European Journal of Pharmaceutical Sciences 47 (2012) 444–450

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Fast mouse PK (Fast PK): A rapid screening method to increase pharmacokinetic throughput in pre-clinical drug discovery

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ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 9 May 2012
Accepted 2 July 2012
Available online 9 July 2012

Keywords:
Pharmacokinetics
High throughput screening
Drug discovery

ABSTRACT

We describe a rapid screening methodology for performing pharmacokinetic (PK) studies in mice called Fast PK. In this Fast PK method, two mice were used per compound and four blood samples were collected from each mouse. The sampling times were staggered (sparse sampling) between the two mice, thus yielding complete PK profile in singlicate across eight time points. The plasma PK parameters from Fast PK were comparable to that obtained from conventional PK methods. This method has been used to rapidly screen compounds in the early stages of drug discovery and about 600 compounds have been profiled in the last 3 years, which has resulted in reduction in the usage of mice by 800 per year in compliance with the 3R principles of animal ethics. In addition, this Fast PK method can also help in evaluating the PK parameters from the same set of animals used in safety/toxicology/efficacy studies without the need for satellite groups.

1. Introduction

Drug discovery companies have employed a battery of new technologies like high-throughput screening (HTS) and parallel synthesis along with automation that have enabled large numbers of active compounds to be identified at early stages of drug discovery. With front loading of DMPK in the drug discovery, the industry has reduced the attrition rate from 40% to 10% (Kola and Landis, 2004; Roberts, 2003). In order to cope up with the increased number of molecules in ADME assays, various *in vitro* experiments have been modified for a high-throughput format with the advent of more sensitive analytical methods like mass spectrometry and liquid handling systems (Kassel, 2004; Tarbit and Berman, 1998). With a better understanding of the DMPK properties many of the compounds synthesised pass the standard *in vitro* tests and qualify to be tested *in vivo*. However, *in vivo* PK studies have been traditionally low-throughput experiments and have failed to provide timely information to influence the design-make-test cycle.

Conventional PK studies use the dosing strategy of a single compound per animal to avoid potential drug–drug interaction problems. This study typically includes two arms (IV and PO) and takes 6–12 terminal blood samples per compound in triplicate and requiring 36–72 mice per study. The high animal usage and labour intensive dosing and sampling poses a major hurdle in the throughput of the assay. Efforts to increase the PK throughput by the use of cassette dosing (*N* in one dosing) were proposed (Berman et al., 1997), but are plagued by inconsistency in the data along with a question of possible drug-drug interactions during the study and this method is not followed widely (Manitpisitkul and White, 2004). Another method to increase throughput is Cassette Accelerated Rapid Rat Screen (CARRS) PK wherein 6 samples are collected in 6 h and analysed by LC-MS/MS by using a mini calibration curve (Korfmacher et al., 2001). This method however is applicable for rats and requires cannulated rats for the study. A more recent Snapshot PK method in mice has been reported, where four samples were collected in duplicate to provide partial PK parameters (Liu et al., 2008). Other methods to improve throughput was by modifying the bio analytical methods like vertical and horizontal sample pooling during sample analysis (Hop et al., 1998; Kuo et al., 1998; Cox et al., 1999).

We describe a Fast PK method, which in addition to increasing the throughput gives complete PK profile with minimum number of mice per experiment ($n = 2$). In this method, eight time points (0.083–24 h) in singlicate were collected using sparse sampling method from two mice to get the full PK parameters (4 time points per mouse), in contrast to the Snapshot PK. The plasma samples from Fast PK studies were analysed by liquid chromatography with tandem mass spectrometry (LC–MS/MS). The results of the Fast PK were compared against Conventional PK and the results obtained

Abbreviations: PK, pharmacokinetics; PD, pharmacodynamics; PO, per oral; IV, intravenous; HTS, high throughput screening; DMPK, drug metabolism and pharmacokinetics; AUC, area under the curve; HPMC, hydroxypropylmethyl cellulose; CMC, carboxymethyl cellulose.

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http://dx.doi.org/10.1016/j.ejps.2012.07.001

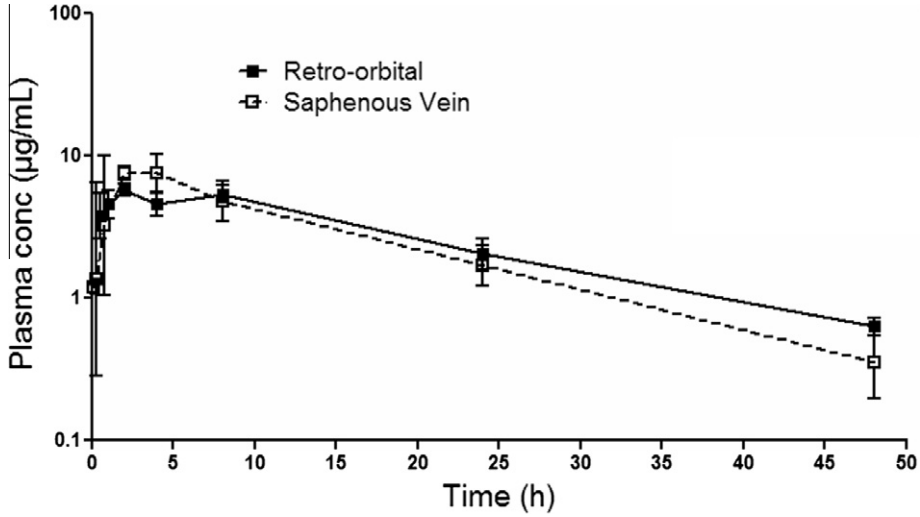

Fig. 1. Comparison of retro-orbital (■) and Saphenous vein (□) bleeding in male BALB/c mice dosed with Rifampicin at 10 mg/kg as a single dose.

Table 1
Comparison of retro-orbital (terminal) and saphenous vein (sparse) bleeding methods in mice after Oral Administration of Rifampicin HCL. Parameters represent Mean ± SD.

Parameters	Sampling routes	
	Retro-orbital	Saphenous vein
Dose (mg/kg)	10	10
C_{max} (µg/mL)	5.73 ± 0.6	7.91 ± 2.4
T_{max} (h)	2.0	4.0
$AUC_{0-\infty}$ (h∗µg/mL)	141.3 ± 9.9	136.8 ± 23.7
$t_{1/2}$ (h)	14	11

Rifampicin was administered to male BALB/c mice at 10 mg/kg as a single dose. The key pharmacokinetic parameters of Rifampicin were compared between retro-orbital and saphenous vein bleeding methods. The C_{max} (5.73 ± 0.6 and 7.91 ± 2.4 µg/mL) and AUC_{inf} (141.3 ± 9.9 and 136.8 ± 23.7 h∗µg/ml) of retro-orbital bleeding method was not statistically different from saphenous vein bleeding method. T_{max} was shifted from 2 to 4 h in saphenous vein bleeding. Terminal half-life was shorter (11 h) in saphenous vein compared to retro-orbital (14 h) bleeding method.

from these studies are described below. In addition, the advantages and disadvantages of this method along with compliance to the 3R principles of animal ethics are described.

2. Materials and methods

BALB/c mice were procured from a vendor approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India). The Institutional Animal Ethics Committee, registered with the Government of India (Registration no. CPCSEA 1999/5), approved all experimental protocols with animals and their use. Six to eight week old BALB/c mice were purchased from Raj Biotech, Pune India and were randomly assigned at five per cage with the restriction that all cage members within a 1–2 g weight of each other in a facility that has 12 h day-night cycle with water and feed provided *ad-libitum*. Mice were allowed 2 weeks of acclimatization before intake into experiments.

Hydroxypropylmethyl cellulose (HPMC) was purchased from Fluka Biochemika, while Rifampicin Hydrochloride, and carboxymethyl cellulose (CMC) were obtained from Sigma-Aldrich. All the other standards, solvents and buffers were procured from commercial sources and the test compounds were synthesised in-house. 96 Well polypropylene plates (v-bottom) were obtained from Greiner Bio-one (Germany).

In conventional PK, compounds were formulated either as a suspension using 0.5% HPMC with 0.1% tween 80 or as a solution using 5% dextrose solution with pH adjustments. Post dosing, the blood samples were collected through retro-orbital plexus puncture and only one sample was collected from one mouse. The experiment was done in triplicate with a total of eight time points (0.083, 0.25, 0.5, 1, 2, 4, 6, 24 h and hence a total of 24 mice were used per experiment. Refinement in blood sampling technique was introduced by the way of saphenous vein bleeding, which allows multiple blood sampling in mice (Hem et al., 1998; Diehl et al., 2001) as against single sample per mouse by retro-orbital plexus puncture.

In Fast PK, the test compounds were formulated either as a suspension using 0.5% HPMC with 0.1% tween 80 or as a solution using 5% dextrose solution with pH adjustments. Formulations were administered by oral gavage in case of oral study and by tail vein dosing in case of intravenous PK. The time points were alternated between the two mice wherein the samples at 0.083, 0.5, 2, 6 h were collected from the first mouse, while 0.25, 1, 4, 24 h were collected from the second mouse. In all the PK studies, the oral dosing volume was kept at 10 mL/kg while the IV was kept at 5 mL/kg. Blood samples (50 µL) were collected into Li-heparin coated Microvette CB 300® tubes (Sarstedt, Germany) and stored at 4 °C until centrifugation. The plasma was separated and stored in 96 well plate at −20 °C until analysis.

Plasma samples (25 µL) were processed by protein precipitation with addition of 225 µL of cold acetonitrile. A 1 mg/mL stock solution of each compound was prepared in dimethylsulfoxide (DMSO) or acetonitrile and was diluted three folds into mouse blank plasma to prepare standards ranging from 8 to 20000 ng/mL. All the samples were extracted using a plate shaker (iEMS incubator/ Shaker, Thermo Labsystems) for 10 min at a shake speed of 650 rpm. The samples were centrifuged in the plate centrifuge (Eppendorff centrifuge 5810R) at 4000 rpm for 20 min at 15 °C. 25 µL of the supernatant was mixed with 225 µL of mobile phase containing the internal standard. A 10 µL of sample was injected into the instrument. In order to further improve the throughput, the samples may be pooled by horizontal pooling depending on the analytical feasibility. The samples were analyzed with triple quadrupole Mass spectrometer coupled to a HPLC system using a reverse phase C-18 analytical column. The chromatographic conditions consists of mobile phase acetonitrile (phase A) and formic acid 0.1% (v/v) or 10 mM ammonium acetate (w/v) in water (phase B) that was run over a 4 min isocratic elution at a flow rate of 0.4 mL/min. A