Toxicology Letters 233 (2015) 172–186

Contents lists available at ScienceDirect

Toxicology Letters

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

Understanding the biokinetics of ibuprofen after single and repeated treatments in rat and human *in vitro* liver cell systems

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HIGHLIGHTS

- IBU biokinetics was described in 3 *in vitro* systems after single and 14 day exposure.
- Modelling predicted the daily kinetic behaviour along the 14 days.
- Rapid uptake, a dynamic equilibrium in 1–2 days, no bioaccumulation were found.
- IBU was metabolised more efficiently in human than in rat hepatic cells as *in vivo*.
- The biokinetic profile could help explaining species differences and dynamics data.

ARTICLE INFO

Article history:
Received 9 November 2014
Received in revised form 5 January 2015
Accepted 7 January 2015
Available online 8 January 2015

Keywords:
Biokinetics
Ibuprofen
In vitro
Primary rat hepatocytes

ABSTRACT

Common *in vitro* toxicity testing often neglects the fate and intracellular concentration of tested compounds, potentially limiting the predictability of *in vitro* results for *in vivo* extrapolation. We used *in vitro* long-term cultures of primary rat (PRH) and human hepatocytes (PHH) and HepaRG cells to characterise and model the biokinetic profile of ibuprofen (IBU) after single and daily repeated exposure (14 days) to two concentrations. A cross-model comparison was carried out at 100 μM, roughly corresponding to the human therapeutic plasma concentration. Our results showed that IBU uptake was rapid and a dynamic equilibrium was reached within 1 or 2 days. All three cell systems efficiently metabolised IBU. In terms of species-differences, our data mirrored known *in vivo* results. Although no bioaccumulation was observed, IBU intracellular concentration was higher in PRH due to a 10-fold lower metabolic clearance compared to the human-derived cells. In HepaRG cells, IBU metabolism increased over time, but was not related to the treatment. In PHH, a low CYP2C9 activity, the major

Abbreviations: ADR, adverse drug reaction; bcell, biliary cell; BSA, bovine serum albumin; C_{cell}, concentration in cell lysate; C_{max}, human therapeutic peak plasma concentration; C_{med}, concentration in assay medium; CV, coefficient of variation; CYP, cytochrome P450; DME, drug metabolising enzyme; DMEM, Dulbecco modified eagle medium; DMSO, dimethyl sulphoxide; FBS, foetal bovine serum; F_{in}, entry rate flow for one cell; F_{out}, exit rate flow for one cell; HMM, hepatocyte maintenance medium; IBU, ibuprofen; ITS, insulin transferrin selenium; k_1, rate constant for binding to medium proteins; k_2, rate constant for unbinding from medium proteins; K_m, Michaelis–Menten constant; LOD, limit of detection; LOQ, limit of quantification; MCMC, Markov-chain Monte Carlo; N_{cell}, number of cells in the assay system; NOAEC, no observed adverse effect concentration; OECD, Organisation for Economic Co-operation and Development; PHH, primary human hepatocytes; PBPK, physiologically-based pharmacokinetics; PRH, primary rat hepatocytes; Q_{cell}, total quantity in cell lysate; Q_{med}, total quantity in assay medium; Q_{prot}, total quantity bound on protein; SD, standard deviation; SW, sandwich; $t_{1/2}$, half-life; TC, toxic concentration; T_{max}, time to reach C_{max}; V_{cell}, volume of a cell; V_{max}, maximal metabolic rate; V_{med}, volume of assay medium.

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http://dx.doi.org/10.1016/j.toxlet.2015.01.006

Primary human hepatocytes
HepaRG cells

IBU-metabolising CYP, led to an increased cytotoxicity. A high inter-individual variability was seen in PHH, whereas HepaRG cells and PRH were more reproducible models. Although the concentrations of IBU in PRH over time differed from the concentrations found in human cells under similar exposure conditions.

1. Introduction

Attrition during drug discovery and development is a major hurdle to the launch of a drug and lack of efficacy and unacceptable toxicity are the two major reasons (Kola and Landis, 2004). Usually, the potential risk for human health of pharmaceuticals is assessed in the early phases of development on the basis of animal testing. However, the extrapolation of observed adverse drug reactions (ADRs) from animals to humans is often difficult. The discrepancies can be frequently attributed to different kinetic behaviours of the compound in the different species (Shanks et al., 2009).

In the last decades, *in vitro* models have improved substantially, resulting in applications accepted as valuable tools to characterise and optimise compounds in terms of efficacy and safety. Most of these *in vitro* models apply a single exposure to deliver discrete information on single endpoints. By contrast, more complex *in vitro* models, for the identification of systemic effects, lack acceptance mainly because they poorly correlate with *in vivo* data (Adler et al., 2011). Differences between *in vitro* and *in vivo* kinetics have been considered as one of the main reasons. Despite this, the implementation of biokinetic information in *in vitro* systems has been mainly ignored. As a consequence, an observed effect indicated by *e.g.* EC_{50} (effective concentration causing the 50% of the effect) or NOAEC (no observed adverse effect concentration) *in vitro* is typically assigned to the applied nominal concentration of the test item, assuming that 100% is available within the cell. On the contrary, a number of abiotic processes affects the fraction of a test chemical that is available for uptake into cells or tissue, reducing its bioavailability. These processes include compound solubility, volatility, stability in aqueous solutions, binding to membrane lipids and proteins in cell culture medium or adsorption to plastic devices. Furthermore, biotic processes, such as mechanisms of cell uptake/extrusion, metabolism, intracellular bioaccumulation (of parent and/or metabolites) as well as saturation of these processes can influence the compound's biokinetic behaviour, affecting the biologically effective dose of test chemical, able to interact with the target or cause toxicity. This hampers the interpretation of *in vitro* data to predict *in vivo* dose–response relationships and compare the true toxic potency of test compounds (Groothuis et al., 2013). Thus, the intracellular concentration is a much more relevant parameter to enable the derivation of a NOAEC *in vitro*. This NOAEC can be then transformed to *in vivo* doses using appropriate modelling techniques, such as physiologically-based pharmacokinetics (PBPK) modelling.

Previous groups have shown that adsorption to plastic devices (Tirelli et al., 2007), binding to macromolecules in the medium (Gülden et al., 2001; Seibert et al., 2002), evaporation of the chemical (Kramer et al., 2012) and the number of cells in the cell system (Gülden et al., 2001, 2010) influence the actual biologically effective concentration and thus the cytotoxic potential of a compound. A recent paper reviewed a number of factors affecting bioavailability of test chemicals in *in vitro* assays and different dose metrics for *in vitro* setups (Groothuis et al., 2013).

To further support this concept, the application of a recently developed model to a set of hypothetical chemicals as well as to 1194 real substances (predominantly from the ToxCast chemical database) shows that the potential range of concentrations and chemical activities under assumed test conditions can vary by orders of magnitude for the same nominal concentration (Armitage et al., 2014).

There is an urgent need for predictive *in vitro* models to identify ADRs in the early phases of drug development, especially for the liver. As the main drug metabolising organ, the liver plays a central role in drug-induced toxicities. Furthermore, repeated drug administration is a more relevant exposure scenario for therapeutics, being usually evaluated in specified *in vivo* repeated-dose toxicity testing. In order to mimic repeated exposures *in vitro*, models retaining *in vivo* characteristics for a sufficiently long time frame should be used. Both hepatotoxicity and repeated exposure were addressed in this work by using different long-term hepatic culture systems.

Primary hepatocytes are the gold standard for metabolism studies because these cells retain *in vivo*-like activities of drug metabolising enzymes (DMEs) (Guillouzo, 1998; Hewitt et al., 2007; Tuschl et al., 2008). However, monolayer cultures of primary hepatocytes lose the activity of some liver-specific enzymes within a few days (Guillouzo, 1998; Tuschl et al., 2009). By contrast, primary rat and human hepatocytes (PRH and PHH, respectively) cultured in a sandwich (SW) configuration with defined medium, maintain their metabolic capacities at acceptable levels over a prolonged time period (Parmentier et al., 2013; Tuschl et al., 2009). The cholangio-hepatocarcinoma derived cell line HepaRG™ has proven itself valuable for many applications, including the prediction of metabolism-dependent hepatotoxicity (Aninat et al., 2006; Anthérieu et al., 2012). This human-derived cell line is a promising system, because after proliferation and differentiation phases it holds adequate and rather stable activity of DMEs throughout long-term culture.

The EU FP7 Project Predict-IV aimed to provide an improved predictability of the non-clinical safety testing by using *in vitro* tests, proposed to integrate dynamics and biokinetics in *in vitro* models after repeated exposure. This paper describes some of the obtained results comparing the three hepatic models described above to study the kinetic behaviour of ibuprofen (IBU), after acute and long-term repeated treatment.

IBU, a non-steroidal anti-inflammatory drug, seldom inducing ADRs in the liver, has been used as model compound, selected on the basis of its physicochemical and metabolic properties.

To the best of our knowledge the *in vitro* biokinetics after single (d0/1) and repeated exposures (d13/14) of a drug are here described for the first time. The integration of biokinetics to well-established rat and human long-term liver culture systems addresses most of the current issues of *in vitro* systems described above. The approach further includes the application of PK modelling, by integrating the kinetic experimental parameters obtained in the different *in vitro* systems, and being a fundamental tool for the extrapolation of *in vitro* data to the *in vivo* situation.

2. Materials and methods

2.1. Chemicals and reagents

IBU was purchased from Sigma–Aldrich (Steinheim, Germany; St. Quentin-Fallavier, France) or Calbiochem (Darmstadt, Germany). For the culture of the PRH in sandwich configuration Collagen from rat tail tendon (Roche, Mannheim, Germany) was used, while PHH were covered with Geltrex™ from Gibco® (Thermo Fisher Scientific, Illkirch, France). The perfusion buffer components were from Merck Chemicals (Merck KGaA) and