important drug-metabolising enzyme pathways. The impact of obesity-related alterations on drug metabolism and its consequences for drug dosing remains largely unknown in both children and adults. An altered drug metabolism may contribute significantly to therapeutic failure or toxicity. The aim of this trial is to investigate the in vivo activity of CYP3A4, CYP2E1 and CYP1A2 substrates in obese versus non-obese children.

METHODS: The CYTONOX trial is an open-label explorative pharmacokinetic trial. We intend to include 50 obese and 50 non-obese children. The primary end points are: in vivo clearance of CYP3A4, CYP2E1 and CYP1A2 substrates, which will be defined by using well-tested probes; midazolam, chlorzoxazone and caffeine. Each of the probes will be administered as a single dose. Subsequently, blood and urine samples will be collected at pre-specified times.

CONCLUSION: The aim of the CYTONOX trial is to investigate the in vivo activity of CYP3A4, CYP2E1 and CYP1A2 in obese and non-obese children. The results are expected to be used in the future as a basis for drug dosing recommendations in obese children.

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In Denmark, 3-5% of children are obese [1]. Obese children have an increased risk of developing chronic diseases like diabetes type II, musculoskeletal disorders, depression, asthma, hypertension, and cancers [2-4], and a higher use of drugs, especially in the 12-19-year age group [5]. Obesity is associated with pathophysiological alterations, such as an altered tissue composition with a relatively higher increase in fat (60%) compared with lean tissue (40%) per kg of total bodyweight as well as alterations in drug binding proteins, cardiac output, organ blood flows and tissue perfusion [6]. 44% have non-alcoholic fatty liver disease which may influence some of the most important drug-metabolising enzymes and cause clinically significant alteration of the hepatic clearance [6-9] and thus contribute significantly to therapeutic failure or toxicity [3, 9].

In adults, obesity was found to influence cytochrome P450 CYP3A4, CYP2E1 and CYP1A2 [8]. CYP3A4 is involved in the metabolism of approximately 50% of all drugs on the market. In obese adults, clearance of CYP3A4 substrates was found to be reduced, while the clearance of CYP2E1 substrates was increased. Well-known examples metabolised by these isoenzymes are acetaminophen via CYP2E1 and antibiotics such as clarithromycin via CYP3A4. CYP2E1 and CYP3A4 clearances have not been studied in obese children despite the fact that drugs metabolised by these enzymes are often administered to children. Drugs with a relatively narrow therapeutic interval including imipramine, carbamazepine and ciprofloxacin are metabolised via CYP1A2. The latter has been investigated in obese children; unfortunately, the study was inconclusive due to lack of statistical power although a trend was observed towards reduced CYP1A2 clearance in obese compared with non-obese children [10]. This is in contrast to the higher CYP1A2 clearance values previously found in obese adults [8]. This discrepancy underlines the fact that the expression and activity of enzymatic pathways in obese children may be different from those of obese adults.

The aim of the present trial is to determine whether the activity of important drug-metabolising enzymes, CYP3A4, CYP2E1 and CYP1A2 is altered in obese children compared with non-obese children.

METHODS

CYTONOX (cytochrome P450 (CYP) 3A4, -2E1 and -1A2 activity in obese – versus non-obese children) is an open-label explorative pharmacokinetic trial. The study protocol was approved by the local Ethical Committee of Region Zealand (SJ-455) and by The Danish Health Authorities (EudraCT: 2014-004554-34).

Study population

A total of 50 obese children aged 11-18 years are recruited from The Children's Obesity Clinic, Department of Paediatrics, Holbæk Hospital, Denmark, and 50 non-obese aged- and gender-matched controls are recruited from local schools in Zealand by the Children's Obesity Clinic. See **Table 1** for inclusion and exclusion criteria. Both parents of each trial participant and all participants

PROTOCOL ARTICLE

1) Department of Clinical Pharmacology, Bispebjerg and Frederiksberg Hospitals
2) Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Germany
3) Children's Obesity Clinic, Department of Paediatrics, Holbæk Hospital, Denmark

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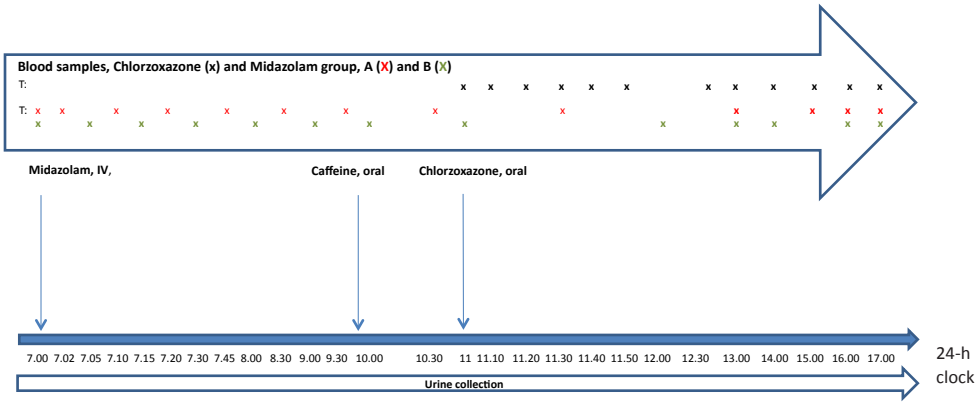
FIGURE 1

Timeline chart, pharmacokinetic trial. In vivo CYP3A4, CYP2E1 and CYP1A2 activity will be determined by: a) the midazolam microdosing method, b) chlorzoxazone multi-sample clearance method and c) the caffeine urinary metabolic ratio method. Each of the probes will be administered as single doses. Subsequently, blood and urine samples will be collected at pre-specified times. In order to minimise blood loss, the use of population pharmacokinetic and sparse sampling based on optimal sampling will be applied. Thus, participants are divided into two equally sized groups (25 obese and 25 non-obese subjects in each group). The former group will have their sampling time postponed compared with the latter group. Baseline blood samples will be taken at admission.



Midazolam: 13 blood samples of 2.7 ml = 35.1 ml
Chlorzoxazone: 12 blood samples of 2 ml = 24 ml
Baseline blood samples = 90 ml
Total = 149.1

CYP = cytochrome P450.

≥ 15 years of age will sign an informed consent form that has been approved by the local ethics committee.

Primary end points

Clearance of CYP2E1-, CYP1A2- and CYP3A4 substrates will be defined by using well-tested probes (midazolam, chlorzoxazone and caffein) as measures of in vivo-iso-specific enzyme activity:

- Chlorzoxazone (CYP2E1) multi-sample fractional clearance method [13-16]
- Caffeine urinary metabolic ratio method (CYP1A2) [10, 17]
- Midazolam micro-dosing method (CYP3A4) [11].

Cmax (maximum plasma concentration), Tmax (time to maximum plasma concentration), AUC (area under the drug concentration/time curve), T1/2 (elimination half time) and Vd (volume of distribution) will be defined as appropriate from blood samples taken at pre-specified times. Each of the probes will be administered as single doses. Subsequently, blood and urine samples will be collected at specified times (**Figure 1**). The metabolism of each of the three drugs used as probes does not affect one other, so the drugs can be administered on the same day.

Secondary end points

From an exploratory point of view, we will investigate whether an altered enzyme activity of CYP3A4, CYP2E1 and CYP1A2 is associated with steatosis and metabolic syndrome.

Markers of steatosis and metabolic syndrome:

- Lipid profile: low-density lipoprotein, high-density lipoprotein, total cholesterol and triglyceride
- Markers of insulin resistance: glycated haemoglobin, blood glucose and insulin
- Inflammatory markers: tumour necrosis factor alpha, interleukin 6, leptin, adiponectin and C-reactive protein
- Liver tests: aspartate amino transferase, alanine amino transferase, alkaline phosphatase, bilirubin, albumin and liver magnetic resonance imaging (to verify steatosis in obese children)
- Three blood pressure measurements.

Intervention

Eligible patients will be admitted to the Children's Obesity Clinic or to the Zelo Phase 1, Clinical Trial Unit, Bispebjerg and Frederiksberg Hospitals after overnight fasting. Baseline blood samples will be taken at admission, and all trial participants will be instructed to empty their bladder. Each trial participant is hospitalised during the initial 10 h of the trial in order to administer the drugs and collect the blood samples. After 10 h, the remaining urine collection will occur in the patients' home setting. For each participant, the trial will have been successfully completed after 24 h.

In order to minimise blood loss, the use of popula-

tion pharmacokinetic and sparse sampling based on optimal sampling will be applied. Thus, participants are divided into two equally sized groups (25 obese and 25 non-obese subjects in each group (Figure 1). In total, the blood sampling will not exceed a volume of 150 ml.

Probe 1, midazolam, CYP3A4: Intravenous midazolam (0.001 mg) will be administered in the morning.

Preparation of midazolam: Shortly before administration, midazolam (Midazolam Hameln 1 mg/ml) is diluted in physiologic saline to a final concentration of 0.1 µg/ml and 10 ml are infused within 5 min.

Blood samples: 2.7 ml blood samples will be collected at pre-specified time periods (Figure 1).

Probe 2, chlorzoxazone, CYP2E1: Tablet (Klorzoxazon, Takeda 250 mg)) will be given orally with 200 ml of tap water. Blood samples of 2.5 ml will be collected for all trial subjects at pre-specified time periods (Figure 1).

Probe 3, caffeine, CYP1A2: all subjects will have a breakfast and a Coca Cola (118.3 ml = 11.5 mg caffeine).

Blood samples will be collected into heparinised tubes and centrifuged for 10 min. at 4 °C at 2,500 g and plasma will be stored at –20 °C until analysis. A 24-h urine collection is performed after midazolam is dosed. The total urine volume is recorded and the urine will be stored in aliquots at –20 °C until analysis.

Criteria for discontinuing or modifying the intervention

Trial participants will be withdrawn if they appear unduly distressed. Immediate intervention is available in case of an acute adverse event, e.g. anaphylactic reaction. Participants can withdraw from the study at any time without consequences for their future care. The sponsor/principal investigator can also withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures.

Ethics

The study will abide by the principles of the Helsinki II Declaration and Act 503 of 1992 on the Scientific Ethics Committee System. The trial will be conducted in accordance with the guidelines of good clinical practice (GCP) and will be monitored by the local GCP.

The parent or legal representative of the child will have an interview with the principal investigator (PI), during which opportunity will be given to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted. They will be provided with written information and contact details of the PI from whom further information about the trial can be obtained, and will be made aware of their right to withdraw the child from the trial at any time. Children will receive information, according to their capacity of understanding, about the trial and its risks and benefits and their assent will be obtained. As appropriate, patients and their families will be provided with copies of the participant information sheets and their signed consent/assent forms.

The investigational medicinal products used in this study are to be prescribed in accordance with their licensed indications. The summary of product characteristics for midazolam and chlorzoxazone are used as reference documents. Furthermore, an update for any new toxicology findings of midazolam and chlozoxazone was performed which brought forth no additional information. Midazolam is administered according to the "midazolam microdosing method". Since these doses are far below the "no observed adverse level", it is an attractive and safe approach for assessing a drug's properties in

TABLE 1

Inclusion- and exclusion criteria for the CYTONOX trial.

Inclusion criteria	Exclusion criteria
Obese children, BMI ≥ 99 percentile	Acute or chronic liver diseases
Healthy non-obese children, BMI ≤ 90 percentile	Other chronic diseases, except from allergic rhinitis, rash etc.
Age 11-18 yrs, block stratified Both genders	Intake of drugs known to induce of inhibit CYP2E1, CYP3A4 and/or CYP1A2, including acetaminophen, ciprofloxacin, azithromycin, ketoconazole, oestradiol etc.
Post menarche, females[a] [11]	Citrus fruit intake within a min. of 2 weeks
Caucasians[b], based on information about parents and on grandparents race	Alcohol intake within a min. of 72 h [9, 12]
Written informed consent from trial participants, age > 15 yrs, and 1 parent	Caffeine (Coca Cola, coffee and tea) intake 48 h prior to administration of the caffeine probe [9]
	Smoking 96 h prior to administration of the caffeine probe [8]
	Kidney disease and dialysis
	Pregnancy (test will be performed)
	Known hypersensitivity to chlorzoxazone, midazolam or caffeine

CYP = cytochrome P450; CYTONOX = CYP 3A4, -2E1 and -1A2 activity in obese- versus non-obese children.
a) Females included will be post menarche (Tanner stage 4) in order to avoid bias as maturation of CYP1A2 activity mirrors pubertal growth, which peaks early in females [11].
b) Caucasians have been chosen in order to avoid bias as a consequence of inter-individual racial differences in enzyme activity.

CYTONOX: An open label, non-randomised, exploratory pharmacokinetic trial comparing 50 obese children and 50 non-obese children and adolescents aged 11-18 years.

vulnerable populations such as children [11].

For blood samples, topical local anaesthetics are offered to all children. The positioning of a venous catheter is common practice at paediatric departments, and the risk of infection is very low. The sampling volume will have no haemodynamic effects on the child or affect the child's growth or development [18].

All adverse events, adverse reactions, serious adverse events (SAEs), serious adverse reactions (SARs) and unexpected serious adverse reactions (SUSARs) will be registered in the patient's record. The time of registration begins once the pharmacokinetic trial starts (t = 0) and ends when the pharmacokinetic trial ends. At this point, 99% of the medicine will have been cleared from the body.

SAEs and SARs will be reported to the sponsor within 24 h. SUSARs will be reported immediately to the sponsor who will inform the health authorities and the ethical committee within seven days. Finally, the end-report will be sent to the health authorities by the sponsor within one year after the trial has ended.

Data management

Information about the subjects is protected in accordance with the Danish Act on Processing of Personal Data. A record will be created on each trial participant, and the record will be stored alongside The Danish Childhood Obesity Biobank's ethical approvals. Personalised, identifiable information and biological samples will be accessible to the project team, authorised by the project sponsor and the PI. Biological material, trial data and personal data will be stored in locked rooms and handled in accordance with the terms and conditions of the Danish Data Agency. Based on study design, missing data will be handled as a complete case analysis in accordance with the European Medicines Agency Guideline on Missing Data in Confirmatory Clinical Trials. Thus, in the case of a trial participant withdrawing, missing values will be excluded from the analyses and the trial participant will be replaced. However, in case of single loss of data, such as missing blood samples, data will still be included in the analyses. Withdrawal due to adverse reactions will be noted.

Sample size calculations and statistical analyses

The level of statistical significance is set at $p < 0.05$. The sample size was calculated on basis of the primary end points and has a power of 80% to detect a minimal difference in clearance (ml/min/mg) of 0.9 for CYP3A4, 0.7 for CYP2E1 and 1.37 [12, 16] for CYP1A2 [10].

Basic descriptive statistics will be used to characterise the study patients. The difference between the means of the groups will be tested for significance using Student's t-test provided data are normally distributed. In case data are not normally distributed, non-parametric statistics will be used. Using multiple regression analysis, the clearance value of CYP3A4 and the clearance as a function of bioavailability for CYP2E1 and CYP1A2 will be correlated with independent variables such as body mass index (BMI), lipid profile, insulin resistance and blood pressure.

Trial registration: EudraCT: 2014-004554-34.

DISCUSSION

Extrapolation from adult observation may give false predictions of clearance values in children. However, extrapolation of results from studies in obese adults to obese children is widely applied in the daily clinic as the impact of obesity on drug metabolism in children is almost non-existing. The main objective of the paediatric regulation by the European Union is to ensure that effective and safe doses are evaluated in children. Yet, it is not mandatory to include obese children in clinical trials when developing a new medicine. Therefore, clinical studies are needed as altered drug biotransformation in obese children can contribute significantly to therapeutic failure or toxicity [8].

One approach is the use of surrogate probes metabolised by known enzyme systems. Studies with such drugs have already provided much information about the regulation of human drug metabolism by gender, exercise, environmental factors, life-style factors such as smoking and interaction with other drugs and to some extent the metabolism in obese adults [17].

In accordance with the ICH E11 guidelines, the present trial protocol includes children between 11 and 18 years as pharmacokinetic studies for dose selection should be performed in paediatric patients in whom the medicinal product is likely to be used [19] or it should be conducted in the paediatric population. Further, females

included will be post menarche (Tanner stage 4) in order to avoid bias as maturation of CYP1A2 activity mirrors pubertal growth, which peaks early in females [20].

The power calculations in this study are based on results from previous studies in adults (CYP3A4 and CYP2E1) [11] and obese children (CYP1A2) [9]. Inclusion of 50 obese and 50 non-obese children is the result of a minimum relevant difference chosen to be 30% based on an expected inter-individual variability and an expected clinically important alteration in the metabolism, especially for drugs with a narrow therapeutic interval. In a future perspective, it could be of interest to investigate other enzymes such as CYP2D6; however, due to gene polymorphism [8], an even larger sample size would be required.

CONCLUSION

This trial is expected to provide new information on the drug metabolism in obese children and thereby contribute with new and important basic scientific knowledge. The results are expected to be used in the future as a basis for drug dosing recommendations in obese children.

CORRESPONDENCE: *Christina Gade.* E-mail: Christina.gade@regionh.dk
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