Falk Workshop

The Challenge of Drug-Induced Liver Injury (DILI)

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Abstracts

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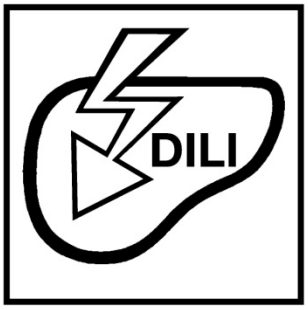
Abstracts of Invited Lectures

Falk Workshop

THE CHALLENGE OF DRUG-INDUCED LIVER INJURY (DILI)



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Session I

Understanding the problem of DILI

DILI as a major risk for new drugs

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Serious drug-induced liver injury (DILI), despite being rare, is a major reason for not approving a drug in development, or for removing one already marketed. With no specific diagnostic biomarker, developing abnormal serum enzyme activities (especially alanine aminotransferase, ALT) remains a signal for possible incipient DILI. Enzyme elevations alone are not harmful, but when associated with jaundice and probably caused by the drug (called “Hy’s law”), there is high probability for acute liver failure, transplantation, or death, DILI accounting for almost 60% of acute liver failure cases in the United States (US). Thus, controlled trials generally include monitoring of serum ALT levels. Previously, trials usually excluded subjects with elevated ALTs, but the arrival of direct-acting antivirals for treating chronic hepatitis C changed that. The threshold of ALT elevation as a warning for possible DILI is uncertain, although generally accepted as 3-fold the “normal” value. In hepatitis C, ALTs may be elevated; if treatment reduces levels, subsequent increases may be compared to a new baseline value. If levels continue to rise, the suspect drug may be discontinued or interrupted for study to exclude all other conditions that mimic DILI. Causality assessment of likelihood and severity is made using expert clinical diagnosis, or a standardized instrument such as RUCAM (**R**oussel-**U**claf **C**ausality **A**ssessment **M**ethod). The US Food and Drug Administration reviews many candidate drugs for DILI using our program “e-DISH” (**e**valuation of **D**rug-**I**nduced **S**erious **H**epatotoxicity, to be described). No drug has been approved since 1998 that later had to be withdrawn for hepatotoxicity.

Dose-dependent versus idiosyncratic DILI: Two different kinds of a problem?

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Drug-induced liver injury (DILI) is a rare but serious clinical problem. A number of drugs can cause severe liver injury and acute liver failure (ALF) at therapeutic doses in a very limited number of patients (< 1:10,000). This idiosyncratic DILI (iDILI), which is currently not predictable in preclinical safety studies, appears to depend on individual susceptibility and the inability to adapt to the cellular stress caused by a particular drug. Progress in understanding the mechanisms are hampered by the absence of reliable animal models. However, “omics” technologies are beginning to develop footprints of subclinical gene expression changes that may assist in identifying drugs with iDILI potential. In striking contrast to iDILI, drugs with dose-dependent hepatotoxicity are mostly detected during preclinical studies and do not reach the market. One notable exception is acetaminophen (APAP, paracetamol), which is a safe drug at therapeutic doses but can cause severe liver injury and ALF after intentional and unintentional overdoses. In fact, APAP overdose is responsible for more ALF cases in the US or UK than all other etiologies combined. Because APAP overdose in the mouse represents a model for the human pathophysiology, substantial progress has been made during the last decade in understanding the mechanisms of cell death, liver injury and recovery. More recently, emerging evidence based on mechanistic biomarker analysis in patients and studies of cell death in human hepatocytes suggest that most of the mechanisms discovered in mice also apply to patients. The rapid development of N-acetylcysteine as antidote against APAP overdose was based on the early understanding of APAP toxicity in mice. However, despite the efficacy of N-acetylcysteine in patients who present early after APAP overdose, there is a need to develop intervention strategies for late presenting patients. In addition, a pressing clinical problem is to better predict on admission who will recover and who will develop ALF and will need a liver transplant to survive. Thus, the different challenges related to DILI are to identify drugs with iDILI potential before they reach the market and for drugs already on the market to identify patients who are at risk to develop iDILI. In contrast, the challenges related to APAP toxicity are to better understand the mechanisms of cell death in order to limit liver injury and prevent ALF and to develop biomarkers that better predict as early as possible who is at risk of developing acute liver failure with poor outcome.

How to diagnose and exclude DILI

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The diagnosis of drug-induced liver injury (DILI) is largely a diagnosis of exclusion because, with the possible exception of protein:drug adducts in paracetamol overdose, there are no laboratory, biopsy, or imaging tests that alone are capable of an establishing an unequivocal diagnosis. However, it is increasing appreciated that drugs that cause DILI typically have characteristic clinical presentations, or "signatures" that can be very useful in the diagnosis of DILI. Indeed, knowing a drug's DILI signature (or sometimes signatures) and the incidence rate of DILI during treatment with that drug, are perhaps the most useful pieces of information in arriving at the diagnosis of DILI. Components of the signature include typical latency from onset of treatment, rate of rise in liver chemistries and rate of resolution when treatment is stopped, whether there are extrahepatic manifestions, whether the injury is hepatocellular, cholestatic or mixed, and sometimes characteristic features on biopsy or serological testing (e.g. liver autoantibodies). A major advance has been the establishment of the LiverTox website (http://livertox.nih.gov/) which provides open access to standardized entries for over 600 different drugs, which include the characteristic clinical presentations when known. LiverTox will also calculate the causality score for individual cases using the RUCAM instrument and case-specific data entered by the site user. However, the problem with standard diagnostic instruments such as the RUCAM is that signatures are not incorporated into the scoring system. The person entering data must therefore subjectively weigh the RUCAM score with the characteristic DILI signature(s) of the drug to arrive at a diagnosis. In the future, it should be possible to construct improved diagnostic instruments that incorporate DILI signatures, data based estimates of the incidence rates of DILI from each drug, and perhaps genetic variants associated with risk of DILI. Of course, these things may not be known in clinical trials of a new drug candidate and the current RUCAM instrument may be most appropriate tool to employ for diagnosis of DILI here, especially for non-hepatologists. However, it is usually the case the severe liver injuries due to new drug candidates occur on a background of much more frequent biochemical abnormalities that have many or all of the characteristics of the severe injuries, including latency and biochemical profile. It is therefore often possible to discern a DILI signature at some point during drug development and retrospectively reassess cases that were adjudicated prior to the identification of the signature.

Preventing DILI – How useful are animal models?

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All new drug candidates have to go through regulatory animal toxicology studies entering into clinical development. However, the value of these studies to predict safety in man is unclear because of the very limited number of studies of animal vs. human toxicity data correlation and the fact that many compounds are stopped following animal toxicology assessment.

DILI is the most common organ toxicity encountered in animal toxicology studies (usually 1–4 week studies in rats and dogs). Contrary to other organ toxicities e.g. kidney, liver toxicity can be monitored by a sensitive biomarker, alanine aminotransferase (ALT), allowing early detection in the clinic at a time when it is fully reversible. Therefore it is not uncommon to move hepatotoxic drug candidates into clinical development when a safety margin has been demonstrated. Occurrence of liver toxicity in animal toxicology studies is correlated with a high risk of DILI during clinical development, usually in phase 1 or 2 trials. On the other hand, false-negative cases remain a major issue in toxicology studies. In a large collaborative retrospective study, approximately 45% of compounds associated with DILI detected during clinical development were negative in animal toxicology studies. Furthermore, most compounds associated with idiosyncratic DILI, mostly detected in large phase 3 trials or post-marketing, are negative in animal toxicology studies. Idiosyncratic DILI is a rare event which is precipitated in an individual by several critical factors occurring simultaneously. These factors may be related to the host (e.g. HLA polymorphism, inflammation), the drug (e.g. reactive metabolites) and/or the environment (e.g. diet/microbiota). Therefore this particular type of toxicity cannot be detected in regulatory toxicology studies and it is very unlikely that the use of new biomarkers including -omics signatures will change this paradigm.

Several animal models have been developed recently that that produce liver toxicity from idiosyncratic DILI-associated drugs e.g. rats pretreated with lipopolysaccharide (LPS), $Sod2^{+/-}$ mice, panels of inbred mouse strains or chimeric mice with humanized liver. These models provide insight into the mechanism of idiosyncratic DILI but are not suitable for screening prospectively new drug candidates.

In conclusion, drug candidates inducing liver toxicity in animal regulatory toxicology studies are correlated with a high risk of DILI in man. However, most compounds associated with idiosyncratic DILI in man are negative in regulatory toxicology studies. No alternative animal model has been shown to predict this particular type of toxicity confidently. Therefore, the best model to predict idiosyncratic DILI is man.

Session II

Dealing with DILI in drug development

Practical issues of pharmacovigilance

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Hepatotoxicity continues to contribute substantially to the attrition of new chemical entities during drug development. Its rarity doesn't allow the detection and accurate estimation of its frequency during phase III clinical trials.

Challenges of pharmacovigilance for hepatotoxicity include inconsistent case definitions in the context of wide range of manifestations of hepatotoxicity which mimic nearly all forms of liver disease, varied threshold to define the adverse reaction compounded by back ground prevalence of raised liver enzymes in patient population and variable latency between onset of exposure to the drug and the manifestation of hepatotoxicity confounded by concomitant medications. Record linkage studies have to overcome inadequate data capturing and incomplete exclusion of alternative explanation to the clinical event; prospective long-term studies are expensive and labour intensive. Therefore, spontaneous reporting of hepatotoxicity has continued to be the major source of label changes by the regulatory authorities. Causality assessment methods still remain the validated tools in pharmacovigilance of drug-induced liver injury (DILI) although these have well recognised limitations.

In the future, robust case control studies should be encouraged following the marketing of new molecular entities to assess the hepatotoxic potential so that the risk benefit ratio can be judged in an individual basis. Algorithms including, drug, host and environmental factors should be developed as a tool for the diagnosis of DILI.

How useful are pharmacogenetics?

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There are several factors impacting the development of Drug-Induced Liver Injury (DILI): Some of them are caused by the environment (co-infections, co-medications, malnutrition, environmental exposures such as alcohol consumption), others are driven by the host (advanced age, female gender, drug exposure, host metabolism, immune responses, liver repair and genetic polymorphisms). Due to the 'multiple determinant hypothesis', the sum of all these factors determines the development of an idiosyncratic drug injury.

Growing areas of DILI-research are focused on exact definitions of unique host factors which could allow a prediction of individual DILIs. One aspect in this context are studies of the effects of genetic variation on the individual response to drugs. It requires the availability of DNA-samples from DILI-patients and the identification of single-nucleotide polymorphisms (SNPs) both in drug-metabolizing enzymes and drug transporters.

Depending on the pathway in which a specific drug is believed to cause DILI (intrinsic, extrinsic or direct pathway), several target genes were identified. Examples for drugs acting along the intrinsic pathway are deficiencies in glutathione-S-transferases (GST) which increase the hepatotoxicity of drugs such as metronidazole, nitrofurantoin or troglitazone. Drugs affecting the extrinsic pathway via a pharmacogenetic correlation are flucloxacillin or ximelagatran.

Despite of being only a part of several factors influencing the occurrence of DILIs, pharmacogenetic studies and gene associations can have promising clinical applications: The final goal are individualized treatment plans for patients. Specific genetic testing has the potential to be utilized as a screening tool prior to administration of a medication with a known potential for DILI as a way to decease DILI events during the development phase of a drug. In addition, it could be a tool to rescue already marketed drugs by excluding a specific small patient population with a high genetic risk of developing DILIs from a specific treatment.

DILI and individual cell models

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Drug-Induced Liver Injury (DILI) accounts for more than 50% of acute liver failures in the USA and is the most common reason for regulatory actions on drugs. Furthermore, DILI is a relevant cause for project terminations in pharmaceutical development. Especially the idiosyncratic form of DILI – affecting only few susceptible patients without clear dependence on dose or duration of therapy – is a threat in late clinical development phases and post-marketing, respectively.

Preclinical approaches to model dose-dependent DILI *in vitro* make use of hepatocytes and/or hepatocyte-like cells in order to predict the liver toxicity of new drugs. Primary human hepatocytes are the golden standard, yet their limited availability and lack of long term stability necessitate the search for alternative sources. Options are hepatic tumor cell lines, immortalized hepatocytes or stem-cell derived hepatocyte-like cells. While advances in availability and long term stability have been achieved, individualization is still a big obstacle for *in vitro* research of idiosyncratic DILI. There are attempts to generate inducible stem cell derived hepatocytes from patients with DILI, yet to our knowledge, no data are available how good these genetically modified cells reflect hepatocyte characteristics of the patient they were derived from.

The major threat for new drugs is the occurrence of only few idiosyncratic DILI cases in late clinical development or post-marketing. In this setting a small number of affected patients may suffice to terminate or withdraw an otherwise promising therapy. Idiosyncratic DILI may be as rare as 1 out of 1 million, but even to exclude a risk higher than 1:1000, cells from over 3000 different donors would have to be tested.

We have developed and investigated MetaHeps®, hepatocyte-like cells generated from peripheral monocytes without genetic modifications. These cells exhibit several hepatocyte-like characteristics and show donor-specific activities of drug-metabolizing enzymes.

With MetaHeps® we have performed *in vitro* investigations in patients with DILI suspicion: By investigating MetaHeps® derived from 23 DILI patients we could show increased *in vitro* susceptibility to the drugs involved in the individual patients. MetaHeps® testing could rule out DILI in 15 patients leading to identification of other causes of acute liver injury. Moreover, MetaHeps® identified the causative agent in polymedicated patients.

In conclusion, *in vitro* research of idiosyncratic DILI requires individual cell models which produce results comparable to the clinical situation. We suggest the MetaHeps® technology as a novel tool to cope with these challenges of DILI.

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