The Pros and Cons of the In-silico Pharmacoc-toxicology in Drug Discovery and Development

1Soodabeh Saeidnia, 1Azadeh Manayi and 1Mohammad Abdollahi
1Medicinal Plants Research Center, Faculty of Pharmacy,
Tehran University of Medical Sciences, Tehran 1417614411, Iran
2Department of Toxicology and Pharmacology,
Faculty of Pharmacy and Pharmaceutical Sciences Research Center,
Tehran University of Medical Sciences, Tehran 1417614411, Iran

INTRODUCTION

The term “In-silico” has been established since 1989 meaning “any biological experiment on or in computer”, and stands comparison with the Latin expressions in vivo, in vitro and in situ, which ascribe to research and test in living organisms, outside the living organisms and where they are found in nature, respectively (Rohrig et al., 2010). This computational method, including databases; Quantitative Structure-Activity Relationships (QSAR); similarity surveys; pharmacophores; homology models and other molecular modeling; data mining; network and data analysis tools, is a comparatively rapid and simple method to predict pharmacology and/or toxicology hypothesis and testing (Ekisn et al., 2007).

In-silico softwares have been ordinary employed to find or to improve a novel bioactive compound, which may exhibit a strong affinity to a particular target. Actually, a world view underlying the theory and methodology of in-silico toxico-pharmacology is still in progress and shows a broad spectrum of opportunities to help the discovery of new targets and finally to result in substances with high affinity and possible biological/pharmacological activity on those tested targets (Ekisn et al., 2007; Tsuchida et al., 2006).

IN-SILICO TOXICO-PHARMACOLOGICAL STUDIES (IN-SILICO TPS) ON NATURAL COMPOUNDS

In the study of natural drug discoveries, one of the applied affinity fingerprints is IC₅₀ data, although it may not detect functional similarities among molecules and is only recommended to find unfair pharmacophores. For instance, it is proved that α- or β-unsaturated ketone moieties are necessary in compounds which act as “ubiquitin isopeptidase inhibitors” such as curcumin (source: Curcuma longa) and punaglandins (source: soft coral like Clavularia viridis). Further studies showed that curcumin is not only an inhibitor of ubiquitin isopeptidase but also an activator of protein-1 and inhibitor of CK2 and PKD as two main parts of COP9 signalosome with the ability to control p53 and c-Jun, which are playing a considerable role in tumor progression. These are just a few samples when cooperation of traditional medicine, modern pharmacology and in-silico approaches may lead to a novel drug discovery (Ekisn et al., 2007; Tsuchida et al., 2006; Fullbeek et al., 2005).

Computational study in natural drug discovery is not only applied to find the new targets and new molecule with high affinity to those targets but also used to determine the metabolic pathways of those active molecules. For instance, camptothecin derivatives (monoterpene-indole alkaloids) have been clinically employed as antitumor drugs. Literature revealed the biosynthetic pathway of camptothecin by in-silico and in vivo investigations, in which adding of glucose into alkaloid have been studied by using the Atomic Reconstruction of Metabolism software, while following the incorporation of glucose into camptothecin with hairy roots of Ophiopogon pumila have been studied (in vitro) by ²H-NMR. Such studies may explain how an in-silico metabolic analysis is able to improve the experimental decorations to gain more comprehensible biological information (Yamazaki et al., 2004).

Literature revealed another comprehensive in-silico evaluation via MetaSite and VolSurf software for two artemisins (a sesquiterpene lactone from Artemisia annua) hybrid-dimers. Regarding to the predictions, Dihydroartemisinin (DHA) can be formed through O-dealkylation pathways and the aliphatic linker was predicted not likely to change. The authors studied
on the five artemisinin metabolites which were predicted to be created without the quinoline moiety (through dimers) or without an artemisinin portion (through quinoline hybrids). They found that all the metabolites consisted of one or two artemisinin functionalities and they concluded that the active compounds have been lightly metabolized but their activity remained (Bray et al., 2005; Lombard et al., 2012).

ROLE OF IN-SILICO TPS IN COSMETICS TOXICITY TESTS

Alongside the above mentioned application of in-silico studies, the European Commission invited industry, Nongovernmental organizations (NGO), EU Member States and the Commission’s Scientific Committee on Consumer Safety to introduce professional and expert scientists in five toxicological fields including toxicokinetics, repeated dose toxicity, carcinogenicity, skin sensitization, and reproductive toxicity to be asked how the computational methods are able to alternate instead of animal experimental and how these replacing methods can be sound. The 7th amendment to the EU Cosmetics Directive prohibits putting animal-tested cosmetics on the market in Europe after 2013. It seems that they have to extend the deadline, because the valid alternative techniques are unavailable until date. Actually, toxicology scientists think that it is not possible to replace animals thoroughly by in vitro and/or in-silico studies in safety examinations in the near future (Fig. 1). Additionally, in vitro tests are not generally reliable because a number of those tests are done on cell lines with abnormal function, in which the main deal is obtaining a measurable activity as an endpoint and how the findings can be associated with human toxicity. Although, animal cell culture has been employed in different sections of medicinal and life science including toxicology and pharmacology, the results gained seem partly invalid and the probability of errors is high due to lack of sufficient controls on temperature, pH, osmotic pressure and so on as well as lack of dynamic status of the biological environment the same as inside organism. For this reason, evaluation of medicines or chemicals on an in vivo animal model of toxicity is highly recommended.

![Diagram](image_url)

Fig. 1: Some alternative methods in order to reduce the animal numbers in oral acute toxicity evaluations of cosmetic products on the basis of OECD; ATP: a kind of cell viability assays widely used to assess the effect of chemotherapeutic drugs on cell lines through detection of adenosine triphosphate. ATP assay is able to detect the lower limit of 1563 cells/well with luminescence (values at least 100 x background readings), while the MTT assay could not detect less than 25,000 cells/well above background readings; Chang liver cell: a normal human liver cell line; HL-60: Human promyelocytic leukemia cell line used for laboratory research on how certain kinds of blood cells are formed; MTD: Minimum toxic dose; OECD: Organization for economic co-operation and development; QSAR: Quantitative structure-activity relationship
In vitro and in vivo tests for evaluation of carcinogenicity and genotoxicity

CTA assay (cytotoxicity may be assessed by evaluation of colony forming ability)

Tg.AC mice, heterozygous (+/-) p53-deficient, and TgHras 2 lines (for mechanistic evaluation)

Gap junction intercellular communication (methods existed in diverse cell types)

In vitro and in vivo tests for evaluation of carcinogenicity and genotoxicity

Fig. 2: Some in vitro and in vivo tests for evaluation of carcinogenicity and genotoxicity. CTA: Cell transformation assay; Tg.AC mice: A kind of transgenic mice using to develop skin tumors in response to specific carcinogens and carry the coding sequence of v-Ha-ras linked to an globin promoter and an SV40 polyadenylation signal sequence; TgHras: is a hemizygous transgenic mouse, approved by regulatory agencies for carcinogenicity assessment; p53: also known as protein 53 or tumor protein 53, is a tumor suppressor protein that in humans is encoded by the TP53 gene
directly from chemical structure and have been encouraged due to rapid and simple application. One of the frequently applied softwares is TOPKAT (Toxicity Prediction by Komputer Assisted Technology; Accelrys Inc., Cambridge), a bio-statistic based and QSAR-containing system. Basically, the systems should retrieve after the analysis of a broad spectrum of findings as toxicologic data from the literature. In such systems, the compounds can be marked by either structural or topological indices, while toxicity data may be categorized by analysis for continuous endpoints. TOPKAT "Model Rat Oral LD₅₀" and "Model for Rat Inhalation Toxicity LC₅₀" are two of the most employed examples (Accelrys Inc., 2002).

IN-SILICO MODELING ON THE BORDERLINE OF NONCLINICAL AND EARLY CLINICAL DRUG DISCOVERY

Previously, the maximum recommended starting dose for First-In-Human (FIH) trials was initiated regarding to none side effect levels, but this process had many limitations such as using allometric scaling (not a valid approach all the time) and arbitrary safety factors. For this reason, a Pharmacokinetic-pharmacodynamic (PKPD) guided approach is now considering to assess the Minimal Anticipated Biological Effect Level (MABEL). This approach is further mechanistic-based to initiate dose selecting according to the predicted PKPD and safety in human. The most important point is that a quantitative prediction model possessing up-to-date clinical data should be available for most of academics and companies to predict pharmacology and safety. It seems that if qualification idea can positively be achieved, it should affect on augmentation of confidence in methodology and consequently in the regulatory requirements for drug discovery (Visser et al., 2013).

IN-SILICO TPS AS A PART OF NON TESTING METHODS

On the basis of the European Chemicals Agency (ECHA) guidance for essential information and chemical safety assessment, non-testing data can be generated by three main approaches that are exhibited in Fig. 3. On the other hand, non-testing methods are considered as two

![Diagram of Non-testing Methods]

Fig. 3: Non-testing methods on the basis of ECHA guidance on information requirements and chemical safety assessment; ECHA: The European Chemicals Agency
main sections including comprehensive (global) and specific (local) ones. As a matter of fact, the first section (well-known as expert systems) formalizes existing knowledge, while specific systems are ordinary employed for a few targets like particular receptors and enzymes. The advantage of the first section is over QSAR techniques, in which prediction is associated with a specific mechanism (Raunio, 2011).

The pharmacokinetic profile of a molecule (absorption, distribution, metabolism and excretion as ADME) can interact with living organisms, which exhibits the fate of that molecule in human body as well as its toxicity. The ADME information of a molecule is considered in finding relations between the toxicological profile of a lead compound and its metabolites, where reactive electrophiles (metabolites) may possibly bind to proteins and DNA, as the primary mechanism of carcinogenesis and adverse effect of idiosyncratic drugs. Moreover, metabolism plays an important role in pharmacotoxicological activity of xenobiotics (Raunio, 2011).

CONCLUSION

As a matter of fact, in-silico approaches should be accompanied by further in vitro and in vivo experiments to verify the biological activities. Unfortunately, there are lots of identified compounds (by in-silico screening methods), which have not been evaluated in vitro or in vivo in order to prove the real positive responses. In-silico molecular approaches are also employed to make modeling for toxicity pathways particularly when there is no essential experimental data available. For instance, metabolizing enzymes are introduced as important targets to involve in clearance of drugs and even activation of their metabolites resulting in probable toxicity. Therefore, determination the correlation between structure and function for p450 enlightens estimating or predicting the possible activities of new compounds (McGovern and Shoichet, 2003, Kavlock et al., 2008).

Moreover, a number of these techniques are able to estimate different physical and chemical properties of the molecules relevant to environmental fate and transport. Interestingly, the interaction between active molecules and proteins is a remarkable group of target-toxicant interactions, which has been identified yet. So far, many in-silico approaches have been achieved and progressed day by day to screen inside molecular libraries to find pharmaceutical applications, especially when these techniques can be combined to structure-based molecular docking with multidimensional quantitative structure activity relationships (McGovern and Shoichet, 2003).

Taking together, the science of toxicology is ongoing to the hallmark achievements, particularly by recent advances in biology, chemistry and computer sciences, the prediction power are being certified. Furthermore, in-silico toxicology is able to provide the essential data, which can help to close gaps existing in some areas. Although, there are extreme developments and ongoing application for toxicogenomics, this area of toxicology generates the main data like the evaluation of gene-environment interactions and development of virtual tissues. Alongside, the high-throughput and genomics technology starts to be employed in toxicology and progressed by the pharmaceutical companies in natural drug discovery.

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REFERENCES


