Review Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2020.24.004071

In vitro ADME Screening Instead of *in vivo* Studies in Preclinical Safety

Mozhdeh Haddadi[1]*, Mohammad Javad Mousavi[2,3], Sara Mohseni[4] and Golnaz Mardani[5]

[1]*Department of Chemical Eng-Bio, Faculty of Chemical-Eng, Amir Kabir University, Tehran, Iran*

[2]*Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran*

[3]*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[4]*Non-Metallic Materials Research Group & Center of Nanotechnology Development, Niroo Research Institute (NRI), Tehran, Iran*

[5]*Department of Food Sciences and Technology, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran*

***Corresponding author:** Mozhdeh Haddadi, Department of Chemical Eng-Bio, Faculty of Chemical-Eng, Amirkabir University, Tehran, Iran

ARTICLE INFO

Received: January 03, 2020

Published: January 13, 2020

Citation: Mozhdeh Haddadi, Mohammad Javad Mousavi, Sara Mohseni, Golnaz Mardani. *In vitro* ADME Screening Instead of *in vivo* Studies in Preclinical Safety. Biomed J Sci & Tech Res 24(4)-2020. BJSTR. MS.ID.004071.

Keywords: *In-vitro*; Pharmaceutical industry; 3R; ADMET

ABSTRACT

Advances of in silico, ex vivo, and *in vivo* testing of the preclinical safety of newly developed pharmaceutical drugs before being administered in humans, is a fundamental step in drug manufacturing. Nowadays, animal testing plays an essential role in the evaluation of drug safety before progression into clinical trials. In recent years, several ex vivo tests have been developed and used in new screening processes to evaluate the toxicity of potential therapeutic molecules. This has led to a great replacement or reduction of in vivo assays. Accordingly, many pharmaceutical industries have a high demand for in vitro assays, and they are inclined to support the primary knowledge of developing novel drugs with adherence to the strategy of 3Rs (reduction, refinement, and replacement). It asserts that in vivo tests should be reduced, refined, and replaced by other preclinical techniques. Recently, using combinational chemistry and high-throughput screening (HTS) has increased the required information on a wide range of candidate molecules in terms of their absorption, distribution, metabolism, excretion, and toxicity (ADMET); which has resulted in a lot of ex vivo ADMET assessments. In this review, we have discussed the methods and tests which have the potential to replace the animal assays; and have addressed their advantages and limitations.

Abbreviations: EMA: European Medicine Agency's ; QSAR: Quantitative Structure-Activity Relationship ; CADD: Computer Aided Drug Design ;CAL: Computer-Assisted Learning ; ADME: Absorption, Distribution, Excretion and Metabolism ; NICEATM: NTP Interagency Center for the Assessment of Alternative Toxicological Techniques ;ICCVAM: Interagency Coordinating Committee on the Validation of Alternative Techniques ;EIA: Enzyme Immunoassay

Introduction

Due to the regulatory prerequisites of its safety, quality, and efficacy; developing a unique medicinal product is regarded as a prolonged and expensive process. Thus, an increasing interest is being designed to promote the effectiveness of drug development, and thereby to introduce novel medications with high levels of quality. Switching to novel toxicity testing techniques mostly focused on non-clinical assessments; is a wise strategy to conserve the time, costs, and animal sources. Although animal testing is practical to assess the toxicity of chemical components of novel pharmaceuticals; simultaneous screening and diagnosis of various compounds using *in vitro* assays have become feasible in many contexts leading to enhancement of drug development. This

replacement offers better ethical and animal safety procedures as well. It should also be internationally evaluated to explore alternative and comprehensive in vitro assays. Alongside, fulfilling the regulatory requirements of in vitro testing necessitates extensive studies to validate the result [1]. Several pieces of evidence show that *in-vitro* studies can prepare a rapid, precise and relevant data than those offered by animal testing.

Furthermore, *in-vitro* tests can predict the specific characteristics of drug and chemical toxicity such as their mutagenesis potential as well as the toxic mechanisms. Also, in a vast range of poisonous events occurred in animal testing, the initial triggers can be minimally identified; while in vitro assays provide a platform to decipher and manipulate such mechanisms. Currently, in-vitro testing of drugs is regarded as a framework to screen their specific harmful characteristics. Preclinical in vitro testing is proceeded in at least two different steps: recognition using a range of distinct tests of the essential biological attributes of the test investigation; and more sophisticated versions of the drug testing. The latter is more likely to proceed through a prolonged duration. Such tests are usually divided into several major categories such as microbial, tissue culture and teratogenicity, fungal assay, cytotoxicity and tissue sensitivity, and sperm analysis. Remarkably, several types of research have evidenced that animal testing is not confident enough in terms of safety assessment of, for example, oral contraceptives (OCs)(Pearson 1986). In this paper, we review the novel efforts and advances in vitro applications to restrict the use of animal tests. Besides, we suggest linking of in silico and in vitro applications as considerable progress toward this perspective goal. We hope these studies offer large scale perspective information for the prediction of drug absorption, bioavailability, and metabolism.

Reduction, Refinement, and Replacement (3Rs)

Different industries such as the pharmaceutical industry have developed novel in vitro experiments which not only has been created for supporting early identification of pharmaceutical candidates but also through regulation needed to adhere with the 3Rs. Currently, the 3Rs strategy is being utilized to reduce, refine, and replace the laboratory use of animals. Various techniques and alternative organisms are practical to perform this strategy [2]. The European Medicine Agency's (EMA) has offered a substitution for animal studies based on in vitro models providing comprehensive information about the conditions and strategies to accept the regulations of 3R alternative techniques (Kienapfel). Furthermore, developing non-animal technologies and using alternative techniques, and enhanced awareness of 3Rs rules have been presented by the UK National Centre for the 3Rs (NC3Rs). The UK government also showed a significant commitment in 2010 to reduce the use of animals as research models and thereby to assist the 3Rs plan. It has also delivered a strategy to decrease the in vivo sources as the research models [3]. Animal substitution is known as applying the non-sentient materials which can substitute conscious living vertebrates used during in vivo experimentation. Relative and absolute changes have been realized as two types of substitutions. Elative substitution is defined as the handling of animals while avoiding exposure to distressful procedures during testing. While the simple change represents the procedures in which the in vivo experimentation is entirely removed from preclinical testing.

In Vitro Techniques for Drug and Chemical Testing

Various methods have been proposed to prevent the use of animal models during experiments. These techniques provide, at least in part, alternative methodologies to test the chemicals and drugs. These technologies have several advantages including timesaving, reduction of human resources, and cost-effectiveness. A detailed characterization of these techniques is as follows:

Using Computers in Various Basic Principles of Biology

Computers have offered a platform by which the biological effects of candidate drugs or chemicals can be simulated independently of assessing animal autopsies. For example, the receptor binding site of a drug molecule can be blocked using computer software known as Computer Aided Drug Design (CADD). By using CADD, scientists can rule out a wide range of putative drug mechanisms, as it eludes testing the unnecessary compounds lacking biological activities [4]. In a mathematical method called Quantitative Structure-Activity Relationship (QSAR), we are also allowed to explain the correlations between physicochemical drug characteristics and their biological activity [5]. Moreover, computer-assisted learning (CAL) program creates a tool to predict molecular interactions without real empirical materials. In a comparative study assessing knowledge gain in students (using test questionnaires, calculations, and interpretation), a better problem-solving attitude was found in those who performed CAL. Also, the novel methods are usually more cost-effective than the previously established laboratory experiments [6].

Substitution of Experimental Animals by Different Model Organisms

Using higher model vertebrates such as monkeys, dogs, rats, and guinea pig in the testing systems is faced with a multitude of ethical issues, suggesting the use of alternative organisms for experimental purposes. In this context, various model organisms were proposed to replace experimental animals [7]. Their larvae and embryos can be expanded and utilized as tools to test drug and chemical responses in cell culture Petri dishes and plates. For instance, recently, due to the availability of a whole genome sequence of Zebra fish, investigators have been attracted toward this model organism for genetic and molecular research. Moreover, another alternative for experimental higher model vertebrates is invertebrate organisms which are largely under focus to be considered for laboratory testing. Also, this platform has another advantage over vertebrate systems which requires lesser housing facilities. For instance, thousands of flies can be superseded in a refuge where only limited numbers of mice can live [8].

Additionally, several genetic and molecular tools have been accessible to take drosophila under study. Owing to the high levels of resemblances in behavioral and development activities, the fruit fly has served as a sensitive and unique pattern for the study of human diseases and genetics [9]. Here are some examples of alternative model organisms which can replace the laboratory use of animals: Prokaryotes like Escherichia coli and Bacillus subtilis have been applied in molecular and genetic studies, and cellular differentiation, respectively. Fungi like Neurospora crassa have been highlighted to be used in genetic studies, circadian rhythm and metabolic regulation studies; and Schizosaccharomyces pombe in molecular genetic research. Among invertebrates, Amphimedon queenslandica has been used in evolution, developmental biology, and comparative genomics; Aplysia sp./sea slug in neurobiology, Caenorhabditis elegans in genetic development studies, Drosophila melanogaster in genetics and neurology research and Hydra in the process of regeneration and morphogenesis.

Using Cell and Tissue Cultures For *In Vitro* Tests

Importantly, using animal experimentation can also be replaced by cell or tissue culture techniques in which the viable cells are grown and proliferated in an in vitro condition. The cells and tissues from the skin, brain, kidney, and liver are picked up from animal sources and can be grown outside the body in appropriate media for several days to months [10].

Development of *In Vitro* Experimentation

In the last decades, the pharmaceutical industries have indicated a particular interest toward in vitro testing. Notably, there has been a constant enhancement regarding the use of in vitro testing by drug companies from 2000 to 2013. It should be noted that the last year of the survey interval (2013) consisted of more than 20% of all in vitro tests during 2000–2013, numerous *in vitro* tests were performed focusing on absorption, distribution, excretion and metabolism (ADME) in safety pharmacology. The majority of the trials included dermal absorption, eye irritation, skin irritation and corrosion (0.1%). A few portions of these experiments are also accounted for aspiration, phototoxicity, endocrine disruption, development, and neurotoxicology tests [3].

Evaluation of Drug Metabolism

Although providing a physiologically relevant platform to examine drug metabolism using animal models can limit the attribution of results to the humans due to the remarkable differences between the various specious. Liver microsomes are applied widespread as a system to test drug metabolism in humans. The concentrations of substrate and enzyme may be considered as selective predictive parameters for either routes or rate of metabolism[11] . In the process of clinical drug development, deficiencies in excretion, metabolism, absorption, and distribution (ADME) are responsible for 39% of attrition; and further 21% of failures are caused by toxicity. Noticeably, drug toxicity is inevitably attributed to metabolic conversion. The former metabolism testing is accomplished during drug development [12]. Also, agencies like the U.S. Food and Drug Administration calls for the in vitro exploring of metabolism and potential drug-drug interactions to be conducted as a regular performance. A range of experiments from in vitro screening of human enzymes to in vivo testing using animal models are available for the measurement of drug metabolism before assessing in humans. Most of the systems are not suitable; nevertheless, it has been recognized a huge inter-species variability in terms of *in vivo* drug metabolism [13]. Hence the need for a human *in vitro* system is also sensed. According to the techniques described by Rane et al. [14], the correlations between *in vitro* and in vivo tests, regarding human drug metabolism, can be realized using *in vitro* systems [14] as well as mathematical refinement [15].

Use of Modeling Systems

Modeling systems are emerging as a great help to decipher and predict the physiological and pathophysiological phenomena. These modeling methodologies are wealthy and various, which besides containing *in vitro* and in vivo prediction tools, involve theoretical or in silico techniques. A combination of relevant factors has highlighted the importance of computational and mathematical cancer modeling. These factors have contributed to the appearance of systems biology techniques in biomedicine resulted from a large volume of molecular information[16-21]. Introduction of cancer modeling has focused on supporting programs at the NIH including the Integrative Cancer Biology Program. It has also led to reducing the expenses of the computational power essential to develop extensive and clinically related simulations [22]. In this context, Anderson et al. assessed the mathematical continuum–discrete hybrid model as in silico-based models. Prospectively, the cancer systems biology, mathematical and computational modeling (i.e., in silico) will be the critical parts of clinical oncology. However, in silico modeling is as challenging as other technical approaches. These barriers should be passed to achieve a genuine potential contribution to transit from the conventional population-based strategies to personalized medicine for cancer [18].

Preclinical Cardiac Safety Testing

Preclinical cardiac safety assessment is in the main part of evolutionary switching which defines the mechanisms beyond the cellular interactions of electrophysiological, contractile and structural cardiac toxicity. As an ideal method to replace the animal models, the human channels and hSC-CMs have created an *in vitro* testing system for the large-scale screening which has been used to evaluate electrophysiological and multi-parametric subcellular and cellular responses. This is usually accompanied by in silico tests describing complicated electrophysiological properties of the cellular responses. Interestingly, these techniques which are mostly based on mechanical approaches could be replaced by traditional 'black box' strategies based on the characterization of the causative influence of numerous compounds which are poorly described on animal models to define testing performance [23].

Challenging in Using Vaccines

The international workshop organized by The NTP Interagency Center for the Assessment of Alternative Toxicological Techniques (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Techniques (ICCVAM), published a list of priorities for the application of the 3Rs-concept. According to the explanation by William Stokes, this includes a list of vaccines each of which needs many experimental animals which causes noticeable pain and distress. A significant biohazard also results from various experiments. Also, vaccines are regarded as a high priority; and in vitro alternatives have been previously described to validate some of the vaccines such as rabies, Clostridium sp., and Leptospira sp. vaccines. A target species control of rabies vaccines is usually conducted using a batch control of these vaccines in mice models as a substitutive examination of vaccine efficacy. During the test performed by the National Institute of Health (NIH), several challenges were presented such as using many animal models and intra-cerebral infection of mice with rabies virus after immunization [24]. In general, these experiments resulted in 50% of the animal's deaths due to the symptoms of rabies, such as severe pain. These conventional strategies often require high numbers of *in vivo* models, which also fail to produce stable and valid results [25].

Furthermore, many test parameters such as target species, virus strains, and route of both vaccinations vary significantly in most of the other challenging models (Matthias König, JLU, Germany). These data support an emergency need for replacing strategies in case of rabies vaccines. As an alternative approach, the serological tests permit the in vitro quantification of neutralizing antibodies in the sera of immunized animal models. This method has been undergoing a few technical modifications since its development in the 1990s [26]. A recombinant compound produced by Merck Company as a novel hepatitis B vaccine known as RECOMBIVAX HB was monitored via a test carried out in mice. As a post-licensing obligation, Merck tried to substitute its mouse authority test using an in vitro protocol for the product released in the US market. Primary studies with a commercial enzyme immunoassay (EIA) yielded variable results. When accompanied by a sample pretreatment step, the experiment showed more predictive and dependable authority in the mouse model. Consistent results were achieved using the EIA compared with the mouse authority according to the evaluation made on manufactured materials that are combined with the tests contrived to lead to a wide range of reactivity in both experiments. This conformity was used for calibration of specific conditions of the in vitro testing to predict satisfactory reply in the in vivo experimentation. Interestingly, the information available from clinical trials appointed confirmative results achieved in terms of human immunogenicity [27].

Assessment of Vaccines Quality

The stability near for Quality Control of Vaccines is an approach for modality reforming control and 3Rs implementation. The concept of constancy near encompasses the applying of test validation, current Good Manufacturing Practice (GMP) rules and final tests of the product. In the condition that final batches of a manufacturing process are consistent with Safety, Quality and Efficacy criteria described in the marketing authorization, substitution of routine in vivo experiments is resulted (Marlies Halder, ECVAM, Italy); De Mattia et al. [28]. Alongside, the organizations that provide regulatory validations of supersede techniques were created including the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM). This led to several in vitro tests to be validated. These assays include the bovine corneal opacity and permeability (BCOP) experiment and cytosensor microphysiometer test utilized for eye irritation testing [3]. Eventually, new technological and particular advances, such as potent in silico models, and "omics" technologies, have simplified the development of many novels' in vitro tests.

Challenging the *In Vitro* Tests

The investigations performed to implement the 3Rs-concept, are exemplarily demonstrated by serological and antigen quantification assays that are successfully established; however, the small number of approved in vitro tests remained unclear. Therefore, the described psychological, regulatory, and technical barriers are discussed which required to be resolved [29]. A challenge of recent in vitro applications is that they require high cost and time to be developed and approved. For instance, five years costs of ELISA analysis for a 10-way bovine vaccine is more than 4-fold lower compared to those of corresponding animal tests. Strikingly, ELISA assay development and validation and work time for transition to *in vitro* would payback after four years. (Marc Lee, Boehringer-Ingelheim Vetmedica, USA).

Full implementation of in vitro applications can indeed take a considerably long time. For example, some severe disadvantages of conventional tests for Leptospira vaccines are animal distress and pain, high costs, and the danger of exposing to pathogens for personnel. The relation of the developed in vitro application with efficiency in animals was shown through the substitution of the conventional challenge assays for Leptospira vaccine. The replacement of the in vivo challenge test by ELISA assay took 19 years with nearly $ 2.0 million costs. With the development of ELISA in 2000, the number of animal tests slightly decreased, while the number of produced vaccines during 1990 and 2009 almost doubled. The slow progress of the 3Rs implementation is apparent due to these hurdles. Because of the possible variations in the production of the "uniqueness" of each vaccine batch as a historical concept remains the main difficulty. Strikingly, batch-wise testing that emphasizes on the final product is required for uniqueness. However, this view has been changed by progressions of vaccine production.

Conclusion

Consistent manufacturing processes which follow quality systems like Good Manufacturing Practice (GMP) determine the

production of new vaccines. The consistency approach is applicable for older products as well. The requirement of replacing in vivo tests by *in vitro*:

a) A novel approach to the fulfillment of an innovative potency test.

b) Proof of consistency needs to replace the required direct relation between potency and efficacy.

c) A close relationship between the regulated industry and regulatory bodies

Consideration of test sets from the aspects of potency/quality/consistency:

a) Acceptance and selection of alternative approaches to in vivo applications on an agent by agent basis.

b) Considering the application of novel and producing technology [29].

c) In the next ten years, producing already started drug in a variety of companies and the automation rate in conventional drug metabolism departments will enhance. Medium and high-throughput in vitro tests that are fully automated will be combined within silico models and data interpretation [30]. In fact, in the next 5–10 years, in silico applications would demonstrate up to 15% of R&D expenditure. They apply to all steps of the drug discovery, development process like predicting the vital properties of lead compounds including solubility, receptor binding, and metabolic stability, and stimulating clinical trials [31].

Conflict of Interest

The authors declare that there is no conflict of interest statement.

References

1. Ukelis U, Kramer PJ, Olejniczak K, Mueller SO (2008) Replacement of *in vivo* acute oral toxicity studies by *in vitro* cytotoxicity methods: Opportunities, limits and regulatory status Regulatory Toxicology and Pharmacology 51: 108-118.
2. Ranganatha N, Kuppast I (2012) A review on alternatives to animal testing methods in drug development International Journal of Pharmacy and Pharmaceutical Sciences 4: 28-32.
3. Goh JY, Weaver RJ, Dixon L, Platt NJ, Roberts RA (2015) Development and use of *in vitro* alternatives to animal testing by the pharmaceutical industry 1980–2013 Toxicology Research 4: 1297-1307.
4. Vedani A (1991) Computer-aided drug design: an alternative to animal testing in the pharmacological screening Altex 8: 39-60.
5. Knight A, Bailey J, Balcombe J (2006) Animal carcinogenicity studies: 3. Alternatives to the bioassay. Altern Lab Anim 34(1): 39-48.
6. Dewhurst D, Hardcastle J, Hardcastle P, Stuart E (1994) Comparison of a computer simulation program and a traditional laboratory practical class for teaching the principles of intestinal absorption Advances in Physiology Education 267: S95.
7. Doke SK, Dhawale SC (2015) Alternatives to animal testing: A review Saudi Pharmaceutical Journal 23(3): 223-229.
8. Wilson Sanders SE (2011) Invertebrate models for biomedical research, testing, and education ILAR journal 52(2): 126-152.
9. Beckingham KM, Armstrong JD, Texada MJ, Munjaal R, Baker DA (2007) Drosophila melanogaster-the model organism of choice for the complex biology of multi-cellular organisms Gravitational and Space Research 18 (2):17-29.
10. Kirouac DC, Zandstra PW (2008) The systematic production of cells for cell therapies Cell stem cell 3: 369-381.
11. Tingle M, Helsby N (2006) Can *in vitro* drug metabolism studies with human tissue replace in vivo animal studies? Environmental toxicology and pharmacology 21(2): 184-190.
12. Kennedy T (1997) Managing the drug discovery/development interface Drug discovery today 2: 436-444.
13. Hucker HB (1970) Species differences in drug metabolism Annual review of pharmacology 10: 99-118.
14. Rane A, Wilkinson G, Shand D (1977) Prediction of hepatic extraction ratio from *in vitro* measurement of intrinsic clearance Journal of Pharmacology and Experimental Therapeutics 200(2): 420-424.
15. Houston JB (1994) Utility of *in vitro* drug metabolism data in predicting in vivo metabolic clearance Biochemical pharmacology 47(9): 1469-1479.
16. Ahn AC, Tewari M, Poon CS, Phillips RS (2006) The clinical applications of a systems approach PLoS medicine 3(7): e209.
17. Coffey DS (1998) Self-organization, complexity and chaos: The new biology for medicine Nature medicine 4(8): 882-885.
18. Deisboeck TS, Zhang L, Yoon J, Costa J (2009) In silico cancer modeling: is it ready for primetime? Nature clinical practice Oncology 6(1): 34-42.
19. Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J (2006) Cancer: a systems biology disease Biosystems 83: 81-90.
20. Kitano H (2002) Computational systems biology Nature 420: 206-210.
21. Liu ET, Kuznetsov VA, Miller LD (2006) In the pursuit of complexity: systems medicine in cancer biology Cancer cell 9: 245-247.
22. Anderson AR, Weaver AM, Cummings PT, Quaranta V (2006) Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment Cell 127: 905-915.
23. Gintant G, Sager PT, Stockbridge N (2016) Evolution of strategies to improve preclinical cardiac safety testing Nature Reviews Drug Discovery 15(7): 457-471.
24. Meslin FX, Kaplan MM, Koprowski H, Organization WH (1996) Laboratory techniques in rabies Pearson R (1986) In-vitro techniques: can they replace animal testing? Human Reproduction 1: 559-560.
25. Wunderli PS, Dreesen DW, Miller TJ, Baer GM (2003) Effects of vaccine route and dosage on protection from rabies after intracerebral challenge in mice American journal of veterinary research 64(4): 491-498.
26. Smith JS, Yager PA, Baer GM (1973) A rapid reproducible test for determining rabies neutralizing antibody Bulletin of the World Health Organization 48: 535.
27. Schofield T (2002) *In vitro* versus in vivo concordance: a case study of the replacement of an animal potency test with an immunochemical assay Developments in biologicals 111: 299-304.
28. De Mattia F, Chapsal JM, Descamps J, Halder M, Jarrett N, et al. (2011) The consistency approach for quality control of vaccines-a strategy to improve quality control and implement 3Rs Biologicals 39: 59-65.
29. Romberg J, Lang S, Balks E, Kamphuis E, Duchow K, et al. (2012) Potency testing of veterinary vaccines: the way from *in vivo* to *in vitro* Biologicals 40: 100-106.
30. Van de Waterbeemd H, Gifford E (2003) ADMET in silico modelling: towards prediction paradise? Nature reviews Drug discovery 2: 192-204.

31. Kienapfel A (2013) A review of the advancements in photo safety testing with regard to ICH's new topic S10: Photo safety evaluation of pharmaceuticals.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2020.24.004071

Mozhdeh Haddadi. Biomed J Sci & Tech Res