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Drug Metabolite(s) Safety Testing

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Disclaimer: Views expressed here are those of the author and not the FDA.

Metabolite Safety Testing

Objectives of this webinar

- Describe what a disproportional or unique human metabolite is in safety testing
- Determine the value of safety testing of metabolites
- Identify when it's necessary to characterize metabolites
- Define the type of metabolite safety studies that may be needed
- Define the exceptions to the rules

Regulatory Guidance Background

- Evolution of regulatory guidelines:
  - Historical approach--case by case evaluation
  - DruSafe subcommittee meeting (2000)
  - PhRMA MIST position paper (Baillie et al, 2002)
  - FDA Guidance for Industry: Safety Testing of Drug Metabolites (2008)
  - ICH-M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (2010, supersedes 2008 FDA guidance)
  - ICH-M3 (R2) Q&A section on metabolites (2013)

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

Disproportional and Unique Metabolites

Definition

- Disproportional human metabolite: Exposure substantially (≥2x) higher in humans than in animals

- Unique human metabolite: Only observed in humans

Why do safety testing of the metabolites

- Historically, toxicological and toxicokinetic evaluations have focused on active pharmaceutical ingredient (API) due in part to insensitive analytical methods and contribution of metabolites to overall drug toxicity
- Metabolites across species are often similar and require no independent toxicity testing (reason not to do testing)
- High doses in animals generally cover exposure levels to disproportional human metabolites (reason not to do further testing)
- Pre-Guidance Metabolite Safety Testing – case by case

Why do safety testing of the metabolites

- Parent and metabolites contribute to target organ toxicity
- Differences in the metabolite exposure can result in different toxicity profile.
- Delayed characterization of disproportional human metabolite may increase safety risk and delay drug registration
- New technologies (HPLC/MS/MS) permit early and accurate characterization of metabolites
- Differences in drug metabolizing enzymes across species

Why do safety testing of the metabolites

Species Differences in CYP450 enzymes

P450	Human	Mouse	Rat	Dog	Monkey
CYP1A2	X	X	X	X	X
CYP2B6	X				
CYP2C9	X (12%)*	X			
CYP2C19	X				
CYP2D6	X (31%)*				
CYP2E1	X	X	X	X	X
CYP3A4	X (50%)*				

* Percentage of drugs metabolized by each CYP enzyme in humans

Martigoni, 2006
Guengerich, 1997

Why do safety testing of the metabolites

- Phase I human metabolites are more likely to pose a safety risk than phase II metabolites (i.e. glucuronide, glutathione conjugates)
- Pharmacologically active metabolite may contribute to overall drug pharmacology and toxicity
- Pharmacologically inactive metabolites are not devoid of toxicity thus require toxicological assessment

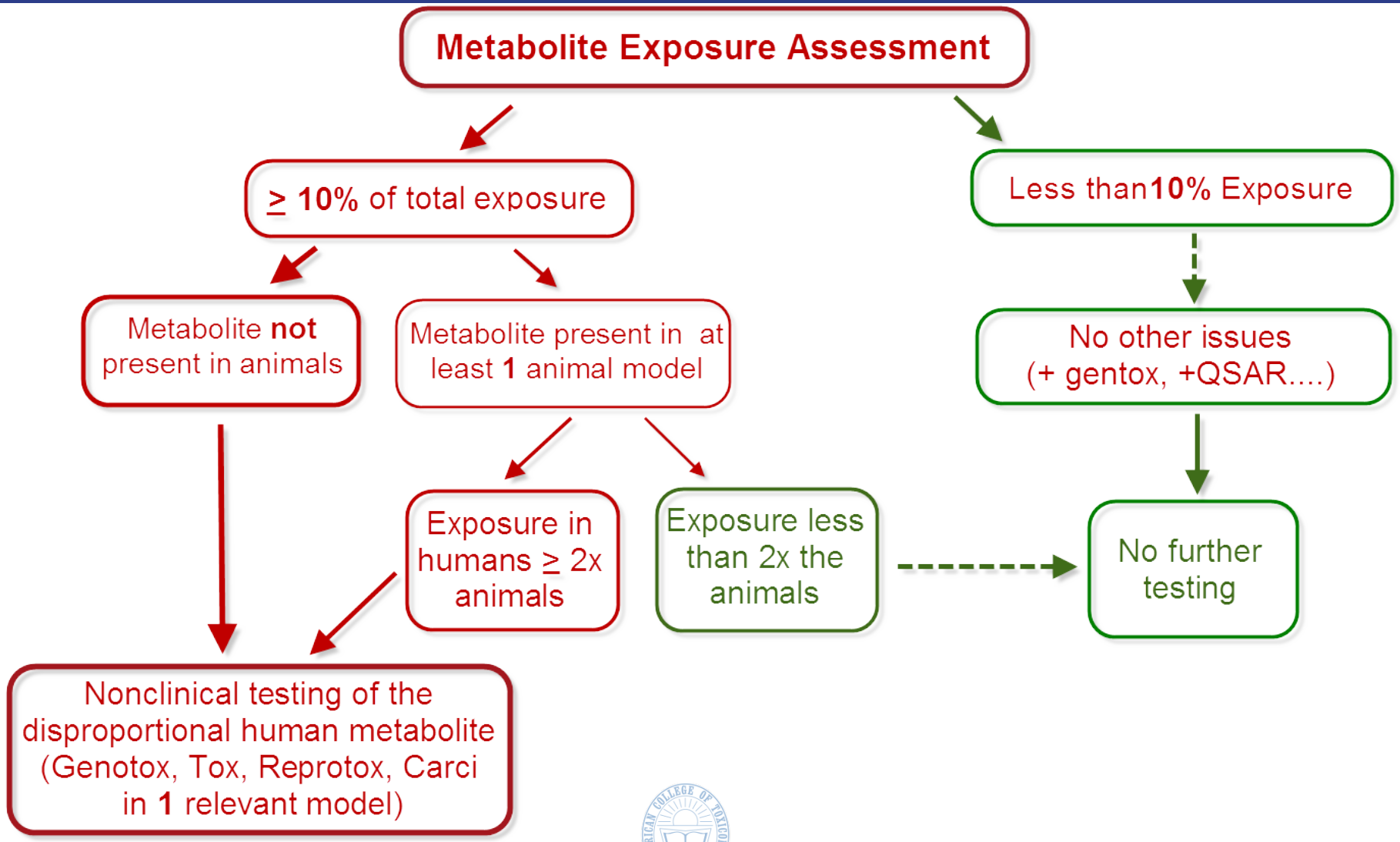
Since 2010 ICH M3(R2) guidance, human metabolites are being dealt with early in the drug development.

Timing of the Drug Metabolite Characterization

Characterizing Metabolites--Timing

- In vitro profiling in test animals and humans before first dose in human study
- In vitro studies may not concur with in vivo metabolite profile
- In vivo metabolite characterization in humans prior to large scale clinical trials
- Compare in vivo metabolite profiles across species
- Steady state metabolite exposure is required when nonclinical data suggest metabolite/drug accumulation
- Single dose radiolabeled study may be used to extrapolate to steady state exposure

Human Metabolite Decision Chart



Metabolite exposure greater than 10% of total drug exposure will need further consideration:

- ❑ Disproportional human metabolite exposure is less than 2x the animal exposure
- ❑ Disproportional human metabolite exposure is ≥2 x animal exposure
- ❑ Metabolite exposure only seen in humans

Disproportional Human Metabolite:

Factors to consider:

- Any known toxicity risk
- Potential for genotoxicity
- Extent of human exposure compared to test species
- Does the metabolite accumulate with repeated dosing?
- Reliability and validation of the analytical assay
- Synthesis of the metabolite
- Alternate animal model
- Background knowledge of the drug class

Unique Human Metabolites

Factors to consider:

- Extent of human exposure
- Single dose vs. multiple dose exposure
- Knowledge of the metabolic pathway
- Biliary exposure in animals
- Alternate animal model
- Any new adverse clinical signal
- Genotoxicity potential

Unique Animal Metabolites

Occasionally animals may form a metabolite(s) not detected in humans that may cause toxicity in animals, not relevant to humans.

Consider

- Number of species the metabolite is formed
- Alternate animal model
- Identification of the metabolic pathway in animals is necessary to exclude toxicity signal relevant to humans

Points to consider before testing

- Metabolite is pharmacologically active or inactive
- Metabolite is unique or disproportional
- In vitro verses in vivo metabolite profile
- For drug doses <10 mg/day, greater fraction of the drug related material might be more appropriate trigger for testing
- Is metabolite a phase I or phase II product
- Phase II products-generally less toxic, more water soluble (glucuronide and gluthathione, metabolites with hydroxyl group) vs. more toxic reactive acyl glucuronide metabolites

Nonclinical Studies to Assess the Safety of Disproportional / Unique Human Metabolites

Nonclinical Studies with Human Metabolite

- Safety pharmacology studies are not needed unless a new signal identified in humans, not observed in animals
- Genotoxicity-
  - ✓ In silico QSAR (Quantitative Structural-Activity Relationship) assessment
  - ✓ In vivo micronucleus or relevant genotox test

- General toxicity study in single relevant species (4 to 13 weeks)
- Embryofetal development study in single relevant species (rat or rabbit)

Nonclinical Studies with the Human Metabolite

- Carcinogenicity study in mouse or rat
  - ✓ Metabolite may be added to parent drug
  - ✓ Metabolite exposure in carcinogenicity study should provide at least 50% of the exposure seen in humans
  - ✓ Use of transgenic animal model may be acceptable for carcinogenicity assessment of human metabolite

Any approach taken should always be based on sound scientific principals and weight of evidence

Possible Exceptions to the Rules

- Some safety testing of metabolites for drugs for life threatening diseases may be waived
- The 10% rule may not apply for drugs that are extensively metabolized with minimal parent drug exposure
- The 10% rule may not apply for metabolites with genotoxicity potential
- Metabolites of the phase II metabolism may exceed the 10% rule (i.e. glucuronide metabolites)
- Previous experience and supportive data for some phase I metabolites may not require rigorous testing (case by case basis)

Possible Exceptions to the Rules

- Embryofetal development study for disproportional metabolite may not be necessary for teratogenic parent drug
- Justified scientifically supported data may be used to wave/reduce metabolite safety testing (i.e. similar to parent drug, low overall exposure, metabolites from similar drug class)

Metabolite Case Examples

A Case Example: Phase II Human Metabolite

- Early in vitro metabolism profile found metabolism among species to be qualitatively and quantitatively similar
- In vivo studies found two disproportionally high glucuronide metabolites in humans with minimal or no exposure in animals.
- In vivo human metabolites were identified as o-glucuronide metabolites, rapidly filtered by the kidney.

Action:

- No further nonclinical assessment was deemed necessary

A Case Example: Phase I and II metabolites

- 2 Disproportional (Phase I and II) human metabolites identified
- Phase I accounted for more than ½ of total drug exposure in plasma
- Phase II metabolite was not considered a safety risk

Action:

- Clinical study was terminated until clarification of the metabolite safety
- Nonclinical assessment of the Phase I metabolite was initiated (genotoxicity, standard toxicology, embryofetal development study..)

A Case Example: Inactive Human Metabolites

- In vitro: 2 primary hydroxylated (M1, M2) and 2 secondary oxidative metabolite (M3, M4) in humans, monkeys, dogs, rats and mice
- In vivo: no notable M4 exposure in rats or mice
- In vivo: monkey M4 exposure 1/3 of parent with new toxicity signal (~60% of the human exposure)
- In vivo: human M4 exposure 4x the parent drug

Action:

- In vitro genotoxicity with M4 (M4 was genotoxic)
- Toxicology studies in rats with M4
- Embryofetal development study in rat with M4
- Carcinogenicity assessment of the M4

Summary

- The unique or disproportional human metabolites are relatively rare.
- In vitro profiling of drug metabolites should be conducted before first dose in humans
- In vivo profiling of metabolites should be resolved before large scale clinical trials.
- Safety testing of drugs with unique/disproportionally higher human metabolites exceeding the 10% the total drug-related product exposure need to be addressed.
- Overall, early human metabolite characterization can lead to quicker resolution of metabolite safety issues.

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