Pediatric microdose study of oral [14C] midazolam; simultaneous metabolic profiling and quantification using HRMS and AMS

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INTRODUCTION

Drug absorption, distribution, metabolism and excretion can differ largely between children and adults, thus causing an increasing interest on studying age related changes in drug metabolism. However, pediatric studies impose additional ethical and analytical challenges. Micrdosing/microtracer research using Accelerator Mass Spectrometry (AMS) overcomes these issues as it allows quantification down to fg/mL levels while using limited sample volumes. Herein, a metabolite in safety testing (MIST) pilot study was performed in children using an oral microdose of [14C]midazolam. A combination of High Resolution Mass Spectrometry (HRMS) and AMS enabled the generation of metabolic profiles leading to their simultaneous identification and quantification.

NOVEL ASPECT

Micrdosing is a safe and informative approach to evaluate metabolism formation in children.

METHODS

MICRODOSING

Children admitted to the pediatric intensive care unit received an oral microdose of [14C]midazolam (20 ng/kg; 60 Bq/kg). Blood samples were taken up to 24 hours after the dosing. Initially, a pool per patient (AUC0–24h) was prepared according to the Hamilton method. Secondly, a pool per age group (0–1 month; 1–6 months; 0.5–2 years; 2–6 years) was generated using equal volumes.

UPLC

Plasma extracts (50 µL) were injected on a UPLC using a linear gradient of acetonitrile (ACN) on 1 mM ammonium formate in MilliQ water + 5% ACN over 30 min at 0.5 mL/min and 50 ºC. Parent drug and metabolite separation was accomplished.

AMS

The remaining 70% of the UPLC flow was diverted to a fraction collector (0–20 min 4 fractions/min, 20–30 min 1 fraction/min). To each fraction at least 25 µg of 12C was added prior to transferring to tin-foil cups. The fractions are dried and subsequently analyzed by automated combustion CO2 AMS, the AMS quantifies 14C levels per fraction. This study design allows simultaneous metabolite identification and quantification from a single UPLC injection.

METABOLIC PROFILES PER AGE GROUP

The UPLC flow was split to allow the 30% of its content to be diverted to an on-line coupled Q-Exactive HRMS for metabolite identification. The Q-Exactive (on-line) provides accurate HRMS and MS/MS data allowing metabolite identification. To each fraction 12C is added prior to transferring to tin-foil cups. The fractions are dried and subsequently analyzed by automated combustion CO2 AMS, the AMS quantifies 14C levels per fraction. This study design allows simultaneous metabolite identification and quantification from a single UPLC injection.

CONCLUSIONS/OUTLOOK

The present work shows the feasibility of using microdose MIST studies with orally available [14C]-labeled compounds to safely generate metabolic profiles in pediatric patients. Stable critically ill children were treated with a microdose of [14C]MOZ in the context of a larger microdosing study. Metabolic profiles were generated for 4 different age groups. In all cases, MOZ, 1-OH-MOZ and 1-OH-MOZ-GLUC could be identified. There is an unidentified fraction of metabolites that is expected to contain MOZ-DG-GLUC and 4-HO-MOZ. The study is ongoing and more patients will be included to investigate whether there are age related changes in the metabolic profiles. Microdose studies allow that medication doses are no longer based only on body surface areas, because knowledge on the metabolism of a drug in that age group is now readily available.

REFERENCES


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Figure 1: MIST workflow.

Figure 2: AMS metabolic profile of plasma samples from pediatric patients from age pool I (0.5-1 years, blue) and age pool IV (2-6 years, orange). Each peak corresponds to a different analyte identified by HRMS (midazolam, 1-hydroxymidazolam and 1-hydroxymidazolam glucuronide). The chemical structure, molecular weight (MW), m/z ratio of the pseudomolecular ion and retention time of each analyte are provided. The corresponding HRMS spectra are also shown.

Figure 3: Metabolic profiles of an oral [14C]midazolam micrortrace in pediatric patients from different age groups. MOZ: midazolam; 1-OH-MOZ: 1-hydroxymidazolam; 1-OH-MOZ-GLUC: 1-hydroxymidazolam glucuronide; MOZ-DG-GLUC: 1-hydroxymidazolamide glucuronide

Figure 4: METABOLIC PROFILES AND HRMS METABOLITE IDENTIFICATION