COMPARATIVE STUDY OF SEVERAL CLASSES OF ORGANIC COMPOUNDS FOR HEPATOTOXICITY. 1. COVALENT BINDING IMPACT

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ABSTRACT: The reactions between the reactive xenobiotic chemicals and cellular components in the living organisms are regarded as non-specific, as opposed to many receptor-binding interactions. Such interactions, however, can disrupt many different cellular processes, causing a wide variety of adverse outcomes such as acute failure of energy flow, loss of nerve function, skin irritation/sensitization, immune system dysfunction, reproductive and developmental impairment, idiosyncratic organ failure and death as well as mutagenicity and carcinogenicity. Hepatotoxicity represents one of many serious adverse health effects, caused by a number of xenobiotic chemicals. Modeling these adverse outcomes is a challenging task, since chemicals from many different structural classes may cause similar biological effects. A fundamental assumption is that there are key molecular triggering events in the xenobiotic toxicity pathway. The covalent binding of chemicals with proteins is recognized as one of these events occurring at molecular level and resulting eventually in manifestation of hepatotoxic effects. Thus the elucidation of the mechanisms involved in these interactions is an essential prerequisite when making classification decisions. Classification conducted in accordance with mechanistic principles (i.e., how a chemical and target organism interact biochemically), and the impact of these events on the nature and the extent of hepatotoxicity could be the key for the predictive applicability of QSAR models. Therefore, the aim of the present study was to evaluate the influence of the chemical structure of different classes of organic compounds to their ability to directly interact with biological macromolecules and elicit hepatotoxicity effects.

Key words: organic compounds, hepatotoxicity, covalent binding

Introduction

The liver is one of the most common organs damaged by xenobiotic chemicals, since it is regarded as the metabolic “factory” in mammalian. Considering that the drug-metabolizing enzymes are mostly concentrated in liver, the latter plays a central role in the metabolism, detoxification and bioactivation of xenobiotics. Thus, it is not surprising that liver can be subjected to hepatotoxic injury; it is often the first major organ to show either adaptive or more severe adverse response to toxic insults [26]. Hepatotoxicity can represent a major threat, which has to be considered in the course of the drug development, and it is one of the principal remaining challenges to drug discovery [6]. The adverse effects that occur may involve neoplastic or non-neoplastic changes to the liver that can seriously compromise hepatic structure and functions, and can be life-threatening. For these reasons, hepatic toxicity has always been considered as an important toxicity endpoint in the safety and risk assessment [18, 29] and drug development. Despite the considerable progress in the understanding of the mechanisms of liver toxicity, in many cases, researchers are still unable to design non-hepatotoxic drugs rationally; the effect of the dose administered is also a major obstacle in this respect. Presently, there are no universal in vitro (molecular or cellular) screening approaches that can be applied systemically for early identification of “hepatotoxic” molecular structures [4]. As a consequence, hepatotoxicity, in most cases, is detected at the later stages of drug development in vivo (whole animal) toxicity studies or at late stages of clinical trials, or even after market release [4].

Several factors can be identified that contribute to the incidence of liver toxicity. The main mechanisms underlying liver injury include the following phenomena [23]: (1) Disruption of membrane integrity or disruption of intracellular ion gradients (i.e. intracellular calcium homeo-
and ATP levels leading to actual cell swelling and cell rupture; (2) Disruption of transport proteins: drugs can affect transport proteins at the canalicular membrane, preventing the excretion of bilirubin and other organic compounds, thus causing cholestasis; (3) Biotransformation: it is sometimes associated with formation of reactive metabolites and enzyme/protein adducts, which can act as immune targets; (4) Cytolytic T-cell activation: the covalent binding of a drug to the CYP450 enzyme can sometimes act as an immunogen, which activates T cells and cytokines, thereby stimulating an immune response; (5) Apoptosis of hepatocytes: the activation of the apoptotic pathways by the so-called tumor necrosis factor α (TNF-α) or Fas receptor can trigger a cascade of intercellular caspases; (6) Mitochondrial disruption: certain drugs inhibit mitochondrial function by binding and disabling respiratory chain enzymes or β-oxidation enzymes, causing oxidative stress. Even though several different mechanisms might be involved in the onset and progression of hepatotoxicity effects of a single substance, there are likely to be only a few principal mechanisms that are activated within the general toxic response of the liver [2].

The actions of xenobiotics in the body exert their specificity, depending on the compounds chemical structure and reactivity. Many physico-chemical and structural descriptors reflect simple molecular properties that can provide insight into the physico-chemical nature of the activity under consideration. Based on this paradigm, QSAR modelling has evolved over the last 100 years [12]. There is an ongoing interest in structure-activity relationships in the field of toxicology. This is because they offer tools for relating toxicological data (or other biological activity end-points) across a spectrum of chemicals. Thus some associations can be made, transcending the peculiarities of single-chemical toxicological experiments and conceivably revealing aspects of toxicological mechanisms that can be generalized across chemicals [24]. The development of QSARs for the toxic effects of reactive chemicals lacks a consistent approach despite the abundance of literature available for QSAR models [34]. A novel concept has been proposed for modelling reactive toxicity, based on reaction mechanism-defined domains and the association of these reactions to cellular targets of toxicological importance. Identification of molecular initiating events such as the covalent reaction with nucleophiles in proteins and DNA that lead to a causal chain of biological effects was suggested to result in improved correlates for toxic effects [34].

It is now recognized that hepatotoxicity induced by xenobiotics can be often attributed to the formation of reactive electrophilic metabolites under the influence of xenobiotic-metabolizing enzymes. Reactive electrophilic substruc-tures (named in accordance with the substructures, representing the electrophilic reactive site) include isocyanates, carbonyl compounds, epoxides, activated carbon-carbon double bonds, alkyl and aryl halides, etc., to name a few [19]. Active, electrophilic metabolites can react with several types of nucleophilic targets. Amino (NH₂), hydroxyl (-OH) and sulfhydryl (-SH) groups are among the most important nucleophilic functionalities from a biological point of view, because they are found in many biological macromolecules such as proteins, and in purine and pyrimidine bases in DNA [19]. The covalent binding of reactive intermediates to macromolecules has the potential to be involved in severe adverse reactions in one of the two principal ways: (1) direct toxicity, where the formation of chemical adduct results in alteration of critical proteins so that the normal cell function of the protein cannot be maintained; and (2) altered immune recognition of target proteins resulting in immune-mediated toxicity [10, 15, 20]. The general association of protein binding with toxicity has led to the covalent binding hypothesis, which suggests that binding to critical cellular proteins may be an initiating event in some target organ toxicities [10].

The interactions between reactive xenobiotics and cellular components are not specific (as opposed to many receptor-binding interactions). However, they can disrupt many different cellular and/or organ/tissue-mediated processes [34], causing a wide variety of adverse outcomes, such as acute failure of energy flow, loss of nerve function, skin irritation/sensitization, immune system dysfunction, reproductive and developmental impairment, idiosyncratic organ failure and death, as well as mutagenicity and carcinogenicity [34]. Modelling these adverse outcomes is a complex challenge because chemicals from many different structural classes of compounds cause similar biological effects. Furthermore multiple pathways lead to multiple biological effects that result in the same adverse outcome [34]. A fundamental assumption is that there are key molecular triggering events in the xenobiotic toxicity pathway. Recognition of at least one of these events as being of principal importance
Covalent binding, although frequently appearing to be the principal cause of toxic phenomena, is rarely possible to be directly associated with a well-defined cell injury. For certain drugs (acetaminophen, bromobenzene), there is a clear correlation between the extent of covalent binding to proteins and the severity of hepatocytes injury [5, 21]. However, with other xenobiotic chemicals, such direct correlation with hepatotoxicity cannot be identified. This is the case with chemicals such as bromophenol, and the paracetamol analog N-acetyl-m-aminophenol, which are able to covalently bind to hepatocytes, yet it does not result in liver damage [5]. On the opposite, covalent binding is inversely correlated with the extent of hepatotoxicity as is the case with diclofenac. In the latter case, binding of the drug to proteins takes place mainly via trans-acylation reaction of the diclofenac glucuronide moiety as a result of phase II metabolic transformation [17]. This process does not cause cytotoxicity per se, whereas the CYP450-catalyzed phase I metabolism of a drug produces toxic metabolites that ultimately cause cell injury [28]. These two metabolic routes co-exist. Thus the more diclofenac is metabolized by CYP450 isoenzymes, the less of it becomes available for covalent binding through the formation of glucuronide [22].

The aim of the present study was to evaluate the influence of the chemical structure, associated with different classes of organic compounds to their ability to directly interact with biological macromolecules and elicit hepatotoxicity effects.

**Materials and Methods**

**Chemicals.** Several classes of organic compounds were selected for discussion (Table 1).

**Electrophilic reaction mechanism domain.** The most common reaction mechanisms associated with different classes of organic compounds are Michael type additions; other nucleophilic (S_N2 and S_Nar) reactions; some acylation reactions; and A_N2 Schiff base formation with aldehydes (Scheme 1).

Identifying the most probable reaction mechanism for certain compounds is sometimes not addressed to modelling of toxicity, which is frequently the cause of many prediction errors [34]. Thus the mechanisms of action are essential when making classification decisions. Classification, according to mechanistic principles (i.e., how a compound and target organism “decide” on the nature and extent of the toxic effect) could be the key for the predictive applicability of QSARs [3].

**OECD (Q)SAR Application Toolbox.** (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical in relation to its molecular structure and have the potential to provide information on hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate the practical application of (Q)SAR approaches in regulatory contexts by governments and industry, and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox [27].
Scheme 1. Reaction mechanisms domains for protein binding. Five major reaction mechanisms for the interaction of an electrophile and nucleophile are selected on the basis of organic chemistry principles and criteria for classification of chemicals in structure-activity relationships.

Results and Discussion

Possible chemical mechanisms of protein binding for different classes of organic compounds, which were collected from the references and predicted by (Q)SAR Application Toolbox and the classification mechanistic principle [3] are presented in Table 1.

Table 1. Probable chemical mechanisms of different classes of organic compounds by (Q)SAR Application Toolbox and the classification mechanistic principle [3].

<table>
<thead>
<tr>
<th>Class of organic compound</th>
<th>Name of compound</th>
<th>Structure of compound</th>
<th>Hepato toxicity log LC$_{50}$ (μM)</th>
<th>Protein binding [ref]</th>
<th>Protein binding [3]</th>
<th>Protein binding by Toolbox prediction [27]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroaromatic compounds</td>
<td>Nitrobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>3.70</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>4-nitroaniline</td>
<td><img src="image" alt="Structure" /></td>
<td>3.54</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td>Halo-nitrobenzenes</td>
<td>2-chloronitrobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>2.85</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>3-chloronitrobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>3.18</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>4-chloronitrobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>3.48</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td>Dinitrobenzenes</td>
<td>1-chloro-2,4-dinitrobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>1.78</td>
<td>[16, 30]</td>
<td>S$_{N}$Ar</td>
<td>Nucleophilic substitution of haloaromatics</td>
</tr>
<tr>
<td>Halobenzene</td>
<td>Bromobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>3.06</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>1,2-dibromobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>2.68</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
<tr>
<td></td>
<td>1,3-dibromobenzene</td>
<td><img src="image" alt="Structure" /></td>
<td>2.69</td>
<td>[16, 30]</td>
<td>No binding</td>
<td>No binding</td>
</tr>
</tbody>
</table>
Nitroaromatic compounds possess multiple modes of toxicity. There is no consensus of opinions as to what the actual mechanism of their toxic action may be [11], since nitroaromatics undergo transformations, characterized by variety of mechanisms, with biological relevance, depending on the experimental systems and species, and the intrinsic reactivity pattern of the chemical under study [32]. Generally, nitrobenzenes require enzymatic reduction of the nitro group to elicit cytotoxicity effects [7].

The α,β-unsaturated carbonyl structures consists of a carbon-carbon double bond (C=C) conjugated to a carbonyl group (C=O). The proximity of a double bond to a carbonyl group activates the C=C, enhancing its electrophilicity [1]. Consequently, a variety of adverse effects can occur by their binding to critical proteins and DNA. These α,β-unsaturated carbonyl compounds can be aldehydes, esters or ketones depending on the principal functionality in their molecular structures. It has been suggested that the position of the carbonyl group within the molecule has an impact on the mechanism of toxicity and that each class should be analyzed as separate entities [33]. For example, α,β-unsaturated aldehydes have a terminal carbonyl (aldehyde) moiety, which, along with its unsaturated counterpart can participate alone or in sequence of chemical reactions with other molecules [14, 33].

Xenobiotics interact with proteins in a variety of ways (Table 1). Some of compounds (parent structures) interact directly with proteins but others have no pronounced electrophilic properties (no protein binding) in Table 1. In the field of hepatotoxicity, one of the most frequently studied interactions between xenobiotics and proteins is that of the enzyme-catalyzed metabolism of substrates. Probably, some of chemicals are metabolized (more particularly, bioactivated) to electrophilic derivatives as reactive metabolites, which may covalently bind to the nucleophilic sites within the cell to produce a toxic effect such as hepatotoxicity. Some amino acids as building blocks of proteins possess nucleophilic functionalities, and electrophiles may react with these nucleophilic sites to produce altered proteins. These interactions are believed to be in the core of the mechanisms, by which many chemicals produce their toxic effects to cells.

### Conclusion

This study is mainly focused on some classes of organic chemicals that interact with the amino acid side functionalities involved in the primary structure of proteins, to produce alterations...
which are believed to be important for eliciting hepatotoxicity effects. In most cases, these interactions occur in the target cells such as hepatocyte, which is the site of formation of reactive species; however, some electrophilic metabolites leave the site of their formation and react with proteins in other cells or tissues. The latter types of interactions are also discussed in order to contribute to our general understanding of how toxic metabolites react with proteins.

References


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