Toxicology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Toxicology

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

Multiscale modelling approaches for assessing cosmetic ingredients safety

Frédéric Y. Bois[a,*], Juan G. Diaz Ochoa[c], Monika Gajewska[b], Simona Kovarich[d], Klaus Mauch[c], Alicia Paini[b], Alexandre Péry[a], Jose Vicente Sala Benito[b], Sophie Teng[a], Andrew Worth[b]

[a] INERIS, DRC/VIVA/METO, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France
[b] European Commission Joint Research Centre, Institute for Health and Consumer Protection, Systems Toxicology Unit, Via Enrico Fermi 2749, Ispra, VA, Italy
[c] Insilico Biotechnology AG, Meitnerstrasse 8, 70563 Stuttgart, Germany
[d] S-IN Soluzioni Informatiche, via G. Ferrari 14, 36100 Vicenza, Italy

ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form 30 November 2015
Accepted 31 May 2016
Available online xxx

Keywords:
PBPK model
Multiscale model
In silico toxicity prediction

ABSTRACT

The European Union's ban on animal testing for cosmetic ingredients and products has generated a strong momentum for the development of *in silico* and *in vitro* alternative methods. One of the focus of the COSMOS project was *ab initio* prediction of kinetics and toxic effects through multiscale pharmacokinetic modeling and *in vitro* data integration. In our experience, mathematical or computer modeling and *in vitro* experiments are complementary. We present here a summary of the main models and results obtained within the framework of the project on these topics. A first section presents our work at the organelle and cellular level. We then go toward modeling cell levels effects (monitored continuously), multiscale physiologically based pharmacokinetic and effect models, and route to route extrapolation. We follow with a short presentation of the automated KNIME workflows developed for dissemination and easy use of the models. We end with a discussion of two challenges to the field: our limited ability to deal with massive data and complex computations.

1. Introduction

The European decision to ban animal testing for cosmetic ingredients has generated a strong momentum for the development of *in silico* and *in vitro* alternative methods. One of the aims of the COSMOS project (funded by the European Commission and by Cosmetics Europe under the 7th Framework Programme) was to develop approaches for the *ab initio* prediction of kinetics and toxic effects through multiscale pharmacokinetic modeling and *in vitro* data integration. We present here a summary of the relevant models and results obtained by the COSMOS team. Our major activities were focused on modeling toxicokinetics and toxicodynamics (effects) *in vitro* and *in vivo*, which are required to perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) (Adler et al., 2011; Bessems et al., 2014; Coecke et al., 2012; Quignot et al., 2014).

Kinetic modeling is a relatively well developed field, with well established compartmental or physiologically based pharmacokinetic (PBPK) models (Corley, 2010; Gibaldi and Perrier, 1982; Peters, 2011). In PBPK models, the transport and overall fate of the substance administered is governed by anatomic and physiological considerations. The models can have a generic structure, which makes them easier to use (no need to develop new equations for a new substance) (Beaudouin et al., 2010; Corley, 2010; Jamei et al., 2009; Willmann et al., 2007). Most of their parameters are physiological, meaning that they do not depend on the chemical considered, but solely on the subject exposed (at least when exposure to the chemical does not alter appreciably the body functions, such as blood flows). Compilations of average parameter values for several species are available, and even values for specific sub-groups (children, pregnant women, elderly people . . .) (Bois et al., 2010). The few remaining parameters, which depend on chemical structure, are sufficiently mechanistic to be obtained by quantitative structure-property relationships (QSPR), or *in vitro* experiments (Hamon et al., 2015). *In vitro* kinetic models are different from PBPK models, in that they are not particularly physiological, but rather represent the *in vitro* system modeled. Some generic models have been proposed for simple *in vitro* systems – including by us, the virtual cell based assay (VCBA)

* Corresponding author.
E-mail address: frederic.bois@ineris.fr (F.Y. Bois).

http://dx.doi.org/10.1016/j.tox.2016.05.026

model, see below – but more complex systems (*e.g.*, bi-compartmental systems, micro-chips) require specific developments (Armitage et al., 2014; Crean et al., 2014; Ouattara et al., 2011; Truisi et al., 2015; Wilmes et al., 2013; Zaldívar et al., 2010).

The state of the art is far less advanced for "extrapolatable" toxicodynamic models. Traditional toxicodynamic models are similar to compartmental models (and in fact extend those with *ad hoc* "effect" compartments). They are data fitted and thus specific for a given experiment or clinical trial (Csajka and Verotta, 2006); they are not designed for extrapolations (except very basic time and dose extrapolations). The equivalent of PBPK models in toxicodynamics are biologically-based models. Among the earliest of such models were the biologically based carcinogenesis models (Armitage, 1985; Moolgavkar and Knudson, 1981). However, given the obscurity and complexity of the cancer process (still not elucidated), those models were at the same time too simple (to avoid criticisms) and too complex (to be used in a regulatory framework). They were never really used for risk assessment, except in the extremely simplified form of the multistage cancer dose-response model (Crump and Howe, 1984). A new generation of models is emerging with "systems biology" models (Geenen et al., 2012; Jusko, 2013) and "physiome" (or virtual human) models (Bassingthwaighte, 2000; Hunter and Borg, 2003). Systems biology models are bottom-up models rooted in biochemistry and benefiting from our increasing understanding of cellular signaling and transcriptional control pathways (facilitated by the explosion of omics data). Physiome models are inherently top-down and multiscale and therefore the closest equivalent to PBPK models (PBPK models can in fact be thought of as vascular body-level solute transport models). They started as high-level descriptions of organ physiology and are increasing their resolution to the cell level (arguably the right level to start understanding the origin of most toxic injuries). Originating from two different research communities (biochemists vs. physiologists) these two approaches are slowly merging as they meet each other at the tissue level. Furthermore, the two approaches did not escape the attention of the members of the 21st century toxicology panel of the US National Academy of Sciences, who placed them at the heart of their vision statement (National Research Council (NRC), 2007), gaining much attention, given the authority of its authors. The ensuing consideration of toxicity pathways and modes of action (MOA) met happily the adverse outcome pathway (AOP) thinking in vogue in the ecotoxicology community (Tollefsen et al., 2014), and is still hesitating about changing name . . . Meanwhile, virtual organ programs have been heavily funded (*e.g.*, by the Virtual Liver project of the German Ministry of Research) (Holzhütter et al., 2012) given their potential impact for predictive drug safety assessment. In short, we are witnessing a convergence of systems biology and virtual organs modeling around the concept of quantitative MOAs, fully amenable to QIVIVE and risk assessment.

This paper follows a bottom-up integration logic: A first section presents our work at the organelle/cellular level. We then go toward modeling cell levels effects (monitored continuously), multiscale PBPK and effect models, and route to route extrapolation. We end with a short presentation of the automated KNIME workflows developed for dissemination and easy use of our models.

2. From organelles to cells

Before manifesting themselves at the cellular level, most toxicity effects start at the scale of organelles. Mitochondria in particular are often targets of toxicity. They perform two critical functions in the cell: the production of more than 90% of the cell's energy, and the control of cell survival as an integral part of programmed cell death (apoptosis). Three general adverse effects result from mitochondrial toxicity: 1. Disrupted energy metabolism; 2. Increased free radical generation; and 3. Altered apoptosis. We addressed the disruption of mitochondrial energy metabolism by measuring and simulating mitochondrial membrane potential (MMP). The measurement of MMP provides information on the mitochondrion's ability to carry out oxidative phosphorylation (which couples electron transfer to ATP synthesis), and transfer ions and substrates across its inner membranes (Nicholls and Ward, 2000). Thus, one of the most common methods to detect mitochondrial toxicity is the monitoring of the cells' MMP. A variety of fluorescent dyes can be used to that effect in high throughput screening. For example, cationic dyes distribute to the mitochondrial matrix in accordance with Nernst's equation (Mitchell and Moyle, 1969), so that the MMP is given by:

$$\mathrm{MMP} = (\alpha \times V)\frac{R \times T}{F}\log\left(\frac{C_{cyt}}{C_{mit}}\right) \quad (1)$$

where R is the gas constant, T the temperature, F the Faraday constant, C_{cyt} the concentration of the chemical in the cell cytosol, C_{mit} its concentration in mitochondria, α a proportionality constant, and V the cell viability. C_{mit} is computed by integration of the following differential equation:

$$\frac{\partial C_{mit}}{\partial t} = K_{mit}(C_{aq} - C_{mit}) \quad (2)$$

where C_{aq} is the concentration in the aqueous phase of the cell, and K_{mit} is a diffusion rate constant dependent on the chemical and cell line used.

We report here results on the *in vitro* MMP disruption of HepaRG cells by caffeine, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), amiodarone and estragole. The MMP was measured and modeled using an extension of the VCBA model (Zaldívar et al., 2011, 2010). That model, like some others (Armitage et al., 2014; Crean et al., 2014; Hamon et al., 2014; Pomponio et al., 2014; Truisi et al., 2015; Wilmes et al., 2013) takes into account the fate of the test compound *in vitro*: partitioning between plastic vial walls, headspace, serum proteins and cells. It includes a model of cell growth and death and has been linked to a threshold model for cell killing. In the course of the COSMOS project, we developed VCBA models for amiodarone, caffeine, FCCP, coumarin, estragole, ethanol, and nicotine. To simulate MMP data, a mitochondrial sub cellular compartment and Nernst's equation were added to the VCBA model. *In vitro* HepaRG MMP data were used to optimize two parameters of Nernst's equation (α and K_{mit}) by least-square minimization. Fig. 1 shows the measured and simulated MMP as a function of the exposure concentration of the four chemicals assayed. The model was able to correctly reproduce the amiodarone and estragole data. The caffeine data show a peak at 0.01 M, which could not be reproduced by the model. The FCCP induced fast decrease of MMP at concentrations lower than 0.1 mM was not well captured by the model either. More experiments with different chemicals will be needed to fully understand the determinants of prediction accuracy.

3. Modeling *in vitro* kinetics and continuously measured cell effects

Two complementary models were used to analyze toxic effects *in vitro*, at the cell level. The first model is the VCBA model introduced above (Zaldívar et al., 2011, 2010). This model is well suited to analyze fixed point cytotoxicity data (as in high-content imaging assays). The second model can be used for continuous cytotoxicity monitoring, using electrical impedance measurements (Xing et al., 2006).

The latter model describes HepaRG cell viability loss following exposure to hepatotoxic molecules. It was applied it to three

Please cite this article in press as: F.Y. Bois, et al., Multiscale modelling approaches for assessing cosmetic ingredients safety, Toxicology (2016), http://dx.doi.org/10.1016/j.tox.2016.05.026