PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

Improved Pharmacokinetic and Bioavailability Support of Drug Discovery Using Serial Blood Sampling in Mice

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ABSTRACT: Pharmacokinetic studies in mice traditionally require one animal per time point, resulting in dosing and euthanizing a large number of animals and producing suboptimal quality of pharmacokinetic data due to inter-animal variability and dosing error. These studies are time-consuming and labor-intensive. To improve the throughput and quality of pharmacokinetic evaluation in mice, we have developed a serial blood sampling methodology using the lateral saphenous vein puncture technique. Two marketed drugs, indinavir and rosuvastatin, were selected for this validation study because of their distinctly different physicochemical and pharmacokinetic properties. Each compound was dosed orally and intravenously in mice using both discrete and serial blood sampling methods. The pharmacokinetic results from serial bleeding are in excellent agreement with those from discrete sampling for both compounds. Compared to the discrete sampling, the serial sampling procedure is a more humane method, allowing for rapid and repeated sampling from the same site without the need for anesthesia. The application of this new method has led to a remarkable reduction in animal and compound usage, a significant increase in throughput and speed, and a drastic improvement in pharmacokinetic data quality. This approach is especially useful for the first-tier *in vivo* pharmacokinetic screening of discovery compounds. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:1877–1884, 2009

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INTRODUCTION

The pharmaceutical industry has been experiencing unprecedented transformation due to skyrocketing research and development cost, expanding regulatory requirements, and expiring patents of major drugs. To remain competitive, pharmaceutical companies are developing paradigm shifting strategies and tactics to cut drug development cost, fill depleting product pipelines and reverse the trend of dropping productivity.[1] As the standard pharmacokinetic evaluation is now routinely performed in the early drug discovery stage to select drug candidates with desirable pharmacokinetic profiles, the attrition rate of development compounds due to poor pharmacokinetic properties has been reduced from about 40% in 1991 to about 10% in 2000.[2] Because of the success of this front-loading

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approach, pharmaceutical companies have continued to embrace this approach to avoid costly late stage failure. With shifting the cost associated with pharmacokinetic studies from the late to early stages of drug discovery, the overall cost has also gone up. One way to lower this cost is to use smaller animals for routine pharmacokinetic evaluation so as to reduce the cost of purchasing larger amounts of starting materials.

The rat has traditionally been used as the rodent species of choice for routine pharmacokinetic screening because of its ease in dosing and serial blood sampling. However, mice have been increasingly used for animal disease models to evaluate efficacy of drug candidates in animals. Genetically modified mice such as certain enzyme or transporter gene knockout mice have also been increasingly employed for mechanistic or drug–drug interaction studies to elucidate drug interactions with a specific drug metabolizing enzyme or transporter.[3,4] In addition, allometric scaling of pharmacokinetic parameters from animals to humans has been shown to be more accurate when mouse data instead of rat data are included in the scaling.[5] Therefore, we decided to investigate the feasibility and applicability of using mouse as the animal species for routine pharmacokinetic studies to reduce the overall study cost and to improve throughput and productivity.

In vivo pharmacokinetic studies in mice are commonly performed using discrete blood sampling because multiple blood sampling of a large volume each would compromise animal physiology and therefore result in unreliable pharmacokinetic data.[6] Because of only one blood draw from each animal, a large number of animals are required for a full pharmacokinetic study. This one animal per time point method has resulted in dosing and euthanizing a large number of animals and producing suboptimal quality of pharmacokinetic data due to inter-animal variability and dosing error. These studies are known to be time-consuming and labor-intensive, causing a significant increase in time, labor, and cost associated with pharmacokinetic studies in mice. Over the years, several research groups have experimented with serial blood sampling from mice with promising results,[7–11] but there have been no reports on direct comparison of comprehensive pharmacokinetic and bioavailability studies in mice dosed orally and intravenously using both serial and discrete sampling methods. To improve the throughput, quality, and turnaround time for *in vivo* pharmacokinetic studies in mice, we have developed and employed a routine serial blood sampling method using the lateral saphenous vein puncture technique.[12] We here present our validation results comparing serial and discrete blood sampling for full pharmacokinetic studies in mice following both intravenous and oral administrations of two marketed drugs that have distinctly different physicochemical and pharmacokinetic properties, indinavir and rosuvastatin. Indinavir is a protease inhibitor for the treatment of HIV infection and AIDS. As a BCS II class compound, indinavir is poorly soluble, highly permeable, and extensively metabolized.[13] In contrast, rosuvastatin is a cholesterol lowering drug for the prevention and treatment of many cardiovascular diseases. As a BCS class III compound, rosuvastatin is highly soluble, poorly permeable, and poorly metabolized.[14] The main objective of this study is to demonstrate how serial blood sampling in mice can be routinely conducted for pharmacokinetic evaluation of drug candidates in the early phase of drug discovery and development.

MATERIALS AND METHODS

Materials

Indinavir and rosuvastatin were purchased from Waterstone Technology (Carmel, IN) and Microsource Discovery Systems (Gaylordsville, CT), respectively. Hydroxypropyl-cyclodextrin, *N*-methyl-pyrrolidinone (NMP), hydroxypropyl methylcellulose, and 0.9% saline were obtained from Sigma–Aldrich (St. Louis, MO) and acetonitrile from EMD Chemicals (Gibbstown, NJ). Solutol HS15 was supplied from BASF (Florham Park, NJ). All other chemicals used were of reagent grade or better.

Animals

Male CD-1 mice (25–30*g*) from Charles River Laboratories (Kingston, NY) were used in all *in vivo* studies. For the discrete blood sampling, 32 and 36 mice were used for oral and intravenous dosing, respectively. For the serial blood sampling, four mice were used in each of the oral and intravenous dosing groups. The animals were fed with a standard laboratory rodent diet (Purina Mills, St. Louis, MO) and housed in individual cages on a 12-h light and 12-h dark cycle with room temperature maintained at $22 \pm 3^{\circ}$C and

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