Metabolic profiling and mass balance studies in pediatric patients using a microtracer approach – a proof of concept

ESTHER VAN DUIJN
DRUG DEVELOPMENT

- Safety
- Efficacy
- Pharmacokinetics of parent drug
- Elimination via Metabolism and excretion
  - Safety of metabolites
  - (Efficacy of metabolites)
  - PK of metabolites
  - Elimination of metabolites
SAFETY ASSESSMENT PRINCIPLES

- Exposure
- Toxicity

Risk

First: Parent drug toxicological assessment in animals

But what about metabolites? FDA guidance on Safety testing of drug metabolites:

- ‘The safety of drug metabolites may need to be determined in nonclinical studies because these metabolites are either identified only in humans or are present at disproportionately higher levels in humans than in any of the animal species used during standard nonclinical toxicology testing.’
- ‘Generally, metabolites identified only in human plasma or metabolites present at disproportionately higher levels in humans than in any of the animal test species should be considered for safety assessment. Human metabolites that can raise a safety concern are those formed at greater than 10 percent of total drug-related exposure at steady state.’
HUMAN METABOLISM DATA

- Generally generated late Phase 2, or Phase 3
  - High 14C dose administration not ethical in early phase

- Lower the 14C dose significantly (e.g. 1000 fold)
  - No ethical concern even if compound is killed
  - No QWBA
FASTER HUMAN DATA

Preclinical

Clinical Phase 1

Clinical Phase 2

Clinical Phase 3

Basic animal safety

Human and Animal Safety

Human metabolism

Animal metabolism
Elemental analyzer (EA) combusts sample. Gaseous CO$_2$ is used instead of graphite.
Combine UPLC, fraction collection, and AMS with high resolution MS/MS-identification
TYPICAL EARLY MIST STUDY DESIGN

- No dosimetry data needed
- No separate clinical trial needed (ICRP class 1 study)
- Include in early clinical trials
- Close to intended therapeutic dose
- Include 100 nanoCi to 1 microCi of $^{14}$C labelled drug
- Sampling blood, urine, faeces
- Profile samples with chromatography
- Count fractions (AMS) and identify with LC-hrMS/MS

- Often combined with mass balance study (discharge from clinic based on AMS data)
WHAT ABOUT CHILDREN?

Back to the FDA guidance?

› ‘The safety of drug metabolites may need to be determined in nonclinical studies because these metabolites are either identified only in children or are present at disproportionately higher levels in children than in any of the animal species used during standard nonclinical toxicology testing.’

› ‘Generally, metabolites identified only in children's plasma or metabolites present at disproportionately higher levels in children than in any of the animal test species should be considered for safety assessment. Children's metabolites that can raise a safety concern are those formed at greater than 10 percent of total drug-related exposure at steady state.’

› High radioactive dose (100 microCi) studies?

› Microtracer studies?
CLASSICAL PAEDIATRIC DEVELOPMENT

- Assess PK differences with adult PK as starting point; single dose if dose-linear in adults (ICH E11)

- Adult therapeutic dose PK data
  - Predict PK of adolescents
  - Adolescent therapeutic dose PK
  - Predict PK of children
  - Children therapeutic dose PK
  - Predict PK of toddlers
  - Toddler therapeutic dose PK
  - Predict PK of infants
  - Infant therapeutic dose PK
  - Predict PK of Neonates
  - Neonate therapeutic dose PK
  - Predict PK of Pre-terms

Time
MICRODOSING-BASED PAEDIATRIC DEVELOPMENT

- Asses PK differences with adult PK as starting point; single dose if dose-linear in adults (ICH E11):
  - With rapid PK: Individual child could benefit from knowing its' individual PK to personalize dosing for the specific child
  - Investigate potential ontogenic differences

Dose linear PK adults from microdose to therapeutic dose

- Microdosing in adolescents
- Microdosing in children
- Microdosing in toddlers
- Microdosing in infants
- Microdosing in neonates

Time
First pediatric [14C]microtracer study to generate metabolite profiles

Midazolam is a widely used marker for CYP3A4/5 activity, with lower activity in neonates than in adults

12 critically ill children included in this substudy (96 patients were eligible, 46 consented)

- Inclusion criteria:
  - Postmenstrual age > 36 weeks
  - Bodyweight > 2.5 kg
  - Clinical need for iv midazolam
  - Indwelling arterial line in place for blood sampling

- Exclusion criteria
  - Anticipated death in 28 hrs
  - Circular/kidney/liver failure
  - Comedication known to interact with midazolam
  - Gastrointestinal disorders
PROOF OF CONCEPT – STUDY DESIGN II

First pediatric [14C]microtracer study to generate metabolite profiles

Oral $^{14}$C-midazolam microtrace 60 Bq/kg, intravenous therapeutic dose of midazolam (0.05-0.3 mg/kg/hr continuous infusion)

Blood sampling up to 24 hr after dosing

Hamilton plasma pool 0-24h per age group
  - 0-1 month (4 patients)
  - 1-6 months (5 patients)
  - 0.5-2 years (1 patient)
  - 2-6 years (2 patients)

Urine and fecal collection up to 72 hrs after dosing
METABOLITE PROFILES

1. 1OH-MDZ-GLU most abundant, followed by unchanged MDZ and 1OH-MDZ.
2. Unspecified metabolites < 10% of total drug related material.
3. MDZ-GLU and 4-OH-MDZ.

\[ \text{MDZ-GLU and 4-OH-MDZ} \]

\[ \text{1-OHMG} \]

\[ \text{1-OHM} \]

\[ \text{Midazolam} \]
### Table 4  Mass balance results after administration of an oral [¹⁴C]midazolam microtracer

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sampling time (hour)</th>
<th>Urine</th>
<th>Feces</th>
<th>Total fraction of the administered dose recovered in urine and feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>155 (Bq)</td>
<td>0.74</td>
<td>6.21 (Bq)</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>124 (Bq)</td>
<td>0.74</td>
<td>31.5 (Bq)</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>81 (Bq)</td>
<td>0.49</td>
<td>64.1 (Bq)</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>330 (Bq)</td>
<td>0.92</td>
<td>7.12 (Bq)</td>
</tr>
</tbody>
</table>

- Longer sampling time for subject 1 may have resulted in a higher recovery
- Adults: 90% urinary recovery after oral dosing
TAKE HOME MESSAGE

- The sample size of this pilot is too small however:
- Microtracers and detection with AMS enable safe dosing to children of $^{14}\text{C}$ labelled drugs
- Avoid delay in drug registration for this vulnerable population

- Radioactivity studies that are being conducted in adults may also be conducted in children:
  - Microdosing can aid in establishing better first therapeutic dosing in children
  - Microtracers combined with cold therapeutic dosing enable
    - Derive absolute bioavailability in children
    - Derive routes of excretion and mass balance
    - Derive metabolite profiles
General Clinical Pharmacology Considerations for Neonatal Studies for Drugs and Biological Products Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within ___ days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm 1001, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document, contact CDER at CDER_CPT@fda.hhs.gov and CBER, Office of Communications, Outreach, and Development at (240) 402-8010

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

July 2019
Clinical Pharmacology
3. **Metabolism**

Drug metabolism commonly occurs in the liver, but may also occur in the blood, gastrointestinal tract, kidney, lung, and skin. Information on the metabolism of specific drugs in neonates is generally limited. Each metabolic pathway has unique ontogenic characteristics that should be considered when designing clinical pharmacology studies in neonates. In addition, some metabolizing enzymes may have higher expression and activity in neonates compared to older populations (e.g., CYP3A7 and CYP3A4), respectively.\(^9\,^{10}\)

Before conducting a clinical pharmacology study in neonates, consider the following:

---

**Contains Nonbinding Recommendations**

*Draft — Not for Implementation*

- To plan neonatal pharmacokinetic studies, a thorough review of the scientific literature should be conducted to obtain information about the metabolic pathways for the specific drug.

- As the postnatal ontogeny of many of these metabolic pathways has not been fully elucidated, it may be necessary to perform additional in vitro or preclinical studies.

- When appropriate, microdosing studies in neonates may be conducted to assess for potential ontogenic differences in the metabolic pathway compared to older populations.\(^11\,^{12}\)
THANK YOU FOR YOUR ATTENTION

Take a look: TIME.TNO.NL

TNO
Wouter Vaes
Marta Pelay
Dimitri Grossouw
Hugo Sandman
Arjan de Vries
Freek Schrander
Rianne de Ligt
Rene Braakman
Daphne de Ruijter
Sabine Bos
Rafael Ochsendorf
Steven Erpelinck
Elwin Verheij
Olaia Alvarez Bermudez
Lotte van Andel

Erasmus Medical Centre/
Bianca van Groen
Miriam Mooij
Dick Tibboel

Radboud University
Saskia de Wildt

esther.vanduijn@tno.nl