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Meeting Report

Meeting Report: Metabolites in Safety Testing (MIST) Symposium—Safety Assessment of Human Metabolites: What's REALLY Necessary to Ascertain Exposure Coverage in Safety Tests?

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Abstract. In the 2012 AAPS metabolites in safety testing (MIST) symposium held in Chicago, IL, USA, on October 15, 2012, regulatory experts and industrial scientists joined together to discuss their perspectives and strategies in addressing contemporary MIST recommendations (FDA 2008, International Conference on Harmonization (ICH) M3(R2), ICH M(R2) Q&A). Overall, these regulatory guidances indicate that metabolites identified in human plasma should circulate at similar or greater concentrations in at least one of the animal species used in nonclinical safety assessment of the parent drug. However, synthetic standards for the metabolites often do not exist or they are intractable to synthesize, thus introducing multiple challenges in drug development for the quantitative comparison of metabolites between human and animals. A tiered bioanalytical strategy for metabolite analysis is a prevalent approach to demonstrate coverage in animals. Recent developments in bioanalytical methodology have yielded several time- and resource-sparing strategies to provide fit-for-purpose approaches that can enable critical decisions related to metabolite quantification and monitoring in plasma. This report summarizes the presentations and panel discussions at the symposium.

KEY WORDS: MIST; safety assessment of human metabolites; metabolite exposure coverage in safety test; ICH M3(R2); LC/MS/MS.

INTRODUCTION AND OBJECTIVES

From 2002 to 2010, considerable discussion has centered around the importance of drug metabolites as potential contributors to drug safety. In 2002, scientists from member organizations of the Pharmaceutical Research and Manufacturers of America issued a position paper proposing instances when metabolites of new chemical entities observed in humans merited a closer look in laboratory animals in nonclinical safety assessment (1). This was followed by other published positions and proposals (2,3), US FDA draft guidance in 2005, final guidance from FDA in 2008, and ultimately culminated with recommendations from the International Conference on Harmonization (ICH) in 2010. The guidance documents laid out criteria describing when a human metabolite needs to be measured in laboratory animal species and defined the circumstances under which direct testing of a metabolite in animal toxicology studies would be needed to provide a risk assessment.

After consensus was reached regarding the 10% of total drug-related material threshold over which metabolites might be considered important, drug metabolism and bioanalysis specialists began to focus on how to measure metabolite exposures in laboratory animals and humans. Developing and validating standard bioanalytical methods for metabolites were feasible (4), although there can be challenges in obtaining authentic standards of metabolites which are difficult to synthesize and measure multiple analytes simultaneously. In response to the bioanalytical challenges posed by these expectations, investigators have proactively developed alternate approaches that can offer assurance of relative exposures to metabolites in animals and humans. These include the application of radiometrically calibrated metabolite standards (5–7) or creating metabolite standards from biological sources that are quantitated using nuclear magnetic resonance (NMR) spectroscopy (8,9). Finally, demonstration of relative exposures to metabolites across species can be accomplished using HPLC-mass spectrometer (MS) peak area comparisons from animal and human plasma extracts (10,11).

The 2012 AAPS symposium of "Safety Assessment of Human Metabolites: What's REALLY Necessary to Ascertain Exposure Coverage in Safety Tests?" held in Chicago, IL, USA on October 15, 2012 consisted of four oral presentations from invited speakers followed by a panel discussion. The speakers were Abigail Jacobs from Office of New Drugs at the US FDA;

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Ronald White from XenoBiotic Laboratories, Inc.; Brian Booth from the Office of Clinical Pharmacology, US FDA; and Hongying Gao from Pfizer, Inc. The symposium brought together regulatory, industrial, academic scientists from multiple disciplines (*e.g.*, toxicology, clinical pharmacology, drug metabolism, bioanalytical sciences, *etc.*) in order to: (1) understand current regulatory expectations for the safety assessment of human metabolites, (2) discuss evolving, fit-for-purpose bioanalytical approaches and methodologies to meet the MIST guidance recommendations, and (3) seek feedback from the scientific community and to identify areas for continuous improvement.

HIGHLIGHTS OF THE PROCEEDINGS

ICH M3(R2) and Metabolite Issues

Abigail Jacobs, CDER/FDA

Sometimes, a metabolite that's "major" in human maybe "minor" in animal test species. Such metabolites could be at greater risk of being disproportionate metabolites. But nonclinical studies in animals are often conducted at a dose level that is far greater (in milligrams per kilogram) than human when normalized by weight; these major human metabolites(s) may still have been acceptably evaluated in the animals with dosing of the parent drug, and no further toxicology studies on the metabolites are warranted.

Before clinical trials, *in vitro* metabolic and plasma protein binding data for animals and humans and preclinical systemic exposure data are usually available. Subsequently, before large numbers of persons are exposed to an investigational drug, nonclinical absorption, distribution, metabolism, and excretion results and *in vitro* biochemical information relevant to potential drug interactions are submitted to the FDA per ICH M3(R2).

The revised International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidance M3(R2) contains a new section on potential metabolite issues. Per ICH M3(R2), evaluation of metabolites is warranted when the metabolites are observed at exposures >10% of total drug-related material exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Not all disproportionate metabolites are of concern, including most glutathione conjugates, most glucuronide metabolites, and metabolites with an additional hydroxyl group.

After ICH M3(R2) was finalized, 12 questions relating to metabolites were received. Thus, a question and answer section was prepared to clarify some issues (12). The questions included topics such as the definition and calculation method of the 10% threshold; how to characterize safety of metabolites; issues relating to characterizing metabolite exposure in a single exposure study; what metabolites are not of toxicologic concern; the need for safety pharmacology studies of metabolites; and issues of prodrugs. The salient points addressed by the questions and answers include:

- The "10% threshold" refers to when a human metabolite comprises greater than 10% of the measured total exposure to drug and metabolites, usually based on group mean area under the curve (*e.g.*, AUC 0–inf).
- The guidance states that the metabolite needs to be at "significantly" greater exposure in human to merit concern. The use of the term "significant" does not refer to statistical significance. Characterization of metabolite toxicity is generally considered adequate when animal exposure is at least 50% the exposure seen in humans at the "to be marketed" dose. However, when a metabolite comprises more than 50% of the total human exposure, it is appropriate for exposure to the metabolite in animals to exceed that in humans at the to be marketed dose.
- It is important to have adequate exposure to the metabolite in one species used in general toxicity evaluation, in one species used in a carcinogenicity study (when carcinogenicity evaluation is warranted or an *in vivo* micronucleus study when carcinogenicity evaluation is not warranted), and in one species used in an embryo–fetal development study.
- The evaluation can be based on single-dose radiolabeled ADME data in humans. However, if exposure data later collected from multiple-dose human studies indicate that steady-state levels of a metabolite exceed 10% of total drug-related material, then additional nonclinical evaluation of metabolite should be considered.
- Because parent drug and metabolites contribute to target organ toxicity in animals at the maximum tolerated dose (MTD), exposure comparisons across species should be conducted at the MTD in animal compared to the maximum exposure in humans at the therapeutic dose, assuming the toxicity of concern can be adequately monitored in humans and does not pose an unacceptable risk. However, if toxicity at the MTD cannot be monitored in humans or poses an unacceptable risk, then the exposure comparison should be conducted at the no adverse effect level for the toxicity of concern.
- Some metabolite types may not be of toxicological concern. With rare exceptions, most glutathione conjugates are formed by conjugation with reactive metabolites to form excretory metabolites that are not of toxicological concern. Most glucuronides are not of concern, except those that undergo chemical rearrangement (*e.g.*, reactive acyl glucuronides). It was noted that generally it is not feasible to test highly reactive metabolites independently because of their instability, but they are assumed to contribute to the overall nonclinical toxicity of the drug.
- Clinical studies assessing safety pharmacology endpoints are conducted during phase I and will have already been assessed in humans before a full characterization of the human metabolites is conducted. Therefore, nonclinical safety pharmacology studies are generally not warranted for the characterization of metabolites. However, if a safety pharmacology signal is seen in humans that was not predicted by nonclinical studies with the parent, then additional safety pharmacology studies of these human metabolites can be considered to better understand the mechanism.
- The guidance does not specifically address prodrugs. If the animal species converts the prodrug to the active metabolite similarly to humans, then a standard testing approach as recommended in ICH M3(R2) can be used. Further, if the active metabolite is not adequately

produced in the animal species, then the target molecule for toxicologic evaluation is the active metabolite and therefore additional testing beyond that recommended for metabolites can be appropriate.

Early Assessment of Mist Liability of a Clinical Drug Candidate Without the use of Radiolabel

Ronald E. White, XenoBiotic Laboratories, Inc.

A consequence of MIST is that analytical chemists have been challenged to find and quantitate human metabolites in plasma during early clinical trials without using a radiolabeled drug. This challenge has now been largely accomplished by applying modern, start-of-the-art mass spectrometers which have the ability to accurately determine molecular weights of ions with mass/charge measurement error <5 ppm. When combined with sophisticated data processing algorithms such as mass defect filtering, isotope pattern filtering, and background subtraction, these methods allow us to discern drug metabolites among the many background compounds present in biofluids. The currently available techniques may be integrated to reliably find and structurally identify the metabolites from the plasma and urine samples that are already collected in typical phase I clinical trials. And semiquantitation of metabolites using liquid chromatography (LC)-UV, LC/MS/MS peak area ratio comparison (10,11,13), radiolabeled calibrant (5–7), and quantitative NMR standards (8,9,14) can be employed to compare the exposures to the metabolites in animals to humans. This allows a sponsor to comply with regulatory expectations for metabolite safety assessment without the need to wait for the conventional ^{14}C-ADME studies that are usually conducted in phases II or III.

MIST: How Do We Deal with Surprises?

Brian Booth, FDA\CDER\OTS\OCP\DCP V

When novel metabolites are discovered in humans to be at significantly higher concentrations than were observed in nonclinical studies, we are faced with several safety-related questions. Is the metabolite active (*e.g.*, SN-38 for irinotecan)? Is there sufficient nonclinical coverage to assure safety? Will we need to monitor this metabolite? If so, how and when will this issue be addressed? These data from humans are used to develop the exposure–response relationships that support approval and guide dose adjustments in different patient settings. However, analytical methods take time to develop and validate, and there is considerable anxiety over when these methodologies are needed. In this case, a fit-for-purpose approach should be used. Frequently, initial phase 1 data are not used to determine the approvability or the labeling for a novel product. Often, the pharmacokinetic behavior of the drug and any metabolites is characterized throughout drug development. This fact allows time for a method for an unexpected metabolite to be validated such that the metabolite can be characterized in later trials. The general rule that can be applied is that when the data are needed for approval and labeling, the assay needs to be fully validated (15). This rule allows flexibility in the time frame for generating the data.

A Simple LC/MS/MS Method for Evaluating Mist Coverage

Hongying Gao, PDM, Pfizer, Inc.

A simple HPLC-MS/MS method was presented whereby quantitative comparisons of exposures to metabolites between animal and human can be obtained in the absence of authentic standards of the metabolites, calibration curves, and other attributes of standard bioanalytical methods (10,13). A statistical analysis showed that if the experimentally determined, animal-to-human MS response ratio is $\geq$2.0 then the actual exposure ratio is unity or greater ($p<0.01$). The confidence level in such a ratio increases exponentially with the measured animal-to-human MS response ratio. This method offers time- and resource-sparing advantages to ascertaining metabolite exposure comparisons between humans and laboratory animal species. It is important to note that pharmacologically active metabolites offer an exception to this approach, as quantitative exposure data for such metabolites are critical to establishing pharmacokinetic/pharmacodynamic relationships. Pharmacologically active metabolites require standard bioanalytical methods using authentic standards and should be included in the bioanalytical method as soon as possible in the drug development timeline. Most metabolites are non-active metabolites, and often, a simple LC/MS/MS measurement is sufficient to demonstrate that an animal toxicology study with the parent drug has covered the safety of the human metabolites, since animals are dosed at a higher level when corrected by body weight. This data-driven bioanalysis strategy would increase the rigor of the bioanalysis accordingly based on the results of the animal-to-human MS response ratio measurements rather than the stage of the drug in the development. Only in rare cases where an actual exposure measurement of a metabolite is needed, then a validated or qualified method using synthetic standard will be needed (16).

PANEL DISCUSSIONS: ALL SPEAKERS

Q: In practice, how could we obtain metabolite data for the day 1 samples in order to establish the stability of the metabolites?

A: Day 1 samples typically in practice are not available, but samples can be available from a date close to day 1 (*e.g.*, day 3). These samples can be used as the baseline readout for the stability test. The most important indicator of stability of a metabolite during storage is the trend of the response of metabolite *vs.* a stability reference normalized by the ratio at the earliest day along the storage time.

Q: If an acyl glucuronide conjugate was identified later in the evaluation, how does one deal with this situation?

A: If the samples were not treated during the sample collection, and an acyl glucuronide conjugate metabolite was found in the samples later, the samples could still be used to evaluate the MIST coverage under the circumstance

described here. If the animal samples have been stored longer than the human samples and the animal *vs.* human ratio was still more than 2, then it is safe to conclude that the animals had more exposure to the acyl glucuronide conjugate than humans. The parent drug, however, may have false coverage in the animal sample since the acyl glucuronide conjugate undergoes conversion during storage; the impact of the conversion can be checked by the Incurred Sample Reanalysis for the drug. Alternately, if the acyl glucuronide had been previously detected in *in vitro* studies, then it may be prudent to treat subsequently collected plasma samples with citric acid during sample collection. Thus, potential acyl glucuronide metabolites will be stabilized.

Q: If the exposures to the metabolites in the animals only need to be 50% of exposures in human, would the LC/MS/MS measurement ratio only need to be 1?

A: Yes. The presentation described how an animal/human ratio of 2 measured using the simple LC/MS/MS methodology is a conservative cutoff to ensure that the animal exposure (AUC) to the metabolites is at least equivalent to that in humans. For metabolites that comprise close to 10% of total drug-related materials, the animal/human ratio measured using the LC/MS/MS methodology needs to be ≥ 1 to assure that the animal exposure to the metabolite has $\geq 50\%$ exposure that of humans.

CONCLUSIONS

Recent regulatory guidance states that metabolites identified in human plasma should be present at equal or greater levels in at least one of the animal species used in safety assessments. This requirement can lead to significant bioanalytical issues, because pure metabolite standards can frequently be challenging to obtain, especially in early phases of drug development. Addressing this need has necessitated some novel scientific strategies. The recent advances in mass spectrometer technology have enabled reliable detection of human metabolites in complex biofluids without requiring radiolabeled drug. Various bioanalytical approaches have been employed in industry to evaluate the exposure of metabolites in animal *vs.* human and can be used in a fit-for-purpose fashion through the drug development timeline. Pharmacologically active metabolites require standard bioanalytical methods using authentic standards, the synthetic standards should be generated, and analysis should be included with the parent drug as early as possible in drug development. For most metabolites, the simple LC/MS/MS peak area ratio comparison is an innovative fit-for-purpose method to demonstrate MIST coverage without synthetic standards or radiolabeled drug, enabling early assessment of human metabolites (*e.g.*, FIH, phase 1). A follow-up discussion on industry practice using innovative bioanalytical approaches to evaluate MIST may be considered later.

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