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Use of physiologically based kinetic modeling-facilitated reverse dosimetry of in vitro toxicity data for prediction of in vivo developmental toxicity of tebuconazole in rats

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HIGHLIGHTS

- A PBK model was developed that describes tebuconazole kinetics in rats.
- It was used to predict developmental toxicity based on in vitro toxicity data.
- The predicted in vivo dose-response data were used to determine a BMDL10.
- The BMDL10 differed only 3-fold from the reported NOAEL values.
- BMDL10 value from predicted toxicity data may serve as a POD for risk assessment.

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ABSTRACT

Toxicological hazard and risk assessment largely rely on animal testing. For economic and ethical reasons, the development and validation of reliable alternative methods for these animal studies, such as in vitro assays, are urgently needed. In vitro concentration-response curves, however, need to be translated into in vivo dose-response curves for risk assessment purposes. In the present study, we translated in vitro concentration–response data of the antifungal compound tebuconazole, obtained in the ES-D3 cell differentiation assay, into predicted in vivo dose–response data for developmental toxicity using physiologically based kinetic (PBK) modeling-facilitated reverse dosimetry. Using the predicted in vivo dose–response data BMD(L)10 values for developmental toxicity in rat were calculated and compared with NOAEL values for developmental toxicity data in rats as reported in the literature. The results show that the BMDL10 value from predicted dose–response data are a reasonable approximation of the NOAEL values (ca. 3-fold difference). It is concluded that PBK modeling-facilitated reverse dosimetry of in vitro toxicity data is a promising tool to predict in vivo dose-response curves and may have the potential to define a point of departure for deriving safe exposure limits in risk assessment.

1. Introduction

The determination of safe human exposure levels of chemicals in toxicological risk assessments largely relies on animal toxicity data. In these toxicity studies, the majority of the animals are used for reproductive and developmental toxicity testing (Van der Jagt et al., 2004). For economic and ethical reasons, there is large interest in the development of in vitro test systems as alternatives for the animal studies. A number of in vitro methods have been developed to screen compounds for potential developmental toxicity, using primary cultures of embryonic cells or embryonic stem cell lines, such as the embryonic stem cell test (EST), as well as test methods using whole embryos, such as the ex ovo assay of chicken embryos or the whole embryo culture (WEC) assay using rat embryos (Adler et al., 2011; Eisenbrand et al., 2002; Genschow et al., 2002; Louisse et al., 2010).

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Among these alternative test systems for the assessment of developmental toxicity, the EST does not require the use of primary animal tissues and can therefore be considered as an animal-free test. The differentiation assay of the EST has been proven useful in the prediction of the in vivo potency ranking of structurally related compounds, such as glycol ethers, retinoids and phenols (De Jong et al., 2009; Louisse et al., 2011; Strikwold et al., 2012). This assay uses the blastocyst-derived embryonic stem cell line D3 (ES-D3) that spontaneously differentiates into contracting cardiomyocytes when cultured as embryoid bodies, and it determines the concentrations of test compounds that inhibit this process as a measure of in vitro developmental toxicity. Recently, we have demonstrated that the ES-D3 cell differentiation assay is useful to predict the relative developmental toxicity potencies of some antifungal compounds in vivo (Li et al., 2015a; Li et al., 2015b). Furthermore, we showed that the correlation between the in vitro effect concentrations and the in vivo effect doses improves when kinetic data on placental transfer are taken into account (Li et al., 2015a; Li et al., 2015b).

Although the ES-D3 cell differentiation assay, in combination with data on placental transfer from the BeWo model, was shown to give a good indication of relative in vivo potencies of antifungal compounds, in vitro data are often not used for risk assessment. An important reason is that in vitro assays provide in vitro concentration–response curves on adverse effects on the cells, without taking into account aspects of absorption, distribution, metabolism and excretion (ADME) which occur in the in vivo situation. Therefore, the implementation of in vitro models as stand-alone in risk assessment frameworks is limited. The application of so-called reverse dosimetry enables the translation of in vitro concentration–response curves into in vivo dose–response curves using physiologically based kinetic (PBK) models, providing a platform to use in vitro toxicity data for risk assessment (Louisse et al., 2016; Louisse et al., 2014; Louisse et al., 2010; Punt et al., 2011; Strikwold et al., 2013). PBK models (also called PBTK (physiologically-based toxicokinetic), PBBK (physiologically based biokinetic) and PBPK (physiologically-based pharmacokinetic) models) quantitatively describe ADME processes of a compound in the body, and can relate external (toxic) doses to internal (toxic) concentrations (Rietjens et al., 2011). In reverse dosimetry approaches aiming to predict in vivo toxic dose levels, in vitro toxic effect concentrations are considered as surrogate tissue or blood concentrations that would cause adverse effect in the in vivo situation. The in vivo toxic dose levels can be predicted using a PBK model to calculate the doses that are needed to reach these internal effect concentrations. PBK modeling-facilitated reverse dosimetry is the only method that enables the quantitative translation of in vitro data to the in vivo situation. Therefore, there is increased interest in using the PBK modeling-facilitated reverse dosimetry approach to derive points of departure (PoDs) for risk assessment, such as Benchmark Dose (BMD) values, based on the translation of in vitro concentration–response data obtained from in vitro toxicity assays (Coecke et al., 2013; Louisse et al., 2012). Our group has shown that PBK modeling-facilitated reverse dosimetry of developmental toxicity data obtained in the ES-D3 cell differentiation assay resulted in the correct prediction of in vivo dose–response curves of glycol ethers, phenol and retinoic acid (Louisse et al., 2014; Louisse et al., 2010; Strikwold et al., 2013). More proofs-of-principle of chemicals from other categories are needed to further evaluate this approach.

In the present study, we assessed whether the PBK modeling-facilitated reverse dosimetry approach could be used to approximate the NOAEL value for developmental toxicity of the antifungal compound tebuconazole in vivo. To this aim we developed a PBK model for tebuconazole in the rat, based on in vitro- and in silico-derived parameter values, and used this model to translate the results of the ES-D3 cell differentiation assay after exposure to tebuconazole to a predicted in vivo dose-response curve for developmental toxicity in rat. For risk assessment purposes, the NOAEL from in vivo studies is an important PoD to set safe exposure levels, and NOAEL values for developmental toxicity of tebuconazole are present in the literature. In more modern approaches, BMDL10 values are used as PoDs to set safe exposure levels. We therefore derived BMD10 and BMDL10 values from our predicted dose-response data in rats and assessed whether the obtained BMDL10 value can serve as PoD in the risk assessment by comparing it with reported NOAEL values.

2. Materials and methods

2.1. Chemicals

Tebuconazole was purchased from Sigma-Aldrich (Steinheim, Germany). Dimethyl sulfoxide (DMSO) was purchased from Acros Organics (Geel, Belgium). Penicillin, streptomycin, L-glutamine, non-essential amino acids, phosphate-buffered saline (PBS) and Hank's balanced salt solution (HBSS) were obtained from Invitrogen (Breda, the Netherlands).

2.2. High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed to quantify the amount of the test compound tebuconazole and its metabolites. The HPLC system used consisted of a Waters (Milford, MA) 600 controller and a 600 pump, equipped with a photodiode array detector set to record absorption of wavelengths between 200 and 400 nm. A Waters 717 plus autosampler was used for sample injection. The temperature of the autosampler was kept at 7 °C.

For analysis of all compounds, 50 μl sample was injected to a C18 5 μm reverse-phase column (150 mm × 4.6 mm I.D.) with a guard column (7.5 mm × 4.6 mm I.D.) (Alltech, Bergen op Zoom, the Netherlands). The mobile phase used for analysis consisted of (A) 0.1% trifluoroacetic acid in nanopure water and (B) HPLC-grade acetonitrile. Elution was at a flow rate of 0.8 ml/min, starting at 22% B with a linear increase to 100% B in 8 min. Subsequently, the gradient returned linearly to the initial condition in 10 min and remained 2 min at this condition prior to the next injection.

2.3. PBK modeling-facilitated reverse dosimetry approach

The PBK modeling-facilitated reverse dosimetry approach to predict in vivo developmental toxicity based on in vitro toxicity data (Fig. 1) applied in the present study consists of six steps, being (1) the determination of in vitro concentration–response data for tebuconazole in the ES-D3 cell differentiation assay, (2) the development of a PBK model for tebuconazole in rat, (3) the evaluation of the predictions made by the PBK model, (4) the translation of in vitro concentration–response data into in vivo dose–response data using the PBK model, (5) the derivation of a BMD(L)10 value based on the predicted dose–response data and (6) the evaluation of the approach by comparison of the predicted BMDL10 value with NOAEL values for in vivo developmental toxicity as reported in the literature.

2.3.1. Determination of in vitro concentration–response data in the ES-D3 cell differentiation assay

The in vitro concentration–response data on the tebuconazole-induced inhibition of ES-D3 cell differentiation into contracting cardiomyocytes were obtained from our earlier study (Li et al., 2015b). The data of three independent experiments of this study are presented in Fig. 1a.