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Biokinetics in repeated-dosing in vitro drug toxicity studies

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ABSTRACT

The aim of the EU FP7 Predict-IV project was to improve the predictivity of in vitro assays for unwanted effects of drugs after repeated dosing. The project assessed the added benefit of integrating long-lived in vitro organotypic cell systems with 'omics' technologies and in silico modelling, including systems biology and pharmacokinetic assessments. RPTEC/TERT1 kidney cells, primary rat and human hepatocytes, HepaRG liver cells and 2D and 3D primary brain cultures were dosed daily or every other day for 14 days to a selection of drugs varying in their mechanism of pharmacological action. Since concentration–effect relationships not only depend on the activity of the drug or the sensitivity of the target, but also on the distribution of compounds in the in vitro system, the concentration of a selection of drugs in cells, microtitre plate plastic and medium was measured over time. Results, reviewed in this paper, indicate that lipophilic drugs bind significantly to plastic labware. A few drugs, including less lipophilic drugs, bind to cell-attachment matrices. Chemicals that reach high concentrations in cells, including cyclosporin A and amiodarone, significantly accumulate over time after repeated dosing, partly explaining their increased toxicity after repeated dosing, compared to a single dose.

1. Introduction

Many derivatives of a lead compound exhibiting a desired pharmacological effect are synthesised early on in the development of a new pharmaceutical entity in order to identify the ones with the most optimal pharmacological response. In order to assess the safety of these lead compounds before the 'first dose in man', toxicity testing of a large number of chemicals on animals is necessary and a costly endeavour (Sasseville et al., 2004). This testing represents one of the major bottlenecks in drug development as toxicity testing in preclinical studies is time consuming and requires large numbers of animals and considerable amounts of test compound. In addition, the high costs of toxicity testing are exacerbated by the high drug attrition rate, where 23% of registered drugs are retracted due to adverse reactions not predicted in animal models (Kola, 2008). These adverse reactions are often idiosyncratic and occur after repeated dosing. Major reasons for the suboptimal correlation between animal and human toxicity are the inter- and intra-species differences in pharmacokinetics (Park et al., 2011).

In light of both ethical and financial costs associated with drug safety testing on animals, human cell-based in vitro assays are increasingly used to screen drug candidates for human-relevant pharmacokinetic properties and molecular mechanisms of toxicity prior to pre-clinical testing in animals. However, the move from using in vitro assays for hazard identification, i.e. the mere potential of a chemical to cause an effect, to hazard characterization in drug development, i.e. dose–response assessment, is still in its early stage of development. It is generally accepted that no single stand-alone in vitro test sufficiently replaces an animal-based toxicity test and thus an integrated strategy is required. Such strategy calls for a battery of in vitro assays employing long-lived, highly functional organotypic cell cultures and a mechanistic understanding of the molecular events leading to adverse health effects (Adler et al., 2011). For such in vitro test battery to be used in a risk assessment procedure, a point of departure needs to be derived from the set of dose–response relationships obtained from these assays and translated into a toxicologically equivalent dose in humans. Indeed, the pharmacokinetics (i.e. the absorption, distribution, metabolism and excretion from a body) of a drug determines the concentration over time of the drug (or its toxicologically relevant metabolite) at the target site, which strongly dictates the drug's toxicity. These processes need to be integrated into a meaningful in vitro-based drug safety testing strategy (Adler et al., 2011).

To improve the predictivity of in vitro systems for unwanted effects of drugs after repeated dosing, the aim of the EU 7th Framework Project, Predict-IV, was to develop such a testing strategy integrating in vitro systems with knowledge of cell biology, mechanistic toxicology and in silico (pharmacokinetic) modelling. The project focussed its efforts on developing testing strategies by using in vitro assays with cells of human origin (whenever possible) and representing target organs most frequently affected by

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poorly predicted drug toxicity, namely the liver, kidney and central nervous system. It opted for using primary human hepatocytes, primary rat hepatocytes and the human hepatoma cell line HepaRG as its main liver models (Mueller et al., 2015), 2D mouse and 3D rat primary brain cell models with an in vitro blood brain barrier (BBB) model to predict neurotoxicity (Culot et al., 2008, 2013; Schultz et al., 2015), and the human renal proximal tubule cell line RPTEC/TERT1 (Wieser et al., 2008; Aschauer et al., 2015b) as its model of choice for predicting nephrotoxicity. Culture conditions were adapted to maintain highly differentiated organotypic cells in culture for 14 days, during which the cells were exposed daily to a selection of 27 drugs varying in their mechanism of pharmacological action and known to cause hepatotoxicity, nephrotoxicity and/or neurotoxicity after repeated use. For a holistic, mechanistic approach, in depth characterization of molecular perturbations induced by the drugs was performed by integrating a suite of 'omics' technologies (e.g. Wilmes et al., 2013). Moreover, exposure conditions and changes within the assays over the 14-day exposure period were monitored and modelled (e.g. Pomponio et al., 2015a,b; Truisi et al., 2015). Dose response analyses and physiologically based pharmacokinetic (PBPK) models were developed to relate daily oral exposure to in vitro derived points of departures (Hamon et al., 2015).

2. Role of in vitro biokinetics in quantitative in vitro–in vivo extrapolation (QIVIVE) studies

Predict-IV uniquely devoted a separate work package (WP3 'Non animal-based models for in vitro kinetics and human kinetic prediction') to propose and apply a step-wise strategy to measure and model cell exposure levels over time of a selected number of drugs in the developed in vitro assays. The aim was to assess whether and how knowledge of the kinetics of drugs in in vitro assays helps to explain the variations in observed effects between drugs, cell types and assay setup, and – in so doing – improve the predictive value of in vitro observed effect concentrations. The inclusion of this work package was based on an increasingly acknowledged problem that the in vitro nominal concentration, the concentration in medium that is added to the cells in vitro and traditionally used to express in vitro concentration–effect relationships, is not necessarily proportional to the biologically effective dose (BED), the concentration at the target site inside the cells, across in vitro assays and chemicals, and between in vitro and in vivo systems (Groothuis et al., 2015). The BED is most closely related to the initial molecular changes caused by the drug in the cell and may represent only a fraction of the nominal concentration (Escher and Hermens, 2004; Paustenbach, 2000). A drug added to an in vitro test system may significantly bind to serum constituents such as albumin and lipids (Seibert et al., 2002), sorb to the plastic of a microtitre plate (Kramer et al., 2012), or evaporate into the headspace (Knöbel et al., 2012; Tanneberger et al., 2013). These processes reduce the in vitro bioavailability of the drug, which determines its concentration at the target site. Moreover, assays may vary in their assay setup (e.g. medium composition) and chemicals may vary in their affinity for in vitro system components, explaining differences in target concentrations across chemicals and assays despite similar concentrations added to the systems (Armitage et al., 2014; Kramer et al., 2012). As such, the nominal concentration in medium is not an adequate indicator of exposure to cells when interpreting and comparing in vitro toxicity data for different chemicals and between different in vitro systems. Indeed, concentration–effect relationships not only depend on the activity of the drug or the sensitivity of the target, but also on the distribution of compounds in the in vitro system (Gülden and Seibert, 2003).

Previous studies have generally focussed on assessing the distribution of chemicals in in vitro systems done after single exposures in simple, metabolically inactive cytotoxicity assays (Gülden and Seibert, 2003; Kramer et al., 2012; Stadnicka-Michalak et al., 2014; Tirelli et al., 2007). It is generally assumed that the test chemical in these assays reaches a chemical equilibrium between the exposure medium, well plate plastic and cell concentration, from which the freely available concentration, generally considered independent of assay setup and more closely related to the BED than the nominal concentration, can be ascertained (Armitage et al., 2014; Gülden and Seibert, 2005; Kramer et al., 2012). However, the assays developed in the Predict-IV project consisted of highly differentiated cells, differing in their metabolic competence and expression of transporters, and dosed repeatedly with test chemicals over a period of 14 days. Simple chemical equilibrium models described in literature estimating the distribution of the drugs are unlikely to suffice. Indeed, differences in observed (cytotoxic) effects between single and repeated dosing may be attributable to an accumulation of the chemical (or its metabolites) in the cells over time, after repeated dosing. In addition, differences in observed effects between cell types, e.g. in vitro kidney, liver and brain models, may be attributable to differences in the uptake and efflux of drugs into cells and its metabolic activation and clearance. By not understanding these differences in distribution of drugs in the Predict-IV in vitro test battery hampers the extrapolation of the observed effects to the in vivo system, where accumulation in cells over time may vary significantly from the in vitro situation. To assess the role of the in vitro distribution of drugs in explaining differences in the toxic potential of drugs across the assays tested in the Predict-IV project, the distribution of a selected number of drugs was measured in kidney, liver, brain and intestinal absorption models (Table 1). The results of these studies are reviewed in this paper.

3. Extracellular concentrations of drugs over time

3.1. Chemical stability

Chemical stability in solution determines the concentration in cells and subsequently its potential to perturb molecular pathways in vitro. The concentration in stock solutions and exposure medium of drugs listed in Table 1 were measured over time. Whereas most drugs were chemically stable in exposure medium as well as in the vehicles used to prepare stock solutions, i.e. distilled water, methanol and DMSO, adefovir dipivoxil hydrolysed significantly in exposure medium of RTPEC/TERT1 cells at 37 °C, hampering the interpretability of their in vitro effect concentrations. Less than 15% of the parent compound was left in solution, highlighting the importance of assessing the effect of hydrolysis products in addition of parent drugs in in vitro toxicity assays (Crean et al., 2015). Amiodarone significantly hydrolysed in distilled water, but not in exposure medium, methanol or DMSO. Stock solutions for amiodarone were therefore only prepared in methanol or DMSO, which were used to directly spike the exposure medium (Pomponio et al., 2015a,b). Data from the Predict-IV project highlight the benefit of measuring the drug concentrations in both stock and working solutions to avoid aberration in cell treatment. The nominal concentration differed from the measured concentration in exposure medium up to 30% for amiodarone and ibuprofen (Pomponio et al., 2015a; Truisi et al., 2015) and even greater differences were reported for cyclosporine A in treating neuronal cells (Bellwon et al., 2015b). As opposed to be being solely attributable to biological variation, variations in effect concentrations between replicate experiments may be attributable to inconsistencies between nominal and measured concentrations of drugs in exposure medium added to in vitro assays.

3.2. Sorption to plastic

Sorption to in vitro system components such as plastic labware and microtitre plates was shown to significantly reduce the freely available drug concentration in in vitro assays for a number of lipophilic drugs. Up to 60% of amiodarone, one of the most lipophilic drugs tested in WP3 of the Predict-IV project, with a $\log D_{7.4}$ of 3.4, was found to bind to plastic labware (Pomponio et al., 2015a). The drug was also found to significantly bind to microtitre plate plastic in a dose- and time-dependent manner (15–35%), suggesting plastic binding saturates at higher concentrations of the drug. The extent of plastic binding was reduced in the presence