Innovating Human Chemical Hazard and Risk Assessment through an Holistic Approach

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Abstract

The innovation of chemical hazard and risk assessment based on *in vitro* and *in silico* modeling of human biology and toxicology is rapidly gaining momentum. Whilst animal studies have been the core basis of chemical safety evaluations for half a century, a wealth of animal-free alternative assays have been developed during that same period but have only scarcely gained implementation in the regulatory arena. The reductionist nature of such as says, and the intent towards one to one replacements of animal studies by alternative assays have limited progress in this area. This paper advocates a human based holistic approach to chemical safety assessment, based on an *in silico* description of human biology, the derivation of the adverse outcome pathway network from that description, its translation into batteries of *in vitro* and *in silico* assays to monitor critical key events in the pathway network, and the integration of the results by modern computational tools to predict health effects. Several ongoing international research projects are described which take on this challenge aiming at providing proofs of principle for the feasibility of this approach. This advance is supported by successes of the application of machine learning in clinical diagnostics and treatment. Crucial elements include the need for quantitative data integration, the management of large scale databases, and overall, the comprehensiveness of the testing strategy as to the coverage of the adverse outcome pathway network represented in the *in silico* system. Apart from the scientific innovation to human safety assessment, the ethical aspect of avoiding the detour of the animal study for determining human safety is an important additional gain of the human based approach. Whilst this innovation meets with practical challenges and possible pitfalls, it takes advantage of rapidly growing computational opportunities and in due course is expected to significantly benefit human health protection.

Keywords: Human biology • Toxicology • Machine learning

Introduction

Ever since the installment of globally agreed animal test protocols, the world of chemical hazard and risk assessment has been moving towards animal-free human-based test systems. Ethical as well as scientific arguments have driven the development of cell culture based assays with which crucial elements of toxicity pathways and the effects of chemicals thereupon could be monitored. In the field of developmental toxicology, an area with relatively high experimental animal use, a broad spectrum of alternative methods have been developed [1,2]. In this area, the embryonic stem cell test and its many variants have been among the most prominent assays both in fundamental mechanistic research and in their application to chemical hazard assessment. Initially built upon the effects on cardiac muscle cell differentiation as the readout parameter, other differentiation routes such as neural and bone cell lineages have been employed to broaden the biological domain of embryonic stem cell based assays [3-8]. A recent review provides a comprehensive overview of the history and current status of these assays [9].

Whilst basic requirements such as standardization, reproducibility, and transferability have generally been met successfully, the level of predictability of *in vitro* tests for *in vivo* developmental toxicity in animal species and in man appeared variable, amongst others depending on the choice of chemicals tested [10-12]. This illustrated the reductionist nature of *in vitro* test systems in general, as effects outside the biological domain of the test are simply not detectable in a given assay. This is one of the main reasons for the lack of acceptance of *in vitro* assays in the regulatory toxicology domain [13]. Batteries of assays with complementary biological domains have been designed in an attempt to enhance the biological domain covered and with that, to improve the overall *in vitro* productivity, with limited success [14]. This is again due to uncertainties about whether the *in vivo* biological system is sufficiently covered by the test battery to exclude false negative results. In addition, given the commonly applied binary nature of scoring compound effects (positive *vs.* negative) the extrapolation of *in vitro* effective concentrations to *in vivo* effective exposures meets with challenges [15]. Moreover, the comparison with animal data meets with the challenge of extrapolation to the human situation. Thus, the bottom-up approach of designing and combining individual *in vitro* assays to achieve a reliable level of toxicity assessment, sufficient for regulatory use, has reached its fundamental limits. Innovation towards reliable animal-free hazard and risk assessment needs a top-down holistic approach, starting from an integral description of the human biology domain that can be disrupted by toxicants [16].

The existing extensive knowledge of human biology and physiology can form the backbone for mapping out the physiologic processes that can be disrupted by toxic exposures to the extent that adverse outcomes are induced. This approach avoids the restriction of looking under the lamppost of most prominent changes and established assays, but raises awareness of collateral pathways that may also be affected and that may also be crucial in determining overall adversity. For instance, simplistic linear one-directional adverse outcome pathway descriptions from receptor binding to adverse health effects can be enhanced by including essential homeostatic feedback mechanisms that codetermine the threshold of adversity. In addition, kinetic modeling may assist in estimating the internal concentration at target of a compound after a given external exposure. Combining dynamic and kinetic modeling in an integrated computational approach founded in an integral description of human physiology allows a hazard and risk assessment that is comprehensive and therefore reliable in the regulatory context. This approach is currently being investigated in several projects.

Literature Review

The EU-Horizon project ONTOX aims at describing biology-based adverse outcome pathway networks for kidney and liver toxicity, as well as for developmental toxicity leading to neural tube closure defects [17]. Ontologies describing physiological parameters and their interaction are constructed to form the backbone for biology-based computational prediction models of toxicity. E.g. for developmental neurotoxicity, a physiologically driven computational model is being developed, which

allows the study of the interaction between the various regulating genes, soluble mediators, and the different cell types expressing them, leading to neural tube closure [18]. It allows for parameter sensitivity assessment of the system that guides the selection of parameters and related assays that need to be assessed after compound exposure. Several successful examples of computational modeling of developmental mechanisms have been shown by US EPA's Virtual Embryo project [19]. Measuring compound-induced changes in e.g. gene expression in assays such as the embryonic stem cell tests provides information that is then used as input for modifying parameter settings in the computational model, which can then predict whether neural tube closure will be compromised under these compound-induced changes in parameter settings. Thus, a holistic developmental model translates individual parameter changes in reductionist *in vitro* assays to the level of adverse effects on the intact developmental process.

In a project sponsored by the Dutch Science Agenda, named The Virtual Human Platform for Safety Assessment (https://vhp4safety.nl/), one case study includes the role of thyroid hormone in brain development, and the effect of disruption of thyroid hormone homeostasis on cognition and motor function development. In this case, the physiological regulatory basis of thyroid-mediated brain development is mapped in detail in a computational model, the adverse outcome pathway network is mapped within the biological framework, the crucial elements translated into the design of a testing battery of *in vitro* assays, and predictions of *in vivo* toxicity will be based on computational data integration. Other case studies in this project focus on kidney toxicity and on neurodegeneration, also by anchoring methodology in biology. These are first cases to be complemented in due course with others to expand the virtual human platform [16].

Related activities are ongoing in the Virtual Physiological Human Project (https://www.vph-institute.org/), which maps human physiology and pathophysiology in the interest of clinical diagnostics and treatment. Crucial elements of this holistic approach are FAIR data collection (https://www.go-fair.org/) [20], its quality control, and its integration into computational models that correctly reflect physiology and correctly predict perturbations by compound exposures at the level of the intact individual. In the clinical setting, successful examples are already in operation of personalized diagnosis and treatment decisions based on individual patient physiological data collection [21]. These decisions are founded on computational decision models, reflecting state of the art understanding of underlying physiology in healthy and disease states, generated through machine learning and continuously updated as new clinical data emerge. The successes of such models suggest that by analogy they can also be instrumental in the field of toxicology. They will function best if applied from a holistic perspective, incorporating the broadest possible up to date knowledge of human biology and physiology to optimize toxicity predictions.

Discussion

With the holistic approach to chemical hazard and risk assessment gaining momentum in innovative international project initiatives, the output of alternative non-animal test systems will be used differently to optimize its use and integration within the novel approach. Chemical risk assessment culminates in the derivation of points of departure and acceptable exposure levels. Thus, the quantitative aspect is crucial. Classical *in vitro* assay validation studies using compound scoring as positive *vs.* negative, have ignored aspects of potency and exposure levels. Rather, concentration-response data of individual assays need to be combined with kinetic modelling, translating *in vitro* effective concentrations to external exposures *in vivo*. In a holistic approach, it is not a single *in vitro* assay that determines hazard and risk, but the computational model that integrates all information of the battery of *in vitro* assays and their kinetic translation to *in vivo* predictions of dose-response [22].

As argued in the above, comprehensiveness of the coverage of human toxicity pathways in an innovated holistic approach to chemical hazard and risk assessment is key to increasing confidence towards regulatory acceptance. It should be realized that the data dossier underlying risk assessment will be in part different between old and new systems, and will never be complete in any system in the sense that all information that could be wished for will be available. This implies that, whereas the existing system based on globally accepted animal studies has data limitations, any new system will have limitations as well, but partly different in nature, which makes direct comparison of old and new difficult. In any case, the holistic basis, as well as the principle of human biology, as the starting points for a risk assessment approach remain persuasive arguments for this innovation. Though mammalian systems show extensive physiological similarity, important differences between species exist in e.g. endocrine homeostasis, developmental toxicity and carcinogenesis, that complicate interspecies extrapolation for risk assessment. This bottleneck is avoided by using human-based test systems and biomonitoring data, cohort data and clinical data.

This human focused approach also meets the ethical reservations related to the current extensive animal use for human safety assessment. The field of reproductive and developmental toxicology requires relatively high animal use, given the need to include both parental and offspring generations in study designs. This has stimulated the search for alternative approaches especially in this area, in which embryonic stem cell based assays have historically gained major attention. As indicated above, a wealth of differentiation routes have been established using stem cells from mice and man, gaining also from experience in the area of regenerative medicine, in which specific differentiated cell types are being generated for transplantation purposes. These assays have significant merits as to the determination of compound-induced disruption of embryonic cell differentiation, both at the molecular and the cellular level. With that they are expected to provide a significant contribution as part of a test battery under a holistic approach governed by human biology.

Conclusion

Revolutionary developments in artificial intelligence and machine learning, combined with expanding knowledge of the molecular mechanisms driving human biology, physiology and disease, warrant an innovative approach to chemical risk assessment. Current initiatives show that the toxicology community is broadening its horizon beyond known mechanisms, and beyond existing animal and non-animal testing, and embracing these developments to modernize its regulatory risk assessment towards a human-based holistic approach, improving human chemical risk assessment whilst reducing animal use. This is a challenging avenue to follow, which is not without possible pitfalls, but promising in its holistic employment of knowledge and emerging technologies, and ultimately of significant benefit for human health protection.

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