Drug Metabolism and Pharmacokinetics 31 (2016) 73–81

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular article

Usefulness of minipigs for predicting human pharmacokinetics: Prediction of distribution volume and plasma clearance

Hiromichi Yoshimatsu [a, *], Yoshihiro Konno [a], Kunikazu Ishii [a], Masahiro Satsukawa [a], Shinji Yamashita [b]

[a] Pharmacokinetics and Safety Department, Drug Research Center, Kaken Pharmaceutical Co., Ltd., Kyoto, Shizuoka, Japan
[b] Faculty of Pharmaceutical Science, Setsunan University, Osaka, Japan

ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form
10 November 2015
Accepted 11 November 2015
Available online 18 November 2015

Keywords:
Predicting human PK
Minipig
Vd
CL
Physiological parameters
Single-species prediction
Allometric scaling approaches

ABSTRACT

In this study, advantages of minipigs to use in preclinical study for new drug development were evaluated in terms of prediction of human pharmacokinetic (PK) parameters of various drugs. Fourteen model drugs having diverse physicochemical properties were selected and intravenously administered to mice, rats and minipigs to obtain their PK parameters. The human volume of distribution (Vd) and clearance (CL) of model drugs were predicted from PK parameters in each animal species.

When Vd of 14 drugs in each species were directly compared with those in humans, minipigs showed the highest correlation. Correction of Vd with an unbound fraction of drugs in tissues further improved the correlation. Allometric scaling that included minipig data resulted in high accuracy in the prediction of human Vd, clearly indicating an importance of minipig data. Minipigs also showed the high predictability of human CL. The prediction of human CL by allometric scaling showed a high accuracy when the data of minipigs were included.

In conclusion, potential advantages of minipigs for predicting human Vd and CL were clearly demonstrated. Reliable prediction of human PK from data of minipigs appears to be possible in preclinical PK study, without relying on PK analysis in other species.

1. Introduction

Recently, the use of minipigs has attracted increasing attention from the pharmaceutical industry as an alternative experimental animal to monkeys or dogs at the preclinical stage of drug development. In addition to the advantages relating to animal care and use requirements, minipigs can offer various benefits derived from the physiological and anatomical similarities with humans. Minipigs have been used routinely for regulatory toxicity testing, typically for general toxicity studies, because of its similar responses to drugs with those of humans [1]. Also, minipigs are used in safety pharmacological studies, particularly for those involving the cardiovascular system. The immune system of the pig has been better characterized than that of dogs, making minipigs an interesting model for the therapeutic study of the immune system manipulation [2].

In addition to pharmacological and toxicological issues, prediction of the human pharmacokinetics (PK) of newly developed drug candidates still remains as a challenging issue to minimize the risk to volunteers in first-in-human studies. For drug-metabolizing enzymes, the comparability of hepatic cytochrome P450 (CYP) subclasses between minipigs and humans was reported, which supports the usefulness of minipigs as an experimental animal to predict a biotransformation pathway in humans [1]. In addition, the gastrointestinal system of minipigs offers some anatomical and functional advantages (in terms of the similarity to humans) over that of dogs [1]. However, a few PK studies have been conducted in minipigs so far.

Several approaches have been developed for the quantitative prediction of human PK and been integrated into all stages of drug development [3,4]. Typically, prediction of the human PK has been achieved either by empirical approaches in which the *in vivo*

Abbreviations: AFE, average-fold error; CL_b, whole blood clearance; CL_p, plasma clearance; CYP, cytochrome P450; f_{up}, unbound fraction in plasma; f_{ut}, unbound fraction in tissues; IVIVE, *in vitro-in vivo* extrapolation; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MLP, maximum lifespan potential; PB-PK, physiologically based-pharmacokinetic; PK, pharmacokinetics; R_B, blood-to-plasma concentration ratio; R_e/i, ratio of binding proteins in extracellular fluid (except plasma) to binding proteins in plasma; ROE, rule of exponent; UGT, UDP-glucuronosyltransferase; Vd, steady-state volume of distribution; V_e, extracellular fluid volume; V_p, plasma volume; V_r, remainder of the fluid volume.

* Corresponding author.
E-mail address: yoshimatsu_hiromichi@kaken.co.jp (H. Yoshimatsu).

http://dx.doi.org/10.1016/j.dmpk.2015.11.001

preclinical data of experimental animals are extrapolated or by mechanistic approaches combining with *in vitro* data. Allometric scaling is one of the most widely used empirical approaches because of its simplicity which typically focuses on interspecies relationships between clearance (CL) or volume of distribution (Vd) and the body weight.

On the other hand, a number of mechanistic approaches have been developed and used for the prediction of human CL. The approach is generally based on a physiologically based pharmacokinetic (PB-PK)-modeling, in which *in vitro* data obtained from human tissues, cells or microsomes are integrated into a physiological model of the human. However, it was reported that the predicted human CL often deviated from the *in vivo* CL. Kilford showed a general trend of a PB-PK approach to underpredict both CYP and UDP-glucuronosyltransferase (UGT)-mediated metabolism [5]. Miners reported, for some drugs, the human CL was under predicted 10- to 30-fold, indicating a difficulty of a quantitative prediction only from the *in vitro* data [6].

In this study, the PK parameters, Vd and CL, of various drugs, 14 drugs those having diverse physicochemical properties, were obtained in minipigs, mice and rats and were compared with those in humans. Human Vd and CL of these drugs were predicted using the single animal species data as well as the allometric scaling of the data of all animals. The accuracy of each prediction was evaluated to demonstrate the potential advantages of using minipigs in preclinical PK studies.

2. Materials and methods

2.1. Materials and reagents

Antipyrine, atenolol, diclofenac sodium, furosemide, ketoprofen, lidocaine and propranolol hydrochloride were purchased from Wako Pure Chemicals (Osaka, Japan); fexofenadine and raloxifene hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA); acetaminophen was purchased from Nacalai Tesque (Kyoto, Japan); felodipine was purchased from AK Scientific, Inc. (Union City, CA, USA); flurbiprofen was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fentanyl injection and lepetan injection 0.3 mg were purchased from Janssen Pharmaceutical K.K. (Tokyo, Japan) and Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. All other chemicals used were reagent grade or better.

2.2. Animals

All procedures on animals were performed according to protocols approved by the Kaken Institutional Animal Care and Use Committee. Male ICR mice (6 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Male Sprague–Dawley rats (6 weeks old) were purchased from Charles River Laboratory Japan (Yokohama, Japan). Male NIBS minipigs (3 months old) were purchased from Nisseiken Co., Ltd. (Yamanashi, Japan). These animals were kept in an experimental animal room with an ambient temperature of 20°C–26 °C and a 12-h/12-h light–dark cycle for at least 6 days (mice and rats) or 1 month (minipigs) before use. Intravenous (IV) administration was performed in 7- to 9-week-old mice, 7- to 9-week-old rats and 4- to 24-month-old minipigs.

2.3. Blood/plasma concentration ratio

The blood/plasma concentration ratio (R_B) was determined *in vitro* after incubation of the compounds with fresh pooled blood from at least 3 mice, 3 rats, 3 minipigs or 3 humans, respectively. Blood was preincubated at 37 °C in a water bath and spiked with the test compounds at 100 ng/mL. The blood samples were then incubated at 37 °C for an additional 10 min. After centrifugation at 14,000 *g* for 10 min at 4 °C, the blood and plasma samples were transferred into 3 volumes of methanol containing ketoconazole (Wako Pure Chemicals, Osaka, Japan), used as an internal standard (IS), and then centrifuged. The concentrations of drugs in the supernatant were determined by LC–MS/MS.

2.4. Unbound fraction in plasma

The unbound fraction of plasma protein (f_{up}) was determined *in vitro* after incubation of the compounds by equilibrium dialysis with pooled plasma from at least 3 mice, 3 rats, 3 minipigs and 3 humans, respectively. Mouse, rat, minipig and human plasma samples containing 10 μg/mL of each drug were incubated at 37 °C for 24 h in an equilibrium dialysis system. The collected samples were transferred into 3 volumes of methanol containing ketoconazole (used as an IS) and then centrifuged. The concentrations of drugs in the supernatant were determined by LC–MS/MS.

2.5. Pharmacokinetic studies

A total of 14 drugs (acetaminophen, antipyrine, atenolol, lepetan injection 0.3 mg, diclofenac sodium, felodipine, fentanyl injection, fexofenadine, flurbiprofen, furosemide, ketoprofen, lidocaine, propranolol hydrochloride and raloxifene hydrochloride) were used in the PK studies. For IV administration, each of the drugs was dissolved in a solution containing dimethyl sulfoxide (Wako Pure Chemicals, Osaka, Japan), ethanol (Wako Pure Chemicals, Osaka, Japan), Kolliphor ELP (BASF Japan Ltd., Tokyo, Japan) and saline (1%, 2.5%, 2.5% and 94% final concentration, respectively). The drugs were then intravenously administered to nonfasting mice and rats at a dose of 1 mg/kg, with the exception of buprenorphine (0.2 mg/kg) and fentanyl (25 μg/kg), and to nonfasting minipigs at a dose of 0.1 mg/kg, again with the exception of buprenorphine (0.02 mg/kg) and fentanyl (5 μg/kg). Following administration, blood samples were collected from the caudal vein of the mice and rats and the sinus venarum cavarum of the minipigs at 0.0833, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h. The plasma samples were separated by centrifugation at 14,000 *g* for 10 min at 4 °C and stored at −30 °C until use. The drug concentrations in the plasma were quantified using LC–MS/MS.

2.6. LC–MS/MS analysis

The LC–MS/MS system consisted of an HTS autosampler (Shiseido, Tokyo, Japan), NANOSPACE SI-2 HPLC and TSQ QUANTUM Vantage mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The following LC conditions were used: column, CAPCELL PAK C_{18} MGIII (2.0 mm I.D. × 50 mm, 5 μm; Shiseido, Tokyo, Japan) or Inertsil® ODS-3 (2.1 mm × 50 mm, 4 μm; GL Sciences, Tokyo, Japan); column temperature, 40 °C; gradient elution at 0.3–0.4 mL/min with methanol or acetonitrile and aqueous 0.1% formic acid or 1 mM ammonium acetate; and injection volume, 10 μL. The following main working parameters for the mass spectrometers were used: ion mode; electrospray ionization, positive (acetaminophen, antipyrine, atenolol, buprenorphine, fentanyl, fexofenadine, ketoprofen, lidocaine, propranolol and raloxifene) and negative (diclofenac, felodipine, flurbiprofen and furosemide); spray voltage, 3000 V; sheath gas pressure, 20 Arb; aux gas pressure, 5 Arb; capillary temperature, 330 °C; selective reaction monitoring method with transitions of *m/z* 151.92 → 109.95 for acetaminophen, *m/z* 189.10 → 77.03 for antipyrine, *m/z* 267.16 → 190.11 for atenolol, *m/z* 468.25 → 396.30 for buprenorphine, *m/z* 293.94 → 250.00 for diclofenac, *m/z* 382.08 → 154.08 for felodipine, *m/z* 337.21 → 188.17 for fentanyl, *m/z* 502.34 → 466.36 for fexofenadine, *m/z* 243.02 → 199.10