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Review

Comparison of minipig, dog, monkey and human drug metabolism and disposition

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ABSTRACT

Introduction: This article gives an overview of the drug metabolism and disposition (ADME) characteristics of the most common non-rodent species used in toxicity testing of drugs (minipigs, dogs, and monkeys) and compares these to human characteristics with regard to enzymes mediating the metabolism of drugs and the transport proteins which contribute to the absorption, distribution and excretion of drugs. **Methods:** Literature on ADME and regulatory guidelines of relevance in drug development of small molecules has been gathered. **Results:** Non-human primates (monkeys) are the species that is closest to humans in terms of genetic homology. Dogs have an advantage due to the ready availability of comprehensive background data for toxicological safety assessment and dogs are easy to handle. Pigs have been used less than dogs and monkeys as a model in safety assessment of drug candidates. However, when a drug candidate is metabolised by aldehyde oxidase (AOX1), N-acetyltransferases (NAT1 and NAT2) or cytochrome (CYP2C9-like) enzymes which are not expressed in dogs, but are present in pigs, this species may be a better choice than dogs, provided that adequate exposure can be obtained in pigs. Conversely, pigs might not be the right choice if sulfation, involving 3-phosphoadenosyl-5-phosphosulphate sulphotransferase (PAPS) is an important pathway in the human metabolism of a drug candidate. **Discussion:** In general, the species selection should be based on comparison between in vitro studies with human cell-based systems and animal-cell-based systems. Results from pharmacokinetic studies are also important for decision-making by establishing the obtainable exposure level in the species. Access to genetically humanized mouse models and highly sensitive analytical methods (accelerator mass spectrometry) makes it possible to improve the chance of finding all metabolites relevant for humans before clinical trials have been initiated and, if necessary, to include another animal species before long term toxicity studies are initiated. In conclusion, safety testing can be optimized by applying knowledge about species ADME differences and utilising advanced analytical techniques.

Contents

Abbreviations: AUC, area under the plasma concentration versus time curve; (B–A)/(A–B), cross-membrane transport rate, basolateral to apical and reverse; CB, covalent binding; CL, clearance; C_{max}, maximal plasma concentration; CYP, cytochrome P450; fu, fraction unbound drug to plasma; F, absolute bioavailability (AUC_{PO}/AUC_{IV}); K_i, inhibition constant; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MALDI-TOF-MS, matrix assisted laser desorption-time of flight- mass spectrometry; NAT, N-acetyl-transferase; NMR, nuclear magnetic resonance (spectroscopy); OAT, organic anion transporter; OCT, organic cation transporter; PAPS, 3-phospho-adenosyl-5-phosphosulphate-sulphotransferase; $T_{1/2}$, half-life; UGT, uridine diphosphate glucuronyltransferase; V_m/K_m, intrinsic clearance.

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1. Introduction

Minipigs have been reviewed from various angles as models for toxicity testing of new medicines and chemicals and ethical and welfare implications of the minipigs, regulatory acceptability and biology, have been reviewed (Bode et al., 2010; Ellegaard et al., 2010; Guest Editor Roy Forster, 2010, The Rethink Project, J.Pharmacol. Toxicol. Methods **62** No. 3; Van der Laan, Brightwell, McAnulty, Ratky, & Stark, 2010; Webster, Bollen, Grimm, & Jennings, 2010). A comprehensive monograph covering all aspects of the minipig in biomedical research including ADME is also available (McAnulty, Dayan, Ganderup, & Hastings, 2011). The draft pig genome sequence (Sscrofa 10.2) has been developed and given insight into the demography and evolution (Groenen et al., 2012) and is likely to result in improved disease models for humans.

The objective of this article is to provide an overview on the ADME properties of non-rodents as a basis for choosing the right species for the toxicity testing of drug candidates being developed for human use. It is also an aim to put these properties in context with the development of drugs (small molecules), the regulatory perspective, and the timing of studies.

Selecting a non-rodent species for non-clinical safety evaluation is sometimes difficult due to large interspecies differences in their susceptibility to the toxicity of xenobiotics (Beasley, 1999; Hengstler, Van der Burg, Steinberg, & Oesch, 1999). Monkey, minipig and dog are the candidates with sufficient background data available for use in toxicology. Therefore, these three species are compared with humans. The liver is the major site of the metabolism of xenobiotics, steroids, cholesterol and bile acids. CYP-mediated metabolism is an important first step (phase I) in the oxidation of a large proportion of known xenobiotics, including drugs. Apart from CYP enzymes, other oxidative enzymes, like FMO and AO enzymes can be involved in the metabolism of xenobiotics. The oxidation step is most often followed by a conjugation step where a polar moiety is added to the modified molecule (phase II). The conjugate formed is easily excreted from the liver or kidneys in a partly membrane-transporter-mediated process.

The biotransformation of xenobiotics (focused on drugs) in both humans and animal species has been described in a comprehensive review (Parkinson & Ogilvie, 2008). The focus on the use of the minipig as an animal model has been growing since the publications of the Rethink Project in 2010. Comprehensive reviews examining the role of CYPs in porcine metabolism of xenobiotics, substrate specificity, inhibition, genetic expression and receptor-driven regulation compared with human data have appeared (Preusse & Skaanild, 2011; Puccinelli, Gervasi, & Longo, 2011). Also the use of swine in drug discovery and development has recently been reviewed (Helke & Swindle, 2013). Even though there have been recent reviews of the role of CYP enzymes in porcine metabolism of xenobiotics, the role of other enzymes like aldehyde oxidase has been lacking. In the context of drug development, it is the exposure of human relevant metabolites that are important as compared to the enzymes involved in the formation of such metabolites. Unfortunately, such data from studies in pigs are scarce in the literature.

The role of membrane transporters in drug disposition of non-rodent animal models is only beginning to emerge along with the increased focus on membrane transporters in clinical development of drugs. The role of membrane transporters in drug disposition of non-rodent animal models is only beginning to emerge along with the increased focus on membrane transporters in clinical development of drugs. Apart from the important role of transporters in cell uptake and efflux of drugs in gut, liver and kidney, the role of transporters in the blood–brain barrier (BBB) has special attention. Due to the tight junctions between cells in the BBB, the uptake of a drug to the brain as well as the efflux must take place by either passive diffusion or by a facilitated or active transport. The following section is aimed at showing the progress made in recent years with in vitro and in vivo studies related to pig metabolism and disposition.

2. Progress in the use of minipigs in ADME studies

2.1. In vitro pig model of BBB

A model of the BBB has been validated using pig brain endothelial cells (Patabendige & Abbott, 2014; Patabendige, Skinner, & Abbott, 2013; Patabendige, Skinner, Morgan, & Abbott, 2013). Such a model will be valuable in projects with the primary target in the brain. If the site of action is supposed to be in the peripheral system, the model could serve as an in vitro model to estimate the exposure to the brain and to develop in vitro–in vivo correlations (IVIVC). It is worth mentioning that there are a number of transgenic pig models for human diseases in the brain.

2.2. Pgp and CYP3A expression in minipigs

The presence of Pgp and CYP3A in livers and small intestines of foetal, neonatal, juvenile, and adult Göttingen minipigs have been determined by immunohistochemical methods. A gene expression similar to that in humans is reported (Van Peer et al., 2014).