In silico Toxicology

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Introduction

„Although toxicology is not the most prominent factor for this high attrition rate and late failures, toxicity still is the cause of ca 20% of the dropouts during late development stages”

„Adverse drug reactions are believed to be one of the leading causes of death (in the United States and are estimated to have occurred in over two million patients in 1994 with more than 100 000 fatalities)”

„…of the 2000 chemicals that are evaluated for their potential hazard each year by the EPA the vast majority are assessed in the absence of experimental toxicity data”

Definition

**Computational Toxicology (In Silico Toxicology):** the application of the tools of computational biology to assess the risk chemicals pose to human health and the environment.

Computational toxicology, an **applied science**, utilizes the latest advances in **mathematics, biology, chemistry, and computer technologies**. *Integrating* all of these sciences into a biologically based computational model enables the researcher to *numerically investigate*, either pharmacokinetically and/or pharmacodynamically, the impact of exposure to environmental chemicals on people.
Toxicology Methods

Certain experiments are IMPOSSIBLE (e.g. human experiments with carcinogens).

Possible methods for the preclinical toxicity predictions:
- **In vitro** toxicology models
- **In vivo** toxicology models (cell cultures, animal models)
- **Computational** toxicology

Drawbacks:
- **in vivo studies** require large amounts of compound
- **in vitro assays** may lack reliable high-throughput
- **in vivo animal models** are expensive and occur suffering and/or distress
- both **in vitro and in vivo models** are unable to correctly predict some human toxicities

Alternatives to animal testing
Objectives of computational toxicology

1. improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes

2. provide predictive models for screening and testing

3. improve quantitative risk assessment

• Impacting attrition rates
• Predict potential adverse drug reactions (ADRs) and certain toxicities
• Highlighting potential hazards of compounds scaling up sufficient quantities
• Assessing the toxicological impact of contaminants
• Predicting potential environmental hazards
In silico toxicology expectations

1. excellent **correlation** with the ‘wet-lab’ data
2. high **sensitivity**
3. high **selectivity**
4. **available** and easy to use

OECD principles:

- Be associated with a **defined endpoint** of regulatory importance
- Take the form of an **unambiguous algorithm**
- Have a defined domain of **applicability**
- Be **associated with appropriate measures** of goodness of fit, robustness, and predictivity
- Have a **mechanistic basis**
Possible endpoints

M - Mutagenicity
C - Carcinogenicity
SS - Skin sensitization
I - Irritancy
T - Teratogenicity
H - Hepatotoxicity
MTD - Maximum tolerated dose
BD - Biodegradation
AT - Acute toxicity
LD_{50} - Lethal Dose, which causes the death of 50 % of a group of test animals
ET - Environmental toxicities
IT - Immunotoxicity
NT - Neurotoxicity
ED - Endocrine disruption
CT - Cardiotoxicity
Toxicological process in Drug R&D

1. Understanding the contribution of disease risk factors
2. Understanding patient susceptibilities
3. Understanding target distribution
4. Understanding the consequences of metabolism and predicting actual outcomes

„On target” toxicity: exaggerated pharmacological effects
„Off target” toxicity: undesired effect, not predicted from pharmacology

Can deleterious site where the therapeutic target is located be IDENTIFIED?

„Mechanism-related” – exaggerated pharmacology:
  • target located in undesired tissue
  • parent component interacting with an undesired target exerting a different mechanism

CAN’T be solved via chemical optimization!!!

„Chemistry related” – the compound or metabolite interacts with an undesired target (e.g. covalent bond)

CAN be solved via chemical optimization!

„Dose-responsive” toxicity: toxicity can be corelated with exposure of the toxic chemical species (correct compartment + sufficient time)

In case of non dose-responsive toxicity

Greater susceptibility of certain individuals may will be sought!
In silico toxicity prediction techniques

I. Data driven systems: derive predictions from a training set of experimentally determined data

II. Expert systems: techniques that mimic human reasoning about toxicological phenomena

III. Molecular modeling techniques (docking): methods that model biochemical events that are relevant for toxicity
I. Data driven systems

Formalized methods for the extraction of prediction models directly from experimental data
  • prediction of further compounds with similar structures possible
  • require experimental data from which predictive models can be derived

Quantitative structure-activity relationship (QSAR) models are typical examples for data driven systems
Generate equations by statistically identifying molecular descriptors and/or substructural molecular attributes that are correlated with toxicity with a suitable algorithm (MLR, PLS, NN etc.)
Number of chemical features is almost unlimited – some of them might be relevant for toxicity
  • easy: to find relevant features
  • hard: to guarantee, that no important features are missing
Data Driven Systems QSAR Softwares

- **OSIRIS** Property Explorer - calculates on-the-fly various drug-relevant properties (Open source)
  

- **lazar** Open source inductive database for the prediction of chemical toxicity
  
  [http://lazar.in-silico.de/](http://lazar.in-silico.de/)

- **MC4PC** Windows based Structure-Activity Relationship (SAR) automated expert system
  

- **TOPKAT** Quantitative Structure Toxicity Relationship (QSTR) models for assessing various measures of toxicity
  
An integral part of Actelion's inhouse substance registration system
Draw a chemical structure and calculates on-the-fly various drug-relevant properties

Prediction results are valued and color coded:
• Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red
• Whereas a green color indicates drug-conform behaviour

http://www.organic-chemistry.org/prog/peo/
OSIRIS Property Explorer

The diagram below shows the results obtained by predicting all available structures of four subsets of the RTECS database. E.g. all structures known to be mutagenic were run through the mutagenicity assessment. 86% of these structures where found to bear a high or medium risk of being mutagenic. As a control set served a collection of traded drugs of which the mutagenicity risk assessment revealed only 12% of potentially harmful compounds.

http://www.organic-chemistry.org/prog/peo/
Example 1.: Application of SAR models in cardiovascular safety pharmacology

Prolongation of the QT interval

An adverse drug reaction, may also lead to the potentially lethal arrhythmia Torsades de pointes (TdP)

- antiarrhythmic drugs (Quinidine, Amiodarone, Sotalol, Procainamide, Ranolazine)
- antihistamines (terfenadine, astemizole)
- macrolide antibiotics (Erythromycin)
- certain Fluoroquinolone antibiotics
- major tranquilizers
- tricyclic antidepressants
- gastrointestinal Motility agents (Cisapride, Domperidone)
Regulatory authorities issued recommendations for the establishment of cardiac safety during preclinical drug development: ICH S7B

**Mechanism of action:**

- blocking the rapidly activating component of the delayed rectifier potassium current, termed $I_{Kr}$
- ion channel protein is encoded by the human ether-a-go-go related gene (hERG)
- drugs that induced TdP in patients were shown to be potent hERG blockers, but not all hERG blockers prolong the QT-interval and induce TdP in humans

Preclinical hERG studies should be accomplished in GLP environment by:

- *In Vitro* electrophysiology studies (whole-cell patch-clamp assays)
- *In Vivo* studies (intact animal models)

Evolution of hERG values in a Roche project after the implementation of a predictive project specific model—the model consists of only 2 calculated parameters such as the number of hydrogen-bond acceptors and the hydrophobic surface area. The initial model was trained with 11 molecules and validated with more than 100 additional results ($r^2 = 0.812; q^2 = 0.732; \text{RMSE} = 0.371$).

Example 1.: Application of SAR models in cardiovascular safety pharmacology

Phospholipidosis: the intracellular accumulation of various phospholipids reflecting a disorder in phospholipid storage in the lysosomes

Drug-induced phospholipidosis: first reported in 1966 when Greselin, an increased number of foam cells in the rat lung after the application of a cholesterol metabolism inhibitor

Pharmacological compounds may induce phospholipidosis:
- antipsychotics
- antidepressants
- antiarrhythmics
- antianginals
- antibacterial
- antimalarials
- cholesterol-lowering agents

Detection of phospholipidosis by the use of trans electron microscopy. Clean tissue (left side) versus affected tissue (right side). Images by B. Lenz.
Amphiphile is a term describing a chemical compound possessing both hydrophilic and hydrophobic properties (Common amphiphilic substances are soap and detergent)

Most of the agents that induce phospholipidosis are so-called cationic amphiphilic drugs (CAD)

Amphiphilicity can be expressed as vector sum (dashed line).

Example 2.: *In silico* screening for drug-induced phospholipidosis

1. Potential of a compound to induce phospholipidosis is characterized by **two calculated physico-chemical properties**:
   - basic pKa value (calculation by commercial programs can be utilized)
   - **amphiphilicity** (the vector sum calculated from the charged group to each atom/residue within a molecule, weighted with respect to its hydrophobic/hydrophilic property)

2. The **sum of the calculated vectors** is calibrated by means of measured amphiphilicities taking into account the conformational effects of the individual molecules.

3. The amphiphilicity of a molecule is **expressed in terms of free energy** (\(\Delta G_{\text{AM}}\)) - A program called CAFCA (CAlculated Free energy of Charged Amphiphiles) was developed and used for the calculation of the amphiphilic properties of molecules.

**Compounds** with calculated basic \(pK_a < 7\) and a \(\Delta G_{\text{AM}} > -6\) kJ/mol have **no potential hazard** in the phospholipidosis assay. With this approach approximately 80% of the positive and negative in vitro findings could be **classified correctly**.

Limitations: sensitivity vs specificity

False positive (type I error): serious of compounds needlessly discarded
False negative (type II error): allow potentially toxic compounds to continue uncovered later (higher expense, particularly patients during phase IV or post market!)

Sensitivity or the recall rate measures the proportion of actual positives which are correctly identified as such (i.e. the percentage of sick people who are identified as having the condition)
Specificity measures the proportion of negatives which are correctly identified (i.e. the percentage of well people who are identified as not having the condition). They are closely related to the concepts of and type II errors
In case of testing for faults, one may be willing to risk discarding functioning components (low specificity), in order to increase the chance of identifying nearly all faulty components (high sensitivity)

False negative >>> False positive!!!
Limitations: data

The *major bottleneck* of QSAR modelling is still the limited public availability of high-quality toxicity-data!

- **Quantity of data:** lack of relevant HUMAN data
- **Quality of data:** testing can change the outcome dramatically, results are often laboratory-specific (differences in dose, distribution characteristics, animal strains etc.)
- **Chemical space:** structural diversity of the training set - only structures existed within the training set or at least share the same mechanism of toxicity must cover predicted structures
Distributed Structure-Searchable Toxicity (DSSTox) Database Network

- DSSTox Database Network is a project of Environmental Protection Agency (EPA), National Center for Computational Toxicology
- A public data foundation for improved structure-activity and predictive toxicology capabilities
- Website provides a public forum for publishing downloadable, structure-searchable, standardized chemical structure files associated with toxicity data

http://www.epa.gov/nheerl/dsstox/index.html
II. Expert systems

Trying to formalize the knowledge of experts who assessed the toxicity of compounds in a computer program. Many of the most successful predictive software are in fact expert systems:

- intuitively appealing, easy access to topological knowledge
- prediction of biotransformations and metabolites
- provide specificity
- even works with very few experimental measurements
- integration of very diverse chemical and biological information

BUT

- accurate and regularly updated database required
- suffer from a moderate sensitivity
- a large number of molecules are ‘flagged’ leaving it uncertain
- requires extensive literature searches

Positive light: these ‘flags’ may trigger a careful evaluation
Expert System softwares

- **DEREK** Expert system for the prediction of toxicity (genotoxicity, carcinogenicity, skin sensitization, ...)  
  [http://www.lhasalimited.com](http://www.lhasalimited.com)

- **METEOR** Expert system for the prediction of metabolic transformations  
  [http://www.lhasalimited.com](http://www.lhasalimited.com)

- **METAPC** Windows based Metabolism and Biodegradation Expert System  
  [http://www.multicase.com](http://www.multicase.com)
Example 4.: Drug bioactivation and hepatotoxicity

**Major reasons for drug failure** are adverse events in man with some toxicities appearing *only during the post-approval period* of a drug, **hepatotoxicity** has been identified as the **major safety concern** for **discontinuation of clinical trials** and either **post-approval withdrawal**

**Bioactivation** of the parent drug molecules to **toxic reactive metabolites** might result in covalent binding to cellular targets resulting in hepatotoxicity via immuno-mediated mechanisms

**Preclinical tools** for the assessment of metabolism are currently available and allows medicinal chemists to find compounds with improved reactive metabolite formation, for example, DEREK and METEOR (Lhasa Ltd.) are available to predict chemistry-associated toxicities and metabolism processes

Example 4.: Drug bioactivation and hepatotoxicity

**Derek for Windows** is an expert knowledge base system that predicts whether a chemical is toxic in humans, other mammals and bacteria.

The computer program that contains expert knowledge rules in toxicology and applies the rules to make predictions about the toxicity of chemicals, usually when no experimental data is available.

The program indicates potential toxicity for many toxicological endpoints, including:

- Carcinogenicity
- Mutagenicity
- Genotoxicity
- Teratogenicity
- Respiratory Sensitisation
- Neurotoxicity
- Skin Sensitisation
- Hepatotoxicity
- Ocular Toxicity
- Irritancy
Example 4.: Drug bioactivation and hepatotoxicity

Molecular structures are entered into the program, either via a chemical editor program or by importing Molfiles, .skc, or SDfiles.

Results are supported by evidence in the form of literary citations, examples and comments allowing:

- Formation of a judgement about whether you agree with the prediction from Derek for Windows
- Consideration of ways the chemical structure could be redesigned to make it less toxic
- More extensive bibliographic search if this is required
Example 4.: Drug bioactivation and hepatotoxicity

Meteor is an expert knowledge based software program for predicting the metabolic fate of chemicals. Covers phase I and II biotransformations. Providing users with access to xenobiotic metabolic biotransformations.

Process a single structure input as a (Molfile or via a drawing). Can batch process multiple query structures and generate multiple reports.

From a query structure input by the user Meteor generates results using reasoning rules within a knowledge base containing biotransformations, intermediates and reasoning rules. Predictions take into account:

- Lipophilicity
- General prevailance of the biotransformation in the literature
- Species
- The relative liklihood of competing biotransformations

http://www.lhasalimited.org/
Example 4.: Drug bioactivation and hepatotoxicity

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Example 4.: Drug bioactivation and hepatotoxicity

METEOR not only add value to the identification of the probable sites of metabolism (metabolic soft spots), but additionally addresses the potential for reactive metabolite formation, guiding the investigators to experimental approaches for the identification of drug metabolites.

Still relative formation rates of metabolites cannot be predicted yet limiting the versatility of this tool.

Important information required for risk assessment like absolute metabolite exposure and target tissue concentration cannot be predicted.

False negative prediction of drug metabolites remains a major drawback, especially when human metabolism is being dealt with.

Generation of additional new local rules specific to a particular chemical space may improve the predictive power.

Molecular modelling techniques (docking)

Assess the interaction of small molecules with biological macromolecules (predominately proteins), by fitting the ligand into the active site of the receptor.

Mainly for pharmaceutical research – can also be applied for toxicological purposes

Can be used to elucidate mechanism and biotransformation and to predict receptor-mediated toxicity

BUT

- only for receptor mediated mechanisms (e.g. cytochrome P450, estrogen receptor)
- receptor structure must be available (homology modelling)
- complex and partially unknown mechanisms are beyond their scope
Example 3.: Predicting non-DNA reactive genotoxic activity of kinase inhibitors

Predictions of clastogenic events mediated via enzymes and receptors, for example kinases, in programming through (Q)SARs and rules yielded low sensitivity values compared to other genotoxicity assays and might therefore require other in silico approaches

- the second largest group of drug targets (after G-protein-coupled receptors), currently account for 20–30% of the drug discovery programs of pharmaceutical companies
- the largest protein family with approximately 500–1000 enzymes being encoded by the human genome
- targeted inactivation of protein kinases is primarily accomplished by using ATP binding site blocking small molecules that hamper enzymatic activity
- off-target kinase inhibition is implicated as a major cause for the induction of chromosomal damage

Careful evaluation of the specificity of any novel compounds that target kinases is needed to proactively address their safe use in the clinic

Example 3.: Predicting non-DNA reactive genotoxic activity of kinase inhibitors

Anaplastic lymphoma kinase (ALK) is a valid target for anticancer therapy.

Potent ALK inhibitors suitable for clinical use are lacking, because the majority of described kinase inhibitors bind in the ATP pocket of the kinase domain.

Computer modeling indicated that docking solutions obtained with a homology model representing the intermediate conformation of the ALK kinase domain reflected closely experimental data.

In the absence of a resolved structure of ALK molecular models are useful tools for the rational design of ALK selective inhibitors.

4-phenylamino-quinoline compounds may have potential as templates for ALK inhibitors.

Importance of considering different conformational states of the kinase domain when performing virtual screens for potential new inhibitors was also highlighted.

Example 3.: Predicting non-DNA reactive genotoxic activity of kinase inhibitors

Docking of inhibitors **SKI-606** (pink) and **PD173955** (cyan) in the intermediate and active conformations of **ALK-L256T** active model

The *activation loop* is colored in red

The DFG motif and the *gatekeeper residue* are shown in color-coded sticks (O, red; C, purple)

Example 3.: Predicting non-DNA reactive genotoxic activity of kinase inhibitors

A. Intermediate ALK-L256T SKI-606

B. Intermediate ALK-L256T PD173955

C. Active ALK-L256T SKI-606

D. Active ALK-L256T PD173955

Schematic diagram of the interactions made by SKI-606 and PD173955 docked in the 3D model of the intermediate and active conformations of the L256T-ALK kinase domain.

Conclusions

*In silico* predictive toxicology techniques are **fast** and **cost efficient alternative or supplement** to bioassays for the identification of toxic effects at an early stage of product development

**QSAR models** are capable to predict one specific endpoint – for a group of compounds (validation!)

**Expert systems** and purchasable data driven systems are capable to predict many endpoint simultaneously

- Out of box predictions for certain structures
- Lack of SAR understanding („black box”)
- Unknown mechanism
Conclusions 2

In Silico Toxicology: Is it for Real? The short answer is „Yes”…

A more relevant question: whether the in silico toxicology applications available to researchers to predict? Yes, BUT…

Results should be taken with criticism

Consideration of benefit (fast) and risk (false positive)

Too early to adequately judge the impact of computational toxicology on ADRs