Mechanism based in silico evaluation of hepatotoxicity

Milen Todorov

Abstract: Efforts to develop new predictive methods to assess the likelihood of a xenobiotic being a hepatotoxicant have been challenging due to the increasing concerns of safety chemical assessments. A huge variety of chemicals exist in the environment without information for their ability to exert hepatotoxic effect. Based on the assumption that damages on DNA may lead to hepatotoxic outcome it is assumed that available models for identification of mutagens can be used for prediction of the effect. In the current study this assumption is investigated by in silico application of DNA reactivity profilers over dataset of chemicals with known hepatotoxic effect. The results suggest that the approach can be used for identification of hepatotoxins with special concern about the influence of their metabolism. The overall predictions show high statistical performance – 86% correct predictions for hepatotoxins. Regarding non hepatotoxins it was found that the role of metabolic detoxification should be taken into account for explanation the effect of in vitro mutagenic chemicals.

Key words: Hepatotoxicity, Mutagenicity, QSAR, Metabolism, Computational tools

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. Its major functions are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being.

Substances capable of producing liver damage and, more specifically, hepatocyte damage are known as hepatotoxins. The effect is known as hepatotoxicity and implies chemical-driven liver damage. The damage caused to hepatocytes can be cytotoxic, genotoxic or metabolic [1]. The first type of injury is a common feature of many intrinsic hepatotoxins. It is evidenced by morphological changes in the structure of hepatocytes. Genotoxins are substances that produce, in first term, DNA damage and show tendency to induce primary hepatocarcinomas. Finally, drugs can also alter the cellular metabolism of hepatocytes without causing cell death. While the detection of cytotoxic and metabolic hepatotoxins requires experimental evidences the effect of genotoxic hepatotoxins is assumed to be predicted by making use of DNA reactivity toxicophores. The latter are defined in a large number of studies by examination of abundance of mutagenicity experimental data (Ames test) [2].

A huge variety of chemicals exist in the environment, and their potential to exert hepatotoxic effect has not been evaluated. In this respect the (quantitative) structure-activity relationships (QSARs) approach which is used successfully in prediction of variety biological/toxic endpoints [3] is considered as promising way in prediction of hepatotoxicity. Nowadays, the focus is set on so called expert based QSARs which allows reliable prediction of toxic or biological response for new chemicals. Such kind of models quantifies features of the investigated chemical structure so that overall toxic/biological properties of the compound can be predicted based on the relationship between structure and activity computed using the knowledge of training data set. There are several excellent reviews on the meaning of QSARs [4, 5], their pitfalls [6], and perspectives [7].

The aim of this study is to investigate the possibility to predict genotoxic hepatotoxicity by making use of in silico tool with encoded structural rules for DNA reactivity.

EXPERIMENTAL

Mutagenicity as a surrogate endpoint

As discussed in introductory section, one of the mechanisms associated with hepatotoxicity is thought to be the formation of a covalent bond between the electrophilic chemicals and the DNA bases. Schultz et al. [8] described a conceptual framework for predicting the toxicity of reactive chemicals where plausible molecular initiating events
were based on covalent reactions with nucleophiles in proteins or DNA and would ultimately lead to a variety of different adverse outcomes including mutagenicity and hepatocyte cytotoxicity. Based on this observation it is expected that the interactions associated with both effects (mutagenicity and hepatotoxicity) can be considered as an outcome of a presence of same toxicophores (structural alerts) responsible for DNA damages. As a logical sequence it is also expected that QSAR models based on mutagenicity experimental data (Ames test) can be used successfully in prediction of hepatotoxic effect of xenobiotics.

**AMES test for mutagenicity**

Mutations arise when the DNA in a cell is damaged in such a way that the information contained in the genetic code is altered. The *Salmonella* reverse mutation test – known as the Ames test [9] has been used for several decades as a useful tool for detection of potentially mutagenic chemicals. Genetically different strains of *S. typhimurium* are used for testing. They all carry some type of defective (mutant) gene that prevents them from synthesizing the amino acid histidine. In the presence of mutagenic chemicals, the defective gene may be mutated back to the functional state, allowing the bacterium to grow on the minimal medium.

Many chemicals are not mutagenic by themselves, but may be metabolized to mutagenic forms [10]. Bacteria and most cultured mammalian cells cannot perform most of the metabolic conversions found in mammals and humans because they do not contain the necessary metabolizing enzyme systems. This limitation has been partially overcome by the development of exogenous metabolic activation systems that can be added to the test procedure. These systems usually consist of homogenates of liver fractions (S9) of rodents, usually rats that had been pretreated with substances to enhance the levels of the preferred metabolic enzymes [10]. A complete in vitro testing protocol includes tests with and without S9 fractions.

**OECD QSAR Tollbox**

This is a software tool especially designated for chemical risk assessment [11]. A key part of the system is so called categorization of chemicals. The categorization allows grouping of chemical substances into chemical categories. The chemical category is such a group of substances possessing similar physicochemical, toxicological and ecotoxicological properties or their fate in environmental and occupational surrounding or they behave using the common pattern as a result of chemical similarity.

An important advantage of the system is the large number of built-in profilers for different biological/toxic endpoints. Each profile consist a set of rules related to specific or general criteria associated to the respective endpoint.

Another advantage of the system is the opportunity to investigate a chemical with account to its metabolic fate. It is well known that the chemical in its parent form may not exert toxic effect however after metabolism a reactive metabolite can be produced which may damage biological macromolecules. This became extremely important in assessment of mutagenic potential of various type of chemicals.

In the following two sections details will be given for current versions of both profilers associated with DNA damages and *in vitro* metabolic simulator incorporated in version 3.2 of the Toolbox.

**Profiling schemes for DNA damages**

Two profiling schemes for screening of potential DNA damaging chemicals are implemented in the Toolbox. The first one is based on Ames mutagenicity model part of OASIS TIMES system [12]. The profiler contains exact definitions of 78 structural alerts responsible for interaction of chemicals with DNA. The second profiler was created following the mapping of existing structural alerts for mutagenicity and carcinogenicity. The
mapping was performed to achieve maximum overlap and usability whilst restricting redundancy in the alerts, and to ensure that the alerts related to the molecular initiating event of covalent DNA binding by OECD [13]. A total of 60 new or re-defined alerts have been created. The scope of both profilers is to investigate the presence of alerts within the target molecules responsible for interaction with DNA.

**Metabolism simulator**

The current in vitro rat liver metabolic simulator represents electronically designed set of 509 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. A training set of 647 xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their metabolism in rodent liver microsomes and S9 fraction. On the whole, the simulator contains 450 – 470 enzymatic phase I transformations, such as aliphatic C-oxidation, aromatic C-hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide hydrolysis, carbonyl group reduction, nitro and azo group reduction, N-hydroxylation, etc. Additionally, 15 – 20 enzymatic phase II transformations, such as glucuronidation, sulfation, glutathione conjugation, N-acetylation, etc. are included with significantly lower priority than phase I ones.

The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit diverse array of in vitro biological effects.

**RESULTS AND DISCUSSION**

The concept of relating mutagenicity data to hepatotoxicity data has been the subject of limited assessment. The relationship between hepatotoxicity and Ames mutagenicity was studied by analyzing experimental data for both effects taken from literature source for hepatotoxicity [14] and Ames data form OASIS genotoxicity database included in the Toolbox. The overlapping 56 chemicals were analyzed and it was found that almost 80% (44/56) were hepatotoxic and mutagenic simultaneously. It should be noted that the number of investigated chemicals is not sufficient for ultimate conclusion, however it is enough to show that there is a link between both effects.

The overall predictions for hepatotoxins as a result of presence of DNA structural alert(s) is presented in Table 1.

<table>
<thead>
<tr>
<th>Hepatotoxicity/# chemicals</th>
<th>Identified DNA alerts</th>
<th>No alert found</th>
</tr>
</thead>
<tbody>
<tr>
<td>-S9</td>
<td>+S9</td>
<td>25</td>
</tr>
<tr>
<td>Hepatotoxins (176)</td>
<td>107</td>
<td>44</td>
</tr>
<tr>
<td>Non hepatotoxins (107)</td>
<td>64</td>
<td>N/A</td>
</tr>
</tbody>
</table>

To assess the possibility to predict hepatotoxins as a result of identified DNA binding alert(s) a set of 281 chemicals [14] with known effect was screened with both DNA profilers in the Toolbox. Initially profilers were applied on parent structures without simulation of metabolism. Better performance shows the OECD profile in terms of identification of DNA binding alerts in 61% (107 chemicals) from all 176 hepatotoxins. Next, the rest 69 chemicals without DNA alert were screened again in combination with in vitro metabolism simulator. The result shows that DNA alert(s) were found in metabolites for 44 chemicals. This finding is not surprising while most xenobiotics are known to be not intrinsically toxic to the liver but can cause injury secondary as a result of production of an
hepatotoxic metabolite(s), as a result of bioactivation [15, 16]. Taken together, prediction results with and without metabolic activation leads to 86% (154/176) correct predicted hepatotoxins. Regarding the group of the chemicals which do not contain any alert (25 chemicals) with and without metabolic activation it is expected that the hepatotoxic effect is probably a result from non genotoxic mechanism, hence it is not possible to be predicted following the logic in this study. In conclusion one may state that the result for hepatotoxins is acceptable and can be used as an evidence for reliability of the predictions obtained by using in silico approach for this effect.

The analysis of non hepatotoxins (105 chemicals) was performed in the same sequence of steps. It is expected that the chemicals from this group lacks DNA binding alert(s) and this was found true for 41 chemicals. The focus of further investigation was set on the rest 64 chemicals - non hepatotoxins in which DNA alert was found. The first direction was to explore the mutagenic effect of these chemicals as response in Ames test with metabolic activation. For part of them (16 chemicals) the mutagenic effect was confirmed with available experimental data in Oasis genotoxicity database in the Toolbox. In this case it is expected that the role of detoxification in metabolism is crucial for non hepatotoxic effect. Inactivation of reactive metabolites was discussed recently by Mekenyan et al. [17] in a study regarding in vitro - in vivo genotoxicity. It is known that in vitro, all generated metabolites are theoretically available and able to interact with macromolecules present in the incubation medium, and thus have the potential to elicit a mutagenic effect [18]. In vivo, enzymes are aggregated in multienzyme complexes and the cells could be protected from reactive metabolites via formation of intermediates between consecutive enzymes. The channeling effect allows the product of one enzymatic reaction to become a substrate of the subsequent enzymatic reaction. It can be therefore assumed that the in vitro active chemicals and/or their active metabolites are not freely available in vivo to cause damage. The majority of these metabolites are considered to be "blocked" across the in vivo detoxification pathways. This hypothesis is successfully applicable for some in vitro mutagens (e.g. aromatic amines possessing polar functional groups) for which has been shown that the parent chemicals and/or their metabolites could be 'inactivated' in liver detoxification pathways.

Based on this consideration it is expected that the results for non-hepatotoxins can be significantly improved if the detoxification logic is implemented as a component in the screening schema. The influence of such addition regarding reliability of the predictions will be a subject of further study of improved approach for identification of hepatotoxic chemicals.

CONCLUSION

Hepatotoxicity (liver injury) is one of the toxic effects which should be evaluated as it is required by many directives for safety assessment of chemicals. A huge variety of chemicals exist in the environment, and their potential to exert hepatotoxic effect is unknown. The possibility to predict the effect by using in silico approach is discussed in this study. Based on the assumption that the DNA reactivity is one of the mechanisms responsible for hepatotoxicity it is assumed that models for mutagenicity can be used for prediction of this effect. This hypothesis was investigated by application of DNA reactivity profilers in the OECD QSAR Toolbox over large number of chemicals with known hepatotoxic effect. The results suggest that this approach can be used successfully with special notice regarding mandatory account of metabolic activation of the investigated chemicals. The overall prediction for hepatotoxins shows high statistical performance – 86% correct predictions. Regarding non hepatotoxins it was found that the role of detoxification should be taken into account for explanation of the non hepatotoxic effect of in vitro mutagenic chemicals.
REFERENCES


About the author:
Milen Todorov, PhD, Department of Inorganic Chemistry, Faculty of Natural Sciences, University "Prof. Assen Zlatarov", 1 Prof. Yakimov str., Burgas, Bulgaria, tel. 056/716 491, e-mail: mtodorov@btu.bg

This paper has been reviewed