American College of Clinical Pharmacology Position Statement on the Use of Microdosing in the Drug Development Process

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In 2004, the US Food and Drug Administration (FDA) introduced its "Critical Path" document[1] highlighting the serious discordance between major advances in science and technology and limited or stagnated new drug development. Despite advances in many scientific disciplines pertaining to drug discovery and development, the registration of new chemical entities (NCEs) has declined. "The medical product development process is no longer able to keep pace with scientific innovation. Only a concerted effort to apply the new biomedical science to medical product development will succeed in modernizing the critical path."[1] This "Critical Path" document and a subsequent exploratory investigational new drug (IND) guidance[1,2] challenge this discordance and offer examples of newer methodologies that might be incorporated early in the drug development process to increase the efficiency of bringing a new therapeutic agent to the bedside. In addition, other regulatory agencies such as the European Medicines Agency have also supported the use of new methodologies in drug development.[3] One such methodology addressed in both documents is microdosing.

The concept behind microdosing is to use extremely low, nonpharmacologically active doses of a drug to define the agent's pharmacokinetic profile in humans. Definitions of microdosing do not differ between European[3] and US[2] regulatory agencies. The European Medicines Agency position paper defines microdose as "less that 1/100th of the dose calculated to yield a pharmacological effect of the test substance to a maximum dose of < 100 micrograms."[3] The US FDA agrees with this definition for drugs.[2] For protein products, the FDA suggests a maximum microdose of <30 nanomoles.[2] The main advantage in drug development is that smaller preclinical safety studies are needed to support a microdosing study in humans compared to the traditional first-in-human study. Relatively few microdosing studies involving only a small number of subjects have been published in peer-reviewed journals.[4-13] It should be emphasized that these published microdosing studies probably represent only a small fraction of all microdosing studies performed because studies done in early drug development (ie, "phase 0" studies) are often not published in peer-reviewed journals.

This position statement was developed to evaluate the available published data on microdosing and to provide recommendations regarding the application of this strategy. The document is not meant to be an exhaustive review of the foundation for this technology; rather, the focus is on its applications

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to the streamlining of new drug development in humans.

MICRODOSING METHODOLOGIES

Any bioanalytical method with sufficient sensitivity can be used to support microdosing studies. Accelerator mass spectrometry (AMS), positron emission tomography (PET), and other highly sensitive analytical techniques, including liquid chromatography with tandem mass spectrometry (LC/MS/MS), are analytical methods that have been used. The application of PET in the context of describing a new drug's pharmacokinetic (PK) profile will not be addressed as some important limitations restrict its use as a microdosing strategy that do not exist for AMS or LC/MS/MS methodologies.[3]

In a typical microdosing study, the drug is given to a small number of human subjects by either the oral or intravenous route of administration.[3,11] Blood and possibly other biologic fluids are collected over several expected drug half-lives. If AMS is used, the drug must be labeled with ^{14}C.

Proponents of microdosing note the following advantages of the technique.[7,8]

1. Only gram quantities of the drug are required for safety testing.
2. A much smaller toxicology package is needed.
3. Pharmacokinetic data for initial dose selection can be generated in 4 to 6 months from obtaining preliminary toxicology data in animals.
4. The cost of a microdosing study is a fraction of a full first-in-human study that typically evaluates a full range of doses. (However, the cost of a microdosing program has not been elucidated in the literature.)

In support of the microdosing concept for first-in-human data, the use of AMS has been compared to liquid scintillation counting of biologic samples[9-11] and, in unlabeled drug microdosing, compared to larger doses for LC/MS/MS.[14] In general, these studies incorporate very small numbers of animals and human subjects to determine drug disposition profiles.[9-13,15-21] This initial experience begs the following question: do these studies using very small numbers of animals or human subjects result in validation of the concept and useable data? Furthermore, can microdosing accurately predict key pharmacokinetic parameter estimates (eg, bioavailability, clearance, elimination rate) observed at much higher therapeutic doses of a drug? Most important, can microdosing predict drug metabolism rates for compounds that are metabolized by polymorphically expressed enzymes and transporters as well as a situation where a compound's oral absorption and/or clearance mechanisms involve saturable pathways? Recognizing that some of these variables may be of greater interest to scientists and clinicians versus drug development investigators in their initial screening process, it would appear that these variables could have varying influences on microdosing-based predictions.

ABILITY TO PREDICT HUMAN PHARMACOKINETICS AT THERAPEUTIC DOSAGES USING MICRODOSING STRATEGIES

A recent study compared nelfinavir microdosed as ^{14}C-labeled intravenous drug (and analyzed using AMS) with therapeutic doses of unlabeled oral drug (analyzed by high-performance liquid chromatography–ultraviolet [HPLC-UV]) in 6 healthy volunteers. The 2 arms of the study gave divergent pharmacokinetic results from day 1 to steady state at day 11.[19] Area under the curve over the dosing interval (AUC) increased from first dose to steady state for the microdosing regimen by 23.7% but decreased in the therapeutic dose regimen by 9.5%, a total difference of nearly 35%. The argument can be made that the small number of subjects contributed to the differences observed, but for drugs with a narrow therapeutic index, could this difference lead to problematic dosing in true phase I PK studies with single and multiple ascending doses? In contrast, Bauer and colleagues,[13] assessing the central nervous system penetration and distribution of an investigational antiamyloid drug, gained substantial insight into the human distribution and preliminary PK characteristics using a PET-based microdosing strategy.

A rigorous assessment of the utility of microdosing studies to accurately predict drug disposition in small groups of humans should include a comparative trial pairing the PK results obtained from paired therapeutic dose and microdose data. One of the most widely commented on trials in editorials and commentaries/reviews[21] was recently published.[12] Lappin and colleagues[12] compared the pharmacokinetics of 5 drugs—warfarin, ZK253, diazepam, midazolam, and erythromycin—in adult volunteers receiving a microdose and a pharmacologic dose. The choice of study drugs was "selected to represent a situation in which prediction of human PKs from nonclinical data might be considered problematic." The study of each drug involved 6 volunteers who underwent blood sampling over a 120-hour period, and drug concentrations were determined using HPLC-AMS

methodology. Different from the authors' conclusions, our assessment suggests that microdosing accurately predicted disposition characteristics only for midazolam and intravenous erythromycin, whereas the diazepam $t_{1/2}$ was ~25% higher and Vd ~30% lower than the intravenously administered pharmacologic dose. We agree that microdosing failed to predict the disposition profile of warfarin. In addition, the bioavailability of oral ZK253 appeared to be determined from data extrapolated from the intravenous (IV) data to "estimate" the oral data when there was no correlation between the observed IV microdose and oral pharmacologic dose data.

On initial assessment, these differences for midazolam and diazepam, described in the article by Lappin et al,[12] may appear acceptable in the early stages of an agent's development. However, these comparative results, though interesting and informative, must be interpreted with caution as the blood sampling strategies used for each study drug "were chosen based on the known therapeutic dose PKs of the drug." A priori knowledge of optimal sampling times for a drug PK study clearly enhances the accuracy and robustness of the final results. Unfortunately, under more realistic conditions of microdose studies, it would appear that human sampling strategies will be based on the results from preliminary animal PK data, which may not accurately predict pharmacokinetic profiles in humans. Although this study provides important information on the utility of a microdose development strategy, a true test will involve the results obtained with investigational agents where optimal sampling strategies are not known until after human pharmacologic dose data subsequently become available. Thus, it would appear that under the conditions outlined, this study[12] accurately predicted the disposition characteristics for 3 of the 5 compounds, although the degree of accuracy depends on an individual's definition of acceptable variability. Clearly, the true utility of phase 0 microdosing strategies lies with the ability to predict under what conditions microdosing approaches will provide data within a predefined "acceptance or target range" of variance between predicted and actual determinations. The study of Lappin and colleagues provides insight into some of the important variables that affect the overall utility of such findings in the decision-making process.

Another question concerns whether microdosing can accurately predict PK parameter estimates for drugs that exhibit nonlinear PK characteristics. A recent study[14] examined the PK of 3 compounds—fluconazole, tolbutamide, and an investigational compound, MLNX—in rats. Recognizing that the real interest with microdosing strategies is data from humans, this study provides insight into the utility of this new drug development approach. This study used microdosing versus traditional dosing (referred to as macrodoses in this report), employing a highly sensitive LC/MS/MS methodology. For fluconazole and tolbutamide, dose linearity was shown regardless of which dosing strategy was used. In contrast, the nonlinear PK of MLNX disposition was accurately identified with the traditional (macro) doses but unrecognized with the microdosing technique. The results noted in this animal study are similar to the disparity in warfarin disposition characteristics described in the CREAM (Consortium for Resourcing and Evaluating AMS Microdosing) trial[12] in humans addressed above. These combined findings suggest that caution be used in drug development decisions employing microdosing strategies for drugs with more complex disposition characteristics until more comparative data are available.

ALTERNATIVES TO MICRODOSING FOR EARLY PHASE I STUDIES

In the recent FDA guidance on exploratory INDs, alternatives to microdosing study designs are outlined.[2] These early phase studies include the following:

- One fourth of the 2-week rodent no observed adverse effect level (NOAEL) on a mg/m^2 basis
- Up to one half of the AUC at the NOAEL in the 2-week rodent study or the AUC in the dog at the rat NOAEL, whichever is lower
- The dose that produces a pharmacologic and/or pharmacodynamic response or at which target modulation is observed in the clinical trial

Given the availability of highly sensitive analytical instruments, this approach offers a viable alternative to the use of labeled drug and microdosing strategies that may be more cost-effective and provide a better representation of the individual compound's pharmacokinetic profile. Obviously, direct comparisons of the observed results from these approaches (including the economic analysis) are needed.

CONCLUSIONS

There is no question that decreasing the time of drug development is essential for bringing new therapies to the patient promptly as well as reducing costs.[22] The precepts of microdosing encompass an exciting new paradigm that can potentially assist in safely

accelerating human new drug development of select therapeutically valuable agents. Capitalizing on the continuing rapid advances in technologies that have direct applications within the analytical laboratory is extremely intriguing. However, from the paucity of reported data describing the strengths but, more important, the limitations of this evolving paradigm, it would appear that more validation of the technique, along with a specific focus on its limitations, needs to be addressed. Such validation studies will need to determine the (statistically) appropriate/optimal sample sizes for microdosing studies in animals and humans that encompass the spectrum of metabolic perturbations observed for drugs and similar compounds. Moreover, microdosing methods capable of accurately accounting for differences in important disposition characteristics (eg, linear vs nonlinear disposition) and polymorphically expressed pathways (eg, metabolism, transport) must be described before this approach can be more widely applied in the new drug development process. We urge investigators refining microdosing methodologies to use a variety of drugs; undertake rigorous, statistically sound validation studies that incorporate appropriate subject numbers; and account for the clinically relevant individual differences inherent to human drug disposition as noted above. Most important, an additional focus of such work should be the timely submission of such data for peer review, allowing critique by the scientific community and continuous refinement of this evolving strategy. In parallel, critical assessment of the overall costs associated with microdosing strategies must be undertaken and this information disseminated within the context of human drug development in order for drug developers to make rational financial decisions about the utility of this method.

Despite identified limitations of the current microdosing paradigm, the concept not only is very intriguing but also appears to be one, after appropriate refinement and validation, that may have an impact on the new drug development process. At present, it would appear that studies using microdosing methodology should not be relied on as the primary or sole approach to screen new drug candidates, as the potential exists with current methodologies to possibly reject important new drugs while possibly accepting drugs that could result in significant safety issues. Until more information is available and has undergone appropriate scrutiny, it would appear that a microdosing strategy could complement standard animal-to-human allometric scaling, refining current phase I study designs. Human drug development is a dynamic process that capitalizes on the continuous advancements realized within the analytical pharmacology and data analysis laboratories, combined with a precise understanding of the integrated pharmacokinetic-pharmacodynamic-pharmacogenomic drug profile. Microdosing methodology appears to be one of the many new viable "tools" in the drug development "toolbox." The exact role and impact that microdosing methodologies will have on new drug development is yet to be fully realized but will surely be continuously refined with continued experience and, above all, data sharing through the publication of studies employing this novel methodologic approach.

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