Pediatric microdose study of oral [^{14}C] midazolam; simultaneous metabolic profiling and quantitation using HRMS and AMS

Esther van Duijn[1], Marta Pelay-Gimeno[1], Bianca D. van Groen[2], Arjan de Vries[1], Miriam G. Mooij[3], Albert D. Windhorst[4], Harry N. Hendrikse[4], Saskia N. de Wildt[5], Wouter H.J. Vaes[1] ([1] TNO, Zeist, The Netherlands; [2] Erasmus University Medical Center, Rotterdam, The Netherlands; [3] Leiden University Medical Center, Leiden, The Netherlands; [4] VU University Medical Center, Amsterdam, The Netherlands; [5] Radboud University, Nijmegen, The Netherlands.)

TNO innovation for life

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. Microdosing/microtracer research using Accelerator Mass Spectrometry (AMS) overcomes these issues as it allows quantification down to fg/mL levels while using limited sample volumes.[1-3]

Herein, a metabolite in safety testing (MIST) pilot study was performed in children using an oral microdose of [^{14}C]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

Microdosing is a safe and informative approach to evaluate metabolite formation in children.

METHODS

MICRODOSING

Children admitted to the pediatric intensive care unit received an oral microdose of [^{14}C]midazolam (20 ng/kg; 60 Bq/kg). Blood samples were taken up to 24 hours after the dosing. Initially, a pool per patient ($AUC_{0\text{-}24h}$) was prepared according to the Hamilton method.[4] 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 ˚C. Parent drug and metabolite separation was accomplished.

Q-Exactive HRMS

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.

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 ^{12}C was added prior to transferring to tin-foil cups. Samples were subsequently analyzed by automated combustion CO_2 AMS,[5] (off-line, 1MV Tandetron) for ^{14}C level quantification.

MIST WORKFLOW

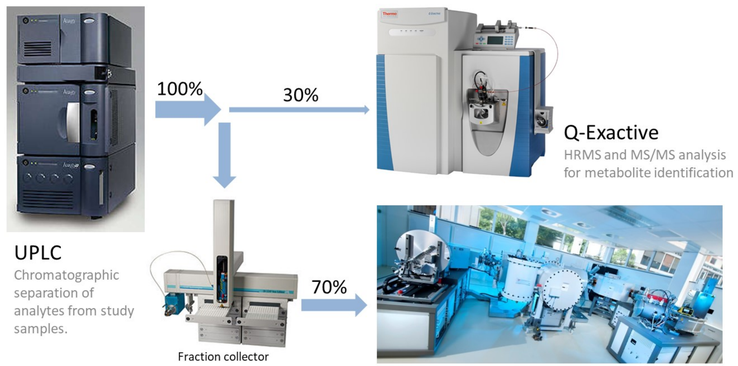

Figure 1: Plasma samples from pediatric patients are pooled according to defined age ranges. After protein precipitation using ACN, the samples are centrifuged and injected on a UPLC. Only 50 µL of plasma were used to generate one MIST profile. The flow is split to the Q-Exactive (30%) and a fraction collector (70%). The Q-Exactive (on-line) provides accurate HRMS and MS/MS data allowing metabolite identification. To each fraction ^{12}C is added prior to transferring to tin-foil cups. The fractions are dried and subsequently analyzed by automated combustion CO_2 AMS. The AMS quantifies ^{14}C levels per fraction. This study design allows simultaneous metabolite identification and quantification from a single UPLC injection.

METABOLIC PROFILES PER AGE GROUP

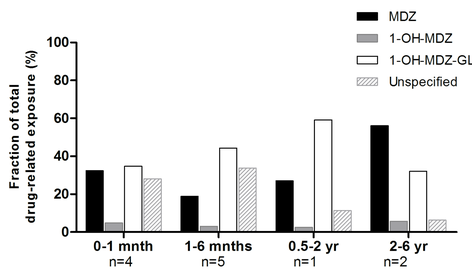

Figure 3: Metabolic profiles of an oral [^{14}C]MDZ microtracer in pediatric patients from different age groups. MDZ: midazolam; 1-OH-MDZ: 1-hydroxymidazolam; 1-OH-MDZ-GLUC: 1-hydroxymidazolam glucuronide.

AMS METABOLIC PROFILES AND HRMS METABOLITE IDENTIFICATION

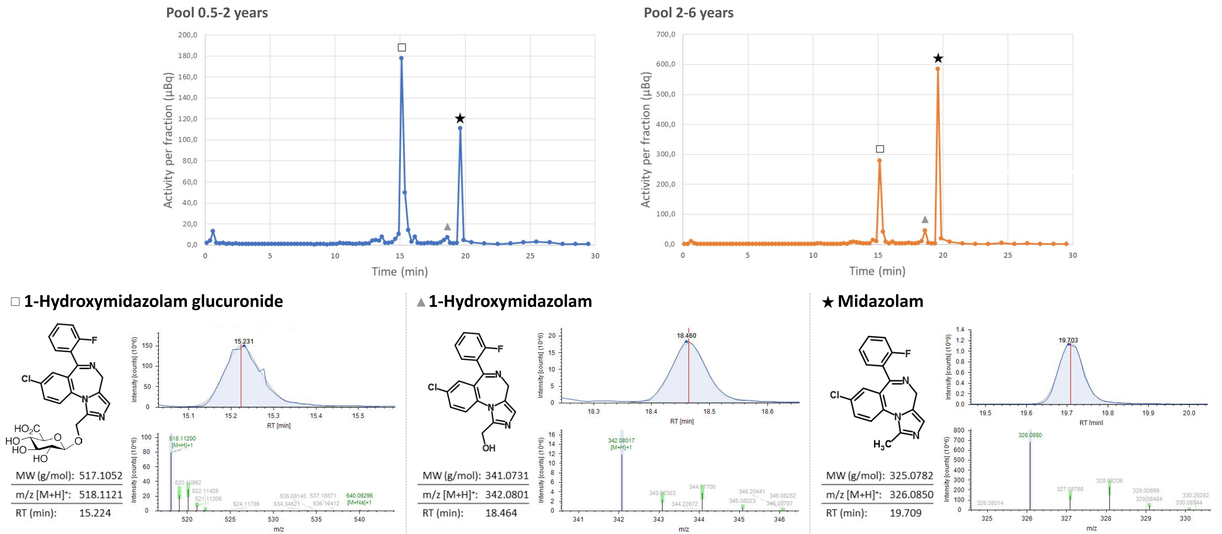

Figure 2: AMS metabolic profile of plasma samples from pediatric patients from age pool III (0.5-2 years, blue) and age pool IV (2-6 years, orange). Each peak corresponds to a different analyte identified by HRMS (★midazolam, ▲1-hydroxymidazolam and □ 1-hydroxymidazolam glucuronide). The chemical structure,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

CONCLUSIONS/OUTLOOK

The present work shows the feasibility of using microdose MIST studies with orally available [^{14}C]-labeled compounds to safely generate metabolic profiles in pediatric patients. Stable critically ill children were treated with a microdose of [^{14}C]MDZ in the context of a larger microdosing study. Metabolic profiles were generated for 4 different age groups. In all cases, MDZ, 1-OH-MDZ and 1-OH-MDZ-GLUC could be identified. There is an unidentified fraction of metabolites that is expected to contain MDZ-GLUC and 4-HO-MDZ. 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

[1] Mooij M.G., van Duijn, E. *et al. Clin. Pharmacokinet.*, **2017**, 56(10), 1185-1195.
[2] Turner, M.A., Mooij, M.G. *et al. Clin. Pharmacol. Ther.* **2015**, 98(3) 234-237.
[3] Mooij M.G., van Duijn, E. *et al. Clin. Pharmacokinet.*, **2014**, 53(11), 1045-1051.
[4] Hamilton, R.A., Garnett, W.R., Kline, B.J. *Clin. Pharmacol. Ther.* **1981**, 29(3), 408-413.
[5] van Duijn, E., Sandman, H. *et al. Anal. Chem.* **2014**, 86(15), 7635-7641.