MOLECULAR MECHANISMS ASSOCIATED WITH HEPATOTOXICITY OF CARBONYL COMPOUNDS

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ABSTRACT

Hepatotoxicity represents a serious adverse health effect caused by a number of xenobiotic chemicals. This effect is generally detected at the later stages of drug development or with in vivo (whole animal) studies. Both the chemical structure and reactivity of an organic compound have significant impact on its mode of action, and, hence, the anticipated hepatotoxic effects. Currently, there is an ongoing interest in the structure-activity relationships in the field of the chemical toxicology. Considering the fact that enzymes, catalyzing the biotransformation of xenobiotics are predominantly concentrated in liver, the latter plays a significant role in the metabolism, detoxification and bioactivation of external chemicals in the living organism. Protein and DNA covalent binding, which represent formation of adducts of the parent chemicals or their active metabolites with the biological macromolecules is considered to be a key initiating step that can be used to predict the potential of a chemical to cause specific harmful effects. The identification of the electrophilic reactivity of xenobiotics and their metabolites towards target biological macromolecules could serve as an important step for the elucidation of many toxic effects, including hepatotoxicity. Therefore, the aim of the present study was to identify the probable chemical mechanisms, associated with the liver injury, caused by an important class of organic chemicals such as carbonyl compounds.

Key words: *α,β-unsaturated aldehydes, molecular mechanism, hepatotoxicity*

Introduction

The liver as the principal xenobiotics-metabolized organ is vulnerable to injury or toxic overload. Considering that the enzymes, catalyzing the biotransformations of xenobiotics are mostly concentrated in liver, the latter plays a central role in the metabolism, detoxification and bioactivation of xenobiotic chemicals. Thus, it is not surprising, that liver represents the first organ to show either adaptive or severe adverse response to toxic insults [15]. Hepatotoxicity is regarded as major threat, which should be considered during drug development, and is quite a challenge to discover a drug devoid of this adverse effect [5]. Most of the so-called "black box warnings" and withdrawals of drugs from the market are associated with hepatotoxicity [20].

Several factors can be indicated that contribute to the incidence of liver toxicity. The main mechanisms, underlying liver injury include the following adverse effects [13]: (1) Disruption of membrane integrity or intracellular ionic gradients (e.g., intracellular calcium homeostasis) and ATP levels leading to action disruption, cell swelling and cell rupture; (2) Disruption of transport proteins: some drugs affect the functions of the transport proteins at the canalicular membrane, preventing the proper excretion of bilirubin and other organic compounds, which in turn causes cholestasis; (3) Biotransformation, more particularly, bioactivation: it is associated with the formation of reactive metabolites that are able to form enzyme/protein/or DNA adducts, which can act as immune targets or cause genotoxicity. (4) Cytolytic T-cell activation: covalent binding of a drug to CYP-450 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 tumor necrosis factor α (TNF- α) or F as receptor can trigger a cascade of intercellular caspases; (6) Mitochondrial disruption: certain drugs inhibit the mitochondrial function by binding to, and disabling respiratory chain or β-oxidation enzymes, causing oxidative stress. Even though several different mechanisms might be involved in the onset

and progression of hepatotoxicity 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 nature of the activity under consideration. Based on this paradigm, QSAR modelling has evolved over the last 100 years [8]. The fundamental assumption in the QSAR approach is that structurally similar chemicals have sufficiently common mechanistic elements to share a common rate-determining step and similar energy requirements for eliciting their biological activity [14]. Therefore, if a mechanistic hypothesis can be proposed that links a group of related chemicals with a particular toxicological endpoint, the hypothesized mechanism can be used to select physical, chemical or reactivity parameters in order to establish a structure-activity relationship [4].

It is now recognized that hepatotoxicity induced by xenobiotics can be, in many cases, attributed to reactive electrophile metabolites formed by the biocatalytic action of drug-metabolizing enzymes. Reactive electrophilic substructures (named in accordance with the substructure representing the electrophilic reactive site) include isocyanates, carbonyl compounds, epoxides, activated carbon-carbon double bonds, and alkyl and aryl halides, to name a few [11]. Electrophilic metabolites can react with several types of nucleophiles. Amino ($-NH_2$), hydroxyl (-OH) and sulfhydryl (-SH) functionalities are among the most important from a biological point of view because they are found in many biological macromolecules such as proteins and DNA [11]. The covalent binding of reactive intermediates to macromolecules as an initial molecular event has the potential to be involved in severe adverse reactions in at least one of two principal ways: (1) direct toxicity where the chemical adduct results in alterations 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 [7, 10, 12]. 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 [7].

In the light of the points discussed above, the aim of the present study was to identify the probable chemical mechanisms, associated with the liver injury, caused by an important class of organic chemicals such as carbonyl compounds, more particularly, α,β-unsaturated aldehydes.

Material and Methods

Compounds. α,β-Unsaturated aldehydes were selected for discussion (Table 1).

Electrophilic reaction mechanism domain. The most common electrophile-nucleophile reaction mechanisms are Michael type reactions, S_N2 reactions, S_NAr reactions, acylation reactions and Schiff base formation. Identifying the most probable reaction mechanism for compounds is often not addressed in modeling toxicity and is frequently the cause of many prediction errors [17]. 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].

QSAR Application Toolbox. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on hazards of chemicals, while reducing time, monetary cost 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 [16].

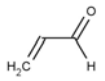
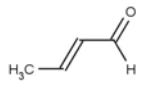
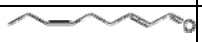
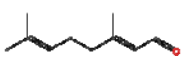
Results and Discussion

The α,β-unsaturated carbonyl structure fragment 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 binding of such compounds to critical proteins and DNA. These α,β-unsaturated carbonyl compounds can be aldehydes, esters or ketones, depending on the electron-withdrawing functionality. 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 analysed as separate entities [18]. α,β-Unsaturated aldehydes possess terminal aldehyde moiety, which, along with its unsaturated C=C counterpart can participate alone or in a sequence of chemical reactions with other molecules [9, 18].

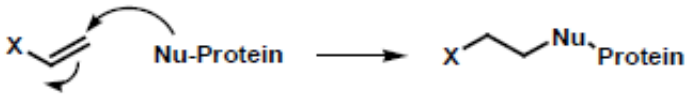
The interactions between reactive xenobiotics and cellular components are not specific (as opposed to many receptor-binding interactions) but they can disrupt many different cellular and/or organ-specified processes [17], causing a wide variety of adverse outcomes such as acute failure of energy flow, 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 [17]. Modelling these adverse outcomes is a complex challenge because chemicals from many different structural classes of compounds cause similar biological effects. Probable chemical mechanisms of α,β-unsaturated aldehydes by QSAR toolbox [16] and the classification mechanistic principles [3] are presented in Table 1.

Table 1. Probable chemical mechanisms of α,β-unsaturated aldehydes

Name of compound	Structure of compound	Rat oral LD_{50}, mg/kg [19]	Mouse oral LD_{50}, mg/kg [19]	Cytotoxicity LC_{50}, μM [6]	Probable mechanism identified by Toolbox [16]	Probable mechanism by [3]	Biotrans formation [6]
2-propenal		26	13.9	40±4	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro-genase oxidation
2-Butenal			240	150±15	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro-genase oxidation
2-Pentenal				227±30	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro genase oxidation
2-Nonenal		5000		130±15	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro genase oxidation
2,4-Nonadienal				110±10	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro genase oxidation
2,6-Nonadienal				180±20	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro genase oxidation
3,7-Dimethyl-2,6-octadienal				226±23	1,4-Michael addition, Schiff base formation	1,4-Michael addition, Schiff base formation	Aldehyde dehydro genase oxidation

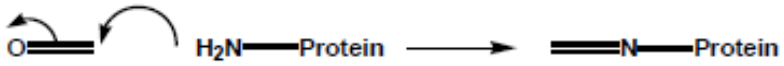
The electrophilic mechanisms of α,β-unsaturated aldehydes, which can be associated with hepatotoxic effects are presented (Scheme 1).

Michael acceptors



Identification characteristics: double or triple bond with electron-withdrawing substitutent X, such as –CHO, -COR, -CO_2R, -CN, -SO_2R, -NO_2.

Schiff base formers



Identification characteristics: Reactive carbonyl compounds such as aliphatic aldehydes, some α,β and α,γ diketones, α-ketoesters. Simple monoketones and aromatic aldehydes can be excluded.

Scheme 1. Electrophilic mechanisms (1,4-Michael addition and Schiff base formation)

Conclusion

The characteristic molecular mechanisms eliciting hepatotoxicity of α,β-unsaturated aldehydes have been generalized. This can serve as a prediction tool for such effects for structurally similar chemicals. The molecular (electrophilic) mechanisms of α,β-unsaturated aldehydes, which were identified for hepatotoxicity are 1,4-Michael addition and Schiff base formation.

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