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Reflection paper on non-clinical evaluation of drug-induced liver injury (DILI)

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Table of contents

1. Introduction

Drug-induced adverse liver effects are frequent events in non-clinical and/or clinical studies during the development of new drug entities. The detection of hepatotoxicity alerts is a continuous process covering all drug development phases, and the identification of a hepatotoxic potential in non-clinical studies has frequently resulted in delayed or discontinued development of drug candidates.

This reflection paper reviews current approaches to the detection of drug-induced hepatotoxicity alerts in nonclinical, regulatory toxicity studies and proposes their integrated risk assessment. The aim of this paper is foremost to provide an overview and guidance to facilitate further discussions on this topic. Based on new developments this paper can be updated and ultimately lead to a specific guidance document.

To date standard non-clinical toxicity studies remain the cornerstone of the prediction of hepatotoxicity in humans. However, new approaches as well as the refinement of existing methods are necessary to improve prediction of drug-induced liver injury (DILI) in humans. Various promising investigative approaches are currently being evaluated for potential screening purposes or use on a case-by-case basis following hepatotoxicity alerts in standard non-clinical toxicity studies. The principles of the present proposal may also be applied to the resolution of adverse reactions in the liver identified by pharmacovigilance or new publications.

Further, a number of industry/academic/regulatory consortia are assessing the utility of new approaches. These consortia are amongst others operating under the Innovative Medicines Initiative (IMI) and the C-path and ILSI/HESI institutes. If one or more of these consortia initiatives leads to the validation and acceptance of new approaches, it may be appropriate to revise this document.

2. Scope

The aim of this paper is to provide a perspective on non-clinical approaches to identify, characterise, and assess the risk of drug-induced hepatotoxicity in humans. A key part of this approach is the collection and interpretation of data generated during the standard drug development studies, which is the basis of the current hepatotoxicity testing paradigm. A thorough assessment of results from standard toxicity tests should clarify the need for additional *ad hoc* investigations and guide the type of investigations that may subsequently be needed. Such investigations should be performed on a case-by-case basis, to improve the understanding of findings observed in standard non-clinical studies and to provide a better understanding of the mechanism(s) involved. Novel, properly evaluated non-clinical investigative studies may also help to improve the prediction or understanding of the risk of hepatotoxicity in humans.

3. Key considerations

Non-clinical animal toxicity studies are designed to detect and characterise target organ toxicities (ICH M3(R2), 2009), and are of proven value for detection of hepatotoxicity caused by many drugs, agrochemicals, industrial chemicals and other compounds (Amacher, 1998; Greaves et al., 2004). Drug-induced adverse liver effects that are reported in non-clinical studies during the safety evaluation of various new drug entities require careful evaluation and risk assessment to determine whether clinical testing of the new drug can be conducted safely. In numerous instances, non-clinical hepatotoxicity alerts have resulted in delayed or discontinued development of drug candidates.

Some candidate drugs may cause isolated adverse liver effects in humans that are not predicted from non-clinical studies. This type of drug-induced liver injury (DILI), termed "idiosyncratic". The mechanism of human idiosyncratic liver effects appears to include an interaction of genetic and non-genetic factors that are not reproduced in standard non-clinical toxicity studies (Ulrich, 2007; Walgren et al., 2005). Retrospective analysis of non-clinical data has provided no evidence that idiosyncratic drug-induced hepatotoxicity in humans could have been predicted from non-clinical toxicity data (Kaplowitz, 2005; Ong et al., 2007, Peters, 2005). Given the current understanding that idiosyncratic drug reactions (IDRs) are rare, human-specific, and most often dose-independent events, it should be emphasised that an improved prediction of idiosyncratic hepatotoxicity may not be achievable on the basis of non-clinical toxicity data. It is conceivable that improved detection and prediction of idiosyncratic drug-induced hepatic injury may be achieved in the future by new predictive biomarkers and/or *in vitro* and/or *in vivo* models, which are currently not available and/or validated. Approaches of individual companies as well as international collaborative research initiatives aiming at incorporating promising innovative tests into the regulatory requirements are continuing (Evans et al., 2004). Taking into account the above considerations, this document will not further discuss investigation of idiosyncratic hepatotoxicity.

Given the large amount of non-clinical and clinical data collected during the course of drug development, it is not surprising that non-clinical hepatotoxicity alerts could be found *post hoc* for some compounds that subsequently displayed hepatotoxicity in the clinic; but when these data were critically evaluated in the context of all other study data, including for positive associations or the lack thereof, most often they had unclear relevance. It is noteworthy that the concordance between clinical pathology changes (mainly serum liver enzyme data) in humans and laboratory animals may be as low as 40%, although the correlation of animal / human toxicity may be improved by incorporation of animal histopathology data (Greaves et al., 2004, Olson et al., 2000).

In the next section current approaches for the detection and evaluation of drug-induced hepatotoxicity alerts in regulatory toxicity studies will be reviewed. Also a rationale for conducting follow-up studies to assess the clinical relevance of hepatotoxicity alerts in non-clinical studies is proposed. The use and interpretation of available data from standard non-clinical toxicity studies will be clarified in order to mitigate hepatotoxic risks during clinical trials and the marketing of new drugs.

4. Evaluation of hepatotoxic effects

The prediction of hepatotoxic potential of a drug requires the identification of the hazard and the assessment of the relevance of the identified hazard to the relative risk to patients. The following step-wise approach provides a useful framework:

1. Detection and characterisation of hepatotoxic potential in standard non-clinical in vivo toxicity studies.
2. Integrated risk assessment which considers dose- and exposure-response relationships, relative severity of effects, intended patient populations, and clinical monitoring strategies.
3. Hypothesis-driven preclinical investigative studies, when warranted, to further clarify risk to patients.

4.1. Detecting and characterising hepatotoxic potential

Conventional *in vivo* toxicology studies, as described in the ICH M3 guidance with standard histopathology and clinical pathology assessment of the liver, may yield a reliable prediction of the potential of candidate drugs to cause human liver toxicity in clinical trials (ICH M3(R2), 2009): non-clinical toxicity studies are designed to evaluate the potential hepatotoxicity as well as the toxicity to other target organs.

When interpreting the results of such studies, consideration must be given to the relevance of the test species, particularly a) whether pharmacokinetics / pharmacodynamics and metabolism are comparable to those in humans, b) the type and severity of the effect(s), and its relationship to dose- and systemic exposure of the drug and/or its metabolite(s), c) comparison with appropriate historical control data, and d) consistency across studies and species, e) whether the test substance belongs to a class of chemically similar compounds known to pose a risk for hepatotoxicity, or whether such signals are described in literature.

Histologically, acute and subacute hepatic toxicity most commonly involves hepatocellular necrosis/inflammation, steatosis or altered glycogen content, biliary alterations, cholestasis, vascular disorders, or multiple lesions. Evaluation of liver histopathology by light microscopy, ultrastructural pathology (as needed), along with standard clinical pathology markers of liver injury (discussed in further detail below), currently represents the most reliable method for the assessment of hepatotoxicity in standard toxicology studies (Olson et al., 2000). The following points should be given attention:

- In rodent studies, the magnitude of the liver effects is generally determined by comparison of group mean data with concurrent controls. However, significant individual deviations should be recorded.
- In non-rodent studies, where the total number per group is generally low, mean values may be less meaningful or reliable. Individual clinical pathology values should be compared to pre-study data and consideration should be given to the possibility that hepatotoxicity alerts observed in small numbers or a single individual of drug-treated animals may be relevant, even when not statistically significant.

There is no consensus or clear evidence that hepatotoxicity alerts observed in non-rodents are of greater relevance to humans than those in rodents. Retrospective analysis suggested that the dog may be a better predictor of hepatotoxicity in man when compared with rodents or non-human primates (Greaves et al., 2004; Olson et al., 2000). However, in some therapeutic classes, e.g. anticancer drugs, rodents and dogs performed equally well; for some candidate drugs, rodents may be equally relevant to human risk assessment. It is therefore important to use both rodent and non-rodent data for a comprehensive risk assessment for humans.

Clinical chemistry parameters, in combination with hematology and urinalysis data, remain a valuable tool to obtain information on liver toxicity. A selected panel of biomarkers listed in Table 1 should be measured in preclinical studies for the identification of hepatocellular or hepatobiliary injury. The panel for hepatocellular injury should include at least two of the following: Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH). Similarly, at least two of the following serum parameters for identification of hepatobiliary injury should be measured: alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), 5'-nucleotidase (5'-NT) and total bilirubin (TBILI).

ALT as a biomarker has limitations, but it is recognised as the principal non-clinical and clinical biomarker driving diagnosis of drug-induced liver injury (Ozer et al., 2009). ALT is considered to be a

more specific and sensitive indicator of hepatocellular injury than AST in rats, dogs and non-human primates (NHPs). When both values are increased as a consequence of hepatic injury, the magnitude of ALT increase is usually greater than that of AST, in part because of the longer half-life of ALT and the greater proportion of AST bound to mitochondria. Evaluation of both ALT and AST can be useful in helping to distinguish liver injury from muscle injury, since the magnitude of AST elevation is greater than the magnitude of ALT elevation following muscle damage (Nathwani et al., 2005; Ramaiah, 2007). Serum SDH and GDH values may be evaluated as additional indicators of hepatic injury. These biomarkers are not assessed by all investigators and their specificity and sensitivity for drug-induced hepatotoxicity has not been fully defined (Boone et al., 2005). However, to monitor injury induced by compounds that mask ALT activity additional biomarkers of drug-induced liver injury would add value (Ozer et al., 2009).

ALP and TBILI values are routinely measured and are valuable in the assessment of hepatobiliary injury (i.e. liver injury affecting the biliary system) in non-clinical as well as in clinical studies. In humans, serum ALP activity increases with cholestatic liver injury but generally remains below 3x the upper limit of normal (ULN) and is elevated to a much lesser extent than ALT or AST (Boone et al., 2005; Ramaiah, 2007). The measurement of most of these parameters, including ALT, AST, ALP, TBILI, and direct bilirubin concentrations, is also recommended for identification of hepatic injury in humans (Dufour et al., 2000; FDA, 2000bc). Guidelines have been published by the US FDA on the interpretation and management of clinical chemistry findings indicative of liver injury in the absence of histologic data, i.e., results from autopsy or liver biopsy (FDA, 2009). Specifically, an elevation of transaminase activities (ALT and/or AST) in excess of 3x the ULN accompanied by 2x increases above the ULN in TBILI concentration may indicate functionally significant liver damage which, in some patients, may have the potential to progress to life-threatening liver failure (Boone et al., 2005; FDA, 2000b). Recently, proposals have been made to enhance the utility of ALT as a reference standard for DILI (Ozer et al., 2009).

Total protein, albumin, triglycerides, cholesterol, glucose, urea nitrogen, activated partial thromboplastin and prothrombin times data are considered to be supplemental indicators of hepatic function. In drug-induced hepatic injury, evaluation of these parameters may be instrumental in the identification of deleterious effects on glucose metabolism and hepatic synthesis of proteins, lipids, and coagulation factors. These parameters are included in the minimal recommendations for clinical pathology testing in non-clinical studies (Boone et al., 2005). Evaluation of total serum bile acids may provide additional information that aids in hazard characterisation and risk assessment, particularly where cholestatic liver damage is proven or suspected (since bile acid formation and excretion is a key component of bile flow) (Kostrubsky, 2003).

Further examples of a number of liver function and liver injury biomarkers, their documented applications in humans or animals, and their potential advantages as well as limitations have been described in the recent literature (Amacher, 1998 & 2002; Marrer & Dieterle, 2010; Ozer et al., 2008 & 2009).

4.2. Integrated risk assessment

When standard toxicity studies identify a hepatotoxicity hazard, an integrated risk assessment should incorporate the following considerations:

- magnitude and nature of histopathological[1] and/or clinical pathology changes;
- dose response and safety margins in different species;
- reversibility of effects;
- metabolic pathways, the production of (possible reactive) metabolites and potential effects on xenobiotic metabolising enzymes (induction or inhibition);
- comparison of clinical data with non-clinical findings;
- identification of clinical biomarkers;
- disease indication and patient population.

Mean increases in serum ALT levels of 2–4x in the dog and/or rat may raise a concern as an indicator of potential hepatic injury unless a clear alternative explanation is found (Boone et al., 2005). Greater than 3-5x mean levels of ALT are considered adverse, even in the absence of histologic changes, unless the pathogenesis indicates to the contrary (FDA, 2000a). An adverse effect identified in a nonclinical study should be considered as an indicator of potential liability for hepatic injury in humans.

Increases in serum TBILI concentration in individual or group mean data, when compared with concurrent control or pre-test values, should also be critically evaluated and compared with other study data to exclude other factors that may affect serum bilirubin values. Concurrent increases in ALT and bilirubin values in non-clinical toxicity studies should be given particular attention since this pattern has been linked with risk of liver failure in humans (Boone et al., 2005). In the absence of changes in other hepatic parameters or in case of hemolysis, increases in TBILI concentrations alone are unlikely to be adverse and may instead reflect inhibition of transporters mediating bilirubin uptake into hepatocytes (OATP1B1) or efflux of conjugated bilirubin from hepatocytes into bile (MRP2), and/or bilirubin conjugation (UGT1A1) (Ah et al., 2008; Lecureur, 2000).

An important outcome of the integrated risk assessment will be the evaluation of the anticipated safety margin between the circulating plasma drug concentration (C_{max} and AUC) observed in the non-clinical test species at a no-adverse effect level (NOAEL) and the respective plasma concentration required to achieve efficacy in man (FDA, 2000a). This evaluation also needs to take full account of the possible variability in pharmacokinetics in non-clinical species and man, the severity and reversibility of the findings and monitorability in man. Additional non-clinical studies may not be required, especially when the apparent safety margin is of large magnitude. In other cases, additional non-clinical data may be needed to improve the risk assessment prior to clinical development. Moreover, not all changes in the liver are adverse. The clinical approach to a drug that causes a non-adverse adaptive change such as peroxisome proliferation in rat is different to the clinical approach to drugs that cause obvious adverse liver toxicity such as massive hepatocellular necrosis or acute liver failure.

Hepatotoxicity may be identified in non-clinical studies, although the overall concordance between experimental animals and humans for hepatotoxicity may be less than 60% (Greaves et al., 2004; Olson et al., 2000). Generally, most compounds found not to be hepatotoxic in animals are not hepatotoxic in humans. False-negative results in animal studies may be related to insufficient systemic

[1] Liver histopathology is an important tool for identifying and characterising liver injury. The presence of significant apoptosis/necrosis should be addressed (the pattern of cellular damage, the presence of cellular infiltrates, and the presence of necrotic and/or apoptotic cells). Ultrastructural pathology conducted on an as- for e.g., mitochondrial changes, drug accumulation, and early indications of cholestasis, necrosis, steatosis, etc.

exposure to the drug for various reasons. Also, the relatively small number of animals used in toxicity studies may make it difficult to detect hepatotoxicity that occurs at a low incidence. However, it has to be emphasised that there is a lack of information on the human correlate for compounds that are severely hepatotoxic in animals because they never proceed to clinical trials (FDA, 2000a).

Table 1. Clinical chemistry variables that are considered useful in identifying liver toxicity

Parameters[2]	**Hepatocellular**	**Hepatobiliary**
alanine aminotransferase (ALT)	X	
aspartate aminotransferase (AST)	X	
alkaline phosphatase (ALP)		X
total bilirubin (TBILI)		X
gamma glutamyltransferase (GGT)		X
glutamate dehydrogenase (GLDH)	X	
sorbitol dehydrogenase (SDH)	X	
lactate dehydrogenase (LDH)	X	
5'-nucleotidase (5-NT)		X
ornithine carbamyltransferase (OCT)	X	
total bile acids (TBA)	X	X
unconjugated bilirubin (UBILI)	X	

In order to prevent unnecessary use of laboratory animals, additional mechanistic non-clinical *in vivo* investigations to broaden the basis of the safety assessment should only be conducted when the effects noted in the animal species are considered to be of possible clinical relevance. Therefore, primarily *in vitro* systems are encouraged to be used on a case-by-case basis as appropriate in a weight-of-evidence approach in order to provide a better prediction of hepatotoxicity or a better mechanistic understanding of the hepatotoxicity.

4.3. Hypothesis-driven investigative approaches

In case of concerns, a variety of experimental assays and model systems (*in vitro* and *in vivo*) have been described suitable for use in mechanistic investigations that may improve human risk assessment. Useful *in vitro* cell model systems include isolated hepatocytes, co-cultures of hepatocytes with other cell types, cell lines that express defined metabolising enzymes or transporters, liver slices and organotypic liver bioreactors (Gronenberg, 2002). Endpoints that may be assessed range from overt cell cytotoxicity to stress responses, high content cell biology endpoints, changes in gene expression and protein expression, and a broad range of other biomarkers of cellular injury or repair (Beger et al., 2009; Blomme et al., 2009). These approaches are of proven value for investigation of mechanisms of liver injury and are therefore appropriate for use on a case-by-case basis to support risk assessment and strengthen the "weight of evidence" for predictive hepatotoxicity. Typically, they use non-standard and variable experimental designs and/or are not adequately validated with respect to sensitivity and/or specificity for the prediction of clinical safety.

Improvements in the metabolic profiling and drug interactions evaluation of drugs using *in vitro* hepatic systems expressing multiple human or animal drug metabolising enzymes during the non-clinical testing phase may in the future enhance the ability to anticipate both intrinsic and idiosyncratic

[2] Only part of the listed parameters are routinely investigated, others may be used on a case-by-case basis, when considered appropriate.

toxic responses. It has been suggested that subtle individual differences in the hepatic detoxification processes, or in the formation of quantitatively minor but highly reactive metabolites in susceptible individuals, may contribute to rare but unpredictable consequences that occur in a proportion of the population exposed to a drug at therapeutic doses (Amacher, 1998; Evans et al., 2004; Zhang et al., 2010). However, it is recognised that the link between bioactivation, the response of the liver to chemical stress and the occurrence of hepatotoxicity is complex (Antoine et al., 2008) and it has been experimentally demonstrated that in vitro bioactivation alone does not predict toxicity, since many drugs which show bioactivation in vitro have not been associated with hepatotoxicity in the clinic (Obach et al., 2008; Bauman et al., 2009; Gan et al., 2009).

A large number of drugs and other chemicals have been shown to induce hepatic microsomal cytochrome P450 (CYP) forms in experimental animals and humans. Most CYP forms are induced by receptor-mediated mechanisms leading to an increase in gene transcription. Important nuclear receptors involved in the induction of CYP1A, CYP2B, CYP3A and CYP4A subfamily forms comprise, respectively, the aryl hydrocarbon receptor, the constitutive androstane receptor, the pregnane X receptor and the peroxisome proliferator-activated receptor alpha. Hepatic CYP induction can be assessed by *in vivo*, *ex vivo* and by *in vitro* methods. Significant species differences can exist in the enzyme induction response to a given chemical that may be expressed in the toxicological consequences of induction. Hepatic CYP form induction in humans may produce clinically important drug–drug interactions (Zhang et al., 2010). In rodents, hepatic CYP form induction can be associated with the formation of tumours by non-genotoxic modes of action in the liver, thyroid and other tissues (Graham & Lake, 2008). Probably due to differences in the responses of rodent and human hepatocytes to cytokines, some clinical hepatotoxicities are not predicted by standard toxicity studies in rodents. One key aspect is whether these species differences are reflected in differential regulation of cytokine networks in rodents and humans. The cytokine changes implicated in human hepatic cell death may be detected at the molecular level in rodent models (Lacour et al., 2005; Ulrich et al., 2001).

The InnoMed Integrated Project 'Predictive Toxicology – PredTox' (EU Framework Programme 6, 2006 - 2009) showed that various 'omics' technologies may be incorporated into standard rodent short-term repeated dose studies. The joint consortium of Industry and Academia provided evidence that potential biomarkers for hepatotoxic endpoints may be identified by applying a novel systems toxicology approach that integrated the analysis of data from different omics technologies and 'classical' investigational parameters in non-clinical safety studies. This approach will be further elaborated within the Innovative Medicines Inititative (Adler et al., 2010; IMI, 2009).

Overall, detection of hepatotoxicity alerts is a continuous process covering all drug development phases. The principles of the present proposal may also be applied to the resolution of adverse reactions in the liver identified by pharmacovigilance or new publications. Obviously, any new information becoming available during any of the development phases should be integrated in the risk management process.

5. Conclusion

To date, standard non-clinical toxicity studies are the cornerstone of preventing of hepatotoxicity in humans, although their predictive power for all hepatotoxic liabilities in man is unsatisfactory. Nevertheless, conventional non-clinical animal toxicity studies identified and eliminated many compounds that could have been hepatotoxic in humans, which resulted in discontinuation of further drug development. However, novel approaches are needed to improve prediction of drug-induced liver injury (DILI) in humans. Various promising investigative approaches are currently being evaluated for potential screening purposes or use on a case-by-case basis to follow up hepatotoxicity alerts in standard non-clinical toxicity studies. Given that non-clinical hepatotoxic alerts may be generated by drugs that are capable of causing severe DILI in humans as well as drugs that have demonstrated clinically a low incidence or degree of liver effects (e.g., aspirin, tacrine, heparin, hydroxyl-methylglutaryl coenzyme A-reductase inhibitors such as statins), new tests with improved sensitivity as well as improved specificity are needed to predict hepatotoxicity in the clinic.

A number of industry/academic/regulatory consortia are assessing the utility of new approaches. If one or more of these consortia initiatives leads to the validation and acceptance of new approaches, it may be appropriate to revise this document.

6. Abbreviations

5'-NT	*5'-Nucleotidase*
ALP	*Alkaline phosphatase*
ALT	*Alanine aminotransferase*
AST	*Aspartate aminotransferase*
DILI	*Drug-induced liver injury*
GGT	*Gamma glutamyltransferase*
GDH	*Glutamate dehydrogenase*
IDR	*Idiosyncratic drug reaction*
MRP	*Multidrug resistance-associated protein*
NHP	*Non-human primate*
NOAEL	*no adverse effect level*
OATP	*Organic anion transport protein*
SDH	*Sorbitol dehydrogenase*
TBA	*Total bile acids*
TBILI	*Total bilirubin*
UGT	*Uridine diphosphate glucuronosyltransferase*
ULN	*Upper limit of normal*

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