Recent developments in in vitro toxicology: perspectives of European research and Tox21

R. Marchan · C. van Thriel · H. M. Bolt

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During the past decades, substantial efforts have been made to establish alternative in vivo techniques to replace animal experiments (Liebsch et al. 2011; Höfer et al. 2004; Hengstler et al. 2012; Stewart and Marchan 2012; Godoy and Bolt 2012; Cadenas et al. 2012; Marchan 2011; Clift et al. 2011). One approach is the establishment of alternative methods for specific toxicological areas, such as skin and eye irritation, carcinogenicity, repeated dose toxicity of specific organs, reproductive toxicity and skin sensitization (Adler et al. 2011; Lilienblum et al. 2008). Recently, an expert panel estimated the time needed for the replacement of animal experiments (Adler et al. 2011). For example, the authors predicted that current animal tests for skin sensitization could be replaced in approximately seven years (Adler et al. 2011). Also, the development of models to predict lung absorption and renal as well as biliary excretion may require a similar time frame. However, for systemic endpoints of repeated dose toxicity, carcinogenicity or reproductive toxicity, a concrete estimation cannot be calculated (Adler et al. 2011). In the past years, articles published in the Archives of Toxicology have usually focussed on in vitro systems for specific organs, such as skin (Fabian et al. 2013; Basketter 2012), liver (Schug et al. 2013; Godoy et al. 2013; Garthoff 2011), kidney (Jennings et al. 2012; Valente et al. 2012), heart (Vávrová et al. 2011) and lung (Glahn et al. 2008), or on specific fields of toxicology, such as developmental toxicology (Strikwold et al. 2013), pharmacokinetics (Mori et al. 2012; Verma et al.

2012) and genotoxicity (Kirsch-Volders et al. 2011; Santovito et al. 2011).

Since 2008, American funding institutions have supported a different concept, known formally as Tox21 (Betts 2013; Attene-Ramos et al. 2013; Bucher 2013). This concept aims at ‘high-throughput screening, toxicity pathway profiling’ and ‘target-specific, mechanism-based’ performance of in vitro assays (Tice et al. 2013). Practically, large numbers of chemicals are tested for relatively simple endpoints, such as cytotoxicity, cell signalling, DNA damage, inhibition of drug-metabolizing enzymes, interaction with nuclear receptors and isolated molecular targets (Tice et al. 2013). A major difference to previous approaches is that a large number of in vitro assays are used to determine the activation of ‘toxicity pathways’ to help prioritize compounds or even develop predictive models for biological response in humans (Tice et al. 2013). In a recent keynote lecture at the Eurotox 2013 in Interlaken, Franz Oesch summarized the current status of Tox21. Unfortunately, the accuracy of most in vitro assays for predicting human or animal toxicity used in Tox21 was not sufficient to replace in vivo experiments. Recently, relatively critical comments have been published, and Tox21 clearly faces difficulties, mainly due to the following reasons:

- Xenobiotic metabolism is lacking in virtually all in vitro assays.
- Interactions between cells are poorly captured.
- Extrapolating from in vitro concentrations to in vivo dose or blood levels is not straightforward.
- Assessing the effects of chronic exposure conditions in vitro is not possible.
- Identifying when a perturbation to a gene or pathway would lead to an adverse effect in animals or humans remains a major challenge (Tice et al. 2013).

R. Marchan · C. van Thriel · H. M. Bolt (✉)
Leibniz Institut für Arbeitsforschung an der TU Dortmund,
Leibniz Research Centre for Working Environment and Human
Factors (IfADo), Ardeystrasse 67, 44139 Dortmund, Germany
e-mail: bolt@ifado.de

In addition to these criticisms, another reason may be that the assays chosen in Tox21 do not sufficiently represent the mechanisms by which adverse effects are caused in vivo. Despite the current difficulties with the Tox21 concept, the basic idea is nevertheless elegant. Two preconditions must be fulfilled to ensure Tox21's success: first, all relevant mechanisms of in vivo toxicity must be covered by in vitro tests. Second, we must be able to extrapolate from in vivo doses to local concentrations at critical target cells. This is needed to determine in vivo relevant concentrations for in vitro testing. It seems that both milestones have not yet been achieved. In response to the shortcomings of Tox21, the funding agencies in Europe have established research programmes to understand and model the in vivo situation with the overall goal to better understand the requirements needed for in vitro tests. An example is the German Federal Ministry of Education and Research-funded network, Virtual liver, which has primarily established models of general cellular functions, such as vesicular transport (Zeigerer et al. 2012) and lipid metabolism (Stewart et al. 2012; Marchan et al. 2012). Another major undertaking of the network has been to simulate how the liver responds to different types of stress, including intoxication (Höhme et al. 2007, 2010). Metabolic pathways and gene expression patterns of in vitro systems have carefully been compared to the in vivo situation to understand which mechanisms observed in vitro are also relevant in vivo (Godoy et al. 2009; Schug et al. 2013; Heise et al. 2012; Seeliger et al. 2013; Knobeloch et al. 2012; Zellmer et al. 2010). Another approach is the FP7 EU-funded project ESNATS. Stem cell-based in vitro systems have been established that recapitulate the development of stem cells to neuronal cells (Krug et al. 2013; Krause et al. 2013; Kadereit et al. 2012; Frimat et al. 2010). A major finding is that the in vitro differentiation process is compromised by well-known compounds, such as valproic acid and methylmercury, leading to developmental toxicity. Perhaps, projects in the future may profit from a combination of Tox21's high-throughput technology with Europe's in vivo relevant in vitro systems and systems biology approaches.

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