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

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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 (Ekins *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 (Ekins *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 IC_{50} 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 (Ekins *et al*., 2007; Tsuchida *et al*., 2006; Fullbeck *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 *Ophiorrhiza pumila* have been studied (*in vitro*) by ^{13}C-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 artemisinin (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

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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 seems 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

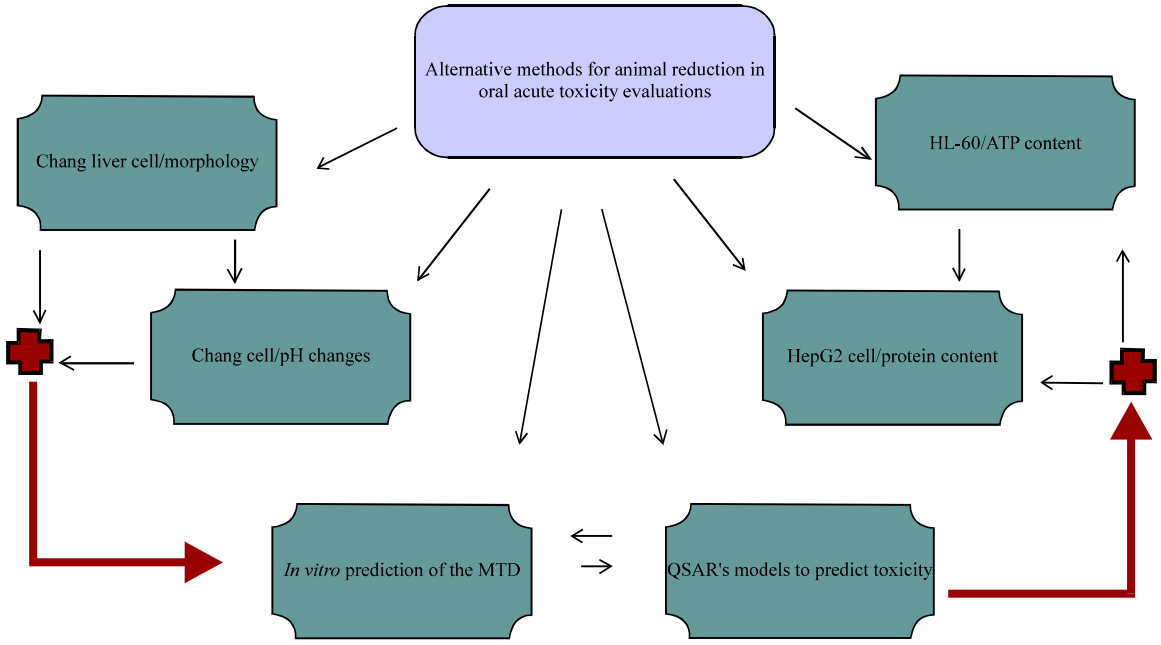

Fig. 1: Some alternative methods in order to reduce the animal numbers in oral acute toxicity evaluations of cosmetic products on the basis of OECD; ATP: a kind of cell viability assays widely used to assess the effect of chemotherapeutic drugs on cell lines through detection of adenosine triphosphate. 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

(Shetab-Boushehri and Abdollahi, 2012). In fact, new methods make it possible to detect changes in cultured cells, whereas the effects of materials on genomics, proteomics and metabolomics could be evaluated. The concern that "what compounds are the primary targets of chemical attack and how they are altered" is considered as important point in generation of computational systems to predict the toxicity on the basis of chemical structure (Adler *et al*., 2011). For instance that controversy raised in the recent years about role of antioxidants as a supplemental chemopreventive or cancer killing. The remarkable point is that whether taking or avoiding antioxidants for chemoprevention and also during chemotherapy is recommendable. Actually, growth and development of cancer cells are correlated to intracellular oxygen. Reversibly, the intracellular hydrogen peroxide increase might lead to further decomposition into water and oxygen, which is effective in chemotherapy of cancer. However, regarding to dual biological role of antioxidants in prevention and therapy of cancers, to reach a conclusions, it is essential to test the antioxidant activity of drugs or chemicals by *in vivo* models due to less reliability of *in vitro* evidences (Adler *et al*., 2011; Saeidnia and Abdollahi, 2013, 2012; Abdollahi and Shetab-Boushehri, 2012). Although, carcinogenesis is a complex biological procedure which makes it difficult to develop alternative *in vitro* tests, i*n vivo* examinations using transgenic animals might lead to reduction and refinement of animal use (Maurici *et al*., 2013).

Another suggestion is the administration of low and harmless dose of compounds in human volunteers due to high sensitivity of human data; in that case blood and urine samples would be used to measure toxicity. In all new methods, animals have not suffered with chemicals so long (Maurici *et al*., 2013). However, different in silico models including Structural Activity Relationship (SAR) and QSAR dedicated to the prediction of carcinogenicity, have been developed so far. Additionally, it has been revealed that the prediction of carcinogenicity may be rarely possible. Therefore, the best suggested models have been established as mechanism-based methods (Fig. 2) obtaining from biological findings (Cronin *et al*., 2003). Actually, a few QSAR studies for skin irritation, eye irritation, genotoxicity and mutagenicity of cosmetics from chemical or natural origins have been reported in the literature until now (Cronin *et al*., 2003; Barratt, 1996).

QSAR SOFTWARES FOR PREDICTION OF TOXICITY FROM CHEMICAL STRUCTURE

There are some computational packages for the prediction of toxicities, in which toxicity can be predicted

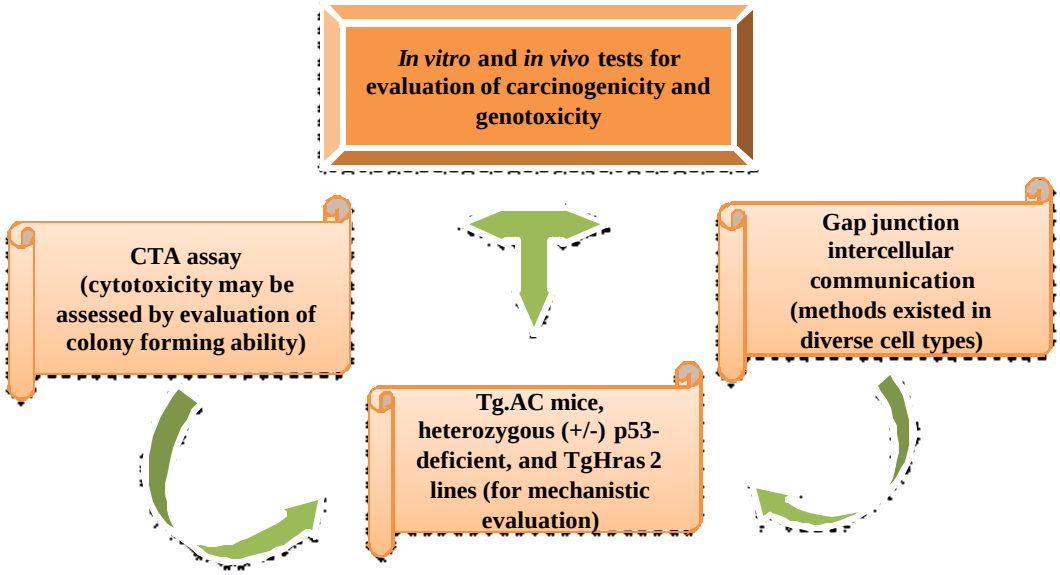

Fig. 2: Some *in vitro* and *in vivo* tests for evaluation of carcinogenicity and genotoxicity. CTA: Cell transformation assay; Tg. AC mice: A kind of transgenic mice using to develop skin tumors in response to specific carcinogens and carry the coding sequence of v-Ha ras linked to a globin promoter 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 LD_{50}" and "Model for Rat Inhalation Toxicity LC_{50}" 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. But 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

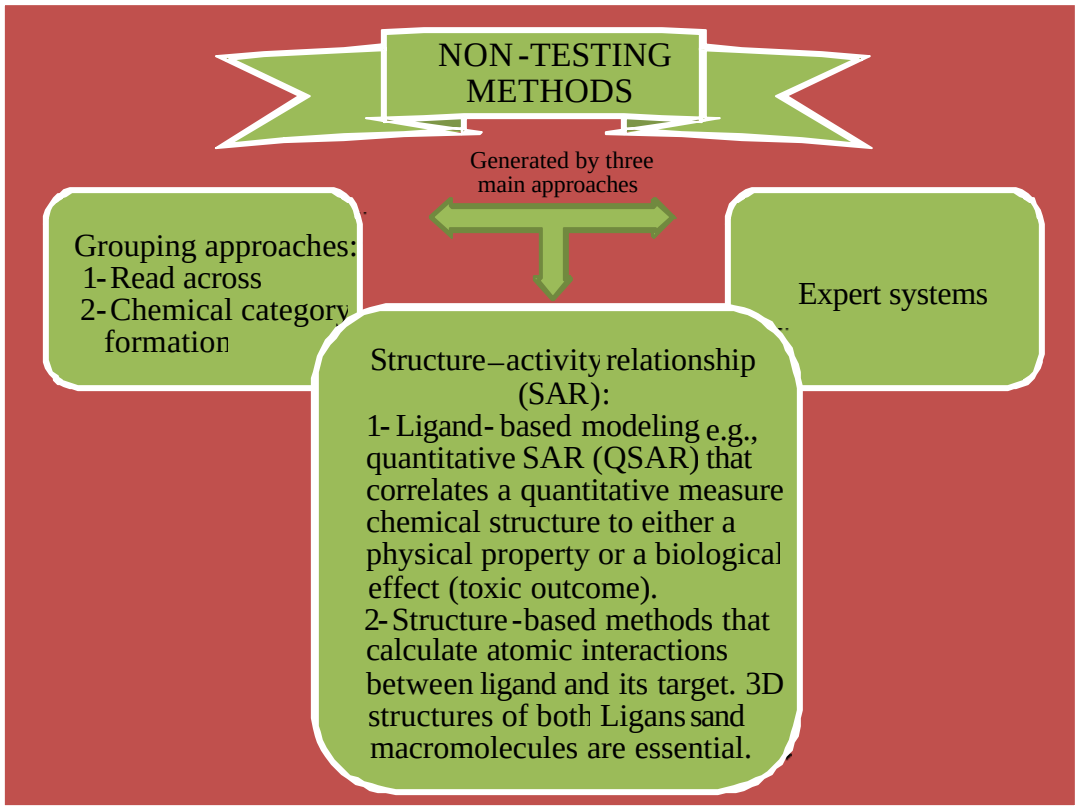

Fig. 3: Non-testing methods on the basis of ECHA guidance on information requirements and chemical safety assessment; ECHA: The european chemicals agency

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 pharmaco-toxicological 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 hallmarked 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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