 DOI: 10.33892/aph.2021.91.144-145

PL-22

ADMET in Drug Development: What is the Role of Animals between *In Silico* – *In vitro* and *In Vivo* Humans?

OLAVI PELKONEN

Research Unit of Biomedicine/Pharmacology and Toxicology, Faculty of Medicine, University of Oulu, Oulu, Finland

Keywords: *ADMET, in silico, in vitro, in vivo, P450*

Traditionally, experimental animal studies have constituted a backbone of drug development, in particular in toxicity evaluation. Almost from the beginning of modern drug development the reliance on animal experiments has been critisised on various grounds, from obvious differences between animals and humans to the emergence of 3R principles (refine, reduce, replace). Increasingly, *in vitro* methodologies have paved the way for more mechanistic and molecular approaches and, even more recently, the application of computational methods have provided platforms for simulating and integrating various approaches. Indeed, some circles even anticipate a more or less rapid disappearance of animal experiments (unless the question is about the animals' own health and disease) or at least their replacement for a majority of current purposes by combined *in vitro* and *in silico* approaches.

It is useful to remember some arguments related to this topic:

1. Variability is a fact of life at all levels of biological organization, be it a population or a gene.
2. Animals are different from humans and thus unreliable predictors without extensive focussed investigations (interspecies differences).
3. 'In vivo at an individual level' constitutes such a complex whole that 'in vivo at a general (group, population etc) level' could provide only approximate predictions (interindividual differences).
4. In vitro-methods can provide answers only to rather specific and well-understood and researched problems (in vitro-in vivo correlations).
5. in silico-tools, especially at a higher level of simulations and machine learning, have to be interpreted and explained in the end at the level of bioscience language and concepts (in this case pharmacology and toxicology).

On the basis of the above arguments one might think that animal studies in drug development (as an example) are not very useful and the reason they are still a prominent part of DD has more to do with historical inertia than with a real necessity. Quite contrary, the use of experimental animals under proper ethical and scientific conditions offers still useful, sometimes absolutely necessary information for the advancement of drug development. In the following, some examples to illustrate this point are briefly described.

Regulatory animal studies on toxicology and toxicokinetics constitute a general framework for understanding *in vivo* effects and behaviour of new chemical entities (NCE) including adverse effects in various bodily functions and organs and in the fate of a specific NCE in a whole organism. Currently the first kinetic study informs on mass balance, extent of absorption and elimination, metabolism, principal metabolites etc, all of which provide an initial view and comprehension of (toxico)kinetic characteristics of an NCE. This view is certainly of importance in the planning of future kinetic studies and in the interpretation of toxicity profile of an NCE. Currently *in vitro* studies in human-derived systems are run in conjunction with animal *in vivo* (and *in vitro*) studies and thus a comparison is possible, with obvious benefits.

In regulatory toxicity studies it is of utmost importance to evaluate administered doses and also internal exposures (concentrations) of an NCE in relation to observed adverse effects, i.e. dose/concentration – response relationships, i.e. which doses/concentrations give rise to particular toxicities. It is not at all sure that similar toxicities at similar doses/concentrations would be expected when an NCE at a later stage (if ever) is given the first time into a human volunteer or patient. However, regulatory toxicity findings would constitute a background and starting point for more in-depth studies *in vivo* or *in vitro*, also employing human *in vitro* systems. It is quite possible that a particular

toxicity found in animals does not emerge in humans, perhaps because of pharmacodynamic or pharmacokinetic interspecies differences. It is possible that more detailed comparative mechanistic studies reveal that the interspecies difference is due to a particular enzyme metabolizing an NCE. In essence, animal *in vivo* studies constitute a model or a platform, where new experiments will be designed and the results obtained will be integrated into a coherent framework, which helps to make decisions to continue (or to terminate the development) to additional studies and ultimately to clinical phases.

Regulatory animal studies, as much as they have been critisized for inflexibility, lack of novelty, animal suffering, etc, have still another bonus to benefit science, a large database of relatively standardized ADMET results of chemicals, both would-be pharmaceuticals and chemicals used for other purposes such as pesticides and biocides. This database has been and still is of vast usefulness for advancing pharmacology and toxicology, offering a large number of well-studied chemicals for studies on chemical space, QSAR etc, and providing valuable reference compounds for various uses.

There are a number of other reasons for the necessity of using animals *in vivo*. Veterinary pharmacology and toxicology is obviously a discipline that is dependent on studies of particular animal species, whose diseases should be treated efficaciously and safely.

In the ADMET research, some topics such as metabolism, especially the role of cytochrome P450 (CYP) in drug-drug interactions, have advanced to the extent that *in vitro* tools helped by pharmacokinetic simulations would provide reliable and robust predictive information for the *in vivo* human studies, Also in other areas of ADMET studies, absorption, distribution and excretion, *in vitro* tools and approaches have advanced to the extent that physiologically-based pharmacokinetic models are at least 'good helpers' for the assessment of advancing to clinical phase. In the area of toxicological studies, kinetic and metabolic information from *in vivo* animal studies is still a necessary part of the assessment of animal-to-human extrapolation.

Some useful sources:

Bessems J.G.: Loizou G., Krishnan K., Clewell H.J., Bernasconi C., Bois F., Coecke S., Collnot E.M., Diembeck W., Farcal L.R., Geraets L., Gundert-Remy U., Kramer N., Küsters G., Leite S.B., Pelkonen O., Schröder K., Testai E, Wilk-Zasadna I., Zaldívar-Comenges J.M.: PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment. Recommendations from a joint EURL ECVAM - EPAA workshop. Regulat Toxicol Pharmacol, 68: 119-139 (2014).

Wilk-Zasadna I., Bernasconi C., Pelkonen O., Coecke S.: Biotransformation in vitro: an essential consideration in the quantitative in vitro-to-in vivo extrapolation (QIVIVE) of toxicity data. Toxicology, 332: 8-19 (2015).

Pelkonen, Olavi: Drug Metabolism – From In vitro to In vivo, From Simple to Complex: Reflections of the BCPT Nordic Prize 2014 Awardee. – Basic and clinical pharmacology and toxicology, 117 (3): 147-155 (2015).

Heinonen T., Pelkonen O., Tähti H.: Toxicology Meets Pharmacodynamics and Pharmacokinetics - New Concepts, Models and In vitro Approaches and Tools. Editorial. Basic. Clin. Pharmacol. Toxicol., 123 Suppl. 5: 3-5 (2018).

Bernasconi C., Pelkonen O., Andersson T.B., Strickland J., Wilk-Zasadna I., Asturiol D., Cole T., Liska R., Worth A., Müller-Vieira U., Richert L., Chesne C., Coecke S.: Validation of in vitro methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study., Toxicol In vitro., 60: 212-228 (2019).