INTRODUCTION

Harmful, unintended reactions to medicines that occur at doses normally used for treatment are called adverse drug reactions (ADRs). ADRs are among the leading causes of death in many countries. Drug manufacturers are required to take action to prevent and detect adverse effects from medicines. ADRs found in clinical trials or post-marketing surveillance occasionally result from metabolites or the process of metabolism. To reduce the safety risk for subjects in clinical trials, the appropriate assessment of safety and pharmacokinetics of metabolites in humans at the early discovery stage is important.

In Japan, the necessity for studies of toxicity and pharmacological effects of all major metabolites was outlined in the notification from the Ministry of Health and Welfare in 1975 (Yakushin No.526, 1975), but the evaluation items had been interpreted on a case-by-case basis because the notification indicated no specific study required for safety assessment (Naito et al., 2007). In 1991, at the first International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the Japanese authorities suggested that assessment of “Metabolites in Safety Perspectives on non-clinical safety evaluation of drug metabolites through the JSOT workshop

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(Received June 5, 2012)

ABSTRACT — The prompt and appropriate safety assessment of drug metabolite(s) was mentioned in regulatory guidances such as an International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance, entitled “Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals” (ICH M3(R2)) implemented in January 1 of 2011 in Japan, and has become a significant issue in the drug development. Upon release of ICH M3(R2) Step 4, a survey was conducted between March and April 2010 on the safety assessment of drug metabolites in 63 member companies of the Japan Pharmaceutical Manufacturers Association (JPMA). The Pharmacokinetics Team in the Non-Clinical Evaluation Expert Committee in JPMA conducted a questionnaire survey and compiled the results to comprehend how safety of drug metabolites are currently assessed at research-based pharmaceutical companies in Japan. The assessment of “Metabolites in Safety Testing” (MIST) can be divided into three stages based on the research purpose as follows: MIST 1 is a stage of estimating human drug metabolites and predicting their potential risks, MIST 2 is a stage of deciding the necessity for non-clinical safety studies, and MIST 3 is a stage of conducting non-clinical safety studies. In this paper, we propose typical approaches on safety assessment of metabolites that meet the purpose of each stage, considering the current level of scientific technology. Our proposals are based on the results from our survey and a symposium about the safety assessment of drug metabolites at the 37th annual meeting of the Japanese Society of Toxicology held in June 2010.

Key words: Metabolites, Safety assessment, JPMA, PMDA, MIST, ICH M3(R2)
Testing” (MIST) was necessary in the following three instances because assessing all major metabolites was unfeasible: when metabolites not found in animal species used in toxicity studies are present in humans (unique human metabolites), when metabolites are formed in particularly large amounts in humans (disproportionate drug metabolites), and when the pharmacological and toxicological activity of metabolites is considered significant (Ohno, 1992). However, this guidance has been interpreted on a case-by-case basis by the tripartite authorities (US, EU, and Japan) regarding the need for optional safety assessments because appropriate abundance thresholds for the metabolite assessments have not been defined clearly. In February 2008, the Food and Drug Administration (FDA) established the Safety Testing of Drug Metabolites guidance (commonly known as the FDA MIST guidance), following the commentary published by the Pharmaceutical Research and Manufactures of America (PhRMA) (Baillie et al., 2002) and some scientific debates in the literature (Baillie et al., 2003; Hastings et al., 2003), which mentioned the abundance threshold for metabolite assessments for the first time. Subsequently, Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH M3(R2)) was established based on the agreement at ICH and proceeded to Step 4 in June 2009. ICH M3(R2) suggests a new general concept based on the consensus reached by the tripartite authorities for the safety assessment of drug metabolites. ICH M3(R2) states that non-clinical safety assessments of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies, unless the metabolite(s) is a unique human metabolite or reactive metabolite that can raise safety concern. If such metabolite(s) is detected, the non-clinical assessments should be conducted to support Phase III clinical trials. For drugs for which the daily administered dose is $< 10 \text{ mg}$, greater fractions of the drug related material might be more appropriate triggers for the safety assessments.

In Japan, ICH M3(R2) Step 5 has been implemented to apply to new drug applications since January 2011. However, the necessity for safety assessments of metabolites is still evaluated based on consultation with the regulatory authority and with reference to the MIST guidance and precedents because details of safety assessments of metabolites are not even described in ICH M3(R2). As for evaluation of the metabolite exposure, which is mentioned in ICH M3(R2), the debate has been actively pursued to establish the appropriate strategy and method that meet the current level of scientific technology. The safety assessment for metabolites mentioned in ICH M3(R2) is currently under review in the Q&A document (ICH M3(R2) Guideline Questions & Answers, June, 2011), but no specific evaluation method is mentioned.

Given such situations, more efforts are required to the pharmaceutical companies and administrative authorities to promptly introduce effective drugs to the clinical setting while reconciling clinical safety. In this review, we propose scientific approaches on safety assessments of metabolites based on the results of our survey on the members of the Japanese Pharmaceutical Manufacturers Association (JPMA) (Minagawa et al., 2010; Furuta et al., 2011) and the discussions at the 37th annual meeting of the Japanese Society of Toxicology (Kawashima, 2010; Nakano, 2010).

**Strategies for the safety assessment of drug metabolites in each developmental stage**

The procedure for the safety assessment of metabolites in the drug development can be divided into three stages based on the research purpose: a stage of estimating human drug metabolites based on the drug characteristics and known information and predicting their potential risks (MIST 1), a stage of deciding the necessity for non-clinical safety studies of drug metabolites found in clinical trials (MIST 2), and a stage of conducting non-clinical safety studies (MIST 3). The flow line related to the safety assessment of drug metabolites is summarized in Fig. 1, showing the typical study contents assumed in each stage. The main study contents in each evaluation stage are detailed in the following sections in a feasible way that the exposure and safety of drug metabolites can be appropriately and efficiently evaluated.

**MIST 1**

At MIST 1, the first stage of drug development, human clinical trials have not been initiated, and the drug manufacturers estimate human metabolites and predict their potential risks with non-clinical studies and *in vitro* studies using human specimens.

ICH M3(R2) suggests that the non-clinical safety assessment of relevant metabolite(s) should be warrant-ed before conducting clinical trials with large numbers of human subjects or with long-term administration of a drug (generally before Phase III). In fact, if serious concerns of metabolites arise in non-clinical safety studies conducted in the late developmental stage, they will severely impact the process of large-scale clinical trials and new drug applications. Therefore, the potential risk evaluation of metabo-
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Fig. 1. Holistic Approaches for DMPK and Safety Studies of Drug Metabolites-MIST Stages 1 to 3. DDI, drug-drug interaction; AM, animal metabolite; HM, human metabolite; BA, bioanalysis; STD, standard; Solid line: an item that is needed in the representative development strategy; Dashed line: an item that is conducted depending on the specific situation of each development strategy.

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ed until a proof of concept is established. Generally, the genotoxic evaluations should be completed before largescale clinical trials. However, if the safety of metabolites is warranted by genotoxic evaluations using a parent drug in which adequate amount of predicted human metabolites are generated, no further evaluation using metabolites is recommended.

According to the FDA MIST guidance and ICH M3(R2) Q&A, adequate systemic exposure of the metabolites, for which characterization is warranted, should be achieved in at least one animal species in each study for general toxicology, embryo-fetal development toxicity, and carcinogenicity (or in vivo micronucleus test without carcinogenicity studies). At MIST 1, finding at least one species that can be used to evaluate qualitative and quantitative metabolic profiles in humans is useful for efficient safety assessments on further development.

Because quantitative evaluation of metabolite exposure is required for its safety assessments in accordance with ICH M3(R2), establishing adequate analytical methods is practical to promptly determine human major metabolites after collecting clinical samples.

**MIST 2**

At this stage, qualitative and quantitative comparison is feasible between in vivo metabolic profiles in animal species and those in humans through the analysis of human samples after the administration of a drug in Phase I clinical trials. The objective of MIST 2 is to determine the necessity of conducting non-clinical safety assessments of metabolites.

ICH M3(R2) states that the non-clinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. ICH M3(R2) Q&A indicates that an exposure to a metabolite is usually presented as a group mean AUC and can be evaluated in the radio-labeled single dose studies or non-radio-labeled single or multiple dose studies, and that an attention should also be paid to metabolites that exceed 10% of drug-related exposure only after multiple dosing. In the survey (Furuta et al., 2011), although about 40% respondents (17/45 companies) answered that the samples for human metabolites identification were obtained in the steady states of multiple dose studies, about half the members (22/45 companies) responded that the samples were obtained after single administration of recommended clinical doses or of doses for which test drugs exhibited linear pharmacokinetics. We considered that metabolic profiles can be evaluated by the results of studies other than those with recommended clinical doses, if their rationales can be explained with scientific acceptability, e.g., in cases where a recommended clinical dose is anticipated to fall within the dose range in which dose proportionality is confirmed in Phase I clinical trials. The survey revealed that many companies used the samples obtained in overseas studies for metabolism analyses because conducting radio-labeled human studies is currently difficult in Japan. The clinical pharmacokinetic studies of radio-labeled drugs are highly useful, especially to assess presence or absence of human unique metabolites. Therefore, to accelerate drug development, improvement of environments is desirable so that such clinical studies can be conducted in Japan.

ICH M3(R2) states that the exposure of human metabolite(s) should be compared with that in animals in toxicity studies, if the metabolite(s) exhibit exposure greater than 10% of total drug-related exposure in humans. A question would inevitably arise as to which toxicity study is appropriate to establish the maximum exposure of relevant metabolites. ICH M3(R2) Q&A suggests that the exposure of relevant human metabolites should be compared with that in animals at the MTD or NOAEL in toxicity studies. ICH M3(R2) Q&A also states that if the toxicity observed at the MTD can be adequately monitored in humans and does not pose an unacceptable risk, the exposure to metabolites in humans should be compared at the MTD; if not, exposure comparisons should be conducted at the NOAEL for the toxicity concern.

Even if drugs are already approved, safety assessments including metabolites should be required when human safety cannot be warranted due to augmented exposure not only to the parent drug but also to metabolites by modification of dosage and route of administration. On the other hand, quantitative assessments of exposure to metabolites may not be necessary if a drug has local effects or a drug administered is excreted as an unchanged form and the quantity of metabolites does not exceed 10% of the dose administered.

At MIST 2, when the necessity for non-clinical safety assessments of metabolites is determined, only a few standard materials of metabolites are available, and analytical methods for metabolites may be rarely validated. Therefore, it is reasonable to add validation parameters in a stepwise fashion to bolster the robustness of analytical methods according to the development stage where exposure to metabolites is assessed. For example, it is generally acceptable to store samples temporarily under the condition in which the parent drug in analytical samples is stable because stability of all metabolites is hard-
ly ensured at initiation of sample storage. Additional data are required to support the stability of the metabolites of which safety is to be assessed at MIST 3. The exposure of stable metabolites in animals can be analyzed retrospectively by the samples obtained from toxicity studies to compare with those in humans. As for the unstable metabolites, such assessment should be conducted on a case-by-case basis. Results of the survey indicated that quantitative analyses using radio-labeled drugs, methods using accelerator mass spectrometry (AMS), methods utilizing peak response factors of mass spectrometry (MS) or nuclear magnetic resonance (NMR) (Yu et al., 2007), tiered validation methods (Leclercq et al., 2009), sample pooling methods (Cheung et al., 2005), and measurement levels by EBF (Timmerman, 2009) are gaining attention as alternatives to quantification using standard materials. Because data for verification of the scientific acceptability of an analytical method depends on its principles, sensitivity and reproducibility as well as on pharmacokinetic properties of the candidate drugs, further consideration would be needed.

**MIST 3**

At the MIST 3 stage, non-clinical safety assessments of metabolites are warranted and conducted before the start of Phase III clinical trials.

Although no details of the non-clinical safety assessment of metabolites have been provided in ICH M3(R2), appropriate safety studies should be conducted considering the possibility including some studies into general toxicity studies with reference to the FDA MIST guidance. Conducting toxicokinetic bioanalysis of metabolites is recommended under GLP conditions for non-clinical safety assessments with the standard materials and validated method (FDA CDER CVM, Guidance for Industry, Bioanalytical Method Validation, 2001).

As for the duration of safety evaluations of metabolites, ICH Q3A(R2) (MHLW, Impurities in New Drug Substances, 2002) and ICH Q3B(R2) (MHLW, Impurities in New Drug Substances, 2003) can be used as a reference. They indicate that the study duration to detect the toxicity of an impurity should be based on available relevant information, and the minimum duration of 14 days and the maximum duration of 90 days are generally considered appropriate. On the other hand, Olson et al. (2000) reported that in repeated-dose toxicity studies, 94% of the primary toxicity can be detected in the first 4 weeks. The results of the survey support this view with more responses for the 4-week study period than the 90-day or longer period (Furuta et al., 2011). Thus, the study duration to detect the toxicity of a metabolite should be appropriately considered on a case-by-case basis, depending on the objective.

According to ICH S7A (MHLW, Safety Pharmacology Studies for Human Pharmaceuticals, 2001), any parent drug and its metabolite(s) that achieve systemic exposure in humans should generally be evaluated in safety pharmacology studies. Because proarrhythmic risk is significant due to its impact to life (MHLW, The Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization [QT Interval Prolongation] by Human Pharmaceuticals [ICH S7B], 2009), many companies answered in the survey (Furuta et al., 2011) that they conduct telemetry analysis and/or human ether-a-go-go-related gene (hERG) inhibition assay at the MIST 3 stage. The FDA MIST guidance does not mention any safety pharmacology study requirements; however, according to ICH M3(R2) Q&A, safety pharmacology endpoints of the parent drug and metabolites are comprehensively evaluated in Phase I trials. Therefore, safety pharmacology studies of metabolites are considered to be important only if a safety pharmacology signal is seen in humans, which was not predicted in non-clinical studies with the parent drug.

According to the results of the survey (Furuta et al., 2011), many companies generally conduct ICH III studies (MHLW, Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (ICH S5(R2), 2000) as reproductive toxicity studies for metabolites in accordance with the MIST guidance. Carcinogenicity studies of metabolites would be considered only if carcinogenicity is concerned based on the results of histopathological examination in general toxicity studies in addition to those of genotoxicity studies and *in silico*-based structural analyses.

**CONCLUSION**

The safety assessment of drug metabolites can be divided into three evaluation stages (MIST 1 to 3) based on the purpose: a stage of estimating human drug metabolites and predicting their potential risks (MIST 1), a stage of deciding the necessity for non-clinical safety studies of drug metabolites (MIST 2), and a stage of conducting non-clinical safety studies of drug metabolites (MIST 3). At MIST 1, major examples of studies conducted are estimation of the metabolite profile in humans by *in vitro* studies including human unique metabolites, estimation of metabolites that should be concerned such as reactive metabolites, analyses about the structural alert by the *in silico* software, determination of animal species used in non-clinical safety studies, and development of analytical methods of metabolite exposures. At MIST 2, the expo-
sures of human metabolites should be compared with the maximum exposure in non-clinical safety studies if the metabolites are observed at exposures greater than 10% of total drug-related exposure in the single or multiple dose clinical trials. Based on the result, whether implementation of non-clinical safety studies of the metabolites is necessary should be decided. At MIST 3, safety studies of metabolites such as genotoxicity, embryo-fetal development toxicity, and carcinogenicity should be appropriately conducted considering the results of general toxicity studies in which such safety issues have been partly evaluated. In the process of MIST 1 to 3, it is crucial to appropriately prepare assay methods and reference standard materials of metabolites, based on the purpose of the evaluation on each stage. Therefore, the appropriate implementation of the assessment in each stage can lead to efficient safety assessments of metabolites.

Currently, no guidelines in Japan including ICH M3(R2) clarify the details about non-clinical toxicity studies of drug metabolites. Although the FDA MIST guidance is useful for safety assessment of drug metabolites, implementation of safety assessment of drug metabolites should be sufficiently discussed with the regulatory agency because a case-by-case approach is generally called for each developmental drug.

ACKNOWLEDGMENTS

The authors thank member companies of JPMA for courtesy in responding to the questionnaires. Support by the Pharmacokinetics Team in the Non-Clinical Evaluation Expert Committee in JPMA are also gratefully acknowledged.

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