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Assessment of inhibition of porcine hepatic cytochrome P450 enzymes by 48 commercial drugs

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ABSTRACT

Drug interactions due to inhibition of hepatic cytochrome P450 (CYP450) enzymes are not well understood in veterinary medicine. Forty-eight commercial porcine medicines were selected to evaluate their potential inhibition on porcine hepatic CYP450 enzymes at their commercial doses and administration routes. Those drugs were first assessed through a single point inhibitory assay at 3 μM in porcine liver microsomes for six specific CYP450 metabolisms (phenacetin o-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, bufuralol 1-hydroxylation, chlorozoxazone 6-hydroxylation and midazolam 1'-hydroxylation). When the inhibition was > 10% in the single point inhibitory assay, IC_{50} values (inhibitory concentrations that decrease biotransformation of selected substrate by 50%) were determined. Overall, 17 drugs showed in vitro inhibition on one or more porcine hepatic CYP450 metabolisms with different IC_{50} values. The potential in vivo porcine hepatic CYP450 inhibition by those drugs was assessed by combining the in vitro data and in vivo C_{max} (maximum plasma concentrations from pharmacokinetic studies of the porcine medicines at their commercial doses and administration routes). Three drugs showed high potential inhibition to one or two porcine hepatic CYP450 isoforms at their commercial doses and administration routes, while seven drugs had medium risk and seven had low risk of such in vivo inhibition. These data are useful to prevent potential drug interactions in veterinary medical practice.

Introduction

A drug pharmacokinetics (PK) and/or pharmacodynamics (PD) can be altered due to co-administration of other drugs, foods, beverages and herbs. These types of drug interactions have been extensively investigated and understood in research and development of human drugs. However, the research and understanding of such drug interactions in veterinary medicine are limited. Co-administration of drugs to animals is a common practice. Long acting formulations and daily administration of veterinary active ingredients in feed or water play significant roles in animal health, production and reproduction. The dosing regimens increase the risk of drug interactions. Therefore, there is a need to understand drug interactions in veterinary medicine for safety and efficacy.

Hepatic cytochrome P450 enzymes (CYP450) belong to a family of enzymes responsible for the biotransformation of many drugs and have significant impact on drug exposure. One type of drug interaction is inhibition of a co-administered drug on one or more CYP450 enzymes, resulting in a change of exposure of the other co-administered drugs (i.e. the victim drugs), which are the substrates of those CYP450 enzymes. This metabolically-based drug interaction can lead to serious safety issues.

Early assessment of CYP450 enzyme inhibition is an important part of the research and development of drugs for human beings (Mouly et al., 2009; Nettleton and Einolf, 2011). There are a variety of approaches to assess the potential CYP450 inhibition using in vitro data and clinical pharmacologic evaluation during the development of drugs for humans (Blanchard et al., 2004; Brown et al., 2005, 2006; Venkatakrishnan and Obach, 2007; Youdim et al., 2007; Fahmi et al., 2008). Evaluation of CYP450 inhibition by commercial veterinary medicines is also valuable to prevent potential drug interactions in veterinary medical practice.

Based on the 2012 FDA Guidance of Drug Interaction Studies,[1] potential drug interactions can be evaluated using basic models, mechanistic static models and more comprehensive dynamic models (e.g. physiologically-based PK (PBPK) models). Without understanding detailed drug disposition, drug interaction mechanisms, and physiological parameters, the basic models have predominantly been used because they are simple and practical.

The most common basic model used to assess the potential of a drug to inhibit CYP450 enzymes is based on IC_{50} or K_i values. IC_{50}

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[1] See: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf (accessed 21 January 2016).

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is defined as an inhibitor concentration that decreases the biotransformation of a substrate by 50% at a single specified substrate concentration (Madan et al., 2002). This value is dependent on a substrate concentration for a competitive inhibitor obtained from in vitro inhibitory assays. K_i is the inhibition constant, a value that is not dependent on assay conditions. For competitive inhibition:

$$K_i = IC_{50} \times K_m/(K_m + [S])$$

in which K_m is the Michaelis–Menten constant for the substrate and [S] is the substrate concentration in the inhibitory assay (Bachmann, 2006). When $[S] \sim K_m$ in a competitive inhibition, $K_i = IC_{50}/2$. The consequence of the presence of an inhibitor is an increased exposure or AUC of a victim drug. The degree of such impact can be expressed as:

$$AUC_i/AUC_0 = 1 + [I]/K_i$$

where AUC_0 is the area under the curve (AUC) of the victim drug in the absence of the inhibitor and AUC_i is the AUC in the presence of the inhibitor. [I] is the concentration of the inhibitor in vivo. Therefore the potential inhibition can be assessed by in vitro IC_{50} assays and in vivo PK [I] values. When $[I]/K_i > 1$, a high possibility of drug interaction due to reversible CYP inhibition is likely. When $0.1 < [I]/K_i < 1$, a medium risk of drug interaction is possible, and when $[I]/K_i < 0.1$, the risk of drug interaction is low (Bachmann and Lewis, 2005).

Values of [I] have been evaluated using in vivo steady state plasma concentration following repeated administration (C_{av}), unbound average plasma concentration ($C_{av,u}$), maximum plasma concentration (C_{max}), and peripheral vein concentration (C_{in}). C_{max} is an in vivo PK parameter available in the literature for most commercial drugs. It is comparable with other in vivo concentrations in such evaluations, although the resultant $[I]/K_i$ ratio would be slightly less conservative (Ito et al., 2003; Bachmann and Lewis, 2005; Bachmann, 2006).

The pig is an important animal protein source globally which represents about 40% of worldwide meat production. There are a variety of marketed porcine medicines, but the information regarding drug interactions of those medicines is limited. Porcine hepatic CYP450 enzymes are similar to human. High homology of each CYP450 isoform has been found between human and pig enzymes, although the relative abundance of those enzymes is different (Achour et al., 2011; Puccinelli et al., 2011). In the current study, in vitro inhibition of 48 commercial porcine medicines on porcine hepatic CYP450 isoforms in porcine liver microsomes was determined. The potential risk of those drugs to inhibit porcine hepatic CYP450 enzymes in vivo was assessed using their in vitro data and published in vivo C_{max}.

Materials and methods

Chemicals and porcine liver microsomes

Active ingredients of commercial porcine medicines were purchased from Sigma-Aldrich, except that desfuroylceftiofur was obtained from Zoetis. Matrix (altrenogest solution 0.22%) was bought from Merck Animal Health. Phenacetin, 4-acetamidophenol, coumarin, 7-hydroxycoumarin, tolbutamide, 4-hydroxy tolbutamide, bufuralol, hydroxybufuralol, chlorzoxazone, 6-hydroxychlorzoxazone, midazolam, 1'-hydroxymidazolam and β-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) were purchased from Sigma-Aldrich. Intact male porcine liver microsomes prepared from 7–8 month old pigs of strain Yorkshire x Duroc x Landrace (Product number Z1001, Lot number 011498A) were bought from Xenotech.

Determination of enzymatic reaction parameters

Michaelis–Menten kinetic parameters, apparent K_m and V_{max} of porcine hepatic CYP450 isoforms were determined using six specific CYP450 isoform substrates in porcine liver microsomes. Eight concentrations of phenacetin (0–1500 μM), coumarin (0–20 μM), tolbutamide (0–2000 μM), bufuralol (0–60 μM), chlorzoxazone (0–2000 μM) or midazolam (0–50 μM) were pre-incubated with 0.1 mg protein/mL porcine liver microsomes in 100 mM phosphate buffer (pH 7.4) at 37 °C for 3 min. The metabolism reactions were initiated with NADPH (1 mM) and terminated after 10 min with cold acetonitrile. The mixtures were centrifuged and the formation of their metabolites in supernatants was quantitatively analyzed using liquid chromatography-mass spectrometry (LC/MS) against the calibration curves of the metabolites (0–5000 nM). The enzymatic reaction parameters of K_m and V_{max} were determined by non-linear fitting of Michaelis–Menten kinetics using Sigmaplot (Systat Software).

Single point inhibitory screening

A single point inhibition using 3 μM of each porcine drug was performed with metabolism of each CYP450 substrate in porcine liver microsomes as an initial screening. Each porcine drug at 3 μM (4–6 replicates) was pre-co-incubated with each CYP450 substrate at concentrations of its K_m value and 0.1 mg pig liver microsomal protein/mL in phosphate buffer at 37 °C. The reactions were initiated with NADPH (1 mM). Blank controls of 26–30 replicates without porcine drugs were incubated simultaneously. Both reactions with and without porcine drugs were terminated after 10 min with cold acetonitrile. The mixtures were centrifuged and the formation of metabolites in those supernatants was monitored with LC/MS. The differences of peak areas between mean values of the incubations with a porcine medicine and the blank controls were compared. When reduction of peak areas of the formed metabolite in the incubations with a porcine medicine was > 10% relative to the controls, the IC_{50} value was determined.

IC_{50} value determination

The incubations were similar to the single point inhibitory screening assay except that eight concentrations (0–30 μM, or 0–300 μM, or 0–600 μM, or 0–1000 μM based upon the percentage of inhibition at the single point inhibitory screening assay) of the selected porcine drugs were incubated with porcine liver microsomes at each CYP450 substrate concentration equal to its K_m. The formation of metabolites was monitored with LC/MS. IC_{50} values were then determined using GraphPad Prism software V. 6.03.

Pharmacokinetics of altrenogest

Five gilts (bodyweight, 131–151 kg) were purchased from Heimerl Farms, OH. Those pigs were dosed by oral gavage with 6.8 mL of Matrix containing 15 mg altrenogest per day for five days. Blood samples were collected at 2 and 7 h post dose every day. The operation was conducted under the animal use protocol (KZ-1829d-2013-03-scs) approved by the company Institutional Animal Care and Use Committee (IACUC) on 18 April 2013, and according to local, state, and national regulations. Altrenogest concentrations in plasma samples were determined using LC/MS against the calibration curve of altrenogest (0–50 ng/mL).

Liquid chromatography-mass spectrometry (LC/MS) analysis

Metabolites formed from phenacetin, coumarin, bufuralol, tolbutamide, chlorzoxazone and midazolam, were analyzed by a LC/MS system consisting of a Waters Acquity UPLC and an Applied Biosystem API4000 Q trap mass spectrometer. The multiple reaction monitoring (MRM) transitions of metabolites using electrospray mode on the mass spectrometer are listed in Table 1. Chromatography conditions for each metabolite are listed in Table 2.

Altrenogest analysis was performed on a LC/MS system consisting of a Waters Acquity UPLC and an Applied Biosystem API4000 mass spectrometer. Separation was accomplished using an Acquity UPLC BEH C18 reverse phase column (1.7 μm, 2.1 × 50 mm) and mobile phases of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Altrenogest was eluted with a 2 min gradient from 60% to 95% B at flow rate 0.5 mL/min. MRM transition of altrengest was monitored from m/z 387 to 327 using positive atmospheric pressure chemical ionization mode on the mass spectrometer.

Results

Michaelis–Menten kinetic parameters, apparent K_m and V_{max}, of phenacetin O-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, bufuralol 1-hydroxylation, chlorozoxazone 6-hydroxylation and midazolam 1'-hydroxylation in porcine liver microsomes are shown in Table 1.

The peak areas of formed metabolites were compared with and without porcine drugs in the single point inhibitory screening assay. The number of replicates of blank controls was five times more than drug-dosed samples in incubation. This minimized the baseline variation to give more precise estimation in the initial screening. When